CORA Cork Open Research Archive
Cartlann Taighde Oscailte Chorcaí

Title	Preclinical atherosclerosis in rheumatoid arthritis
Authors	O'Sullivan, Miriam Jane
Publication date	2014
Original Citation	O'Sullivan, M. J. 2014. Preclinical atherosclerosis in rheumatoid arthritis. PhD Thesis, University College Cork.
Type of publication	Doctoral thesis
Rights	© 2014, Miriam J. O'Sullivan. - http://creativecommons.org/licenses/by-nc-nd/3.0/
Download date	2026-10-17 09:37:59
Item downloaded from	https://hdl.handle.net/10468/1908

UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Preclinical Atherosclerosis in Rheumatoid Arthritis

A Thesis Presented To The National University Of Ireland, Cork

For the Degree of Doctor of Philosophy

by

Dr Miriam Jane O'Sullivan MB BCh BAO MMedSc

Department of Medicine

January 2014

Head of Department: Prof Fergus Shanahan MD, BSc, FRCP(UK), FRCPI, FACP, FRCP(C)
Professor of Medicine, National University of Ireland, Cork

Supervisors: Dr Sinead Harney MB, Bch, MMedSc, D.Phil (Oxon)
Consultant Rheumatologist, CUH

Prof David Kerins MD, FRCPI, FACC, FAHA, FASE
Professor of Therapeutics and Consultant Cardiologist
UCC and MUH

Acknowledgements

This work would not have been possible without the help and support of a number of people. Firstly, I would like to thank all the patients who participated and volunteered their time so willingly.

My rheumatology supervisor, Dr Sinead Harney, having completed her own PhD in recent years, was very supportive and understanding of the trials and tribulations that come with clinical research. Her positive attitude and ability to see the silver lining in all situations throughout the course of the work was uplifting. My cardiology supervisor, Professor David Kerins, provided invaluable expertise with respect to the cardiovascular aspects of this study. He was also very supportive and patient in the final writing up period of this thesis.

Professor Michael Molloy introduced me to rheumatology and encouraged me to undertake this PhD. I am grateful for the years of support and wisdom he has provided me in my career thus far.

I thank Mary Daly who helped me continuously throughout this research, both with patient recruitment and follow-up and as a supportive friend over the last number of years. Also, thanks to Lindsay Horgan was always so helpful with administration. I am grateful to Rita Lynch in the department of medicine for her expertise and guidance in the area of medical administration.

Professor Joseph Eustace was an excellent guide in my often turbulent journey through medical statistics. Thank you also to Dr Cathy Dewhurst and Professor Michael Maher for providing the radiology support for this project, and to Mary

Stapleton and her colleagues in the biochemistry department for their support and expertise. Orla Murray was a very patient teacher when it came to showing the ropes of echocardiography to a rheumatologist. I also have to thank Dr Pat Barry for persevering in his task of teaching me the skill set required for assessment of flow mediated dilatation. He had a difficult job.

Drs Sinead Kinsella and Eanna Falvey, who were also writing their theses at the same time as myself, provided moral support on numerous occasions. In particular, thanks to Sinead who somehow seemed to have the answer to all my questions, no matter how many or what time of the day or night I asked her.

I would like to thank my Dad, who died during the early stages of this research and whose last conversation with me was of the importance of sticking to my goals and believing in myself. I have called on this advice numerous times over the last few years. Thanks to my wonderful mum, Mary, who has always been there to listen to and support me. At this stage, she must wonder if my education will ever be complete! Also thanks to my brothers, John and Andrew and my sister, Karoline, for their support and good company.

Finally, I must express my gratitude to three very important men. My husband, Shomik, whose ongoing support and advice kept me going. His belief in me and unfaltering encouragement were what got me over the final hurdle. Without him, this would not be complete. To my sons, Searlan and Jehan, who make me laugh out loud every day and whose expert ability to sleep deeply, allowed me to burn the midnight oil in the final months of writing. They make me realise what is really important in life.

Contents

Declaration of Original Work 12

Abstract 13

Chapter 1. Introduction 16

Rheumatoid Arthritis 16

Cardiovascular Disease 17

Heart Failure 23

Cardiovascular Disease in RA 27

Traditional and non-traditional risk factors for CVD in RA 27

Carotid IMT in RA 32

PVD in RA 32

Endothelial dysfunction in RA 33

Haemostasis in RA 33

Diastolic dysfunction in RA 34

Risk assessment in RA 36

Osteoarthritis 37

Reasons for undertaking this research 38

Hypothesis 39

Aims 39

References 41

Chapter 2. Materials and Methods 59

Patient Recruitment 59

Baseline Demographic Data 60

Phlebotomy 61

Brain Natriuretic Peptide 62

Plasminogen Activator Inhibitor-1 62
Cytokine and Adhesion Molecule Analysis 63
Ankle Brachial Index 64
Transthoracic Echocardiography 65
Limitations of echocardiography in assessment of diastolic function 70
Limitations to echocardiographic measurements in this study 71
Carotid Artery Ultrasound 72
Flow Mediated Dilatation of Brachial Artery 74
Endothelium-Dependent Dilatation 75
Endothelium-Independent Vasodilatation with Nitroglycerin 76
Limitations of FMD technique 77
Edge Detection/FMD Analysis Software 78
Statistical Calculations 82
References 83

Chapter 3. Demographics, Disease Activity Scores and Baseline Bloods 85
Introduction 85
Aims 88
Methods 88
Statistical Methods 89
Demographic Results 90
Gender 93
Age 94
Disease Duration 95
Education Level 97
Occupation 98

Medications 100
DMARD use 100
Biologic use 101
NSAIDs 102
Prednisolone 107
Statins 108
Other Cardiac Medications 109
Non-cardiac Medications 109
Family History 110
Smoking status 111
Smoking Pack Years 112
Alcohol Consumption 113
Other medical conditions 114
Menopause 116
Clinical Examination 117
Weight 119
Height 120
Body Mass Index 121
Waist Hip Ratio 122
General Health Measures and Disease Activity Scores 123
Short Form 36 124
Physical component score 124
Mental Component Score 126
Health Assessment Questionnaire 127
Visual Analogue Scale 130
Disease Activity Scores for Rheumatoid Arthritis 131
Western Ontario and McMaster Universities Osteoarthritis Index 137

Baseline Phlebotomy 139
Serum Concentrations of Antibodies in RA 143
Fasting Lipid and Glucose Profiles 144
Metabolic Syndrome 151
Electrocardiogram 151
Discussion 152
Demographics and Disease Activity 152
Baseline Phlebotomy 155
ECG 159
Conclusions 159
References 162

Chapter 4. Serum, Plasma and Urinary Markers of Inflammation, Endothelial Dysfunction and Thrombosis 168
Introduction 168
Traditional Inflammatory markers in CVD 169
Adhesion molecules in RA and CVD 170
Cytokines in RA and CVD 171
Markers of thrombosis in RA and CVD 173
Urinary protein as a marker of vascular disease in general and RA 174
Aims 176
Methods 177
Cytokine and Adhesion Molecule Analysis 177
PAI-1 analysis 178
Urine for albuminuria measurement 179
Statistical Methods 179
Results for Routine Inflammatory Markers 180

Comparison of ESR, CRP and Urate in RA and OA 180
ESR, CRP and Urate Levels and RA Serological Markers and Anti-Rheumatic Medications 180
ESR, CRP and Urate levels and Markers of Cardiovascular Disease in RA 182
ESR, CRP and Urate in OA 187
Adhesion molecules Results 189
Comparison of Adhesion Molecule Concentrations in RA and OA 189
Adhesion Molecule Concentrations and RA Serological Markers and Anti-Rheumatic Medications 190
Adhesion Molecule Concentrations and Cardiovascular Markers in RA 192
Osteoarthritis and Adhesion Molecule Concentrations 197
Cytokine Results 198
Comparison of Cytokine Concentrations in RA and OA 198
Cytokine Concentrations and RA Serological Markers and Anti-Rheumatic Medications 200
Cytokine Concentrations and Cardiovascular Markers in RA 209
Osteoarthritis and Cytokine Concentrations 212
Results for Markers of Thrombosis 214
Comparison of Thrombotic Markers in RA and OA 214
Markers of Thrombosis and RA Serological Markers and Anti-Rheumatic Medications 216
Markers of Thrombosis and Cardiovascular Markers in RA 218
Osteoarthritis and Markers of Thrombosis 222
Results for Urinary Protein-Creatinine Ratio and Microalbuminuria 222
Discussion 224
Comparison of ESR, CRP and Urate in RA and OA 224
Comparison of Adhesion Molecules in RA and OA 225

Adhesion Molecule Concentrations and RA Serological Markers and Anti-Rheumatic Medications 227
Adhesion Molecule Concentrations and Cardiovascular Markers in RA 228
Osteoarthritis and Adhesion Molecule Concentrations 230
Comparison of Cytokine Concentrations in RA and OA 232
Cytokine Concentrations and RA Serological Markers and Anti-Rheumatic Medications 232
Cytokine Concentrations and Cardiovascular Markers in RA 235
OA and Cytokine Concentrations 235
Comparison of Thrombotic Markers in RA and OA 236
Markers of Thrombosis and RA Serological Markers and Anti-Rheumatic Medications 238
Markers of Thrombosis and Cardiovascular Markers in RA 239
Osteoarthritis and Markers of Thrombosis 240
Urinary Protein Measures in RA and OA 241
Conclusions 242
References 246

Chapter 5. Macrovascular Endothelial Function in RA 264
Background 264
Aims 268
Methods 268
Statistical Methods 270
Results 271
Comparison of FMD and NMD in RA and OA patients 271
Comparison of FMD and NMD in Males and Females 274
RA Disease Activity and Macrovascular Endothelial Function 275

Assessment of Relationship Between Macrovascular Endothelial Function and Markers of CVD in RA 277
Assessment of FMD and NMD in OA patients 280
Discussion 282
Macrovascular function in RA and OA patients 282
Comparison of FMA and NMD in males and females 283
RA Disease Measures and Endothelial Function 284
CVD risks and subclinical markers in RA patients and macrovascular endothelial function 286
Macrovascular Endothelial Function in Osteoarthritis 289
Conclusions 290
References 292

Chapter 6. Sub-Clinical Carotid Atherosclerosis in RA 299
Introduction 299
Aims 301
Methods 301
Statistical Methods 300
Results 303
Comparison of Carotid Intima-Media Thickness in RA and OA groups 303
Carotid IMT and Characteristics of RA patients 303
Markers of CV disease and Carotid IMT measurements in RA 306
Results for Presence of Carotid Plaque 311
Carotid Plaque and Lipid Profile 311
Carotid Plaque and Diastolic Dysfunction 312
Carotid Disease in the OA group 314
Discussion 315

Comparison of Carotid Intima-Media Thickness in RA and OA groups 315
Carotid IMT and Characteristics of RA patients 316
Markers of CV disease and Carotid IMT measurements in RA 318
Carotid Plaque and Lipid Profile 321
Carotid Plaque and Diastolic Dysfunction 321
Conclusions 322
References 324

Chapter 7. Peripheral Vascular Disease in RA 331
Introduction 331
Aims 334
Methods 332
Statistical Methods 335
Results 336
Discussion 342
Inflammation in RA and ABI 344
ABI and Markers of subclinical CVD and traditional CV risks in RA patients 345
Conclusions 348
References 349

Chapter 8. Diastolic function in Rheumatoid Arthritis 355
Introduction 355
Aims 358
Methods 358
Transthoracic Echocardiography 358
NT-proBNP analysis 359
Statistical Methods 359

Results 360

Systolic Function 360

Diastolic Function 365

Disease activity, anti-rheumatic medications and diastolic function 372

Cardiovascular risks and diastolic function 374

Discussion 380

Conclusions 391

References 393

Chapter 9. Summary and Concluding Remarks 402

Summary of our findings 402

Traditional CV risks and Markers of Inflammation 402

Subclinical Atherosclerosis 404

Endothelial Dysfunction 406

Diastolic dysfunction: 407

Salient points for the practicing clinician 408

Recommendations for the Future 409

Study Limitations 411

Future plans 412

References 413

Appendices

Appendix A (Study Questionnaire) 414

Appendix B (Short Form 36) 420

Appendix C (FMD Protocol) 424

Appendix D (Abstracts)
426i

Declaration of Original Work

I declare that this thesis is my own work and has not been submitted for another degree at University College Cork or elsewhere.

Abstract

Introduction:

There is accumulating evidence of an increased risk of cardiovascular morbidity and mortality in rheumatoid arthritis patients. A combination of both traditional cardiovascular risks and rheumatoid specific factors appear to be responsible for driving this phenomenon. Rheumatoid arthritis has been an orphan of cardiologists in the past and rheumatologists themselves are not good at CVD screening. Identifying the extent of preclinical atherosclerosis in RA patients will help us to appreciate the magnitude of this serious problem in an Irish population.

Methods:

We undertook a cross-sectional study of 63 RA patients and 48 OA controls and compared the 2 groups with respect to 1) traditional CV risks factors, 2) serum biomarkers of inflammation, including CRP, TNFα, IL6 and PAI-1, 3) carotid intima-media thickness (cIMT), carotid plaque and ankle-brachial index (ABI) as markers of pre-clinical atherosclerosis, 4) biochemical and ultrasonic measures of endothelial dysfunction and 5) serum and echocardiographic measures of diastolic dysfunction. Within the RA group, we also investigated for associations between markers of inflammation, subclinical atherosclerosis and diastolic dysfunction.

Results:

Prevalence of traditional CV risks was similar in the RA and OA groups. A number of biomarkers of inflammation were significantly higher in the RA group: CRP, fibrinogen, IL-2, -4, -6, TNFα. PAI-1, a marker of thrombosis, correlated with disease activity and subclinical atherosclerosis in RA patients. With regard to subclinical atherosclerosis measures, RA patients had a significantly lower ABI than OA patients. Carotid plaque and cIMT readings were similar in RA and OA patients. Assessment of endothelial function revealed that RA patients had significantly higher concentrations of adhesion molecules, in particular sero-positive RA patients and RA smokers. Adhesion molecule concentrations were associated with markers of diastolic dysfunction in RA. Urine PCR, another marker of endothelial dysfunction also correlated with diastolic dysfunction in RA. Assessment of endothelial function with flow mediated dilatation (FMD) found no difference between the RA and OA groups. Disease activity scores in RA patients were associated with endothelial dysfunction, as assessed by FMD.

Conclusions:

We did not find significant differences in measures of subclinical atherosclerosis, flow mediated dilatation or diastolic function between RA and OA patients. This is most likely in part due to the fact that there is increasing evidence that OA has an

inflammatory component to its pathogenesis and is associated with metabolic syndrome and increased CV risk.

We reported a significant association between urinary PCR and measures of diastolic dysfunction. Urinary PCR may be a useful screening tool for diastolic dysfunction in RA. The association between RA disease activity and measures of vascular function supports the theory that the excess cardiovascular burden in RA is linked to uncontrolled inflammation.

Chapter 1

Introduction

Rheumatoid Arthritis:

Rheumatoid arthritis (RA) is the most common inflammatory arthritis. It affects 0.5% to 1% of the general population worldwide. The incidence in women is twice that in men. In Ireland, the exact prevalence has not been established, however studies in Dublin indicate a prevalence rate could be estimated at 0.5% (1). A more recent estimate from Arthritis Ireland (2009) suggests there are 40,000 individuals with rheumatoid arthritis in Ireland which would equate to a prevalence of 1.22% (2) Arthritis Ireland (2009) estimates that 70% of those affected by the disease in Ireland are women. Peak age of onset worldwide is between 35 to 45 years, again with geographic variances (3).

The disease primarily affects synovial joints in a symmetrical pattern, particularly the small joints of the hands and feet. Chronic inflammation produces joint damage mediated by cytokines, chemokines and metalloproteases and results in pain, deformity and functional limitation, causing substantial morbidity and accelerated mortality (4).

Along with the synovial based inflammatory events, RA is associated with a number of important extra- articular manifestations, which significantly impact upon patient morbidity and mortality. Chief amongst these is the increase in cardiovascular risk amongst RA patients, which has been highlighted from an early stage in the disease process (5)

Excess mortality in RA is well recognized with standardised mortality ratios 2-3 times those of the general population, a statistic unchanged over 40 years despite considerable advances in the treatment of both RA and cardiovascular disease. It is considered that up to 50% of the excess mortality in RA is attributable to a cardiovascular event (6). A large US health study showed that women with RA were twice as likely to suffer from a myocardial infarction as age and sex matched controls (5). Furthermore, RA is associated with premature cardiovascular disease and shortens life expectancy by 3-18 years (5). Thus the failure to reduce mortality in patients with RA is doubly concerning in view of the major advances in the treatment of RA and in our understanding of the pathophysiology and treatment of coronary artery disease over the past four decades.

Cardiovascular Disease:

Cardiovascular disease (CVD) is the leading cause of death worldwide. There are approximately 18 million deaths worldwide from CVD each year and 2 – 3 times that many experience non-fatal cardiovascular events (7).

Cardiovascular disease is the most common cause of death in Ireland. A significant proportion of these deaths are premature. According to the central statistics office, in 2010 34% of all deaths in Ireland were due to diseases of the circulatory system (8). They occupied the 3rd highest number of acute hospitals' bed days and were the 5th most common reason for discharge from acute hospitals (9). The institute of Public Health in Ireland have predicted a significant rise in the prevalence of hypertension,

diabetes, coronary artery disease and stroke for the years 2015 and 2020 relative to 2007 figures (10). Conditions of the cardiovascular system account for the largest fraction of the annual expenditure by the general medical services scheme and the drug payment scheme (11).

Cardiovascular disease is the result of a pathological process known as atherosclerosis, which causes disease of the coronary, cerebral and peripheral arteries (12). Atherosclerosis begins in childhood with the development of fatty streaks, which are the initial histological phase and represent focal thickening of the intima with an increase in smooth muscle cells and extracellular matrix (13). These fatty streaks contain macrophages which become engorged with lipids and are known are foam cells, the hallmark of early atheroma. Fatty streaks evolve to form fibrous plaques and eventually, advanced lesions with a necrotic lipid rich core which can ultimately calcify (14).

There are numerous factors involved in the development of atherosclerosis including endothelial dysfunction, inflammatory cytokine and adhesion molecule activation and traditional risk factors, including smoking, hypertension and dyslipidaemia.

The initial stage of atherosclerosis is persistent endothelial activation which results in endothelial dysfunction. LDL particles enter the intima from the vessel lumen and are modified and oxidized and then play an important role in inducing endothelial cell activation (15). This dysfunction of the endothelial lining is characterized by an increased production of adhesion molecules, such as V-CAM and I-CAM and pro-

inflammatory cytokines, such as MCP-1, interleukin-1 (IL1) and tumour necrosis factor-alpha (TNFα) and a reduction of nitric oxide levels (16).

A number of cytokines, including IL-1 and TNFα, have a multitude of atherogenic effects (17). TNFα, a pro-inflammatory mediator, plays a critical role in driving the inflammation in RA and atherosclerosis. It triggers the upregulation of IL1, IL6, IL8, matrix metalloproteinases and prostaglandins (18).

Inflammatory cytokines enhance the expression of cell surface molecules such as ICAM-1 and VCAM-1 on endothelial cells, smooth muscle cells, and macrophages. They are also involved in inducing cell proliferation and contributing to the production of reactive oxygen species. Other cytokines, such as interleukin-4 and interleukin-10, are antiatherogenic. Still others, such as interferon-gamma, have a variety of actions with both pro- and anti-atherogenic consequences.

MCP-1 works by interacting with the CCR2 receptor on the surface of the monocytes. Within the intima, monocytes are then modified into macrophages. An important mediator of this process is macrophage colony-stimulating factor. The macrophages are capable of engulfing lipoproteins, in particular, oxidized LDL and then take on a foamy appearance (foam cells). Foam cells proliferate within the intima to form plaques. They produce a number of active substances, including metalloproteinases (MMPs), which can destroy the arterial extracellular matrix (19).

T lymphocytes are another important cell in atheroma formation. Although they are much less abundant than macrophages in plaque, they actively participate in progression of the lesion. They appear to have a regulatory role over the plaque monocytes and macrophages. The Th1 cell subset of T lymphocytes are pro-inflammatory, while Th2 cells tend to exert anti-inflammatory functions. (20).

After atheroma formation, inflammation is still further implicated in the atherosclerosis story. T lymphocytes, after migration into the intima, produce the pro-inflammatory CD40 cytokine, which in turn results in production of extracellular MMPs and the procoagulant tissue factor (TF). This tissue factor promotes a thrombogenic plaque (21). T lymphocytes also exert a negative effect on the collagen support system that keeps the fibrous cap of a plaque stable. Smooth muscle cell production of collagen is inhibited by interferon- γ produced by T lymphocytes within the plaque (22).

A formed plaque is made up of a lipid core covered by a fibrous cap of extracellular matrix and smooth muscle cells. The base of the lesion contains lymphocytes and foam cells. A stable plaque typically contains a small lipid core and has a strong fibromuscular cap. A vulnerable or unstable plaque has a much larger lipid core, a larger quantity of active T cells and foam cells at the base, and a thin protective cap. The exact mechanism of plaque rupture is not known but MMPs, produced by macrophages may disrupt the thin cap of unstable plaques, leading to rupture.

The traditional cardiovascular risk factors, including smoking, dyslipidaemia and diabetes play critical roles in the development of atherosclerosis.

Smoking impacts on all stages of atherosclerosis, from the earliest stages of endothelial dysfunction, right through to acute clinical thrombotic events (23). It interferes with the body's haemostatic processes by altering the function of endothelial cells (24). It has also been found to be associated with a number of inflammatory markers, including CRP, IL6 and TNF (25).

Abnormalities of lipid profiles play a critical role in atherosclerosis development and progression (26). Epidemiologic studies conducted in countries around the world have shown an increasing incidence of atherosclerosis when serum cholesterol concentrations were above 3.9 mmol/L (27, 28). Dyslipidaemia initially affects the endothelial cells, which upon activation express new adhesion molecules and chemotactic factors that provoke an inflammatory process (29).

The pro-atherogenic lipid profile associated with diabetes is thought to be one of the main mechanisms by which diabetes increases cardiovascular risk. Diabetic patients have an increased production of VLDL in the liver and research has shown that this increased VLDL production is the main defect in atherogenic dyslipidaemia (30).

The presence of type II diabetes mellitus is associated with acceleration of pre-existing atherosclerosis to clinical cardiovascular events (31).

Because of the seriousness of the acute manifestations of atherosclerosis, it is useful to detect it early in its course. The gold standard for imaging the coronary arteries is a coronary angiogram; however, there are a number of less invasive imaging techniques that are useful surrogates.

Use of carotid ultrasound to measure intima-medial thickness (IMT) and the presence of plaque is a non-invasive method of assessing extra-coronary atherosclerosis. B-mode ultrasound is an easily assessable method of imaging the carotid arteries. A number of studies, including the Rotterdam study and the Cardiovascular Health Study have shown an association between carotid artery IMT and the risk of coronary heart disease events (32-34).

Measurement of carotid artery IMT and plaque appears to be a valuable screening tool; however, the above studies did not find evidence that treating patients based on IMT measurements would reduce their future risk of CVD events.

Peripheral arterial disease can be detected by measuring the ankle brachial index. Ankle brachial index (ABI) is not only a marker of peripheral artery disease but also of generalized atherosclerosis. Reductions in ABI have been associated with the presence of cardiovascular risk factors and with higher rates of coronary and cerebrovascular disease (35, 36). In 2008, a meta-analysis performed by the Ankle Brachial Index Collaboration, reported that ABI provided independent risk information compared with the Framingham risk score (FRS) and also, an ABI < 0.9 in combination with FRS, approximately doubled the risk of all cause and cardiovascular mortality and major coronary events across all Framingham risk groups (37). The advantage of ABI measurement is that it is easy to learn and can be performed in the doctor's office, without having to refer to specialist centres, It is also a well-tolerated procedure and non- invasive.

Endothelial dysfunction, one of the earliest steps in the development of atherosclerosis, is measured in research studies most commonly by non-invasive brachial artery ultrasound to assess flow-mediated dilatation (38). This procedure is much less invasive and better tolerated than the gold standard for diagnosis of endothelial dysfunction, intracoronary infusion of acetylcholine. It involves measurement of the change in diameter of a conduit artery after a period of induced ischaemia. It is primarily nitric oxide mediated and is reduced in people with CV risk

factors and atherosclerosis (39). It has been shown to correlate well with the endothelial vasodilator function of coronary vessls (40).

Measuring markers of coagulation dysfunction can also provide useful information regarding cardiovascular risk. There is increasing evidence that markers of impaired fibrinolysis are associated with the development of coronary heart disease (41). Intravascular thrombosis is induced by the plasminogen activator / plasmin system. The most important inhibitor of this system is plasminogen activator inhibitor-1 (PAI-1). PAI-1 has been shown to be associated with increased CVD risk in the general population (42). High circulating levels of PAI-1 have shown to significantly predict the onset of myocardial infarction (43) and hypofibrinolysis due to high PAI-1 concentrations can be found in patients with high thrombotic risk, such as those with the metabolic syndrome and obesity (44).

Heart Failure:

Systolic heart failure has seen improvements in survival over recent years, however, heart failure overall is the only cardiac disorder that has not seen reductions in prevalence or significant improvements in survival over the last decade. It is the most common medical cause for hospitalization. Its prevalence increases with age and it affects about 8% of females and 10% of males over the age of 60 years (45). In 2008, there were more than 20,000 patients admitted with heart failure in Ireland, 90% of which were emergency admissions (46).

Primary diastolic dysfunction is an important cause of heart failure, as it is often a silent alteration preceding systolic dysfunction. When diastolic dysfunction leads to symptoms of heart failure, it is known as heart failure with preserved ejection fraction (HF-PEF), previously known as diastolic heart failure. There is preservation of the ejection fraction in patients who have isolated diastolic heart failure. Diastolic heart failure accounts for up to 40 to 50% of all cases of heart failure. It is estimated that mortality rates for patients with diastolic heart failure are 4 times higher than those of the healthy general population (47). There are a number of clinical conditions which can result in primary diastolic heart failure. These include hypertension, obesity diabetes mellitus, coronary artery disease and restrictive and hypertrophic cardiomyopathies (48).

Diastole refers to the phase of the cardiac cycle when the heart is relaxed. It is classically divided into 4 stages: isovolumetric relaxation, rapid filling, slow filling and atrial contraction(48). Diastolic function is basically a measure of left ventricular filling ability. In normal sinus rhythm, diastolic flow from the left atrium to the left ventricle across the mitral valve has 2 components: the E wave, which represents early diastolic filling and the A wave, reflecting atrial contraction in late diastole. The E wave is influenced by the relative pressure gradient between the left atrium (LA) and left ventricle (LV) and the A wave is primarily influenced by LV compliance and LA contractility. Two other important components of diastolic flow are the isovolumetric relaxation time (IVRT) and the deceleration time (DT). The IVRT is the time interval from cessation of left ventricular outflow to the onset of mitral valve inflow. Deceleration time of the E velocity is the interval from peak E to

a point of intersection of the deceleration of flow with the baseline. It correlates with the time of pressure equalization between the LA and LV (49).

Defects in distensibility, filling or relaxation of the left ventricle result in diastolic dysfunction (50). These defects lead to an increase in ventricular resistance as the ventricle loses its ability to accept an adequate blood volume. The LV becomes stiff and less compliant. The result is a rise in left ventricular diastolic pressure.

The Mayo Clinic Echocardiography Laboratory uses a 4 stage grading system for diastolic dysfunction. Grade 1 is impaired myocardial relaxation. There is a prolongation of the isovolumetric relaxation time and the deceleration time. The mitral E velocity, which is a measure of the early transmitral gradient**,** is reduced and the A velocity or A wave, which represents atrial contraction, is increased. This results in an abnormal E/A ratio of less than one. During grade 2 of diastolic dysfunction, an increase in left atrial pressure combined with the abnormal myocardial relaxation of grade 1, gives rise to a pattern known as pseudonormalisation of mitral flow filling pattern. Here the deceleration time returns to normal and the E/A ratio is between 1 and 1.5. There are a number of techniques which can be utilised at the time of echocardiography to distinguish between a "true-normal" and "pseudo- normal" pattern, these include: positioning the patient in the upright-, sitting position, asking the patient to perform Valsalva manoeuvre or asking them to take sublingual nitroglycerin. These techniques decrease the pre-load and help to unmask any relaxation defect of the left ventricle. In grade 3, the abnormal E / A ratio reversal is associated with a restrictive pattern, whereby the E wave

deceleration time is reduced to less than 130 msec, but this normalises with the Valsalva manoeuvre. This is known a reversible restrictive diastolic dysfunction. Grade 4 occurs when this restrictive pattern is no longer reversible.

The pulsed wave Doppler technique is used at the time of echocardiography, to detect diastolic dysfunction. In an apical 4-chamber view, a sample volume of 1 to 2 mm is placed between the mitral leaflet tips during diastole to measure mitral velocities. A sample volume of 3-4 mm placed again between the tips of the mitral leaflets, measures the isovolumetric relaxation time. Pulsed-wave Doppler has a drawback whereby it is affected by tachycardia, atrio-ventricular block and changes in pre- and after-load.

Using tissue Doppler imaging (TDI) adds additional information regarding diastolic function. It involves recording an early diastolic mitral annulus velocity, e'. For this measurement the sample position must be at the mitral annulus and the velocity recorded at end-expiration. TDI allows measurement of velocities that represent longitudinal contraction (positive systolic wave, Sa) and relaxation (early negative diastolic wave, e' and late negative diastolic wave, a') (51). The e' wave reflects the velocity of ventricular lengthening in early diastole.

Tissue Doppler imaging appears to be independent of heart rate and pre-load which make it superior to traditional pulsed wave measurements. Using both the conventional pulsed wave velocity and TDI, the E/e' ratio can be calculated and this is a validated reliable index for the estimation of pulmonary capillary wedge pressure. This non-invasive tool allows assessment of left ventricular pressures.

Cardiovascular Disease in RA:

Similar to diabetic patients, people with RA have a 1.5 - 2.0-fold increased risk of developing coronary artery disease compared with the general population (52, 53).
In a retrospective study of 603 RA patients, Maradit-Kremers et al found that RA patients has a significantly higher rate of myocardial infarction prior to the incidence of RA compared with non-RA controls . This CVD event risk was found to be independent of traditional cardiovascular risk factors. This suggests that the CVD risk associated with inflammatory arthritis precedes the diagnosis of RA. They also found that RA patients were less likely to receive coronary artery bypass grafting and had a higher rate of sudden death (54). Dhawan et al., reported that RA patients also have a two-fold increased risk of stroke compared with controls, and this risk rises to three-fold in those with the disease for a decade or more (55). Nicola et al reported that RA patients, in particular those who are rheumatoid factor positive, are twice as likely to develop heart failure as controls.

Traditional and non-traditional risk factors for CVD in RA:

As in the general population, the traditional cardiovascular risk factors such as male sex, age, diabetes, metabolic syndrome and dyslipidaemia are also implicated in CVD in RA patients (56). It is still unclear whether hypertension is more common in RA patients than the general population (57). However, it appears to be underdiagnosed and undertreated in RA (58). A number of factors associated with RA, such as inactive lifestyle, increased body fat composition relative to muscle

mass and medications, in particular, corticosteroids, are obvious potential causes of hypertension in RA. A direct link between inflammation and hypertension in RA has not been studied in detail, however; control of disease activity has been associated with improvements in blood pressure (59).

An elevated BMI is considered a traditional cardiovascular risk factor. S. Gabriel and colleagues retrospectively studied 822 RA patients and 603 age and gender matched non-RA controls. They collected information on cardiovascular risks and outcomes (60). They found, as expected, that a low BMI in the non-RA cohort, was not associated with an increased risk of cardiovascular death. Conversely, they found a threefold increase risk of cardiovascular death associated with low BMI in the RA group. Even after correcting for traditional risk factors, including hypertension, smoking and diabetes mellitus, this association was still significant.

Patients with RA have alterations in body composition, due to factors such as a sedentary lifestyle causing accumulation of fat and activation of the NF-κB pathway stimulating muscle breakdown. This can result in rheumatoid cachexia, which is characterised by low muscle mass and high fat mass (61). Perhaps this cachectic state, resulting in a low BMI, is in part due to chronic active inflammation, therefore suggesting that inflammation in RA may play a role in cardiovascular death.

In the general population, elevations in total cholesterol and LDL cholesterol and decreased levels of HDL cholesterol are associated with cardiovascular events. This is not the typical lipid profile of a patient with active RA. Active RA is a catabolic state mediated by TNFα and other pro-inflammatory cytokines. These inflammatory mediators are associated with low total cholesterol and low HDL cholesterol.

The lipid pattern in RA is consistent with that seen in several other inflammatory conditions. Typically, RA patients have low total cholesterol, a low HDL and an elevated triglyceride level, resulting in an unfavourable ratio of total cholesterol to HDL (62). Markers of inflammation such as ESR and CRP have been shown to have an inverse association with HDL cholesterol, in RA patients (63). Lower total and LDL cholesterol levels have been shown to be associated with higher CV risk (64).

.

Smoking is the strongest known environmental risk factor for RA (65). The prevalence of cigarette smoking appears to be higher in RA patients as reported in a recent meta-analysis (OR 1.56, 95% CI 1.34, 1.80) (66). RA patients who smoke are more likely to be sero-positive for rheumatoid factor and it is these RF positive RA patients who tend to do worse from a cardiovascular point of view. They also have more severe joint disease and have an increased DMARD requirement (67).

Current smoking status was found to contribute to CV risk in RA patients in a study by Gonzalez et al. However, its contribution to overall CV risk was less in RA patients than controls (68). Similar to low BMI and low total cholesterol being associated with increased CV risk in RA, this finding demonstrates that traditional CV risk factors appear to have a weaker association with CV events in RA patients compared to controls.

A study in 2006 looked at Framingham risk score and coronary calcium scores in RA patients and controls. It found that patients with longstanding RA had a greater probability of having higher coronary calcium scores than controls with any level of

Framingham risk. It was concluded from the study that factors other than those in the Framingham risk score, contribute to the accelerated atherosclerosis of RA patients (69). Chung's group have also found an association between the metabolic syndrome and RA. This association was found to be independent of body mass index, which suggests an inflammatory driving force for the development of metabolic syndrome (70).

A number of disease markers for RA, including elevated ESR, CRP, anti-CCP-antibodies, joint swelling and destructive changes in joint radiographs, have been shown to be significantly associated with increased risk of CV disease and death (71-73). Subjects who have tested positive for rheumatoid factor have a significantly increased risk of MI and heart failure (74).

Medications used for the treatment of RA can also affect CV risk. Glucocorticoid use dampens inflammation and helps mobility however; their use is associated with an increased risk of a number of CV risk factors, such as altered body fat composition, insulin resistance and dyslipidaemia. Presence of carotid plaque has also been reported to be associated with steroid use (75, 76).

Goodson et al reported that NSAID use was not associated with increased CV death in patients with inflammatory arthritis (77). Disease control with both synthetic and biologic DMARDS appears to reduce CV risk. Methotrexate, the most commonly prescribed synthetic DMARD for the treatment of RA, was found to be associated with 21% fewer CV events, according to a recent meta-analysis (78).

Barnabe et al found that anti-TNF therapy appears to be associated with reduced risk of CV events (79). Factors that promote atherosclerotic complications such as elevated platelet levels, circulating cytokine levels and leucocyte trafficking are all

reduced by blockade of TNF-α (80). TNF inhibitors also seem to have beneficial effect on insulin resistance (81), lipid profile and carotid atherosclerosis (82, 83). Studies so far on the effect of TNF blockade on endothelial function in RA show short-term benefits only and more long-term follow-up studies are required to assess this further (84, 85).

Another theory that may explain the increased prevalence of CVD in RA is that both diseases may share common genetic risk factors. The main susceptibility gene for RA development in northern European populations is HLA-DRB1 (86). This gene has been shown to be associated with disease severity. Specific HLA-DRB1 genotypes, including HLA-DRB1*0404 and HLA-DRB1*0401 are associated with severe disease and extra-articular manifestations (87). In 2008, Farragher et al reported that the HLA-DRB1 genotype predicted all-cause and CVD mortality in RA patients. In particular, the HLD-DRB1*0404 and *0401 alleles were associated with a high hazard ratio for CVD mortality (88). The fact that these alleles are associated with more severe RA, suggest that the link reported above with CVD mortality could be due to a high inflammatory burden driving both diseases.

Carotid IMT in RA:

A number of studies have found that cIMT, a marker of subclinical atherosclerosis, is increased in RA patients compared with healthy controls due to the inflammatory burden of the disease (89-92). One study also reported that the annual rate of progression on cIMT was higher in RA than non-RA patients and correlated with markers of RA disease activity (93).

Carotid IMT is associated with longstanding disease (94) but there is also evidence that it is abnormal within 1 year of RA symptom onset (95). Gonzalez-Gay et al followed up RA patients without clinical evidence of CVD and found that cIMT had a high predictive power for development of CV events over a 5 year period (96).

Plaque, as well as IMT, is also more common in RA patients than controls (91).

PVD in RA:

Studies examining the relationship between RA and peripheral vascular disease have been very limited. Perhaps this is because researchers have tended to concentrate on coronary artery disease instead, due to the increased mortality associated with it. Also, clinicians may erroneously attribute symptoms of peripheral vascular disease to the arthritis itself.

The prevalence of PVD in RA has been reported as 10% (97). Similar to the general population, this rate increases with age and in the presence of coronary artery disease. Del Rincon et al did report a higher rate of arterial obstruction in RA patients compared with controls, independent of traditional CV risks (98). Contrary

to this study, a 10 year follow-up study of over 800 RA patients did not find a difference in the incidence of PVD compared with that in non-RA controls (99).

Endothelial dysfunction in RA:

Patients with RA have been shown to have evidence of abnormal flow mediated dilatation, a measure of endothelial dysfunction, early in the course of their disease and this is present prior to any cardiovascular symptoms (100). Markers of disease activity and cardiovascular risk are associated with abnormal brachial artery flow mediated dilatation in RA (101, 102). There is also evidence that treatment of inflammation in RA patients is associated with improvements in brachial artery flow, however it is not yet proven whether these effects are long-lasting (103).

Microalbuminuria, another marker of endothelial dysfunction, is a more frequent finding in RA patients than in non-RA controls (104). However, whether it is associated with other markers of CV disease in RA patients, has not being investigated extensively.

Haemostasis in RA:

Studies in the general population have found the process of atherosclerosis is associated with alterations of biomarkers of haemostasis. Patients with acute MI and unstable angina have elevated levels of markers of thrombosis (105). In an earlier

study, Johansson et al found that the tPA/PAI-1 complex was independently associated with the development of a first-ever stroke, supporting the hypothesis that disturbances in fibrinolysis precede cerebrovascular events (106).

In RA populations, there is evidence that CVD risk correlates with abnormalities of the coagulation system. Having a diagnosis of RA has been found to predict increased levels of PAI-1 and fibrinogen, and inhibitors of the fibrinolytic system are elevated in RA patients (107). Some earlier studies of coagulation in RA found similar results (108, 109). Sodergren et al reported that carotid intima-media thickness and endothelial dysfunction in RA patients were associated with markers of haemostasis (110). An increase in biomarkers of haemostasis leads to a pro-thrombotic state and these alterations may be one of the mechanisms by which RA patients are at an increased risk of CV events.

Diastolic dysfunction in RA:

Disease duration and the age of onset of rheumatoid arthritis have been shown to correlate with the presence of diastolic dysfunction on echocardiography (111, 112).

Rexhepaj et al found a higher prevalence of LV diastolic dysfunction in RA patients without visible evidence of CV disease, compared to controls. They looked at LV diastolic function in 80 RA patients and found a significantly lower early diastolic flow velocity and E/A ratio in the RA group (113).

Heart failure in RA is more likely to be associated with a preserved ejection fraction and less likely to display the typical symptoms and signs of heart failure, compared to hear failure in non-RA patients. One suggested reason for this is that diastolic

function in RA patients may be inherently different from that in non-RA groups (114).

A limited number of studies have assessed the incidence and prevalence rates for congestive heart failure (CHF) in rheumatoid arthritis. In a population-based study by Gabriel et al, medical records of 450 RA patients were compared to those of 450 age and sex matched non-RA controls. Over a thirty-year period they found 78 cases of incident CHF in the RA group, compared with 54 cases in the non-RA group, relative risk 1.6 (95% CI 1.12-2.27). This risk of incident CHF did not hold through for osteoarthritis (OA) patients compared to non- OA controls (115). In a follow-on study of the same cohort of patients, Nicola et al found an increased risk of incident CHF in rheumatoid factor positive and negative patients compared to non-RA controls (hazard ratio 2.29 and 1.34) (116).

In 2003, Wolfe et al published data on the lifetime prevalence of CHF in RA patients. Using a combined cohort of RA patients from more than 700 community-based rheumatology practices, they found an adjusted lifetime relative risk of CHF of 1.43 (95% CI 1.24-1.33) in the RA group compared to OA patients and an adjusted lifetime prevalence of CHF of 2.34% in the RA group compared to 1.64% in the OA group (117).

There is an excess of CHF related mortality in RA patients that was first described by Mutru et al in 1989. They found that RA patients who were age- and gender-matched to controls, had a higher CHF- attributed mortality in both males and females ($p = 0.004$, $p = 0.042$, respectively) (118).

Risk assessment in RA:

In 2009, the CARRE investigation, a cross-sectional study looking at CVD prevalence in RA and diabetic patients found that cardiovascular affection in RA was much higher than in controls and similar to that of diabetic patients (119). Van Halm et al reported a CVD prevalence of 5% in the control group, 12.4% in the diabetic group and 12.9% in the RA group. Compared to the non-diabetic controls, the relative risk of CVD was 2.3 in diabetic patients and 3.1 in RA patients. After adjustment for traditional CV risks, the relative frisk of CVD was still high, 2.0 in diabetic patients and 2.7 in RA patients. The authors concluded that CV risk factors only partially explained the increased risk in the RA group and that RA itself, like diabetes, should be considered a separate risk factor. This highlights that CV screening and treatment in RA patients needs to be a priority for clinicians.

Because of findings from the CARRE study and others mentioned above, the European League Against Rheumatism, in 2010, developed a set of evidenced-based recommendations for CV risk management in RA patients (120). These recommendations include that RA should be regarded as a condition associated with a higher CVD risk, due to increased prevalence of traditional risk factors and the inflammatory burden. They advise that adequate control of disease activity is necessary to lower CV risk. If there are no national guidelines for CV risk assessment, the Systematic Coronary Risk Evaluation

(SCORE) model or the Framingham risk score should be used to assess risk. Peters et al advised that score models should be adapted for RA patients by introducing a

1.5 multiplication factor when the RA patient fits 2 of the following criteria: disease duration of greater than 10 years, anti-CCP antibody or rheumatoid factor positive and presence of certain extra-articular manifestations (vasculitis, pericarditis and pleuritis).

Because of the potential anti-inflammatory role of statins, ACE-inhibitors and angiotensin blockers, these medications should be used when treatment for dyslipidaemia and /or hypertension is required. Caution is also advised by the expert group with respect to use of NSAIDs, particularly in those RA patients with documented CV risk. They advise using the lowest possible corticosteroid dose.

The EULAR expert committee reiterated that although rheumatologists are now starting to acknowledge the increased CV risk in RA, attention needs to be given to detecting and managing these risks.

Osteoarthritis:

Osteoarthritis (OA) is a common and disabling degenerative joint disease with increasing prevalence and socioeconomic impact. It is the most common chronic joint disorder, resulting in pain, deformity and eventually, disability. The American College of Rheumatology defined OA as " a heterogeneous group of conditions that lead to joint symptoms and signs, which are associated with defective integrity of articular cartilage, in addition to related changes in the underlying bone at the joint margins" (121).

The age-standardized and sex-standardized incidence of hand, hip and knee OA is 100/100,000 person-years, 88/100,000 person-years and 240/100,000 person-years, respectively (122).

The incidence of OA in Ireland is increasing. There are 2 main reasons for this, one is the aging population, who have an increased prevalence of OA and the other is the obesity epidemic, which is a major risk factor for development of OA.

Reasons for undertaking this research:

We undertook this study to investigate the degree of sub-clinical atherosclerosis in our RA patients. Cardiovascular disease is common but is under-diagnosed and under-investigated in RA patients. Given the extent of the excess cardiovascular morbidity and mortality associated with RA we felt it was important to characterise the degree of this serious extra-articular manifestation in our RA cohort. The magnitude of pre-clinical atherosclerosis in RA has not been studied extensively in an Irish population to date and we fell that this area should be high priority for rheumatologists.

Heart disease and other chronic diseases have been described by Ban Ki Moon as a "public health emergency in slow motion" (123).

Hypothesis:

Our hypothesis was that patients with rheumatoid arthritis are more likely to display evidence of pre-clinical atherosclerosis compared to patients with osteoarthritis, due to the inflammatory effects of their disease on vascular function.

Our aims were as follows:

To conduct a cross-sectional comparison of patients with rheumatoid arthritis and patients with a non-inflammatory arthritis, namely osteoarthritis, attending a hospital based rheumatology clinic in order to:

A) Quantify the difference in prevalence between the 2 groups regarding:

A1) Presence of subclinical atherosclerosis using 1) ultrasound measured carotid intima- media wall thickness and presence of carotid plaque and 2) measurement of ankle-brachial index as a marker of peripheral vascular disease

A2) Presence of functional impairment of the vasculature by assessment of endothelial dysfunction as measured by 1) brachial artery ultrasound to measure forearm flow mediated dilatation 2) a panel of vascular active factors, including plasminogen activator inhibitor type 1, E-selectin, P-selectin and intracellular adhesion molecules and 3) spot urine albumin creatinine ratio

A3) Presence of established end organ damage by assessment of left ventricular systolic and diastolic dysfunction using transthoracic echocardiography and measurement of brain natruretic peptide (BNP).

B) Determine the Presence, Strength, Significance and Independence of the association of RA with above outcomes using binary logistic regression and adjusting for established and novel cardiovascular risk factors,.

C) Quantify the agreement and explore the Potential Cost Effectiveness of

(i) BNP as a measure of left ventricular dysfunction

(ii) Spot urine albumin creatinine ratio as a measure of endothelial dysfunction.

These investigations conducted by a collaboration of highly experienced cardiologists, rheumatologists and radiologists will provide important mechanistic insights into occurrence and potential determinants of cardiovascular disease and facilitate the training of the applicant in rigorous, high quality patient centered clinical research.

References:

1. Power D, Codd M, Ivers L, Sant S, Barry M. Prevalence of rheumatoid arthritis in Dublin, Ireland: a population based survey. Irish journal of medical science. 1999;168(3):197-200. Epub 1999/11/30.

2. 'Services for People with Rheumatoid Arthritis: International Comparisons'. 2009;Availablefrom: http://www.nao.org.uk/publications/0809/services_for_people_with_rheum.aspx.

3. Woolf AD, Akesson K. Can we reduce the burden of musculoskeletal conditions? The European action towards better musculoskeletal health. Best practice & research Clinical rheumatology. 2007;21(1):1-3. Epub 2007/03/14.

4. Cush J, Weinblatt, M. Kavanaugh, A. Rheumatoid Arthritis: Early Diagnosis and Treatment: Blackwell (Professional Communications Inc): New York; 2008.

5. Solomon DH, Karlson EW, Rimm EB, Cannuscio CC, Mandl LA, Manson JE, et al. Cardiovascular morbidity and mortality in women diagnosed with rheumatoid arthritis. Circulation. 2003;107(9):1303-7. Epub 2003/03/12.

6. Gabriel SE, Crowson CS, O'Fallon WM. Mortality in rheumatoid arthritis: have we made an impact in 4 decades? The Journal of rheumatology. 1999;26(12):2529-33. Epub 1999/12/22.

7. Nieuwlaat R, Schwalm JD, Khatib R, Yusuf S. Why are we failing to implement effective therapies in cardiovascular disease? European heart journal. 2013;34(17):1262-9. Epub 2013/02/05.

8. Office CS. Vital statistics fourth quarter and yearly summary 2010. 2011.

9. Institute EaSR. Activity in acute public hospitals in Ireland: annual report 2010. 2011.

10. Balanda KP BS, Fahy S, McLaughlin A Making chronic conditions count: hypertension, stroke, coronary heart disease, diabetes. Institute of Public Health. Ireland. 2010.

11. Executive HS. Primary Care Reimbursement: statistical analysis of claims and payments 2009. 2011.

12. Faxon DP, Fuster V, Libby P, Beckman JA, Hiatt WR, Thompson RW, et al. Atherosclerotic Vascular Disease Conference: Writing Group III: pathophysiology. Circulation. 2004;109(21):2617-25. Epub 2004/06/03.

13. Davies MJ, Woolf N, Rowles PM, Pepper J. Morphology of the endothelium over atherosclerotic plaques in human coronary arteries. British heart journal. 1988;60(6):459-64. Epub 1988/12/01.

14. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W, Jr., et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation. 1995;92(5):1355-74. Epub 1995/09/01.

15. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. The New England journal of medicine. 2005;352(16):1685-95. Epub 2005/04/22.

16. Ross R. Atherosclerosis--an inflammatory disease. The New England journal of medicine. 1999;340(2):115-26. Epub 1999/01/14.

17. Szmitko PE, Wang CH, Weisel RD, de Almeida JR, Anderson TJ, Verma S. New markers of inflammation and endothelial cell activation: Part I. Circulation. 2003;108(16):1917-23. Epub 2003/10/22.

18. Maini RN, Elliott MJ, Brennan FM, Feldmann M. Beneficial effects of tumour necrosis factor-alpha (TNF-alpha) blockade in rheumatoid arthritis (RA). Clinical and experimental immunology. 1995;101(2):207-12. Epub 1995/08/01.

19. Libby P. Inflammation in atherosclerosis. Nature. 2002;420(6917):868-74. Epub 2002/12/20.

20. Hansson GK, Libby P. The immune response in atherosclerosis: a double-edged sword. Nature reviews Immunology. 2006;6(7):508-19. Epub 2006/06/17.

21. Mach F, Schonbeck U, Bonnefoy JY, Pober JS, Libby P. Activation of monocyte/macrophage functions related to acute atheroma complication by ligation of CD40: induction of collagenase, stromelysin, and tissue factor. Circulation. 1997;96(2):396-9. Epub 1997/07/15.

22. Amento EP, Ehsani N, Palmer H, Libby P. Cytokines and growth factors positively and negatively regulate interstitial collagen gene expression in human vascular smooth muscle cells. Arteriosclerosis and thrombosis : a journal of vascular biology / American Heart Association. 1991;11(5):1223-30. Epub 1991/09/01.

23. Ambrose JA, Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. Journal of the American College of Cardiology. 2004;43(10):1731-7. Epub 2004/05/18.

24. Barua RS, Ambrose JA. Mechanisms of coronary thrombosis in cigarette smoke exposure. Arteriosclerosis, thrombosis, and vascular biology. 2013;33(7):1460-7. Epub 2013/05/21.

25. Bermudez EA, Rifai N, Buring JE, Manson JE, Ridker PM. Relation between markers of systemic vascular inflammation and smoking in women. The American journal of cardiology. 2002;89(9):1117-9. Epub 2002/05/04.

26. Steinberg D, Witztum JL. Oxidized low-density lipoprotein and atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology. 2010;30(12):2311-6. Epub 2010/11/19.

27. Stamler J, Wentworth D, Neaton JD. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). JAMA : the journal of the American Medical Association. 1986;256(20):2823-8. Epub 1986/11/28.

28. Romm PA, Green CE, Reagan K, Rackley CE. Relation of serum lipoprotein cholesterol levels to presence and severity of angiographic coronary artery disease. The American journal of cardiology. 1991;67(6):479-83. Epub 1991/03/01.

29. Manduteanu I, Simionescu M. Inflammation in atherosclerosis: a cause or a result of vascular disorders? Journal of cellular and molecular medicine. 2012;16(9):1978-90. Epub 2012/02/22.

30. Arca M, Pigna G, Favoccia C. Mechanisms of diabetic dyslipidemia: relevance for atherogenesis. Current vascular pharmacology. 2012;10(6):684-6. Epub 2012/12/25.

31. Saely CH, Rein P, Vonbank A, Huber K, Drexel H. Type 2 diabetes and the progression of visualized atherosclerosis to clinical cardiovascular events. International journal of cardiology. 2013;167(3):776-80. Epub 2012/04/10.

32. Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo M, Sharrett AR, et al. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987-1993. American journal of epidemiology. 1997;146(6):483-94. Epub 1997/09/18.

33. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK, Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. The New England journal of medicine. 1999;340(1):14-22. Epub 1999/01/08.

34. del Sol AI, Moons KG, Hollander M, Hofman A, Koudstaal PJ, Grobbee DE, et al. Is carotid intima-media thickness useful in cardiovascular disease risk assessment? The Rotterdam Study. Stroke; a journal of cerebral circulation. 2001;32(7):1532-8. Epub 2001/07/07.

35. Newman AB, Siscovick DS, Manolio TA, Polak J, Fried LP, Borhani NO, et al. Ankle-arm index as a marker of atherosclerosis in the Cardiovascular Health Study. Cardiovascular Heart Study (CHS) Collaborative Research Group. Circulation. 1993;88(3):837-45. Epub 1993/09/01.

36. van der Meer IM, Bots ML, Hofman A, del Sol AI, van der Kuip DA, Witteman JC. Predictive value of noninvasive measures of atherosclerosis for incident myocardial infarction: the Rotterdam Study. Circulation. 2004;109(9):1089-94. Epub 2004/03/03.

37. Ankle Brachial Index C, Fowkes FG, Murray GD, Butcher I, Heald CL, Lee RJ, et al. Ankle brachial index combined with Framingham Risk Score to predict cardiovascular events and mortality: a meta-analysis. JAMA : the journal of the American Medical Association. 2008;300(2):197-208. Epub 2008/07/10.

38. Al-Qaisi M, Kharbanda RK, Mittal TK, Donald AE. Measurement of endothelial function and its clinical utility for cardiovascular risk. Vascular health and risk management. 2008;4(3):647-52. Epub 2008/10/02.

39. Joannides R, Haefeli WE, Linder L, Richard V, Bakkali EH, Thuillez C, et al. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. Circulation. 1995;91(5):1314-9. Epub 1995/03/01.

40. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, et al. Close relation of endothelial function in the human coronary and peripheral circulations. Journal of the American College of Cardiology. 1995;26(5):1235-41. Epub 1995/11/01.

41. Kohler HP, Grant PJ. Plasminogen-activator inhibitor type 1 and coronary artery disease. The New England journal of medicine. 2000;342(24):1792-801. Epub 2000/06/15.

42. Fibrinogen Studies C, Danesh J, Lewington S, Thompson SG, Lowe GD, Collins R, et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. JAMA : the journal of the American Medical Association. 2005;294(14):1799-809. Epub 2005/10/13.

43. Juhan-Vague I, Pyke SD, Alessi MC, Jespersen J, Haverkate F, Thompson SG. Fibrinolytic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. ECAT Study Group. European Concerted Action on Thrombosis and Disabilities. Circulation. 1996;94(9):2057-63. Epub 1996/11/01.

44. Franchini M, Targher G, Montagnana M, Lippi G. The metabolic syndrome and the risk of arterial and venous thrombosis. Thrombosis research. 2008;122(6):727-35. Epub 2007/11/13.

45. Braunwald E. Chronic heart failure: a look through the rear view mirror. European heart journal. 2013;34(19):1391-2. Epub 2012/10/30.

46. "What is heart failure?" Irish Heart Foundation and HSE 2013.

47. Gary R, Davis L. Diastolic heart failure. Heart & lung : the journal of critical care. 2008;37(6):405-16. Epub 2008/11/11.

48. Oh JK SJ, Jamil Tajik A. The Echo Manual. 3rd ed2006. p. 120-1.

49. Lester SJ, Tajik AJ, Nishimura RA, Oh JK, Khandheria BK, Seward JB. Unlocking the mysteries of diastolic function: deciphering the Rosetta Stone 10 years later. Journal of the American College of Cardiology. 2008;51(7):679-89. Epub 2008/02/19.

50. Aurigemma GP, Gaasch WH. Clinical practice. Diastolic heart failure. The New England journal of medicine. 2004;351(11):1097-105. Epub 2004/09/10.

51. Sitia S, Atzeni F, Sarzi-Puttini P, Di Bello V, Tomasoni L, Delfino L, et al. Cardiovascular involvement in systemic autoimmune diseases. Autoimmunity reviews. 2009;8(4):281-6. Epub 2008/09/27.

52. Solomon DH, Goodson NJ, Katz JN, Weinblatt ME, Avorn J, Setoguchi S, et al. Patterns of cardiovascular risk in rheumatoid arthritis. Annals of the rheumatic diseases. 2006;65(12):1608-12. Epub 2006/06/24.

53. Peters MJ, van Halm VP, Voskuyl AE, Smulders YM, Boers M, Lems WF, et al. Does rheumatoid arthritis equal diabetes mellitus as an independent risk factor for cardiovascular disease? A prospective study. Arthritis and rheumatism. 2009;61(11):1571-9. Epub 2009/10/31.

54. Maradit-Kremers H, Nicola PJ, Crowson CS, Ballman KV, Gabriel SE. Cardiovascular death in rheumatoid arthritis: a population-based study. Arthritis and rheumatism. 2005;52(3):722-32. Epub 2005/03/08.

55. Dhawan SS, Quyyumi AA. Rheumatoid arthritis and cardiovascular disease. Current atherosclerosis reports. 2008;10(2):128-33. Epub 2008/04/18.

56. Dessein PH, Joffe BI. Suppression of circulating interleukin-6 concentrations is associated with decreased endothelial activation in rheumatoid arthritis. Clinical and experimental rheumatology. 2006;24(2):161-7. Epub 2006/06/10.

57. Panoulas VF, Metsios GS, Pace AV, John H, Treharne GJ, Banks MJ, et al. Hypertension in rheumatoid arthritis. Rheumatology. 2008;47(9):1286-98. Epub 2008/05/10.

58. Panoulas VF, Douglas KM, Milionis HJ, Stavropoulos-Kalinglou A, Nightingale P, Kita MD, et al. Prevalence and associations of hypertension and its control in patients with rheumatoid arthritis. Rheumatology. 2007;46(9):1477-82. Epub 2007/08/21.

59. Klarenbeek NB, van der Kooij SM, Huizinga TJ, Goekoop-Ruiterman YP, Hulsmans HM, van Krugten MV, et al. Blood pressure changes in patients with recent-onset rheumatoid arthritis treated with four different treatment strategies: a post hoc analysis from the BeSt trial. Annals of the rheumatic diseases. 2010;69(7):1342-5. Epub 2010/05/18.

60. Gabriel SE. Heart disease and rheumatoid arthritis: understanding the risks. Annals of the rheumatic diseases. 2010;69 Suppl 1:i61-4. Epub 2010/01/09.

61. Stavropoulos-Kalinoglou A, Metsios GS, Smith JP, Panoulas VF, Douglas KM, Jamurtas AZ, et al. What predicts obesity in patients with rheumatoid arthritis? An investigation of the interactions between lifestyle and inflammation. International journal of obesity. 2010;34(2):295-301. Epub 2009/10/28.

62. Toms TE, Panoulas VF, Smith JP, Douglas KM, Metsios GS, Stavropoulos-Kalinoglou A, et al. Rheumatoid arthritis susceptibility genes associate with lipid levels in patients with rheumatoid arthritis. Annals of the rheumatic diseases. 2011;70(6):1025-32. Epub 2011/03/15.

63. Lee YH, Choi SJ, Ji JD, Seo HS, Song GG. Lipoprotein(a) and lipids in relation to inflammation in rheumatoid arthritis. Clinical rheumatology. 2000;19(4):324-5. Epub 2000/08/15.

64. Toms TE, Symmons DP, Kitas GD. Dyslipidaemia in rheumatoid arthritis: the role of inflammation, drugs, lifestyle and genetic factors. Current vascular pharmacology. 2010;8(3):301-26. Epub 2009/09/18.

65. Costenbader KH, Feskanich D, Mandl LA, Karlson EW. Smoking intensity, duration, and cessation, and the risk of rheumatoid arthritis in women. The American journal of medicine. 2006;119(6):503 e1-9. Epub 2006/06/06.

66. Boyer JF, Gourraud PA, Cantagrel A, Davignon JL, Constantin A. Traditional cardiovascular risk factors in rheumatoid arthritis: a meta-analysis. Joint, bone, spine : revue du rhumatisme. 2011;78(2):179-83. Epub 2010/09/21.

67. Albano SA, Santana-Sahagun E, Weisman MH. Cigarette smoking and rheumatoid arthritis. Seminars in arthritis and rheumatism. 2001;31(3):146-59. Epub 2001/12/12.

68. Gonzalez A, Maradit Kremers H, Crowson CS, Ballman KV, Roger VL, Jacobsen SJ, et al. Do cardiovascular risk factors confer the same risk for cardiovascular outcomes in rheumatoid arthritis patients as in non-rheumatoid arthritis patients? Annals of the rheumatic diseases. 2008;67(1):64-9. Epub 2007/05/23.

69. Chung CP, Oeser A, Avalos I, Gebretsadik T, Shintani A, Raggi P, et al. Utility of the Framingham risk score to predict the presence of coronary atherosclerosis in patients with rheumatoid arthritis. Arthritis research & therapy. 2006;8(6):R186. Epub 2006/12/16.

70. Chung CP, Avalos I, Oeser A, Gebretsadik T, Shintani A, Raggi P, et al. High prevalence of the metabolic syndrome in patients with systemic lupus erythematosus: association with disease characteristics and cardiovascular risk factors. Annals of the rheumatic diseases. 2007;66(2):208-14. Epub 2006/08/12.

71. Maradit-Kremers H, Nicola PJ, Crowson CS, Ballman KV, Jacobsen SJ, Roger VL, et al. Raised erythrocyte sedimentation rate signals heart failure in patients with rheumatoid arthritis. Annals of the rheumatic diseases. 2007;66(1):76-80. Epub 2006/07/05.

72. Liang KP, Kremers HM, Crowson CS, Snyder MR, Therneau TM, Roger VL, et al. Autoantibodies and the risk of cardiovascular events. The Journal of rheumatology. 2009;36(11):2462-9. Epub 2009/10/17.

73. Rho YH, Chung CP, Oeser A, Solus J, Asanuma Y, Sokka T, et al. Inflammatory mediators and premature coronary atherosclerosis in rheumatoid arthritis. Arthritis and rheumatism. 2009;61(11):1580-5. Epub 2009/10/31.

74. Tomasson G, Aspelund T, Jonsson T, Valdimarsson H, Felson DT, Gudnason V. Effect of rheumatoid factor on mortality and coronary heart disease. Annals of the rheumatic diseases. 2010;69(9):1649-54. Epub 2009/07/25.

75. Dessein PH, Joffe BI, Stanwix AE, Christian BF, Veller M. Glucocorticoids and insulin sensitivity in rheumatoid arthritis. The Journal of rheumatology. 2004;31(5):867-74. Epub 2004/05/05.

76. Hafstrom I, Rohani M, Deneberg S, Wornert M, Jogestrand T, Frostegard J. Effects of low-dose prednisolone on endothelial function, atherosclerosis, and traditional risk factors for atherosclerosis in patients with rheumatoid arthritis--a randomized study. The Journal of rheumatology. 2007;34(9):1810-6. Epub 2007/08/19.

77. Goodson NJ, Brookhart AM, Symmons DP, Silman AJ, Solomon DH. Non-steroidal anti-inflammatory drug use does not appear to be associated with increased cardiovascular mortality in patients with inflammatory polyarthritis: results from a primary care based inception cohort of patients. Annals of the rheumatic diseases. 2009;68(3):367-72. Epub 2008/04/15.

78. Micha R, Imamura F, Wyler von Ballmoos M, Solomon DH, Hernan MA, Ridker PM, et al. Systematic review and meta-analysis of methotrexate use and risk of cardiovascular disease. The American journal of cardiology. 2011;108(9):1362-70. Epub 2011/08/23.

79. Barnabe C, Martin BJ, Ghali WA. Systematic review and meta-analysis: anti-tumor necrosis factor alpha therapy and cardiovascular events in rheumatoid arthritis. Arthritis care & research. 2011;63(4):522-9. Epub 2010/10/20.

80. Feldmann M, Maini RN. Lasker Clinical Medical Research Award. TNF defined as a therapeutic target for rheumatoid arthritis and other autoimmune diseases. Nature medicine. 2003;9(10):1245-50. Epub 2003/10/02.

81. Tam LS, Tomlinson B, Chu TT, Li TK, Li EK. Impact of TNF inhibition on insulin resistance and lipids levels in patients with rheumatoid arthritis. Clinical rheumatology. 2007;26(9):1495-8. Epub 2007/01/24.

82. Allanore Y, Kahan A, Sellam J, Ekindjian OG, Borderie D. Effects of repeated infliximab therapy on serum lipid profile in patients with refractory rheumatoid arthritis. Clinica chimica acta; international journal of clinical chemistry. 2006;365(1-2):143-8. Epub 2005/09/24.

83. Del Porto F, Lagana B, Lai S, Nofroni I, Tinti F, Vitale M, et al. Response to anti-tumour necrosis factor alpha blockade is associated with reduction of carotid

intima-media thickness in patients with active rheumatoid arthritis. Rheumatology. 2007;46(7):1111-5. Epub 2007/04/24.

84. Tikiz H, Arslan O, Pirildar T, Tikiz C, Bayindir P. The effect of anti-tumor necrosis factor (TNF)-alpha therapy with etanercept on endothelial functions in patients with rheumatoid arthritis. Anadolu kardiyoloji dergisi : AKD = the Anatolian journal of cardiology. 2010;10(2):98-103. Epub 2010/04/13.

85. Mazzoccoli G, Notarsanto I, de Pinto GD, Dagostino MP, De Cata A, D'Alessandro G, et al. Anti-tumor necrosis factor-alpha therapy and changes of flow-mediated vasodilatation in psoriatic and rheumatoid arthritis patients. Internal and emergency medicine. 2010;5(6):495-500. Epub 2010/09/17.

86. Thomson W, Harrison B, Ollier B, Wiles N, Payton T, Barrett J, et al. Quantifying the exact role of HLA-DRB1 alleles in susceptibility to inflammatory polyarthritis: results from a large, population-based study. Arthritis and rheumatism. 1999;42(4):757-62. Epub 1999/04/22.

87. Turesson C, Schaid DJ, Weyand CM, Jacobsson LT, Goronzy JJ, Petersson IF, et al. The impact of HLA-DRB1 genes on extra-articular disease manifestations in rheumatoid arthritis. Arthritis research & therapy. 2005;7(6):R1386-93. Epub 2005/11/10.

88. Farragher TM, Goodson NJ, Naseem H, Silman AJ, Thomson W, Symmons D, et al. Association of the HLA-DRB1 gene with premature death, particularly from cardiovascular disease, in patients with rheumatoid arthritis and inflammatory polyarthritis. Arthritis and rheumatism. 2008;58(2):359-69. Epub 2008/02/02.

89. van Sijl AM, Peters MJ, Knol DK, de Vet HC, Gonzalez-Gay MA, Smulders YM, et al. Carotid intima media thickness in rheumatoid arthritis as compared to

control subjects: a meta-analysis. Seminars in arthritis and rheumatism. 2011;40(5):389-97. Epub 2010/10/05.

90. Chatterjee Adhikari M, Guin A, Chakraborty S, Sinhamahapatra P, Ghosh A. Subclinical atherosclerosis and endothelial dysfunction in patients with early rheumatoid arthritis as evidenced by measurement of carotid intima-media thickness and flow-mediated vasodilatation: an observational study. Seminars in arthritis and rheumatism. 2012;41(5):669-75. Epub 2011/11/01.

91. Roman MJ, Moeller E, Davis A, Paget SA, Crow MK, Lockshin MD, et al. Preclinical carotid atherosclerosis in patients with rheumatoid arthritis. Annals of internal medicine. 2006;144(4):249-56. Epub 2006/02/24.

92. Pieringer H, Schumacher S, Stuby U, Biesenbach G. Augmentation index and large-artery remodeling in patients with longstanding rheumatoid arthritis compared with healthy controls. Seminars in arthritis and rheumatism. 2009;39(3):163-9. Epub 2008/10/01.

93. Nagata-Sakurai M, Inaba M, Goto H, Kumeda Y, Furumitsu Y, Inui K, et al. Inflammation and bone resorption as independent factors of accelerated arterial wall thickening in patients with rheumatoid arthritis. Arthritis and rheumatism. 2003;48(11):3061-7. Epub 2003/11/13.

94. Gonzalez-Juanatey C, Llorca J, Testa A, Revuelta J, Garcia-Porrua C, Gonzalez-Gay MA. Increased prevalence of severe subclinical atherosclerotic findings in long-term treated rheumatoid arthritis patients without clinically evident atherosclerotic disease. Medicine. 2003;82(6):407-13. Epub 2003/12/10.

95. Hannawi S, Haluska B, Marwick TH, Thomas R. Atherosclerotic disease is increased in recent-onset rheumatoid arthritis: a critical role for inflammation. Arthritis research & therapy. 2007;9(6):R116. Epub 2007/11/08.

96. Gonzalez-Juanatey C, Llorca J, Martin J, Gonzalez-Gay MA. Carotid intima-media thickness predicts the development of cardiovascular events in patients with rheumatoid arthritis. Seminars in arthritis and rheumatism. 2009;38(5):366-71. Epub 2008/03/14.

97. Guellec D, Bressollette L, Gueguen F, Jousse-Joulin S, Marhadour T, Devauchelle-Pensec V, et al. Is routine ankle-brachial pressure index evaluation useful in rheumatoid arthritis? Joint, bone, spine : revue du rhumatisme. 2013;80(1):111-3. Epub 2012/07/31.

98. del Rincon I, Haas RW, Pogosian S, Escalante A. Lower limb arterial incompressibility and obstruction in rheumatoid arthritis. Annals of the rheumatic diseases. 2005;64(3):425-32. Epub 2004/07/24.

99. Bacani AK, Gabriel SE, Crowson CS, Heit JA, Matteson EL. Noncardiac vascular disease in rheumatoid arthritis: increase in venous thromboembolic events? Arthritis and rheumatism. 2012;64(1):53-61. Epub 2011/09/10.

100. Hannawi S, Marwick TH, Thomas R. Inflammation predicts accelerated brachial arterial wall changes in patients with recent-onset rheumatoid arthritis. Arthritis research & therapy. 2009;11(2):R51. Epub 2009/04/07.

101. Sarli B, Baktir AO, Cebicci M, Dogan Y, Demirbas M, Kurtul S, et al. Predictors of Endothelial Dysfunction in Patients With Rheumatoid Arthritis. Angiology. 2013. Epub 2013/10/01.

102. Sandoo A, Kitas GD, Carroll D, Veldhuijzen van Zanten JJ. The role of inflammation and cardiovascular disease risk on microvascular and macrovascular endothelial function in patients with rheumatoid arthritis: a cross-sectional and longitudinal study. Arthritis research & therapy. 2012;14(3):R117. Epub 2012/05/19.

103. Bosello S, Santoliquido A, Zoli A, Di Campli C, Flore R, Tondi P, et al. TNF-alpha blockade induces a reversible but transient effect on endothelial dysfunction in patients with long-standing severe rheumatoid arthritis. Clinical rheumatology. 2008;27(7):833-9. Epub 2007/12/14.

104. Pedersen LM, Nordin H, Svensson B, Bliddal H. Microalbuminuria in patients with rheumatoid arthritis. Annals of the rheumatic diseases. 1995;54(3):189-92. Epub 1995/03/01.

105. Tousoulis D, Antoniades C, Bosinakou E, Kotsopoulou M, Tsoufis C, Marinou K, et al. Differences in inflammatory and thrombotic markers between unstable angina and acute myocardial infarction. International journal of cardiology. 2007;115(2):203-7. Epub 2006/06/22.

106. Johansson L, Jansson JH, Boman K, Nilsson TK, Stegmayr B, Hallmans G. Tissue plasminogen activator, plasminogen activator inhibitor-1, and tissue plasminogen activator/plasminogen activator inhibitor-1 complex as risk factors for the development of a first stroke. Stroke; a journal of cerebral circulation. 2000;31(1):26-32. Epub 2000/01/08.

107. Dimitroulas T, Douglas KM, Panoulas VF, Toms T, Smith JP, Treharne GJ, et al. Derangement of hemostasis in rheumatoid arthritis: association with demographic, inflammatory and metabolic factors. Clinical rheumatology. 2013;32(9):1357-64. Epub 2013/05/16.

108. Wallberg-Jonsson S, Cvetkovic JT, Sundqvist KG, Lefvert AK, Rantapaa-Dahlqvist S. Activation of the immune system and inflammatory activity in relation to markers of atherothrombotic disease and atherosclerosis in rheumatoid arthritis. The Journal of rheumatology. 2002;29(5):875-82. Epub 2002/05/23.

109. Kamper EF, Kopeikina LT, Trontzas P, Potamianou A, Tsiroglou E, Stavridis JC. The effect of disease activity related cytokines on the fibrinolytic potential and cICAM-1 expression in rheumatoid arthritis. The Journal of rheumatology. 2000;27(11):2545-50. Epub 2000/11/28.

110. Sodergren A, Karp K, Boman K, Eriksson C, Lundstrom E, Smedby T, et al. Atherosclerosis in early rheumatoid arthritis: very early endothelial activation and rapid progression of intima media thickness. Arthritis research & therapy. 2010;12(4):R158. Epub 2010/08/18.

111. Udayakumar N, Venkatesan S, Rajendiran C. Diastolic function abnormalities in rheumatoid arthritis: relation with duration of disease. Singapore medical journal. 2007;48(6):537-42. Epub 2007/06/01.

112. Wislowska M, Jaszczyk B, Kochmanski M, Sypula S, Sztechman M. Diastolic heart function in RA patients. Rheumatology international. 2008;28(6):513-9. Epub 2007/10/25.

113. Rexhepaj N, Bajraktari G, Berisha I, Beqiri A, Shatri F, Hima F, et al. Left and right ventricular diastolic functions in patients with rheumatoid arthritis without clinically evident cardiovascular disease. International journal of clinical practice. 2006;60(6):683-8. Epub 2006/06/30.

114. Davis JM, 3rd, Roger VL, Crowson CS, Kremers HM, Therneau TM, Gabriel SE. The presentation and outcome of heart failure in patients with rheumatoid arthritis differs from that in the general population. Arthritis and rheumatism. 2008;58(9):2603-11. Epub 2008/09/02.

115. Gabriel SE, Crowson CS, O'Fallon WM. Comorbidity in arthritis. The Journal of rheumatology. 1999;26(11):2475-9. Epub 1999/11/11.

116. Nicola PJ, Maradit-Kremers H, Roger VL, Jacobsen SJ, Crowson CS, Ballman KV, et al. The risk of congestive heart failure in rheumatoid arthritis: a population-based study over 46 years. Arthritis and rheumatism. 2005;52(2):412-20. Epub 2005/02/05.

117. Wolfe F, Freundlich B, Straus WL. Increase in cardiovascular and cerebrovascular disease prevalence in rheumatoid arthritis. The Journal of rheumatology. 2003;30(1):36-40. Epub 2003/01/01.

118. Mutru O, Laakso M, Isomaki H, Koota K. Cardiovascular mortality in patients with rheumatoid arthritis. Cardiology. 1989;76(1):71-7. Epub 1989/01/01.

119. van Halm VP, Peters MJ, Voskuyl AE, Boers M, Lems WF, Visser M, et al. Rheumatoid arthritis versus diabetes as a risk factor for cardiovascular disease: a cross-sectional study, the CARRE Investigation. Annals of the rheumatic diseases. 2009;68(9):1395-400. Epub 2008/08/14.

120. Peters MJ, Symmons DP, McCarey D, Dijkmans BA, Nicola P, Kvien TK, et al. EULAR evidence-based recommendations for cardiovascular risk management in patients with rheumatoid arthritis and other forms of inflammatory arthritis. Annals of the rheumatic diseases. 2010;69(2):325-31. Epub 2009/09/24.

121. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis and rheumatism. 1986;29(8):1039-49. Epub 1986/08/01.

122. Oliveria SA, Felson DT, Reed JI, Cirillo PA, Walker AM. Incidence of symptomatic hand, hip, and knee osteoarthritis among patients in a health

maintenance organization. Arthritis and rheumatism. 1995;38(8):1134-41. Epub 1995/08/01.

123. Secretary-General, in concluding remarks to forum, emphasizes importance of partnerships in race to meet health related Millennium Development Goals [15 June 2009. SG/SM/12314 DEV/2746]. New York, NY: United Nations.Retrieved from: http://www.un.org/News/Press/docs/2009/sgsm12314.doc.htm.

Chapter 2

Materials and Methods

Patient Recruitment:

Ethical approval for this study was granted by the Ethics Department for Teaching Hospitals on Cork, Ireland. The study commenced in July 2007. Study patients were recruited consecutively from the rheumatology and orthopaedic outpatient departments, of a tertiary referral centre, Cork University Hospital. Patients with a diagnosis of rheumatoid arthritis, as defined by the ACR criteria (1) for 3 years or more were eligible for study inclusion. Osteoarthritis patients were recruited as the control population. Exclusion criteria for both groups were age less than 18 years and greater than 65 years, a previous diagnosis of coronary artery disease, cerebrovascular disease, peripheral vascular disease, carotid artery disease, previous cardiac bypass surgery or coronary artery stenting, current pregnancy or pregnancy in the previous 3 months, current or previous diagnosis of malignancy.

Baseline Demographic Data:

After written informed consent was given, each recruit was assigned an individual study number and identity was known only to the primary physician. The patients completed an in-depth questionnaire (appendix A), with assistance from a physician. Details of background medical history, current and previous treatments, family and social history, and traditional cardiovascular risks were recorded. Disease activity and impact on quality of life were assessed using validated measures. These included a Health Assessment Questionnaire (HAQ), short form-36, physician and patient visual analogue scales and a disease activity score (DAS-28), which is a calculation involving tender and swollen joint count, general health status and ESR.

A systematic chart review was also carried out to quantify duration and mean doses of each anti- rheumatic treatment used and to look for documented evidence of extra-articular manifestations of RA and complications. A physical examination was carried out by the physician. An electrocardiogram was performed on all participants and an early morning urine specimen was collected for calculation of microalbumin:creatinine ratio**.** A database was established for storage of and access to information collected from the questionnaires and chart review.

Phlebotomy:

Each study participant donated a morning sample of 30mls of blood, after a 10 hour fast. Venous blood was drawn for:

a) Traditional risk factors and baseline bloods:

1. Fasting cholesterol – HDL, LDL, Triglycerides
2. Fasting Glucose, HBA1c
3. Urea, Creatinine and electrolytes, calculation of estimated GFR by MDRD
4. Thyroid function tests
5. Full Blood Count

b) Rheumatoid specific factors

1. IgM rheumatoid factor
2. Anti- CCP antibody
3. ESR, high sensitivity CRP
4. ANA, ENA

c) Vascular specific factors and markers of clotting

1. N-terminal pro-Brain Naturietic peptide
2. Plasminogen activator inhibitor type 1
3. Adhesion molecules: Intracellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM), E- selectin, P- selectin, L- selectin
4. Cytokines: Il-1α, Il-1β, Il-2, Il-4, Il-6, Il-8, Il-10, TNFα, INFγ, VEGF, EGF, MCP-1
5. D-dimers, fibrinogen

Brain Naturietic Peptide:

Blood was collected at the time of transthoracic echocardiography for measurement of serum NT-pro BNP. Samples were collected in a clotted bottle and centrifuged at 5000 rpm for 20 minutes at 4 degrees Celsius. The serum was pipetted into aliquots and stored at minus 80 degrees and samples were analysed in batches. The Roche cobas proBNP II kit was used on an Elecsys 1010 analyzer in the department of biochemistry, in Cork University Hospital, to analyse all serum samples. Samples were analysed according to the manufacturers' standard operating procedures.

Plasminogen Activator Inhibitor-1:

A blood sample was taken at the time of echocardiography and collected in a plastic tube containing sodium citrate to measure PAI-1 activity and PAI-1 antigen levels. Blood samples were centrifuged at 5000 rpm for 20 minutes at 4 degrees Celsius. The upper 2/3 of plasma was extracted from the sample and this was re-centrifuged for a further 20 minutes to remove any platelets. Again the upper 2/3 was removed and divided into aliquots. Three 500ul aliquots were collected for each patient. Aliquots were transferred on ice for storage in a minus 80 degree Celsius freezer and stored for analysis at a later stage.

When samples were collected for all the study recruits, the aliquots of frozen plasma were transported on dry ice to Professor Nuala Booth, Department of Molecular and Cell Biology, University of Aberdeen, Institute of Medical Sciences. PAI-1 antigen

level was measured by quantitative ELISA and a specific activity assay was utilized to measure PAI-1 activity levels according to the manufacturer's instructions.

Cytokine and Adhesion Molecule Analysis:

The cytokine profile of each serum sample was analysed quantitatively using a multiplex chemi-luminescent array (Randox), a high fidelity system capable of determining the concentration of IL-1α, -1β, -2, -4, -6, -8, -10, TNFα, IFNγ, VEGF, EGF and MCP-1. The protocol followed the manufacturer's instructions and entailed the incubation of 100 µl of un-diluted sample, calibrator or control on a biochip pre-coated with immobilized capture antibody, to which 200 µl of assay diluents had been added. Following a 1 hour incubation at 37°C on a thermoshaker (370rpm) each well was washed twice with wash buffer (Tris buffered saline) and 300µl of conjugate (HRP-labelled detection antibodies) was added. This was incubated for a further hour at 37 °C on the thermoshaker (370 rpm) prior to 2 further washes. At this time 250 µl of a 1:1 combination of luminal and peroxide was added and incubated in the dark for 2 minutes. Samples were then analysed using the EVIDENCE Investigator platform. EVIDENCE software was then used to determine the intercalated x-values from the resultant standard curve, the results expressed as pg/ml.

The same multiplex chemi-luminescent immunoassay (Randox) was used to analyse the adhesion molecule profile. The Randox adhesion molecule biochip array simultaneously and quantitatively measured E-selectin, L-selectin, P-selectin,

intracellular adhesion molecue-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1).

Ankle Brachial Index:

Ankle brachial index was performed in the usual manner. Blood pressure was recorded in both arms after a period of at least 15 minutes rest. The highest systolic reading of the 2 was used in the index calculation. For ankle pressure measurement, the cuff was placed around the calf and it was then inflated 20mmHg above the upper limb systolic pressure. Using a handheld Doppler ultrasound probe over the dorsalis pedis, the pressure at which arterial pulsation was first heard, was recorded and used in the calculation. ABI for each leg was calculated as a ratio of ankle pressure to upper limb systolic pressure. A mean of both lower limb ABIs was then calculated. A reading of 0.96 or higher was taken as normal.

$$\frac{\text{Ankle Pressure (mmHg)}}{\text{Highest Arm Pressure (mmHg)}}$$

Transthoracic Echocardiography:

Prior to commencement of patient recruitment, the technique of transthoracic echocardiography was learned. Transthoracic echocardiography and tissue Doppler imaging were studied over a 12-month period. Seven hundred and eighty echocardiograms were performed under supervision of the chief cardiac physiologist. Two courses in echocardiography were completed as part of pre-study training: Philips Echocardiography Course (2006) and Echocardiography in Practice, Waterford Regional Hospital (2006). The Echo Manual by Jae K. Oh was used as educational tool and reference point (2). Transthoracic echocardiography was performed on all patients by the PhD student. Intra- and interobserver variability was accounted for by co- imaging at the time of echocardiography, by a blinded echocardiographic physiologist and reporting was conducted by a cardiologist unaware of patients' diagnoses. Recommendations from the Guidelines and Standards Committee of the American Society of Echocardiography in collaboration with the European Association of Echocardiography, for the use of echocardiography in clinical trials (3) and for the evaluation of left ventricular diastolic function by echocardiography (4) were followed.

A Vivid–I GE-Medical portable echocardiography machine was used with a 2.5Hz probe. The patients were positioned in left lateral decubitus position to obtain parasternal and apical views. A customized echocardiography bed was used. The patient's identification details were entered prior to commencing the ultrasound and a sharp electrocardiographic tracing with clear R and P waves was available on screen prior to scanning. All images were acquired at end-expiration. Depth, size and gain settings were adjusted during each study as required.

The examination began by placing the transducer in the left parasternal region, at the level of the 3rd or 4th intercostal space. A long-axis view image of the left ventricle was recorded. The aortic root, aortic valve leaflets, left atrium and mitral valve leaflets were also visualised in this view. Colour flow imaging in this plane was used to assess mitral and aortic valve competency. M-mode echocardiography was performed in this parasternal long axis view. An M-mode cursor was placed across the mid-ventricular line, at a level just below the free edge of the anterior mitral leaflet, to calculate left ventricular diameter in systole, LVDS (mm), left ventricular diameter in diastole, LVDD (mm), inter-ventricular septal wall thickness in diastole, IVSTD (mm) and left posterior wall diameter in diastole, LPWDD (mm), (figure 2.1). Ejection fraction and fractional shortening were calculated based on the above measurements.

A second M-mode cursor was placed across the aortic valve level of the long-axis parasternal view. This was used to measure the dimension of the left atrium, LA (cm) at end-systole. LA dimension was taken as the largest distance between the leading edges of the posterior aortic wall and the posterior LA wall. Peak early (E) and late (A) opening of the mitral valve were recorded by passing an M-mode cursor across the anterior mitral leaflet, using the long-axis parasternal view again**.**

The transducer was then rotated 90 degrees clockwise into a parasternal short axis (PSAX) view, so the plane of the ultrasound beam was perpendicular to the plane of the long axis of the LV. The LV was assessed in a cross-sectional plane at the level of the mitral leaflets. By tilting the transducer inferiorly, the anterolateral and posteromedial papillary muscles were visualised. Left ventricular wall motion was assessed in this plane. Tilting further inferiorly, the LV apical wall motion was

assessed in a transverse plane. Changing to a superior tilt on the transducer, the trileaflet aortic valve and right ventricular outflow tract were visualised.

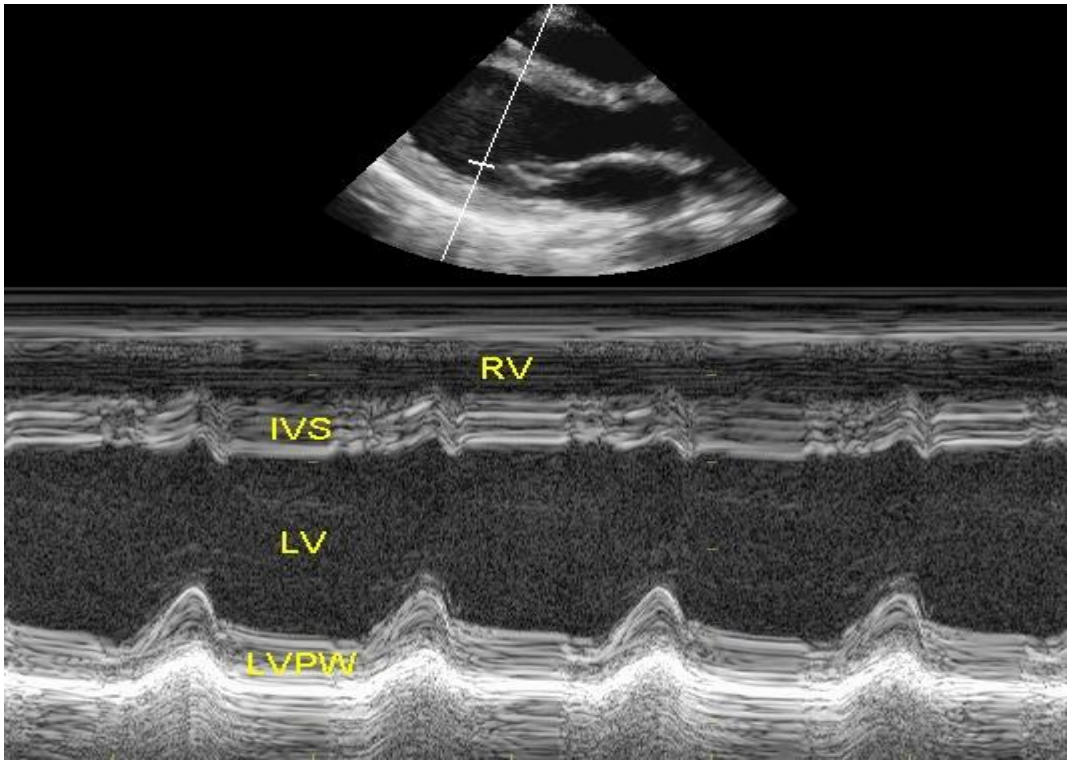


Figure 2.1 M-mode echocardiogram across the left mid-ventricle, with parasternal long-axis 2D echocardiographic guidance. RV, right ventricle; IVS, interventricular septum; LV, left ventricle; LVPW, left ventricular posterior wall. (5)

Apical images were also recorded with the patient in the left lateral decubitus position. The transducer was placed over the point of maximal apical impulse. An apical 4 chamber view was found and the image orientated so that the apex was visualised at the top of the image. The notch on the transducer was pointing superiorly so that the left ventricle was displayed on the right side of the image. All 4 chambers were assessed in this plane, the atrial septum was visualised in its entirety and the inferior pulmonary views were seen emptying into the left atrium. All LV

segments were reassessed in this view, particularly the apex, which was often seen more clearly here than in the previous parasternal views (figure 2.2).

Tilting the transducer slightly superior and a small clockwise rotation at the same time, revealed the apical 5-chamber, long axis view. This allowed assessment of the left ventricular outflow tract and the aortic valve. Colour flow was used to assess the mitral, tricuspid and aortic valve competencies in the apical images. A further clockwise rotation of the transducer displayed an apical 2-chamber view of the left side if the heart. In particular, it was useful for visualising the inferior LV wall.

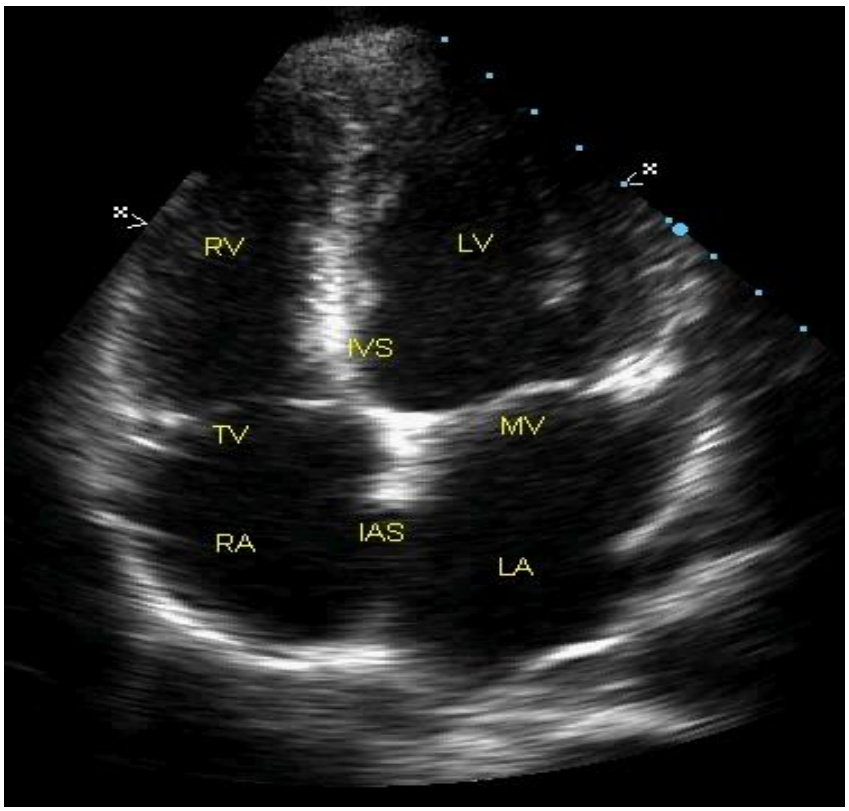


Figure 2.2 2D echocardiographic image of an apical 4-chamber view. LV, left ventricle; LA, left atrium; RV, right ventricle; RA, right ventricle; MV, mitral valve; TV, tricuspid valve; IVS, interventricular septum; IAS, interatrial septum.

Returning to the apical 4-chamber view to assess diastolic function, both Pulsed-wave Doppler (PWD) and Tissue Doppler Imaging (TDI) were utilised. Mitral inflow was measured with PWD to assess LV filling. A 1 to 2 mm sample volume was placed at the mitral leaflet tips during diastole to measure the inflow velocity pattern. Spectral gain and wall filter settings were optimized to display a crisp waveform. Sweep speed was set at 100mm/s, at end-expiration. Measurements recorded were early diastolic velocity (E wave) m/s, late diastolic filling velocity (A wave) m/s, the E/A ratio and deceleration time in milliseconds (DT). Valsalva maneuver was performed by each subject, in the sitting position, to unmask any pseudonormal pattern of filling. A standard grading system for diastolic dysfunction was used for grouping subjects' results: grade 1 is impaired relaxation with normal filling pressure; grade 2 is a pseudonormalized pattern; grade 3 represents restrictive filling (figure 2.3).

Tissue Doppler imaging is the method of choice for recording longitudinal velocities of the mitral annulus (6).

In the apical 4-chamber view, the ultrasound beam was positioned perpendicular to the lateral annulus and a 5 mm sample volume was placed over the lateral portion of the mitral annulus. Doppler gain was reduced to minimise background noise and obtain a clear signal. Spectral recordings were again taken at end-expiration, at a sweep speed of 50-100mm/s and measurements reflected an average over 3 consecutive cardiac cycles. The waveform generated 3 distinct velocities: systolic (S'), early diastolic (E') and late diastolic (A') velocities. A smaller sample volume of 2mm was then positioned over the septal (medial) portion of the mitral annulus and 3 medial anular velocities generated.

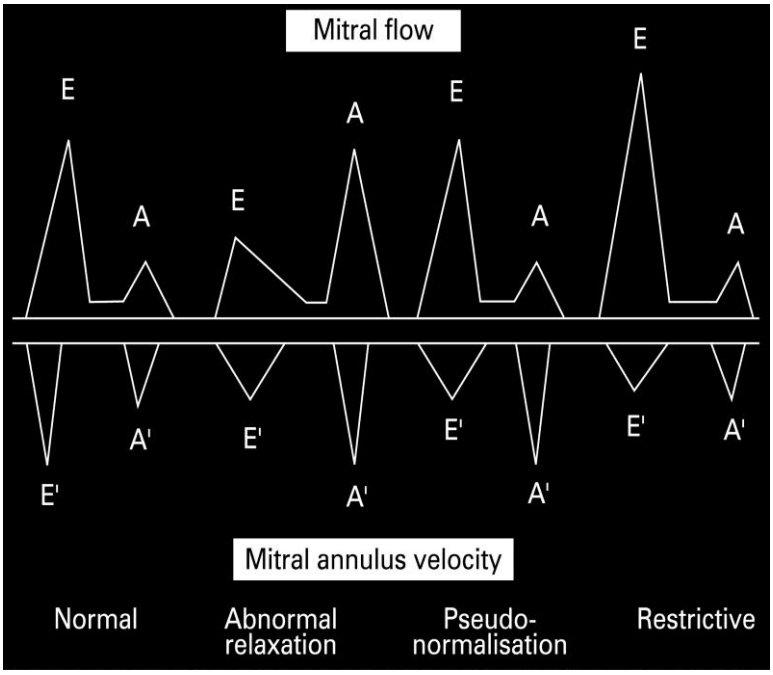


Figure 2.3 Schematic representation of normal and abnormal mitral inflow and mitral annulus velocities. E, E wave, peak early diastolic velocity; A, A wave, late diastloic filling velocity; E', E prime, early diastolic annular velocity; A', A prime, late diastolic annular velocity.

Limitations of transthoracic echocardiography in assessment of diastolic function:

Doppler echocardiographic measurements of diastolic function may vary from day to day in the same patient, depending on preload and afterload. Assessing LV filling with PW doppler of mitral inflow can give rise to a difficulty in recognising pseudonormalization and diastolic heart failure in patients with a normal ejection

fraction, because similar values for LV filling patterns can be seen in healthy normal subjects and patients with cardiac disease (4).

For this reason, the American Society of Echocardiography recommend using multiple measures to assess diastolic function . Sinus tachycardia and first-degree AV block can result in fusion of the early (E) and late (A) atrial velocities, this may result in a reduction in E/A ratio and deceleration time. Use of the Valsalva maneuver to identify a pseudonormal mitral inflow pattern can be difficult for patients to perform and it is not standardized. It is another reason to combine Tissue Doppler Imaging with mitral inflow velocities in the overall assessment of diastolic function. With regard to TDI, there are potential technical limitations, if proper attention to sample size, sample location, gain and filter is not ensured.

Limitations to echocardiographic measurements in this study:

Pulmonary venous flow was not assessed as part of the workup for diastolic function in this study. High quality images of the pulmonary vein are difficult to obtain and measurements derived from pulmonary venous flow are no longer felt to add significant information to assessment of diastolic function, when parameters such as mitral inflow velocity and tissue doppler imaging are available (3).

Carotid Artery Ultrasound:

Patients attended the radiology department for carotid ultrasound examination. B mode ultrasound using a Toshiba Xario system and linear array 7mHz probe was used and scans were performed by a trained radiographer.

IMT (intima-media thickness) values were obtained at specific points for the right and left common carotid artery and for both the right and left internal carotid artery (RCCA, RICA, LCCA, LICA), (figure 2.4). An average of three readings was taken for each variable and a composite value, expressed in mm was recorded. Each of the 4 vessels was assessed for presence of plaque. Plaque was defined as a distinct protrusion > 1.5mm into the vessel lumen. This was recorded as either present or absent. If present, plaque size was recorded and expressed in mm. Both the radiographer performing the examination and the radiologist reading and interpreting the scans, were blinded to the identity of the study subjects and are unaware of whether they formed part of RA or OA group.

Based on recommendations from the American Society of Echocardiography (7, 8), carotid intimal medial thickness (cIMT) values were reported as a mean of the cIMT from the left and right common carotid arteries. A mean cIMT of greater than 0.9mm was considered abnormal (9).

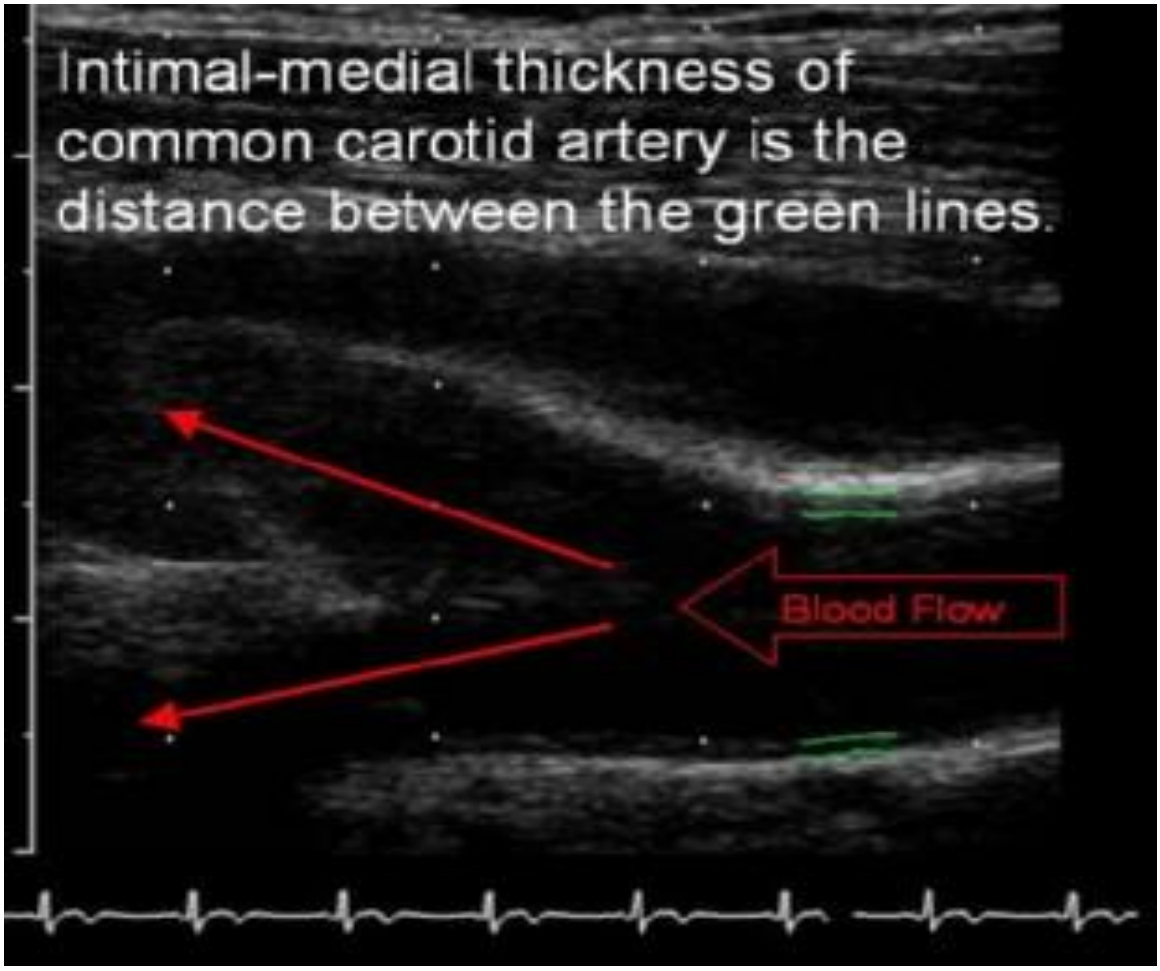


Figure 2.4 IMT of Common Carotid Artery

Flow Mediated Dilatation of Brachial Artery:

Prior to commencement of patient recruitment, the technique of flow-mediated dilatation (FMD) of the brachial artery was learned. Thirty brachial artery ultrasounds for flow mediated dilatation were observed and ninety practice scans were then performed under supervision by a trained technician in the field, prior to scanning study patients.

Subjects presenting for baseline measurement of FMD fasted for at least 8 hours prior to the study and were studied in a quiet temperature controlled room (22°C). Advice was given to withhold all vasoactive medications for at least 4 half lives. Subjects were also advised to avoid exercise and foods which have clear-cut FMD effects such as caffeine, high fat foods and Vitamin C, and (where relevant) to desist from smoking for at least 6 hours prior to the study.

A Philips HDI 3000 Ultrasound machine was used for all studies (Philips Healthcare, Netherlands). A linear array transducer with a broadband multiple frequency of 7-12 MHz, attached to a high-quality mainframe ultrasound system, was used to acquire images with sufficient resolution for real time analysis by the image acquisition software. Timing of the cardiac cycle was carried out by the image acquisition software analysis of continuous real-time digital ECG input; this will be described later.

Each subject was positioned supine with the dominant arm in a formed foam mould to allow the subject to rest comfortably for the duration of the study. A standard automated blood pressure cuff (Omron^{TM} 705 IT, Omron Healthcare Europe B.V.)

was attached to the non-dominant (non-study) arm and blood pressure and heart rate were measured on a regular basis throughout the study. The brachial artery was imaged above the antecubital fossa in the longitudinal plane – See Figure 2.5.

A segment with clear anterior and posterior intima lumen interfaces was selected for continuous 2D grey-scale imaging. A stereotactic probe-holding device was custom-developed and used for all of the FMD studies. During image acquisition, anatomical landmarks such as veins and fascial planes were used to help maintain the same image of the brachial artery throughout the study.

Endothelium-Dependant Dilatation

To create a flow stimulus in the brachial artery, a blood pressure cuff is placed below the antecubital fossa on the mid-forearm. A baseline, resting 2D ultrasound image is then acquired for 2-5 minutes. Following this, the blood pressure cuff is inflated to 50mm Hg above the recorded stable systolic blood pressure for complete occlusion of arterial forearm inflow for 5 minutes. This forearm ischaemia produces dilation of downstream resistance vessels via auto-regulatory mechanisms. On release of the cuff, a brief hyper-perfusion state through the brachial artery is induced to accommodate the dilated resistance vessels. The resulting increase in shear stress in the brachial artery causes the brachial artery to dilate and the longitudinal image of the artery is recorded continuously for the next 2 minutes. The FMD, defined as the percentage increase in mean diameter over a 10 second interval, 55 seconds after

tourniquet deflation, is automatically averaged and calculated by the software from diastolic vessel diameters, using the following formula:

$$\frac{\text{Maximum diameter} - \text{baseline diameter}}{\text{baseline diameter}} \times 100\%$$

The accepted normal FMD routinely reported in the scientific literature is 4% change or more from baseline for the brachial artery. One not infrequently reported measurement is absolute change of brachial artery diameter (in mm). However, the most widely cited FMD measurement in the literature is percentage change of FMD from baseline.

Endothelium-Independent Vasodilatation with Nitroglycerin

Following a rest period of 10 minutes to allow a return of brachial artery diameter to resting, steady state perfusion, a further baseline scan is obtained. An exogenous NO donor is then administered in the form of a single dose of sublingual nitroglycerin spray (0.4 mg). This is given to determine the maximum obtainable vasodilator response, and to serve as a measure of endothelium-*independent* vasodilation which indicates vascular smooth muscle function. Peak vasodilation occurs 3-4 minutes after nitroglycerin administration and the brachial artery image is continuously captured during this time period.

Limitations of FMD technique

High resolution ultrasound assessment of brachial artery reactivity is technically challenging. To ensure satisfactory application of the FMD technique, the author was initially trained in principles and technical aspects of 2D and Doppler ultrasonography, especially of the upper limb. Venous and arterial ultrasonography was observed during the training period. Multiple training scans (>50) were performed on normal healthy subjects in order to accurately identify normal brachial artery anatomy and also to perfect the FMD technique prior to initiation of the studies described. Training in use of the edge detection software and its interpretation was also carried out prior to research data collection. Training scans were carried out under supervision and intra-observer and inter-observer variability was established (until an acceptable reproducibility with a mean difference of 2% to 3% in FMD over time was achieved). In training and test subjects, all baseline scans were repeated within 7 days of the initial scan to ensure satisfactory reproducibility. Use of objective edge detection and analysis software reduced observer bias. Standardised documentation for data collation was developed and protocols were derived to standardise FMD measurement and analysis.

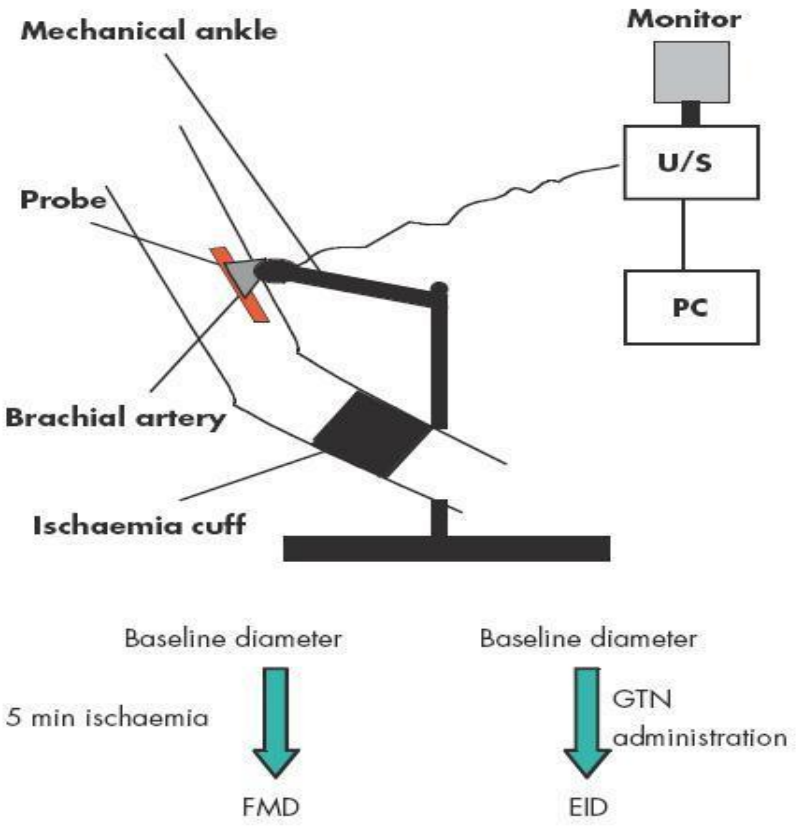


Figure 2.5 – Flow Mediated Dilatation is evaluated by the use of high resolution ultrasound. EID, endothelium dependant dilatation; GTN, Glyceryl Trinitrate; PC, personal computer with image acquisition software; U/S, high resolution ultrasound

Edge Detection/FMD Analysis Software

Edge or image acquisition software – Vessel Image Analysis (V.I.A.) from M.D. Medic Ltd., UK - allows the real-time capture of vessel diameter by the use of a personal computer equipped with a frame grabber software device connected to the ultrasound system. Integral to the software is an artificial neural network vessel wall detection software system which automatically detects and tracks the anterior and

posterior artery walls within an area of interest defined by the sonographer. – see **Figure 2.6**

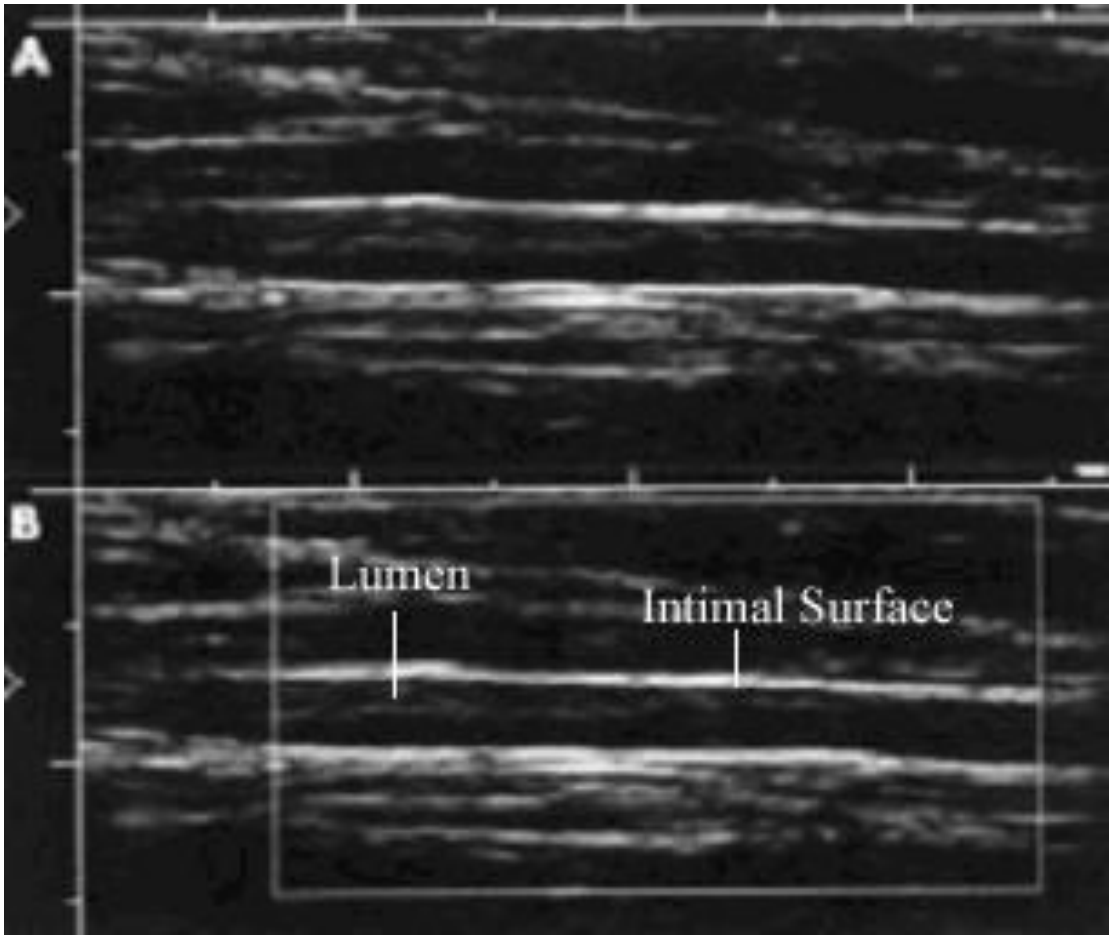


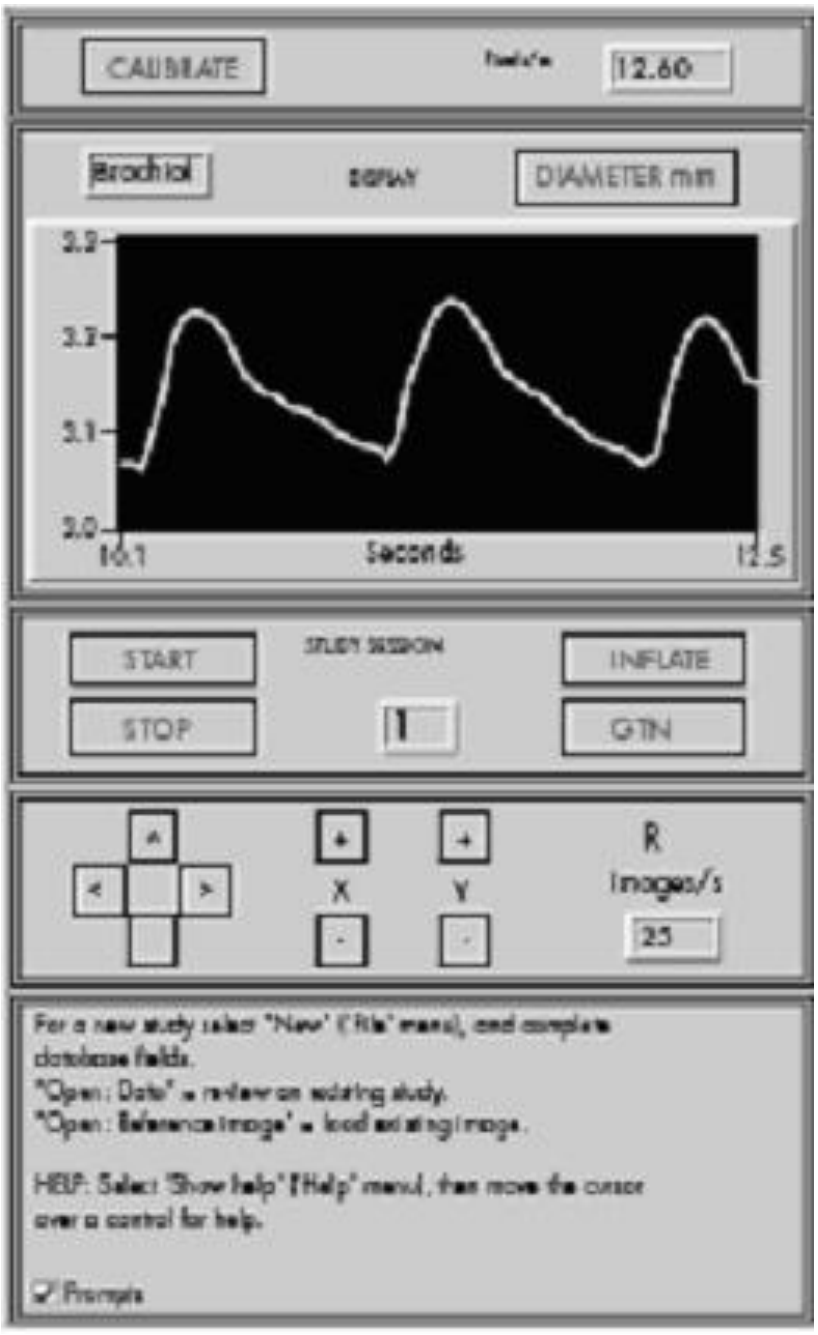
Figure 2.6

(A) The brachial artery is imaged such that the media–lumen interface is clearly seen.

(B) The arterial media–lumen interface is automatically located and tracked within the region of interest.

The vessel diameter is then determined by averaging a large number of local vessel diameters (determined by the region of interest specified by the sonographer). The B

mode images are processed at 25 frames/second and the vessel diameter, including diameter changes over the cardiac cycle is displayed in real time – **see Figure 2.7**



The wave-form shown represents the range in artery diameter from maximum at peak-systole

Figure 2.7

The mean vessel diameter within the region of interest is displayed in real time at 25 frames/second. This allows the operator to optimise image quality continually.

At a sampling rate of 25 frames/second, it is possible to optimise ultrasound imaging parameters at the start of the scan. A stereotactic clamp is used to hold the transducer and a fine movement screw gauge is incorporated into the stereotactic clamp to allow for fine adjustments of the transducer (to accommodate subject movement). The software also saves a two dimensional image of the brachial artery over the scanning area to allow for comparison with repeated tests.

There are distinct advantages to the use of automated image analysis software. It allows wall tracking to be optimised throughout the study, avoiding loss of data because of limited image quality. Other commonly used techniques require vessel images to be stored on a computer disk or videotape for later off line analysis. Image storage for post-hoc analysis carries the risk of image degradation from video recording and from working through the image processing steps. Operator intervention to optimise images stored conventionally is also at risk of observer/interpreter bias.

Statistical Calculations:

A power calculation was performed prior to the study for 80% power. Exploratory data analysis on the data set was conducted including examination of significant outlying or clinically improbable values. Internal consistency checks on repeated measurements were also performed. The distribution, central tendency and variance of variables in the overall study population and of subgroups defined by the primary explanatory variable were examined using both tabular and visual approaches. Independent t-tests were used to compare normally distributed data and the non-parametric test, Mann Whitney-U was used to compare non-normally distributed data. Chi^2 testing was employed to compare categorical variables. Bivariate correlation was used to investigate for statistically significant associations between continuous variables. Binary logistic regression was used to investigate for evidence of an association between a number of outcome variables and independent covariates. $P < 0.05$ was taken as significant.

Analyses were conducted using SPSS® version 20 statistical software, with the advice and assistance of Prof Joseph Eustace (Director, Clinical Research Facility, University College Cork and Associate Professor of Statistics, Johns Hopkins Department of Biostatistics).

References:

1. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis and rheumatism. 1988;31(3):315-24. Epub 1988/03/01.

2. Oh JK SJ, Jamil Tajik A. The Echo Manual. 3rd ed2006.

3. Gottdiener JS, Bednarz J, Devereux R, Gardin J, Klein A, Manning WJ, et al. American Society of Echocardiography recommendations for use of echocardiography in clinical trials. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography. 2004;17(10):1086-119. Epub 2004/09/29.

4. Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. European journal of echocardiography : the journal of the Working Group on Echocardiography of the European Society of Cardiology. 2009;10(2):165-93. Epub 2009/03/10.

5. Van de Veire NR, De Sutter J, Bax JJ, Roelandt JR. Technological advances in tissue Doppler imaging echocardiography. Heart. 2008;94(8):1065-74. Epub 2008/01/31.

6. Sohn DW, Chai IH, Lee DJ, Kim HC, Kim HS, Oh BH, et al. Assessment of mitral annulus velocity by Doppler tissue imaging in the evaluation of left ventricular diastolic function. Journal of the American College of Cardiology. 1997;30(2):474-80. Epub 1997/08/01.

7. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography. 2008;21(2):93-111; quiz 89-90. Epub 2008/02/12.

8. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Desvarieux M, et al. Mannheim intima-media thickness consensus. Cerebrovascular diseases. 2004;18(4):346-9. Epub 2004/11/04.

9. Corrales A, Parra JA, Gonzalez-Juanatey C, Rueda-Gotor J, Blanco R, Llorca J, et al. Cardiovascular risk stratification in rheumatic diseases: carotid ultrasound is more sensitive than Coronary Artery Calcification Score to detect subclinical atherosclerosis in patients with rheumatoid arthritis. Annals of the rheumatic diseases. 2013;72(11):1764-70. Epub 2013/07/16.

Chapter 3

Demographics, Disease Activity Scores and Baseline Bloods

Introduction:

At the time of initial screening of patients for study entry, we collected data on patient demographics. This information included disease duration, current and previous medication use, family and personal medical history, baseline traditional cardiovascular risk factors and arthritis disease activity scores.

Of particular interest to us were patients' traditional cardiovascular risk factors.

It is still unclear whether hypertension is more common in RA patients than the general population (1). A recent met-analysis of 7 RA case-control studies did not find an increased prevalence of hypertension in RA patients compared with controls (2). The relative risk of hypertension in RA needs to be investigated with longitudinal studies in the future.

The prevalence of dyslipidaemia does not appear to be higher in RA patients compared with controls, despite the increased risk of CVD in RA (2, 3). Inflammation associated with RA and some of the treatments for RA are associated with changes in lipid profiles.

Consistent with other inflammatory conditions, RA is associated with low total cholesterol, low HDL concentrations and elevated triglyceride levels (4). The

proinflammatory cytokines driving the inflammatory effects of RA are linked with a low total and HDL cholesterol.

Changes in lipid profile as a result of treatment with TNF inhibitors and anti-IL6 are common. TNF blockers appear to elevate total cholesterol and HDL levels but do not affect LDL levels (5).

This dual effect results in a stable atherogenic profile. The improvement in lipid profile with TNF inhibitors occurs mainly in treatment responders, suggesting that the benefit for lipids is via control of inflammation rather than a direct effect on cholesterol by anti-TNF therapies(6). Treatment of RA with anti-IL6 therapy, tocilizumab, is associated with elevations in total and LDL cholesterol. Whether these effects are long standing or not and whether they impact on CV risk is currently being investigated (Roche H-L. A study of the effect of tocilizumab on markers of atherogenic risk in patients with moderate to severe rheumatoid arthritis (7).

Statin therapy in RA has been shown to have a dual benefit. Expected reductions in total cholesterol and LDL levels have occurred along with improvements in disease activity and inflammatory markers (8).

Insulin resistance appears to be associated with RA. Chung et al studied 124 RA patients and found that 54% of them have evidence of insulin resistance (9). This is higher than rates of 40% which have been recorded in the general population (10). Data is conflicting with regard to whether diabetes mellitus has a higher prevalence in RA patients compared to the general population. RA treatments have different

effects on glucose control. Corticosteroids increase the risk of diabetes; however hydroxychloroquine and TNF inhibitors have been shown to be associated with a reduced risk of future diabetes mellitus (11).

Smoking rates are decreasing in the general population; however it is still an important modifiable CV risk factor. It is also the strongest known environmental risk factor for RA (12). Sero-positivity in RA is linked to smoking and also to a more aggressive disease (13).

With regard to baseline phlebotomy, we were particularly interested in lipid and glucose profiles in the RA group for reasons mentioned above.

Aims:

We aimed to characterise patients' baseline demographic details and compare demographics and disease activity scores in RA and OA patients. We aimed to assess patients' traditional cardiovascular risk factors at baseline and compare these in RA and OA patients.

At the time of study entry blood samples were drawn for baseline routine bloods and fasting lipid and glucose profiles. We aimed to compare lipid and glucose readings in RA and OA patients.

Methods:

After informed consent was given and inclusion and exclusion criteria were met, each patient completed a study questionnaire regarding length of diagnosis, current treatments, smoking status, education level and family history. A number of disease activity and quality of life measures were also assessed at this time (see chapter 2 for more detail). A review of each patient's medical notes was performed to cross check background medical information.

A physical examination was performed on all recruits. Weight, height, waist circumference, hip circumference, blood pressure and heart rate were recorded.

Blood was drawn for full blood count, coagulation screen and d-dimers, renal and liver profile, thyroid function, fasting lipid and glucose profile, rheumatoid factor and anti-CCP antibodies.

Baseline ECG was also performed in all patients to screen for previous silent myocardial events.

Statistical Methods:

All demographic variables and blood result variables were checked for normal distribution. If variables were normally distributed, means were quoted and t-tests utilised to compare groups. If variables were not normally distributed, medians were quoted and non-parametric analysis, in the form of Mann-Whitney U test was performed for comparisons. Bivariate correlations and binary logistic regression were used to assess for associations between fasting glucose and lipid profiles and markers of atherosclerosis.

Demographic Results:

347 patients were screened for suitability to enter the study. After inclusion and exclusion criteria were applied as discussed in chapter 2, a total of 111 subjects were recruited for the study. The majority of exclusions were due to either a documented prior history of cardiovascular disease or were currently being investigated for cardiac sounding symptoms and signs.

Sixty-three recruits had a diagnosis of rheumatoid arthritis (RA) and 48 had osteoarthritis (OA).

A summary of demographic findings are presented in table 3.1.

Characteristics	Rheumatoid Arthritis n = 63	Osteoarthritis n = 48	P value
Age,mean (SD), yrs	50.57 (7.57)	50.58 (7.14)	0.993
Female, n (%)	40 (63.5)	39 (81.2)	0.057
Duration of disease, median (IQR)	7 (4-11)	6 (3-9)	0.227
Current Smoker (%)	31.7	20.8	0.200
Smoking Pack Years,median (IQR)	20 (10-30)	15 (10-20)	0.193
Alcohol units/wk, median (IQR)	7.5 (3-10)	7 (3-12)	0.787
Not working due to disease, n	9	3	0.226
Current NSAID use, n	28	12	0.035*
Current selective COX2 NSAID use, n	4	3	0.983
Current steroid use, n (%)	29 (46)	NA	
Mean steroid dose, mg (SD)	7.93 (3.32)	NA	
Current DMARD use, n (%)	46 (73)	NA	
Current Biologic use, n (%)	34 (53.97)	NA	
Current Statin, n (%)	11 (17.5)	11 (22.9)	0.475
Hypercholesterolaemia, n (%)	12 (19)	14 (29.17)	0.212
Hypertension, n (%)	12 (19)	10 (20.8%)	0.815
Diabetes, n	1	0	
Joints Replaced, n (%)	5 (7.9)	2 (4.2)	0.418

Family History of MI, n (%)	16 (25.4)	29 (60.4)	<0.001*
Weight (kg), mean (SD)	72.55 (14.54)	75.21 (13.56)	0.323
BMI (kg/m^2), mean (SD)	26.20 (3.48)	28.05 (4.31)	0.017*
Waist/Hip ratio, mean (SD)	0.86 (0.10)	0.85 (0.06)	0.287
HAQ, median (IQR)	0.375 (0.125-0.75)	0.250 (0-0.625)	0.135
SF36 PCS, mean (SD)	48.15 (7.42)	48.04 (8.73)	0.296
SF36 MCS, mean (SD)	53.25 (5.83)	52.43 (5.94)	0.473
General Health Score, mm, median (IQR)	30 (17.5-37.5)	20 (10-30)	0.025*
Physician VAS disease activity(mm), median (IQR)	20 (10-30)	10 (10-20)	<0.001*
Patient VAS disease activity (mm), median (IQR)	20 (10-30)	10 (3-20)	0.004*
WOMAC, median (IQR)	NA	8 (3-12.5)	
Tender Joint Count, median (IQR)	3 (0-6)	NA	
Swollen Joint Count, median (IQR)	4 (1.5-6)	NA	
DAS28ESR, mean (SD)	3.62 (1.33)	NA	
DAS28CRP, mean (SD)	3.55 (1.24)	NA	

Table 3.1: Baseline demographic and disease activity scores for RA and OA patients. P <0.05 is taken as significant. RA = rheumatoid arthritis, OA = osteoarthritis, SD = standard deviation, n = number of patients, IQR = interquartile range, MI = myocardial infarction, BMI = body mass index, HAQ = health assessment questionnaire, SF36 PCS = short form 36 physical component score, SF36 MCS = short form 36 mental component score, VAS = visual analogue scale, WOMAC = Western Ontario and McMaster Universities osteoarthritis index, DAS 28 = disease activity score for 28 joints.

Gender:

71.2% of the total study population were female. Commencing the study were 40 females with rheumatoid arthritis and 23 males. 39 females and 9 males with a diagnosis of osteoarthritis were included (table 3.2, figure 3.1).

	Male (%)	Female (%)	Total (%)
RA	23 (36.5)	40 (63.5)	63 (100)
OA	9 (18.8%)	39 (81.2)	48 (100)
Total	32	79	111

Table 3.2: Breakdown of study groups by gender

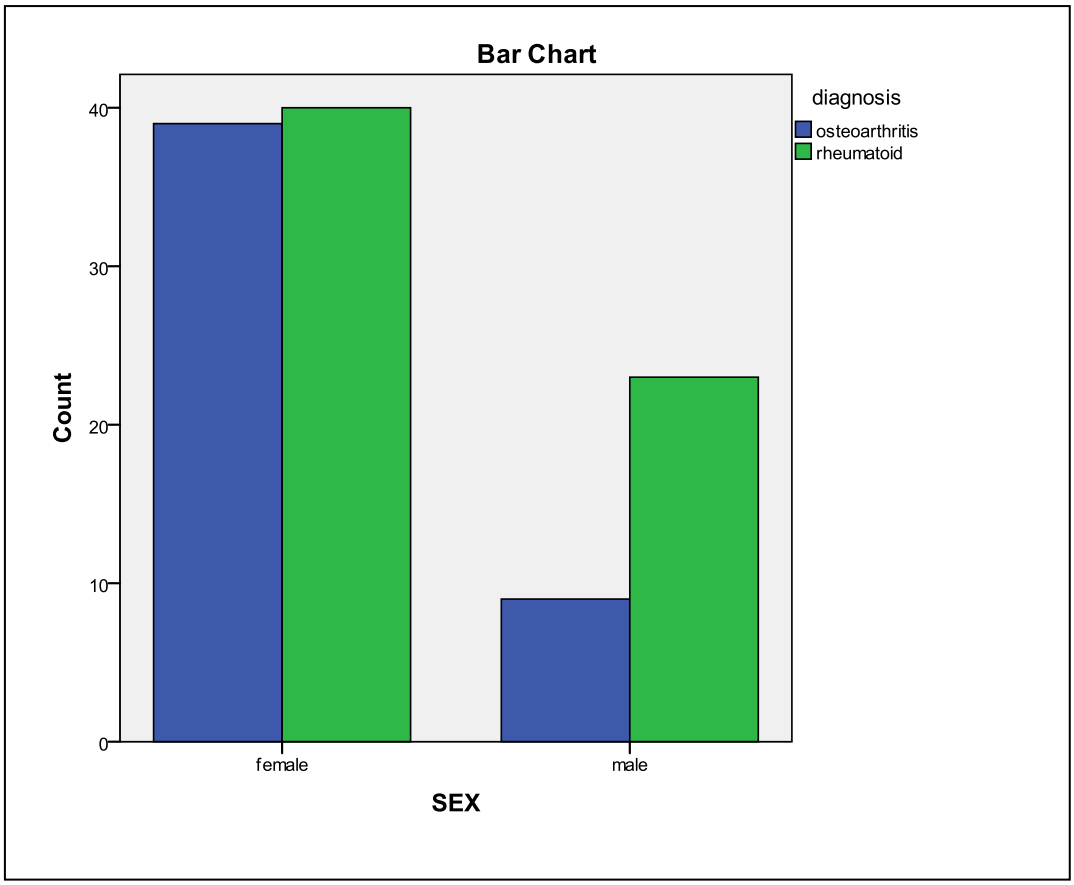


Figure3.1: Gender distribution according to diagnosis

Age:

Mean age of the total study group was 50.58years (+/- 7.35SD), range 30 to 63 years. Mean age in the osteoarthritis group was 50.58 years (+/- 7.136SD), range 32 to 62. In the rheumatoid group, 50.57 years (+/- 7.566SD) was the mean age and ages ranged from 30 to 63 years, figure 3.2. There was no significant difference in ages between RAs and OAs, p = 0.993.

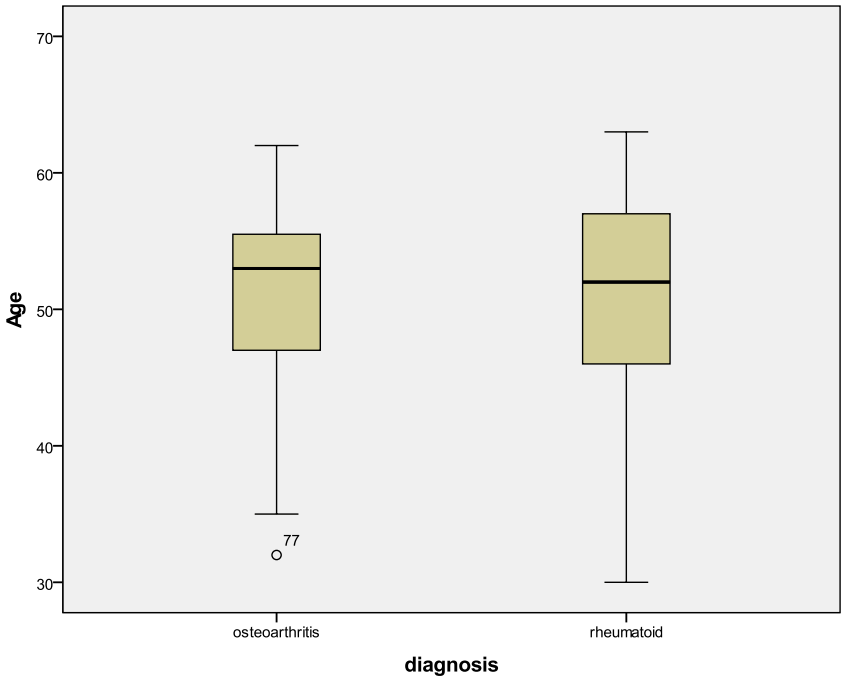


Figure 3.2: Boxplot of age in RA and OA

Disease Duration:

Disease duration was not normally distributed. Median duration of diagnosis for RA and OA groups inclusive was 6yrs (IQR4-9.5; range 1 to 28 years). The median length of diagnosis at study entry point was 6yrs (3-9) for osteoarthritis (range 1 to 20 years). 7yrs (4-11) was the median duration of disease for the rheumatoid arthritis group (range 1 to 28 years). Using a Mann-Whitney U test, we found no difference in disease duration between RA and OA patients, p=0.227.

66.7% of cases of rheumatoid arthritis and 14.6% of OA cases were diagnosed by a consultant rheumatologist. The remainder were given a diagnosis by their GP (figure 3.3)

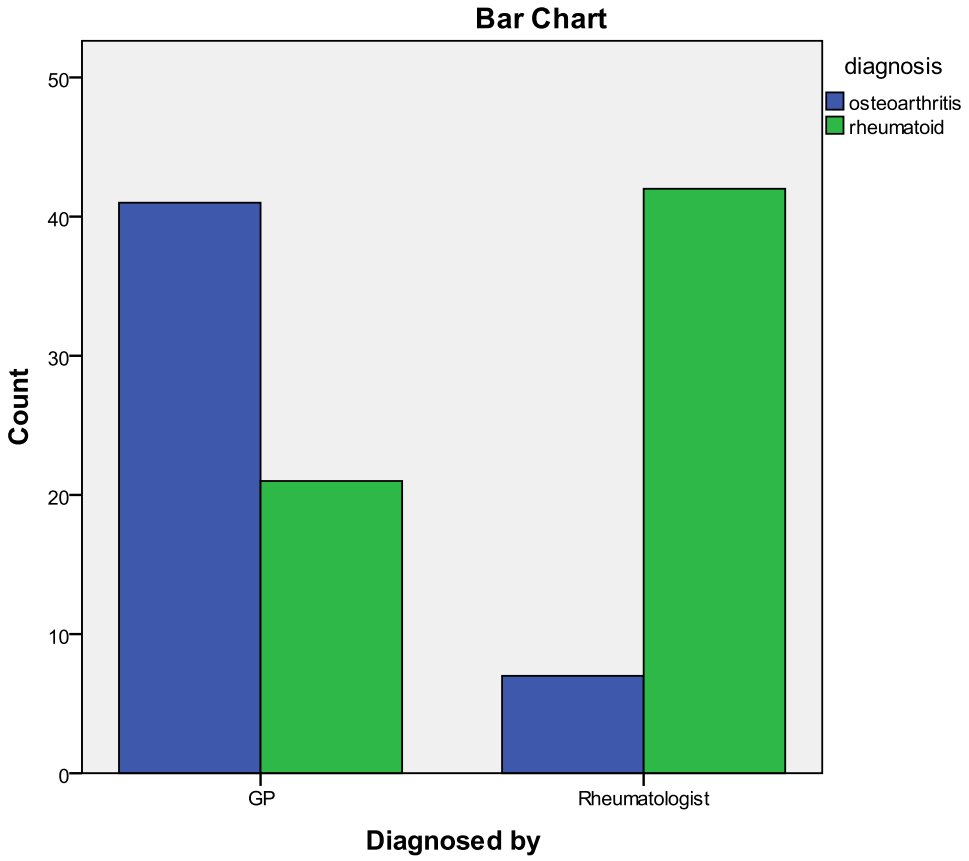


Figure 3.3: Breakdown according to source of diagnosis

Education Level:

There was no significant difference between RA patients and OA patients with regard to education level when all levels of education were analysed together, however there was a significant difference in the number of RA patients and OA patients attending 3rd level education, (2 vs. 8, respectively), p = 0.014, (table 3.4).

Education Completed	RA %	OA %	p
Primary school	7.9	4.2	NS
Junior certificate	41.3	39.6	NS
Leaving certificate	47.6	39.6	NS
3rd level education	3.2	16.7	0.014*

Table 3.4: Comparison of RA and OA patients' education level, p, significance level

Occupation:

Occupation types were similar between the 2 groups, p = 0.429 (table 3.5). 14.3% of the RA group and 14.6% of the OA group were unemployed (not disease-related). A further 14.3% of the RA group and 6.3% of the OA group were unable to work due to their disease. This difference between RA and OA patients was not significant, Fisher's exact test, p =0.226. 3.2% of the RA group and 6.3% of the OA group were retired. There was no significant difference between the 2 groups with regard to employment status, p = 0.515, (figure 3.4).

Occupation	RA n = 63	OA n = 48
Working in the home	12	8
Labourer	25	16
Semi-professional	20	13
Professional	4	8
Self-employed	2	3

Table 3.5: Groups according to occupation

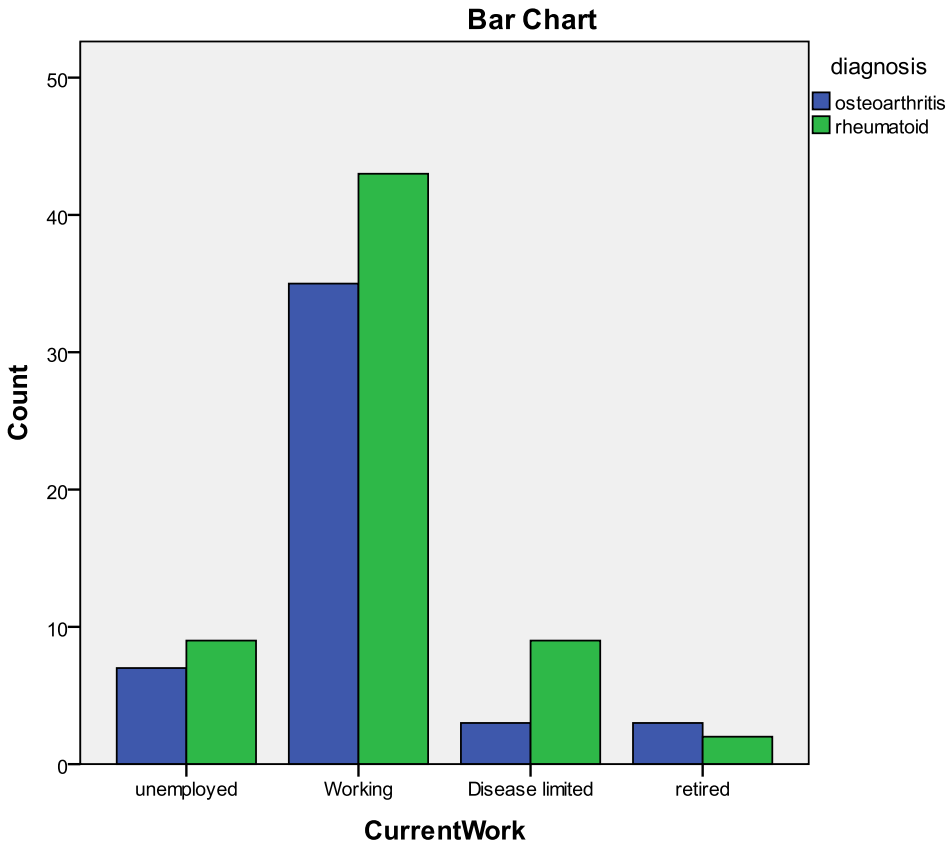


Figure 3. 4: Comparison of current employment status

Medications:

DMARD Use:

Within the RA group, methotrexate was the most commonly prescribed disease modifying anti-rheumatic drug (DMARD). 39 of 63 RA patients were taking methotrexate at the time of the study. All patients taking methotrexate were also on folic acid. A small percentage of RA patients were taking an alternative DMARD (table 3.6 and figure 3.5). 27% (17/63) of the RA group were not taking any DMARD at the time of the study. 54% of RA patients had been on a different DMARD in the past, methotrexate again being the most common one.

DMDMARD	Frequency	%
Methotrexate	39	61.9
Leflunomide	3	4.8
Hydroxycholoquine	1	1.6
Sulphasalazine	1	1.6
Azathioprine	2	3.2
None	17	27

Table 3.6: Summary of DMARDs used in RA patients

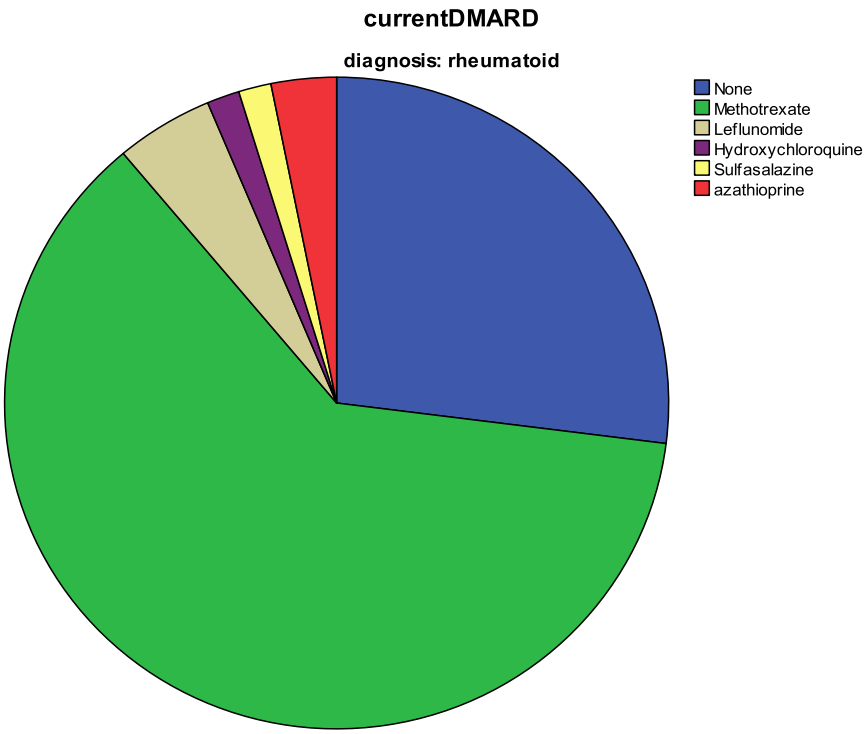


Figure 3.5: Current DMARD use in RA group

Biologic use:

34 (53.97%) RA patients were on a biologic agent for treatment of their disease at the time of the study. 21 were taking etanercept, 11 adalimumab and 2 rituximab (figure 3.6). 19% of the group had been on another biologic prior to their current treatment.

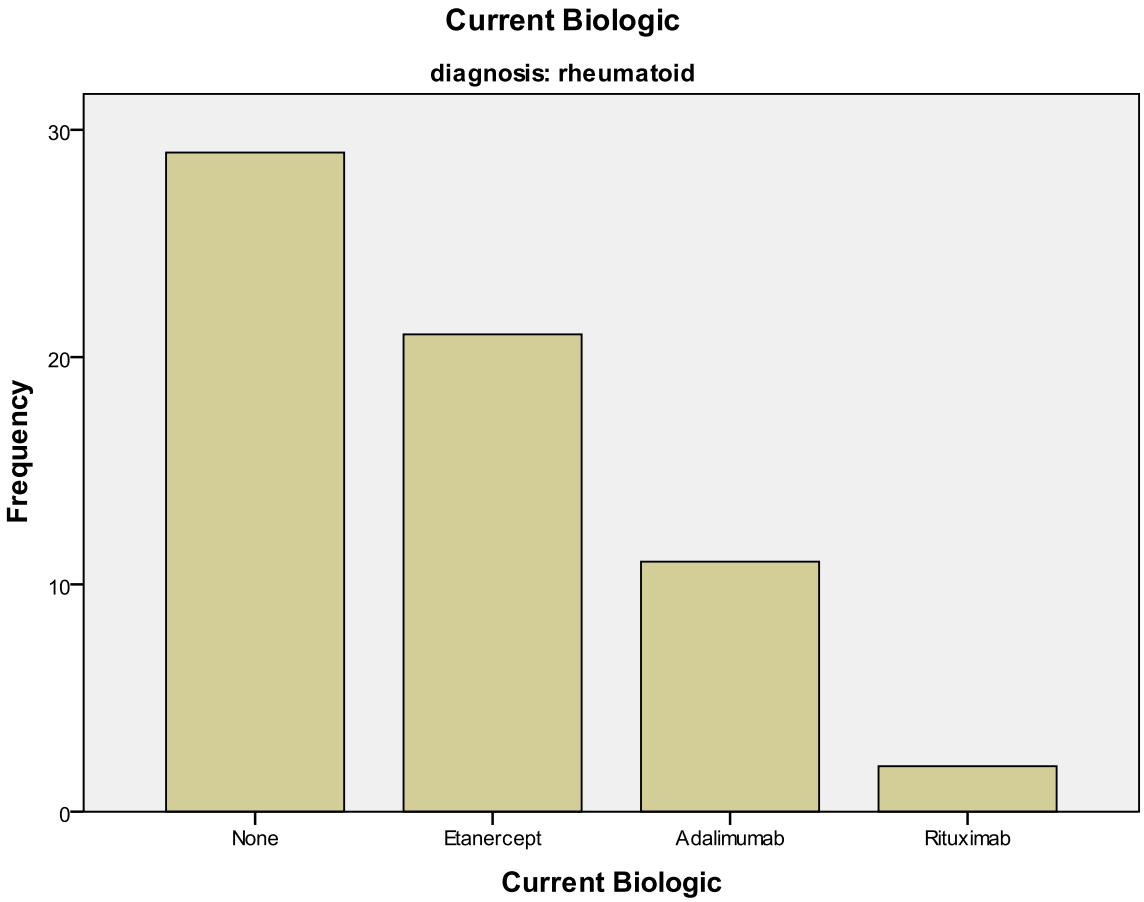


Figure 3.6: Current biologic usage

NSAIDs:

Of the total study group of 111 patients, 40 were taking a non-selective COX 2 nonsteroidal anti-inflammatory (NSAID) medication when entering the study. 28 of these patients had rheumatoid arthritis. This was significantly higher than the number of OAs currently taking an NSAID, p = 0.035 (figure 3.7). Diclofenac was the most commonly prescribed non-selective COX 2 NSAID. Naproxen was the 2nd most commonly prescribed (table 3.7, figure 3.8).

15 RA patients and 24 OA patients had never been on a non-selective COX2 NSAID for their disease, prior to the study. Diclofenac was the most commonly prescribed non-selective COX2 NSAID prior to the study, followed by ibuprofen (table 3.8, figure 3.9).

4 RA patients and 3 OA patients were on a selective COX2 NSAID at the time of study entry. They were either taking celecoxib or etoricoxib. 3 of the RA group had been taking rofecoxib in the past (table 3.9).

OA patients who were taking an NSAID regularly were found to have a significantly higher weight and waist circumference than OA patients not taking an NSAID, $p=0.045$ and $p=0.036$, respectively. Blood pressure did not differ significantly between patients taking a regular NSAID and those not, $p=0.567$.

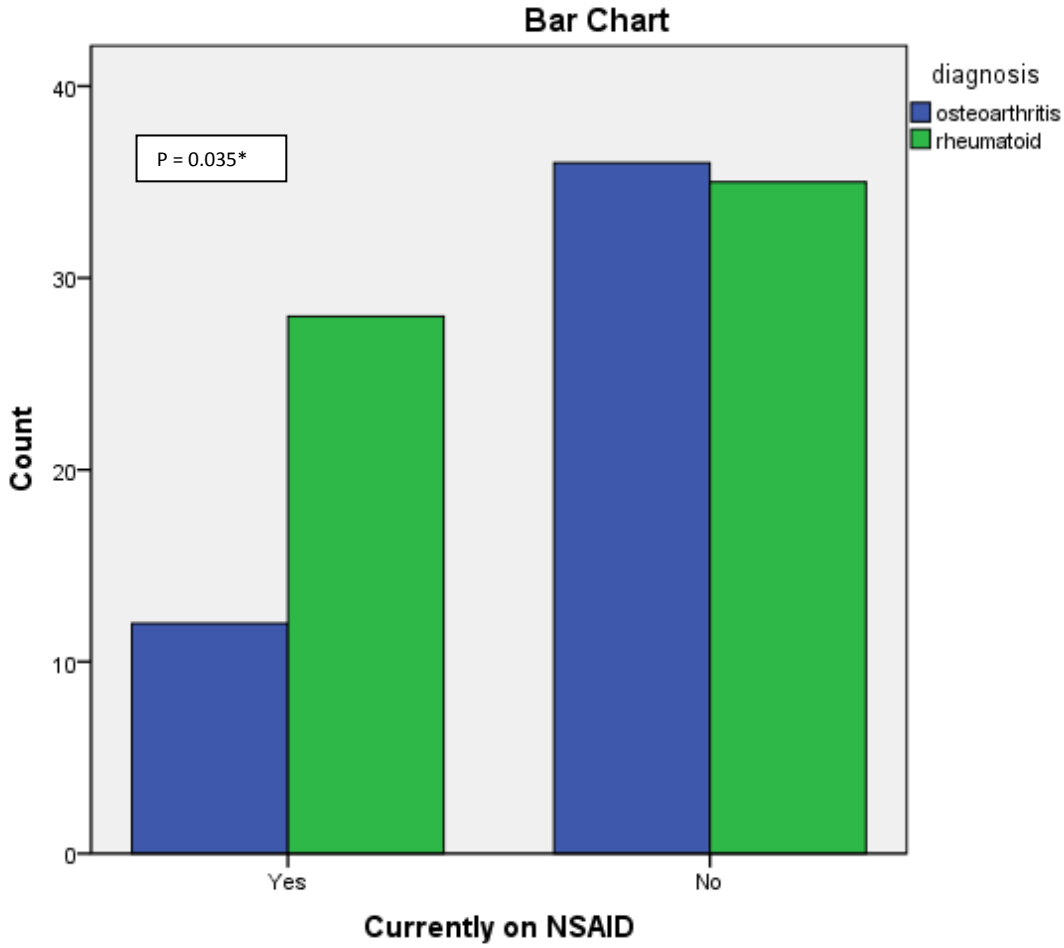


Figure 3.7: Difference in current NSAID use between RA and OA patients

NSAID	RA	(%)	OA	(%)	Total	(%)
None	35	(55.6)	36	(75)	71	(64)
Diclofenac	15	(23.8)	9	(18.8)	24	(21)
Naproxen	9	(14.3)	1	(2.1)	10	(9)
Ibuprofen	2	(3.2)	2	(4.2)	4	(3.6)
Meloxicam	2	(3.2)	0	(0)	2	(1.8)

Table 3.7: Breakdown of current non-selective

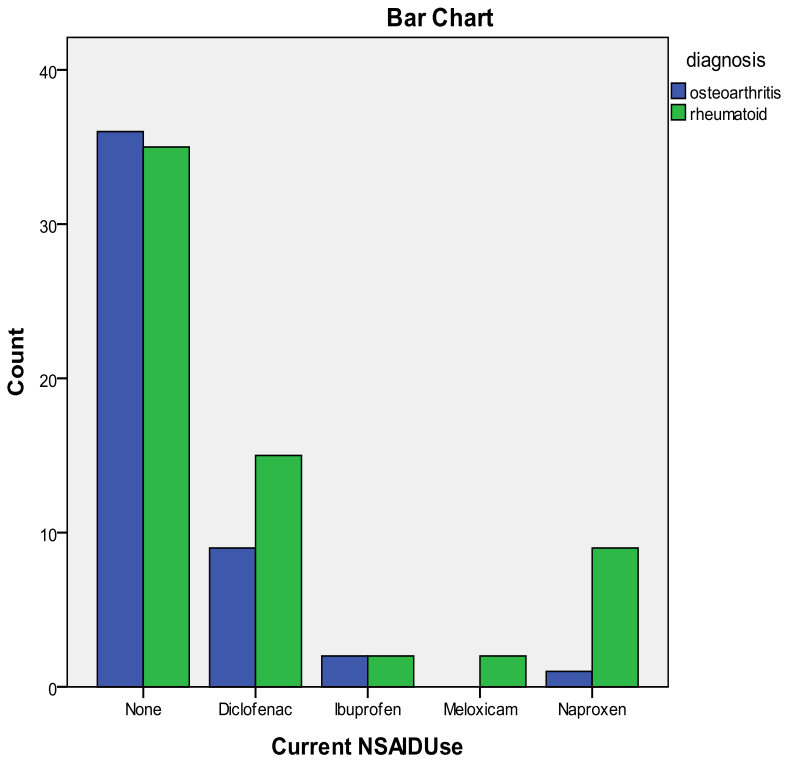


Figure 3.88: Graphic representation of nonselective COX 2 NSAID use

NSAID	RA	(%)	OA	(%)	Total	(%)
None	15	(23.8)	24	(50%)	39	(35.1)
Diclofenac	29	(46)	15	(31.3)	44	(39.6)
Ibuprofen	13	(20.6)	7	(14.6)	20	(18)
Meloxicam	0	(0)	2	(4.2)	2	(1.8)
Naproxen	3	(4.8)	0	(0)	3	(2.7)
Nimesulide	3	(4.8)	0	(0)	3	(2.7)

Table 3.8: Breakdown of previous n on-selective COX2 NSAID use

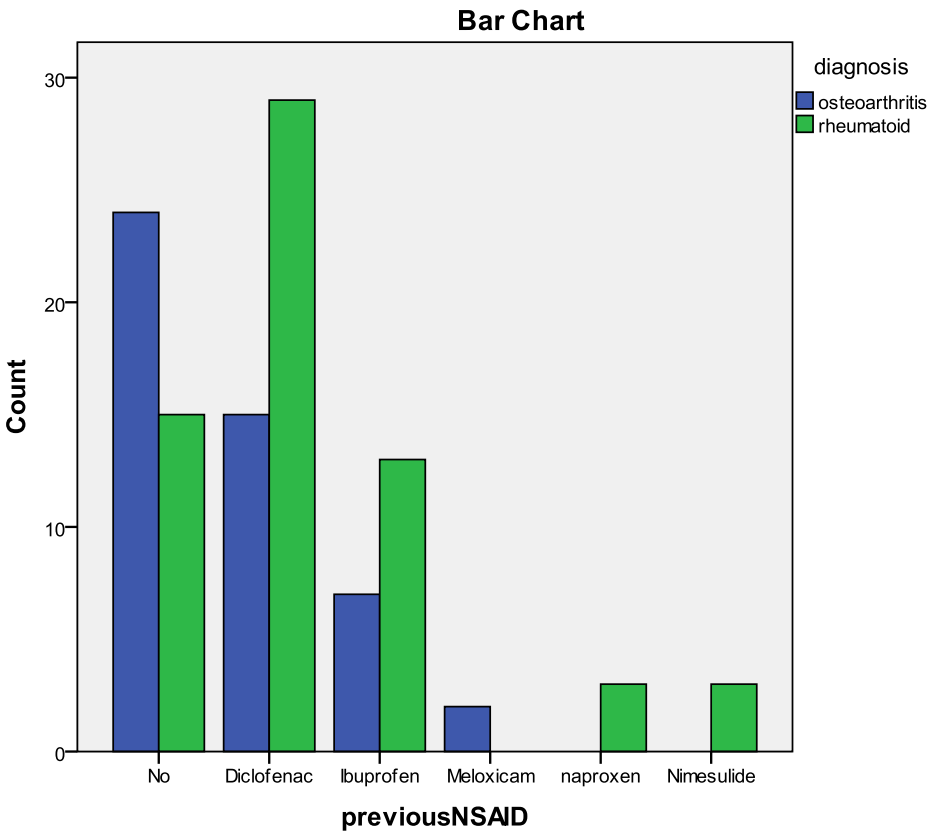


Figure 3.9: Graphic representation of prior use of non-selective COX2 NSAIDS

	RA n	OA n
Current COX2		
Celecoxib	1	1
Etoricoxib	3	2
Previous COX2		
Celebrex	6	0
Etoricoxib	3	2
Rofecoxib	3	0

Table 3.9: current and previous COX2 NSAID use

Prednisolone:

29 people (46%) in the RA group were on steroids at study entry. The mean dose of prednisolone was 7.93mg (+/- SD 3.32). Maximum dose of current steroid use was 15mg. None of the OA group was taking steroids. 52 (82.5%) of the RA group had been steroids at some stage since their diagnosis. Mean prednisolone dose in female patients with RA was 7.5mg (+/-2.99), compared with 8.89mg (+/03.97) in male RAs, p = 0.368. Data was also collected on the maximum steroid dose for each patient. Steroid doses ranged from 0 – 40mg. There was no significant difference in

males and females with regard to maximum steroid doses (20 [10-20] vs. 15[10-20]), p = 0.382.

Statins:

There was no significant difference between number of RA patients and OA patients taking statins. 11 (17.5%) of the RA group and 11 (22.9%) of the OA group were taking a statin. Atorvastatin was the most commonly prescribed in both groups (table 3.10). Nobody was taking a fibrate or ezitimibe at the time of the study.

Statin Type	RA (%)	OA (%)	Total (%)
None	52 (82.5)	37 (77.1)	89 (80.2)
Atorvastatin	9 (14.3)	5 (10.4)	14 (12.6)
Pravastatin	0 (0.0)	2 (4.2%)	2 (1.8%)
Simvastatin	0 (0.0)	1 (2.1%)	1 (0.9%)
Rosuvastatin	2 (3.2)	3 (6.3%)	5 (4.5%)

Table 3.10: Breakdown of Statin types between the groups

Other Cardiac Medications:

There was no significant difference between the 2 groups with regard to use of cardiac medications. 2 RA patients and 2 OA patients were taking NS Aspirin. In the RA group, 3 were taking an ACE inhibitor and 3 an angiotensin receptor blocker. In the OA group, 2 were taking an ACE inhibitor and 5 people were on an angiotensin receptor blocker. One OA patient and 4 RA patients were on beta-blockers when entering the study. 2 RA patients and 1 OA patient were taking a calcium channel blocker. None of the RA group was taking a loop diuretic but 4 were on a thiazide diuretic at study entry. In the OA group, 1 patient was on a loop diuretic and 6 were taking a thiazide diuretic.

Non-cardiac Medications:

30.2% of the RA group were taking calcium and vitamin D supplementation and 14.3% were on a bisphosphonate. In the OA group, 6.3% were on calcium and vitamin D supplementation and 1 patient (2.1%) was on a bisphosphonate.

There was a significant difference between the 2 groups with regard to bisphosphonate use, $p = 0.026$ and calcium/vitamin D use, $p = 0.02$.

There was also a significant difference in use of proton pump inhibitors (PPI) between the 2 groups, $p < 0.001$. 33/63 of RA group were on a PPI and 5/48 of the OA group. Antidepressants were being taken by 5 of the RA group and 2 of the OA group.

Family History:

30.2% (19/63) of the RA group and 16.7% (8/48) of the OA group had a first degree relative with a diagnosis of rheumatoid arthritis, p = 0.1. There was a significant difference in family history (1st degree relative) of myocardial infarction between the groups (table 3.11).

RA patients with a family history of MI had a significantly higher systolic blood pressure than those with no family history of MI, 140.73 mmHg (SD14.47) versus 129.23 mmHg (SD 13.03), p=0.031.

Family Cardiac History	RA (%) N=63	OA (%) N=48	P value
Myocardial Infarction	16 (25.4)	29 (60.4)	<0.001*
Cardiac angioplasty+/- stenting	2 (3.2)	1 (2.1)	0.725
Coronary artery bypass grafting	2 (3.2)	6 (12.5)	0.060
Stroke	10 (15.9)	13 (27.1)	0.149
Peripheral artery disease	1 (1.6)	0 (0)	0.381

Table 3.11: Comparison of cardiac family history between the groups

Smoking status:

54% (29/63) of the RA group were either current (31.7%) or former (22.2%) smokers. 30% of the female RA patients were current smokers and 15% were former smokers. 34.8% of male RA patients currently smoked and the same number were ex-smokers.

52.1% of the OA group were either current (20.8%) or former (31.3%) smokers. 20.5% OA females currently smoke and 30.8% were previous smokers. 22.2% OA males currently smoke and 33.3% of them were previous smokers.

There was no significant difference between the number of smokers in the RA and OA groups (p = 0.355), also of note, there was no significant difference between the numbers of female RA and OA smokers (p = 0.222) or male RA and OA smokers (p = 0.705) (tables 3.12 and 3.13).

Smoking distribution

			diagnosis		
			osteoarthritis	rheumatoid	Total
Smoker	Never	n	23	29	52
		%	47.9%	46.0%	46.8%
	Current	n	10	20	30
		%	20.8%	31.7%	27.0%
	Former	N	15	14	29
		%	31.3%	22.2%	26.1%
Total		N	48	63	111
		%	100.0%	100.0%	100.0%

Table 3.12: Illustrating % of smokers and ex-smokers in the study group.

Smoking distribution between the sexes in the RA group

			SEX		
			female	male	Total
Smoker	Never	N	22	7	29
		%	55.0%	30.4%	46.0%
	Current	N	12	8	20
		%	30.0%	34.8%	31.7%
	Former	N	6	8	14
		%	15.0%	34.8%	22.2%
Total		N	40	23	63
		%	100.0%	100.0%	100.0%

Table 3.13: Illustrating % of male and female smokers in the RA group

Smoking Pack Years:

The median smoking pack years for the total study group is 20yrs (IQR 10-27.5), for the RA group 20yrs (IQR 10-30) and 15yrs (IQR 10-20) for the OA group. There was no significant difference between the 2 groups ($p = 0.193$).

The median smoking pack years for both female and male OAs was 15yrs (IQR 10-20). The median smoking pack years for both female and male RAs was 20yrs (IQR

15-30). There was no statistically significant difference in smoking pack years between females in the OA group or RA group (p = 0.645) or between males in the OA and RA groups (p = 0.189).

Alcohol Consumption:

57.7% (64/111) of the total study group drank alcohol. 54% (34/63) of RA patients consumed alcohol and 62.5% (30/48) of the OA group consumed alcohol. The median units of alcohol consumption per week in the RA group was 7.5 (IQR 3 – 10) and 7 (IQR 3 – 12) in the OA group. There was no significant difference between the 2 groups (p = 0.787). The median weekly alcohol consumption in females was 4.5 units (IQR 2 – 9.5) and in males was 12units (IQR10 – 20), this difference was significant, p <0.001 (figure 3.10).

Within the RA group, median alcohol consumption among females was 4 units/wk (IQR2 2 – 10) and 10 units/wk (IQR 10 – 16) among males, p = 0.001. Within the OA group, median alcohol consumption among females was 5 units/wk (IQR 2.5 - 8.5) and 20units/wk (IQR 12 -20) in the males, p = 0.005. Examining females separately, there was no significant difference in alcohol consumption rates between RA patients and OA patients (p =0.611). The same was found for male RA patients versus male OA patients (p = 0.337).

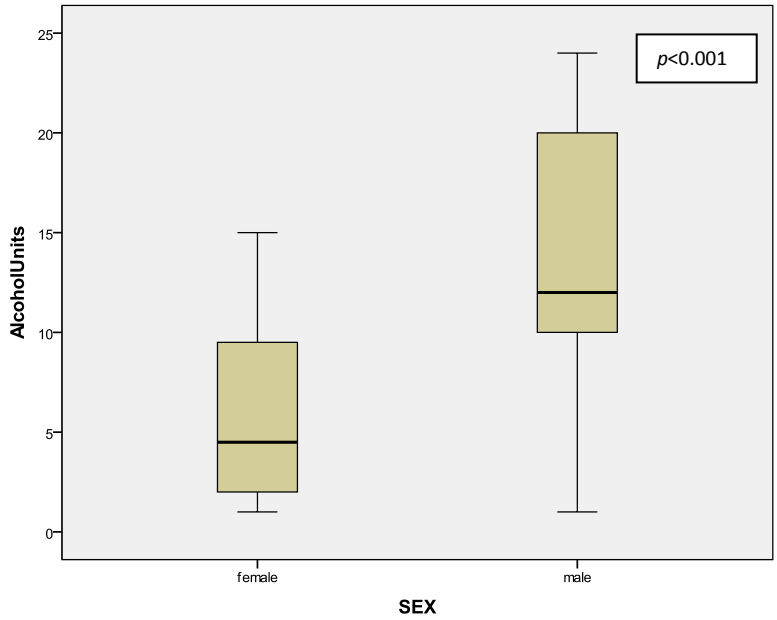


Figure 3.10: Comparison of alcohol consumption between all females and males

Other medical conditions:

One male in the RA group had type 2 diabetes mellitus (DM). Nobody in the OA group had DM. 19% (12/63) of the RA group and 20.8% (10/48) of the OA group had a diagnosis of hypertension (HTN) prior to entering the study, this difference was not significant, p =0.815 (table 3.14). 23.4% (26/111) of the total study group had a diagnosis of hypercholesterolaemia, 12 patients (8 females and 4 males) were from the RA group and 14 patients (11 females and 3 males) from the OA group. There was no significant difference between the numbers of RA and OA patients with an elevated cholesterol, or between females and males with hypercholesterolaemia (p = 0.212, p =0.806 respectively).

Information was also collected on presence of coexisting autoimmune conditions.

Nobody in the study had a diagnosis of Addison's disease, alopecia, autoimmune hepatitis, coeliac disease, vitiligo or Sjogren's syndrome. There was no history of Felty's syndrome.

7 RA patients (4 females and 3 males) had a diagnosis of Raynauds syndrome, compared with none in the OA group ($p = 0.017$). 9 people had a diagnosis of hypothyroidism (2 RAs and 7 OAs, $p = 0.038$) (figure 3.11). There was nobody with a history of hyperthyroidism in the study.

With regard to family history of autoimmune diseases other than RA, one OA patient had a 1st degree relative with systemic lupus erythematosus, as did 1 RA patient. 3 OA patients had a 1st degree relative with coeliac disease and 2 RA patients had a positive family history for coeliac disease.

7.9% (5/63) RA patients and 4.2% (2/48) OA patients had a history of joint replacement performed because of their disease.

	RA	OA
Hypertension present, n (%)	12 (19%)	10 (20.8)
No diagnosis of hypertension, n (%)	51 (81)	38 (79.2)

Table 3.14: Hypertension in the OA and RA groups

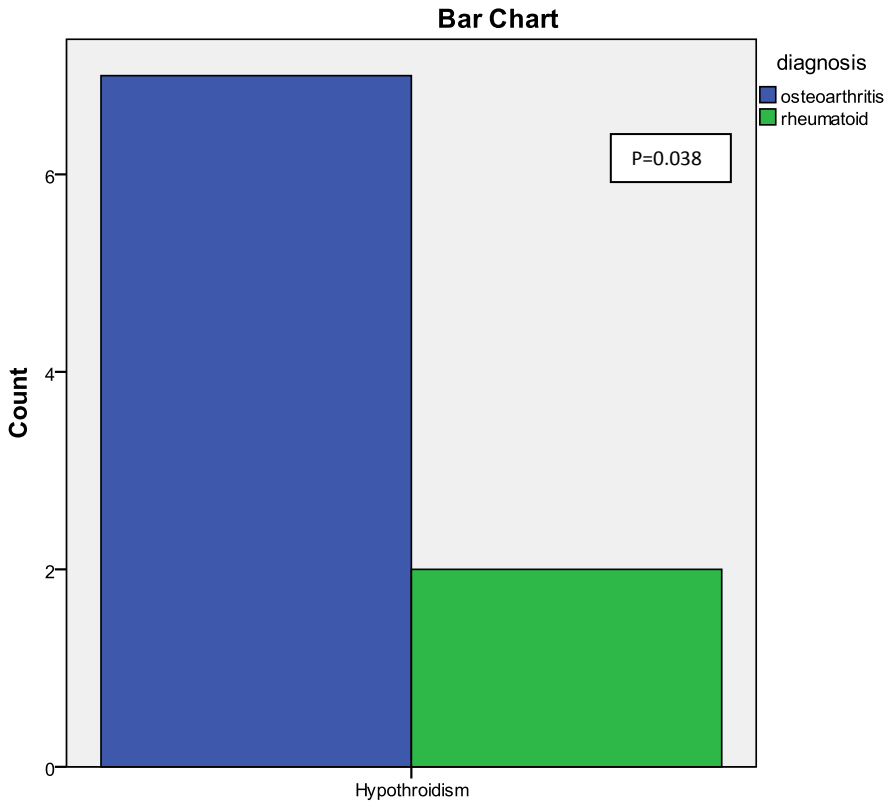


Figure 3.11: Hypothroidism in OA vs RA

Menopause:

27/40 (67.5%) of the female RA patients and 25/39 (64%) of the female OA patients were postmenopausal. The mean age of menopause in the RA group was 48.4yrs (SD+/-2.97) compared with 48.1yrs (SD+/-2.93) in the OA group, p ,= 0.727.

Clinical Examination:

Mean heart rate in the RA group was 73bpm (13.06), compared with 74bpm (6.85) in the OA group, p = 0.472. Heart rates were similar among males in the RA and OA groups (RA 74bpm+/-10.18, OA 74bpm+/- 5.36), *p* = 0.802, and also among females (RA 72bpm+/-14.52, OA 75bpm+/- 7.21), *p* = 0.361.

Blood pressure was measured after a 15 minute period of rest. Mean blood pressure in the RA group was 128.86/79.83 (SD 11.03, SD 7.77) and 131.71/79.47 (SD14.04, SD8.35) in the OA group. There was no significant difference in systolic or diastolic blood pressure readings between RA and OA patients, p=0.276 and p=0.829, respectively. Males with RA had higher systolic and diastolic blood pressure compared to females with RA, p=0.000 and p=0.000. OA males had a significantly higher diastolic blood pressure than females, p=0.049 (table 3.15).

One RA patient had a joint effusion at the time of examination. Seven RA patients had subcutaneous nodules associated with their disease, while 1 OA patient had evidence of Heberden's nodes on hand examination.

		Mean systolic blood pressure, mmHg (SD)	p	Mean diastolic blood pressure, mmHg (SD)	p
RA	Males	141.32 (10.29)	0.000*	85.89 (5.78)	0.000*
	Females	120.00 (12.89)		75.66 (7.27)	
OA	Males	134.33 (10.46)	0.102	83.44 (5.03)	0.049*
	Females	127.36 (10.85)		78.85 (8.14)	

Table 3.15: Comparison of systolic and diastolic blood pressure in male and female RA and OA patients. SD, standard deviation; p, significance level.

Weight:

Weight was normally distributed among the study group. It ranged from 50 to 115kg. Mean weight in the RA group was 72.55kg (SD+/- 14.54) compared with 75.21kg (SD+/- 13.56) in the OA group (p = 0.323). Mean weight for all the females was 68.95kg (SD+/- 12.58), compared with 85.43Kg (SD+/- 10.48) for males (p <0.001). Difference in weight between the sexes was also significant within the disease groups (RA p<0.001, OA p<0.001). There was a significant difference in weight between female RA patients and female OA patients (table 3.16).

Sex	RA	OA	P value
Female	65.32kg(SD+/- 10.49)	72.67kg(SD+/-13.56)	0.009
Male	85.12kg(SD+/-11.85)	86.22kg(SD+/-6.2)	0.734

Table 3.16: Comparison of weight between the sexes in RA and OA

Height:

Height was normally distributed in the study group. Mean height of females was 161cm (SD+/- 6.79), compared with 174cm (SD+/- 5.97) for males, p <0.001 (figure 3.12). Height is the RA group (165.76cm+/-8.98) was similar to that in the OA group (163.60+/-8.36), *p* =0.215. Also, there was no significant difference in height between females with RA or OA (p = 0.845) or between males with RA or OA (p = 0.452).

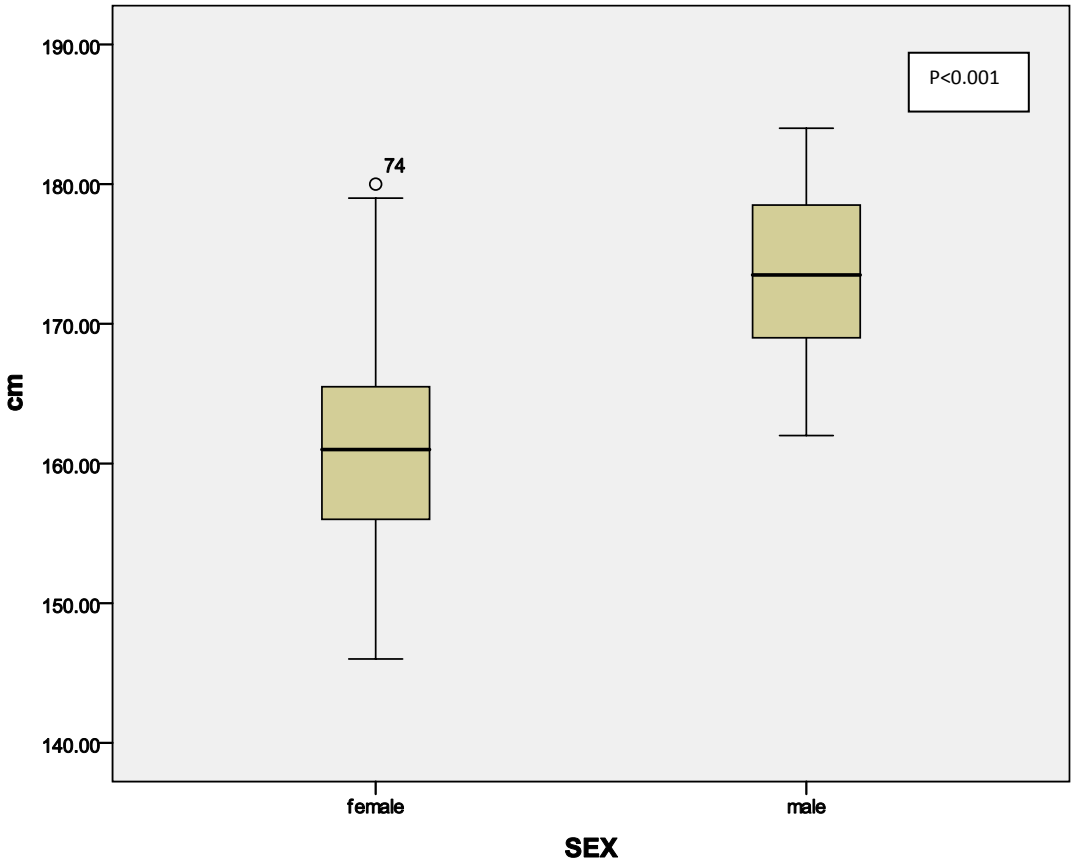


Figure 3.12: Boxplot comparing male and female height

Body Mass Index:

Mean body mass index (BMI) for the study group was 26.99 kg/m^2 (+/- 3.95). Mean BMI in the RA group was 26.20 kg/m^2 (+/- 3.48) compared with 28.05 kg/m^2 (+/- 4.31) in the OA group, p = 0.017 (table 3.17). Mean BMI for all the females was 26.50kg/m^2 (+/- 4.13) compared with 28.23kg/m^2 (+/- 3.21) for all males, *p* = 0.021. For a breakdown of BMI within the diagnostic groups see table 3.18.

	Mean BMI (kg/m^2) in females (SD)	Mean BMI (kg.m^2) in males (SD)	P
RA	25.02 (3.01)	28.24 (3.34)	0.000*
OA	28.01 (4.58)	28.21 (3.02)	0.876
P	0.001*	0.982	

Table 3.17: Mean BMI kg/m^2 (SD) within the groups.

Patients' BMI was also categorized into underweight / normal weight / overweight / obese. A BMI of less than 18.5kg/m^2 was considered underweight; BMI between 18.5 kg/m^2 and 25kg/m^2 was considered normal, BMI greater than 25kg/m^2 but less than 30kg/m^2 was overweight and BMI greater then 30kg/m^2 was obese. The RA group were more likely to have a normal BMI compared with the OA group, p = 0.006 and female RA patients were more likely to have a normal BMI compared

with male RA patients, $p = 0.016$. There was a significant difference in the number of male and female obese patients in the RA group, $p = 0.008$.

BMI category	RA female n	RA male n	OA female n	OA male n
Underweight	0	0	1	0
Normal	23	6	9	1
Overweight	16	11	20	5
Obese	1	6	9	3

Table 3.18: Number of patients in each BMI category

Waist Hip Ratio:

Mean female waist circumference was 86.06cm (+/- 10.64), compared with mean male waist circumference of 97.28cm (+/- 8.29), $p < 0.001$. Within the female population there was a statistically significant difference in waist circumference between the RAs and OAs, (83.56cm [11.14] and 88.62cm [9.58], $p = 0.034$). Hip circumference was also significantly smaller in RA females compared with OA females (101.40cm [7.13] and 106.18cm [10.47], respectively), $p = 0.021$. Male hip circumference was significantly smaller in the RA group compared with the OA group, (105.35cm [6.57] and 109.78cm [3.87] respectively), $p = 0.027$. This was not the case for male waist circumference, $p = 0.866$.

However, waist hip ratio (WHR) was similar between the RA group (0.86+/-0.10) and the OA group (0.85+/-0.06), p = 0.287. Within the diagnostic groups, there was no significant difference in waist hip ratio in females or in males (table 3.19).

	RA	OA	P value
Female WHR	0.83 (+/-0.08)	0.84 (+/-0.05)	P = 0.447
Male WHR	0.93 (+/-0.09)	0.89 (+/-0.08)	P = 0.260

.

Table 3.19: Comparison of Waist Hip Ratio in male and females with RA and OA

General Health Measures and Disease Activity Scores:

Median general health (GH) score for the total group, as measured on a 100mm visual analogue scale, was 25 (IQR 10-30). The median OA GH score was significantly better than the RA score (OA 20mm [10-30], RA 30mm [17.5-37.5]), *p* = 0.025. There was no statistically significant difference in GH scores between males and females (table 3.20).

RA patients on steroids has a significantly worse general health score compared with RA patients not taking steroids, 30mm (20-40) compared with 20mm (10-30), p=0.037. Within the RA group, CRP correlated significantly with GH score, r=0.336, p=0.007.

	RA	OA	P value
Female GH score	30 (10-40)	20 (10-30)	P = 0.110
Male GH score	30 (20-32.5)	15 (10-20)	P = 0.057
P value	P = 0.994	P = 0.324	

Table 3.20: Difference in median (IQR) GH score between females and males in RA and OA groups

Short Form 36

Physical component score:

The mean physical component scores (PCS) were similar in the RA and OA groups (RA 48.15+/- 7.42, OA 48.04+/- 8.73), p = 0.946, and between males and females (males 49.32+/- 7.62, females 47.61+/- 8.11), p = 0.296. Examining females separately, the mean PCS was similar in the RA (47.89+/-7.93) and OA (47.31+/- 8.39) groups, p = 0.753. The same was true for male RA patients compared with male OA patients (48.59+/- 6.58, 51.19+/- 10.00, respectively), p = 0.486.

PCS did not differ between the different treatment groups with rheumatoid arthritis, except in the case of current steroid use where those RA patients taking steroids has

a significantly worse PCS, 45.03 (SD7.77) compared to those not on steroids, 50.80 (SD6.03), p=0.002, (figure 3.13).

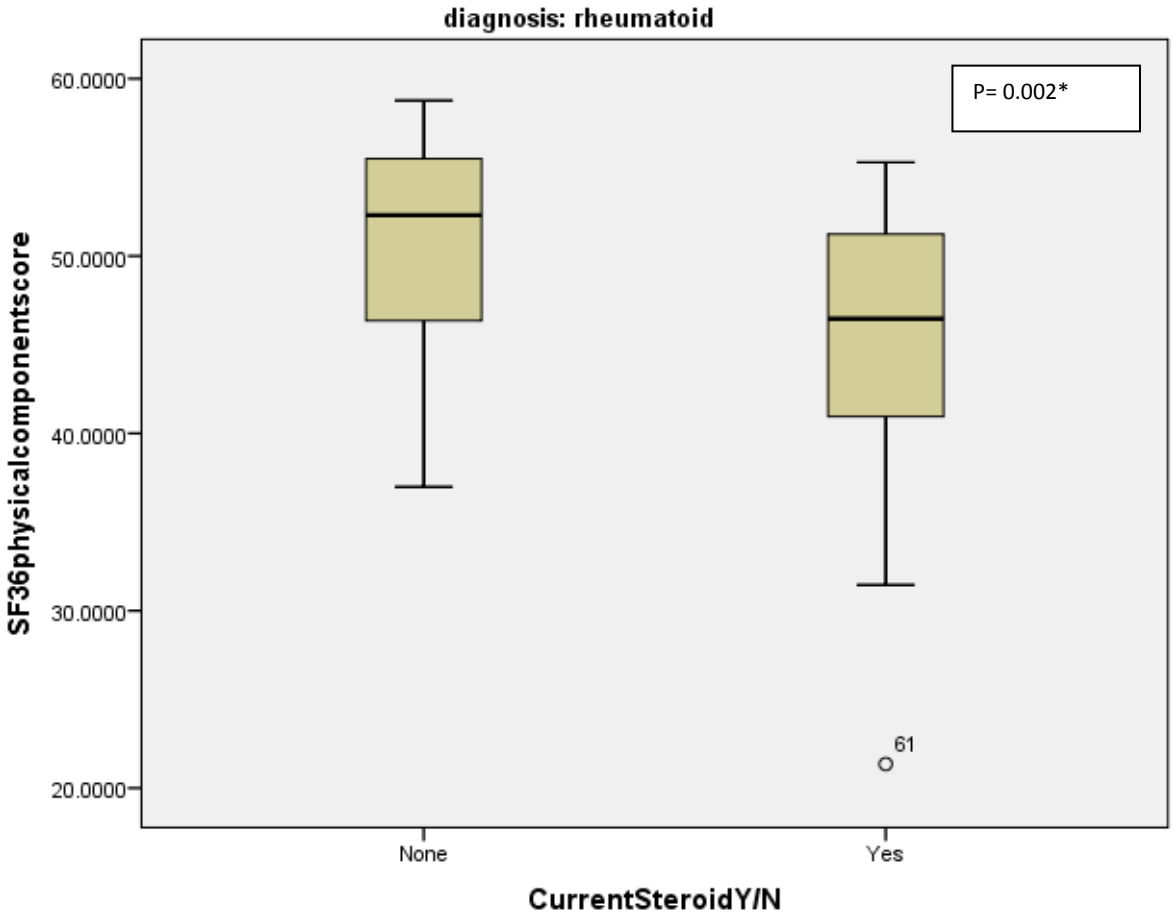


Figure 3.13: Boxplot comparing physical component score in RA patients taking steroids with RA patients not taking steroids.

Mental Component Score:

Mean mental component scores (MCS) were not significantly different between the RA and OA groups (53.25+/- 5.83, 52.43+/-5.94, respectively), p = 0.473, or between males and females (53.18+/-3.47, 52.78+/-6.61 respectively), p = 0.673. For a breakdown of MCSs within the RA and OA groups see table 3.21. RA patients who were on a DMARD or an NSAID at the time of the study had a significantly worse MCS than RA patients not taking a DMARD or NSAID, p=0.005 and p=0.031, respectively. However, those RA patients on a biologic agent had a better MCS than RA patients not taking a biologic DMARD, p=0.01.

	RA	OA	P value
Female mean MCS (SD)	53.35 (6.77)	52.19 (6.28)	0.436
Male mean MCS (SD)	53.06 (3.82)	53.50 (2.50)	0.704
P value	0.827	0.329	

Table 3.21: Comparison of mean MCSs in males and females with RA and OA

Health Assessment Questionnaire:

Median health assessment questionnaire (HAQ) score for the RA group was 0.375 (0.125-0.750), compared with 0.25 (0-0.625) for the OA group, $p = 0.135$.There was a significant difference between HAQ scores of males and females (0.25 [0-0.375], 0.5 [0.125-0.75], respectively), $p = 0.002$. HAQ scores within the diagnostic groups for males and females are detailed in table 3.22 and figure 3.14.

RA patients on steroids had a significantly higher HAQ than RA patients not on steroids, 0.5 (0.25 – 1.25) versus 0.25 (0.125 – 0.625), p=0.025. RA patients with a family history of CVD had a significantly higher HAQ than those with no family history of CVD, 0.625 (0.25 – 1.125) versus 0.25 (0.125 – 0.625), p=0.032.

In the OA group, the HAQ was significantly worse in patients who were overweight or obese, p=0.029.

	RA	OA	P value
Median HAQ score (IQR) in females	0.563 (0.188-0.938)	0.375 (0.125-0.688)	0.033*
Median HAQ score (IQR) in males	0.25 (0-0.375)	0 (0)	0.381
P value	0.002*	0.101	

Table 3.22: HAQ scores in RA and OA patients

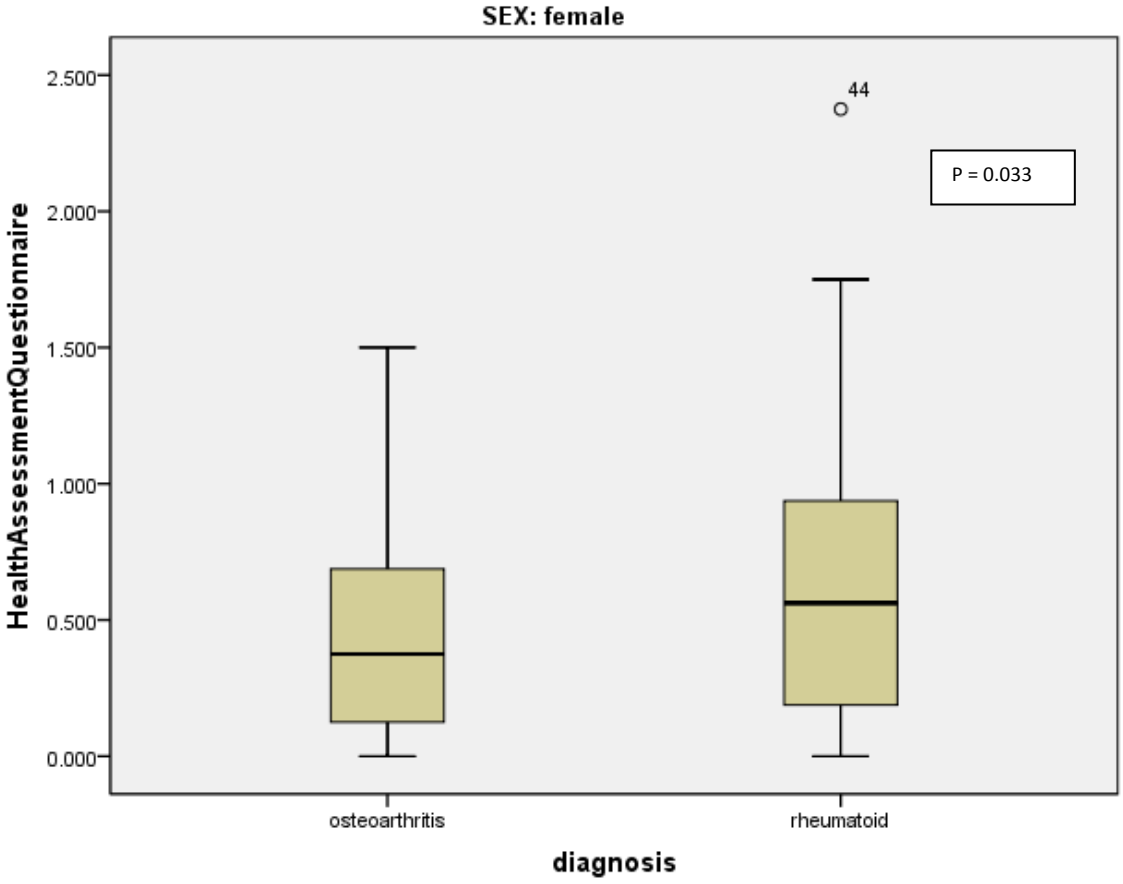


Figure 3.14: Boxplot comparing HAQ scores in RA and OA females

Visual Analogue Scale:

A visual analogue scale (VAS) of physician's assessment of disease activity revealed a median VAS for the total study group of 10mm (10-30). The median OA physician's VAS was 10mm (10-20), compared with 20mm (10-30) for the RA group, $p < 0.001$. When all females were compared, there was a significant difference between the RA and OA groups (20mm [10-30], 10mm [10-20], respectively), $p = 0.003$. The same was found when comparing RA males to OA males (20mm [15-25], 10mm [10-10], respectively), $p = 0.011$.

Patients scored a visual analogue scale as a self- assessment of pain. Median VAS for pain was 20mm (10-30) in both males and females. The scores were significantly worse for RA patients compared with OA patients and also for female RA patients compared with female OA patients (table 3.23).

	RA	OA	P value
Median pain VAS in females (IQR)	20 (10-30)	10 (10-20)	0.033*
Median pain VAS in males (IQR)	20 (15-30)	10 (10-20)	0.044

Table 3.23: Median (IQR) VAS for self-assessment of pain in both groups.

A patient self-assessment for disease activity was also scored on a visual analogue scale. Results are reported as medians (interquartile range). RA patient scores for disease activity were significantly worse than OA patient scores (20mm [10-30], 10mm [3-20], p = 0.004). Male RA patients also had a significantly worse disease activity VAS than male OA patients (RA 20mm [15-25], OA 10mm [0-10], *p* = 0.017). There was no significant difference between female RA patients activity VAS and female OA patient activity VAS (20mm [10-30], 10mm [7.5-20], respectively), p = 0.06.

Disease Activity Scores for Rheumatoid Arthritis:

In the RA group, tender and swollen joint counts were recorded for calculation of the disease activity score 28 ESR (DAS28ESR) and CRP (DAS28CRP).

28 female RA patients and 17 male RA patients had 1 or more tender joints on clinical examination. The median tender joint count for all the RAs was 3 (0-6). The median tender joint count (TJC) was 2.5 (0-7) for females and 4 (0.5-4.5) for males, there was no significant difference between males and females, p = 0.839.

RA patients who were on a DMARD at the time of the study had a higher median tender joint count than those not on a DMARD (3.5[0-6] vs. 2 [1-6]), however the difference was not statistically significant, p = 0.844. Number of tender joint in those currently on a biologic agent compared to those RA patients not currently on a biologic agent was not significantly different (3.5 [1-7], 3 [1-6], respectively), p = 0.967.

There was no significant difference in number of tender joints in RA patients currently taking an NSAID compared with those not currently on an NSAID (2.5[0-7.5], 4 [0-5.5], respectively), p = 0.933).

With regard to steroid use, the number of tender joints was similar in RA patients currently on prednisolone and those not currently taking prednisolone (3[1-7], 2.5[0-6], respectively), p = 0.208. Current smokers had a TJC of 3.5(1-6), compared with 3(0-6.5) in the non-smokers, p = 0.549.

33 females with RA and 18 males with RA had 1 or more swollen joints on examination. Median number of swollen joints for the total RA group was 4 (1.5-6). Median number of swollen joints in the females was 4.5 (2-6.5) and 3 (1-4.5) in the males, p = 0.094. RA patients currently on a DMARD, biologic, steroid or NSAID did not have a statistically significant difference in median swollen joint count compared with those RA patients not taking any of the above medications. Current smoking status did not affect the median number of swollen joints.

DAS28 ESR was normally distributed in the RA group. Mean DAS 28 ESR for the total RA group was 3.62 (+/- 1.33). There was no significant difference between male and female DAS28 ESR scores, p = 0.23. DAS28 ESR scores were also analysed based on current medications and smoking status (table 3.24).

		DAS28ESR Mean(SD)	P value
Current DMARD	Yes	3.49 (1.25)	0.226
	No	3.99 (1.51)	
Current Biologic	Yes	3.62 (1.51)	0.993
	No	3.62 (1.11)	
Current NSAID	Yes	3.66 (1.20)	0.835
	No	3.59 (1.44)	
Current Steroid	Yes	3.94 (1.05)	0.077
	No	3.36 (1.49)	
Current Smoker	Yes	3.92 (0.99)	0.171
	No	3.45 (1.45)	

Table 3.24: Breakdown of DAS28 ESR scores based on medications and current smoking status

Disease activity was also examined as a categorical variable, using a DAS28ESR of >3.2 as a measure of active disease, to compare patients with moderate or high disease activity in RA to those with low disease activity or remission. 43 of the 63 RA patients had a DAS28ESR of greater than 3.2. RA patients with a high DAS28ESR had significantly worse scores for general health, health assessment questionnaire and visual analogue scales (table 3.25).

	DAS28ESR < 3.2	DAS28ESR > 3.2	P
General health score	20 (10 – 27.5)	30 (20 – 40)	0.000*
HAQ	0.188 (0 – 0.375)	0.5 (0.25 – 0.75)	0.005*
VAS physician assessment of disease activity	10 (10- 20)	20 (20 – 30)	0.000*
VAS patient assessment of pain	10 (9 – 20)	20 (20 -35)	0.001*
VAS patients assessment of disease activity	10 (5 – 20)	20 (15 – 40)	0.000*

Table 3.25: Comparison of health and disease activity scores in RA patients with low and high disease activity using DAS28ESR. Results are all median values with inter-quartile range.

DAS28 CRP was also measured in the RA group. Mean DAS28 CRP for the RA group was 3.55 (+/-1.12). DAS28 CRP scores were similar among males and females (3.45 [+/- 0.88], 3.60 [+/- 1.24], respectively), p = 0.579. DAS28 CRP scores were also analysed based on current medications and smoking status (table 3.26). There was a statistically significantly higher DAS28 CRP in RA patients currently on steroids compared with those not on steroids, p = 0.03 (figure 3.15).

		DAS28CRP Mean(SD)	P value
Current DMARD	Yes	3.52 (1.09)	0.801
	No	3.61 (1.23)	
Current Biologic	Yes	3.49 (1.17)	0.646
	No	3.62 (1.07)	
Current NSAID	Yes	3.58 (1.09)	0.825
	No	3.52 (1.15)	
Current Steroid	Yes	3.87 (0.92)	0.030*
	No	3.27 (1.21)	
Current Smoker	Yes	3.66 (0.91)	0.563
	No	3.50 (1.21)	

Table 3.26: Breakdown of DAS28 CRP scores based on medications and current smoking status

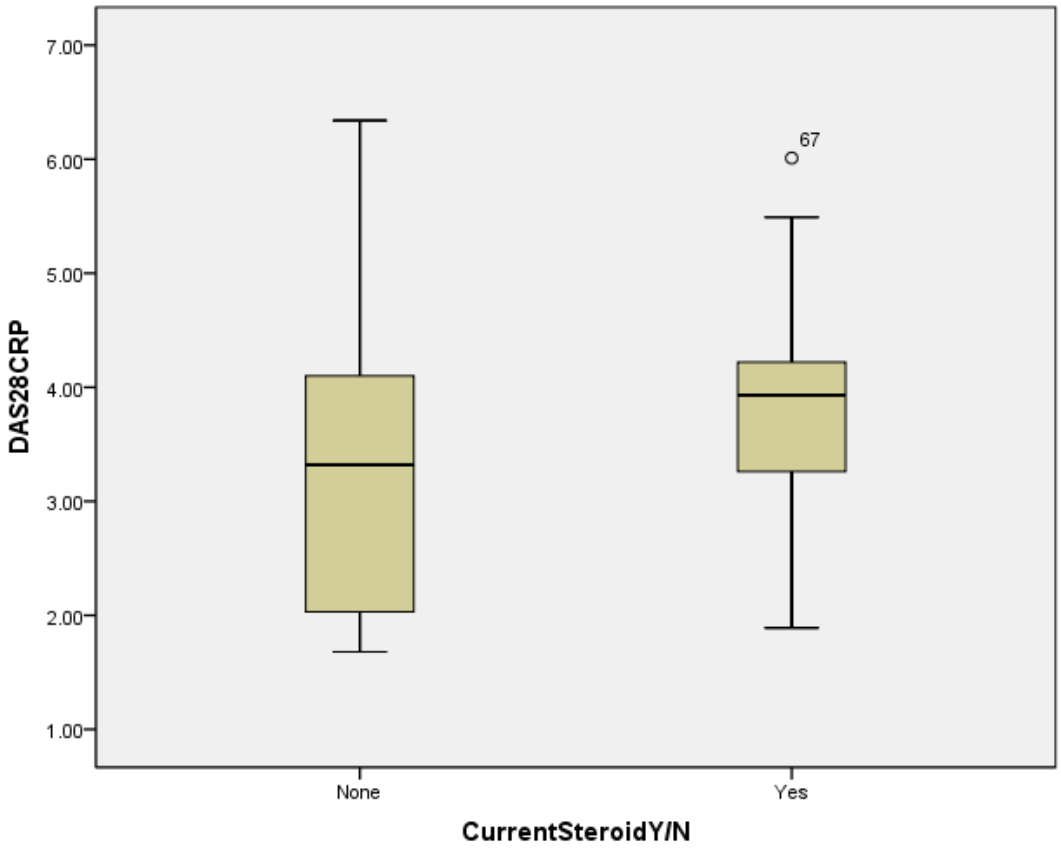


Figure 3.15: Boxplot comparing DAS28 CRP scores in RA patients on steroids with those not on steroids

Western Ontario and McMaster Universities Osteoarthritis Index:

Impact of disease on OA patients was assessed using a scale called Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). This resulted in a score for pain, stiffness, physical function and total score for each patient.

Looking at WOMAC pain score as an ordinal variable, the median score was 2 (1 -4) for all OAs. Median score for pain in those OA patients currently on an NSAID was not significantly different from that of patients not currently taking NSAIDs, (2 [1-5], 1 [1-3], respectively), $p = 0.134$ (table 3.27).

Median score for stiffness in the total OA group was 1 (IQR 1-2) out of a possible maximum of 8. Median stiffness score in OA patients currently on an NSAID was 1.5 (1-2.5) compared with a score of 1 (1-2) in those not on an NSAID, p = 0.183.

The median score for physical function in the total OA group was 5 (1-8.5). 12 of the OA group were on an NSAID at the time of the study and their median physical function score was 5.5 (2-12) compared with a score of 5 (1-8) in those not on an NSAID, p = 0.451. Pain, stiffness and physical function scores were combined to give a total WOMAC score for each OA patient. Median total WOMAC score was 8 (3-12.5) for the total OA group. Being on an NSAID or not did not affect the total WOMAC score, p = 0.404. OA patients who were overweight or obese had a significantly worse total WOMAC score, p=0.041.

	Current NSAID	No current NSAID	p
WOMAC total score, median (IQR)	9 (4.5 – 11)	8 (3 – 11)	0.260
WOMAC pain score, median (IQR)	2 (1- 5)	1 (1-3)	0.134
WOMAC stiffness score, median (IQR)	1.5 (1-2.5)	1 (1 -2)	0.183
WOMAC physical function score, median (IQR)	5.5 (2-12)	5.0 (1-8)	0.451

Table 3.27: Comparison of WOMAC score components in OA patients on an NSAID and those not taking an NSAID. WOMAC, Western Ontario and McMaster Universities osteoarthritis index; p, significance level.

Baseline Phlebotomy:

All patients had bloods taken for full blood count, urea, creatinine and electrolytes (table 3.18). Blood samples were taken to measure d-dimers, as a marker of coagulation, however, IgM Rheumatoid factor positivity interferes with the d-dimer assay, so these results not analysed as they not were felt to be accurate measurements of coagulation in our RA group.

Serum calcium was significantly higher in the OA group, p=0.001. Within the OA group, smokers had a significantly higher serum calcium than non-smokers, 2.52mmol/l (SD 0.09) compared with 2.45mol/l (SD 0.09), p=0.032.

Glomerular filtration rate (eGFR) was calculated using the validated MDRD formula. eGFR was significantly lower in the OA group, 73.98ml/min (SD 11.53) compared with79.51ml/min (SD13.57) in the RA group, p=0.022.

	RA	OA	p
Hb (g/dl)	13.52 (SD 1.24)	13.79 (SD 1.07)	0.225
WCC ($x10^9$/l)	7.63 (SD 2.16)	6.55 (SD 1.79)	0.005*
Plts ($x10^9$/l)	266 (SD 60.03)	274 (SD 65.33)	0.556
Urea (mmol/l)	5.9 (SD 1.73)	5.3 (SD 0.78)	0.020*
Creatinine (umol/l)	81 (SD 13.54)	81 (SD 9.32)	0.854
Calcium (mmol/l)	2.40 (SD 0.09)	2.46 (SD 0.09)	0.001*
Sodium (mmol/l)	141 (SD 2.14)	141 (SD 2.24)	0.487
Potassium (mmol/l)	4.5 (SD 0.39)	4.5 (SD 0.51)	0.815
Albumin (g/l)	37.5 (SD 3.12)	39.7 (SD 2.73)	0.000*

Table 3.28: Comparison of mean values for routine bloods in RA and OA patients. SD, standard deviation; p, significance level; RA, rheumatoid arthritis; OA, osteoarthritis

Within the RA group, values for routine bloods were similar across the different treatment groups, except in the case of current steroid use. This group had a significantly higher total white cell count than RA patients not on steroids, 8.31 $x10^9$/l (SD 2.31) compared with 7.05 $x10^9$/l (SD 1.87), p=0.025.

RA patients with low disease activity, as characterised by a DAS28ESR less than 3.2, had significantly lower white cell counts and higher albumin level compared with RA patients whose DAS28ESR was greater than 3.2 (table 3.29). There was a

significant correlation between ESR and haemoglobin level and ESR and albumin in the RA group, r = -0.413, p=0.001 and r= -0.432, p=0.000. Albumin also correlated with Il6 and Il8 concentrations, r= -0.376, p=0.004 and r = -0.315, p=0.017. Platelets in the RA group correlated with a number of adhesion molecules (table 3.30) and also with E/A ratio on transthoracic echocardiogram, r = -0.307, p=0.017.

Thyroid function was assessed by measuring TSH and T4 levels. These were not normally distributed so medians are quoted and non-parametric tests used for comparisons. There was no significant difference in TSH or T4 levels between RA and OA patients (table 3.31). TSH levels in the RA group correlated significantly with general health status, r=0.320, p=0.011.

	DAS28ESR <3.2	DAS28ESR > 3.2	p
WCC (x 10^9/l)	6.66 (SD 1.54)	8.08 (SD 2.27)	0.016*
Albumin (g/l)	38.75 (SD 2.55)	36.88 (SD 3.22)	0.029*

Table 3.29: Comparison of WCC and albumin concentration in RA patients with low and high disease activity. DAS28ESR; disease activity score 28 joint count with ESR; SD, standard deviation.

	Correlation with platelets, r	p
VCAM	0.025	0.844
ICAM	0.256	0.050*
E-selectin	0.396	0.001*
P-selectin	0.404	0.001*
L-selectin	0.281	0.027*

Table 3.30: Correlation between platelet count and adhesion molecules in RA group. R, correlation coefficient; p, significance level

	Median TSH, mIU/l (IQR)	p	Median T4, pmol/l (IQR)	p
RA	1.54 (0.96 – 2.640	0.115	16.35 (14.80 – 18.50)	0.938
OA	2.04 (1.24 – 3.32)		16.55 (15.10 – 18.20)	

Table 3.31: Comparison of serum TSH and T4 in RA and OA patients. IQR, interquartile range

Serum Concentrations of Antibodies in RA:

Anti-CCP antibody and rheumatoid factor levels were checked in all patients. They were not normally distributed so median values are reported.. Median anti-CCP antibody in the RA group was 144AU/ml (27.5 – 500) and median rheumatoid factor level was 114IU/ml (29.75- 323.75). Nobody in the OA group tested positive for anti-CCP antibody. The median rheumatoid factor level in the OA group was 1.9IU/ml (0 -10).

RA smokers had significantly higher anti-CCP antibody and rheumatoid factor levels than non-smokers (table 3.32). Smoking pack years correlated significantly with anti-CCP antibody and rheumatoid factor levels, r = 0.386, p=0.002, and r = 0.410, p=0.001.

	Smokers	Non-smokers	P
Anti-CCP antibody, AU/ml (IQR)	411.50 (202 – 800)	94 (6.1 – 306.5)	0.001*
Rheumatoid factor, IU/ml (IQR)	187 (57.1 – 604)	91 (11.85 – 304)	0.033*

Table 3.32: Difference between RA smokers and non-smokers with respect to anti-CCP antibody and rheumatoid factor levels.

Fasting Lipid and Glucose Profiles:

Fasting glucose and lipid profile are reported as means. There was no significant difference in mean fasting glucose, cholesterol, LDL, HLD or triglycerides between RA and OA patients (table 3.33). 47 (75%) patients with RA had a fasting cholesterol of greater than 5mmol/l compared with 31 (66%) patients with OA, p =0.323. Of the 78 patients with an elevated serum cholesterol, only 15 (19%) were taking a statin at the time of study entry. No patients were taking a fibrate or ezetimibe.

	RA	OA	p
Mean fasting glucose, mmol/l (SD)	4.92 (0.91)	4.92 (0.56)	0.998
Mean total cholesterol, mmol/l (SD)	5.43 (0.80)	5.41 (0.81)	0.889
Mean LDL, mmol/l(SD)	3.25 (0.58)	3.22 (0.79)	0.804
Mean HDL, mmol/l (SD)	1.59 (0.46)	1.58 (0.44)	0.945
Mean triglycerides, mmol/l (SD)	1.50 (0.89)	1.31 (0.68)	0.205

Table 3.33: Comparison of fasting lipid and glucose profile in RA and OA. SD, standard deviation

RA males had a significantly higher fasting glucose and triglyceride level than females with RA, p=0.003 and p=0.005, respectively. Serum HDL was significantly higher in females than males, in both the RA and OA groups, p=0.000 and p=0.000, respectively. Fasting glucose and lipid profiles were similar in RA patients on disease modifying therapy or steroids and RA patients not on these medications.

Fasting glucose and triglyceride concentration were higher in RA patients who were current or exsmokers, compared with non-smokers (table 3.34). RA and OA patients who had a high BMI, had a significantly higher triglyceride concentration than their counterparts with a normal BMI, p=0.026 and p=0.088, respectively. There was a significant correlation between HDL and markers of body mass in both the RA and OA groups (table 3.35). Triglyceride concentration was also significantly higher in anti-CCP antibody positive RA patients compared with anti-CCP antibody negative RA patients, 1.60mmol/l (SD0.99) versus 1.21mmol/l (SD0.36), p=0.033.

OA and RA patients with a high PAI-1 activity result had a significantly lower HDL compared with OA and RA patients with PAI-1 activity in the normal range (table 3.36). PAI-1 antigen correlated with HDL and triglyceride concentrations in both RA and OA patients (table 3.37)

	Positive smoking history	Non-smokers	p
Mean fasting glucose, mmol/l (SD)	5.27 (1.04)	4.50 (0.45)	0.000*
Mean fasting cholesterol, mmol/l (SD)	5.53 (0.83)	5.31 (1.04)	0.269
Mean LDL, mmol/l (SD)	3.34 (0.58)	3.16 (0.59)	0.256
Mean HDL, mmol/l (SD)	1.53 (0.54)	1.66 (0.35)	0.296
Mean triglycerides, mmol/l (SD)	1.79 (1.00)	1.15 (0.35)	0.003*

Table 3.34: Comparison of fasting glucose and lipid profile in RA smokers and non-smokers. SD, standard deviation; p, significance level.

		Correlation with HDL concentration, r	p
RA	Waist circumference (cm)	-0.327	0.014*
	Weight (Kg)	-0.391	0.003*
	BMI (kg/m^2)	-0.284	0.034*
OA	Waist circumference (cm)	-0.482	0.000*
	Weight (kg)	-0.437	0.002*
	BMI (kg/m^2)	-0.382	0.009*

Table 3.35: Correlations between markers of body mass and serum HDL concentration

	Elevated PAI-1 activity	Normal PAI-1 activity	p
HDL, mmol/l (SD) In OA group	1.19 (0.03)	1.58 (0.44)	0.000*
HDL, mmol/l (SD) In RA group	1.32 (0.32)	1.65 (0.49)	0.014*

Table 3.36: Comparison of serum HDL concentrations in patients with high and normal PAI-1 activity, SD, standard deviation, p, significance level

		Correlation with PAI-1 antigen, r	P
RA	HDL, mmol/l	-0.363	0.009*
	Triglyceride, mmol/l	0.335	0.015*
OA	HDL, mmol/l	-0.304	0.047*
	Triglyceride, mmol/l	0.321	0.036*

Table 3.37: Correlation between PAI-1 antigen concentration and lipid profile in RA and OA patients

RA patients with evidence of atherosclerotic plaque on ultrasound had a more abnormal lipid profile than those with no plaque (table 3.38). RA patients with evidence of diastolic dysfunction on transthoracic echocardiogram had a less favourable lipid profile than RA patients with normal diastolic function (table 3.39).

In both RA and OA patients, there was a significant correlation between lipid profile and E/e', as a marker of diastolic dysfunction on echocardiography (table 3.40).

	Evidence of carotid plaque	No evidence of carotid plaque	P
Serum LDL, mmol/l (SD)	3.67 (0.50)	3.15 (0.54)	0.033*
Serum HDL, mmol/l (SD)	1.33 (0.31)	1.65 (0.46)	0.040*

Table 3.38: Comparison of lipid profile in RA patients with and without carotid plaque

	Evidence of diastolic dysfunction	Normal diastolic function	p
Total cholesterol, mmol/l (SD)	5.79 (0.64)	5.37 (0.79)	0.048*
Serum LDL, mmol/l (SD)	3.55 (0.54)	3.14 (0.59)	0.025*

Table 3.39: Comparison of lipid profile in RA patients with and without evidence of diastolic dysfunction

		Correlation with E/e', r	p
RA	Total cholesterol	0.255	0.077
	LDL	0.508	0.001*
OA	Total cholesterol	0.316	0.034*
	LDL	0.227	0.139

Table 3.40 Correlation between E/e' and lipid profile in RA and OA.

Using logistic regression analysis, diastolic dysfunction, peripheral vascular disease and endothelial dysfunction were not found to be associated with fasting glucose or lipid profile, in either the RA or OA group (table 3.41). However, carotid subclinical atherosclerosis was found to be associated with fasting glucose in the RA group, OR (95%), 5.03 (1.08, 23.43), p=0.039. After simultaneous adjustment for hypertension, serum cholesterol and smoking, this association was no longer significant, OR (95%), 3.50 (0.80, 15.30), p=0.096.

		Fasting lipid profile OR (95% CI)	p	Fasting glucose OR (95%CI)	p
RA	Endothelial dysfunction	0.24 (0.00, 62.49)	0.611	0.98 (0.46, 2.07)	0.954
	Peripheral vascular disease	1.15 (0.02, 70.07)	0.948	1.32 (0.51, 3.40)	0.565
	Subclinical atherosclerosis	0.59 (0.14, 2.49)	0.472	5.03 (1.08, 23.43)	0.039*
	Diastolic dysfunction	0.77 (0.19, 3.19)	0.721	1.38 (0.62, 3.08)	0.434
OA	Endothelial dysfunction	1.29 (0.51, 3.26)	0.590	0.16 (0.39, 6.73)	0.493
	Peripheral vascular disease	1.81 (0.11, 28.99)	0.676	1.84 (0.08, 44.42)	0.708
	Subclinical atherosclerosis	1.94 (0.69, 5.40)	0.208	1.76 (0.39, 8.02)	0.465
	Diastolic dysfunction	0.378 (0.08, 1.87)	0.234	0.91 (0.24, 3.48)	0.892

Table 3.41: Association between markers of atherosclerosis and diastolic dysfunction and fasting lipid and glucose profiles in RA and OA patients. OR, odds ratio; CI, confidence interval; p, significance level

Metabolic Syndrome:

Based on the National Cholesterol Education programme adult treatment panel III (NCEP ATPIII) criteria (14), we investigated whether any of our cohort had a diagnosis of the metabolic syndrome. No one had a prior diagnosis of the metabolic syndrome. We found that 7 RA patients and 7 OA patients fitted the diagnostic criteria, $p = 0.775$, Fisher's Exact test.

Electrocardiogram:

We found no evidence of ischaemic changes on the ECGs of our RA and OA groups. Also, none of our study group had evidence of atrial fibrillation or other arrhythmias.

Discussion:

Demographic and Disease Activity:

Mean age was 50.58 years (SD 7.35) and was similar in RA and OA patients. Over 2/3's of our study group were females. Disease duration did not differ significantly between RA and OA patients.

Methotrexate was the most commonly prescribed DMARD for RA, with 62% of patients taking it. Just over half of the RA group were taking a biologic DMARD; etanercept was the most commonly prescribed one. Use of NSAIDs was significantly higher in the RA group and diclofenac was the most commonly prescribed NSAID. Quite a significant proportion of RA patients were taking corticosteroids at the time of study recruitment, however, mean doses were low and the maximum daily dose was 15mg. Statin use was approximately 20% in both groups. Use of other cardiac medications was similar in RA and OA patients. Bisphosphonates and calcium supplements were more commonly prescribed in RA patients

A significantly higher proportion of OA patients had a family history of myocardial infarction. Later in the study we corrected for this, using logistic regression analysis, when comparing RA and OA patients with respect to presence of subclinical atherosclerosis.

Unemployment rates were similar in the 2 groups, with just under 15% of RA patients unable to work to due to their disease, compared with 6% of the OA group. OA patients were more likely to have attended 3rd level education. There is evidence

that level of education is linked to functional status in RA patients and inability to work due to disability is related to both the physical and psychological impact of a chronic illness (15, 16).

Just over 50% of RA patients were either current or former smokers. Rates were similar in OA patients. A meta-analysis by Boyer et al found that cigarette smoking rates are higher in RA patients than the general population (2). Smoking cessation strategies need more attention in these groups and patient education regarding smoking risks should come not only from public health campaigns and general practitioners but also from their rheumatologist.

Approximately 20% of both RA and OA patients had a prior diagnosis of hypertension. Prevalence of hypercholesterolaemia was similar in the 2 groups. There was 1 RA patient with diabetes mellitus and nobody in the OA group had it.

A number of anthropometric measurements differed between the 2 groups. OA patients had a significantly higher BMI than RA patients and females with OA had a larger waist circumference, however waist hip ratio was similar in the 2 groups.
Research has shown that low rather than high BMI in RA patients is associated with CV risk. Gabriel et al found a threefold increase in risk of cardiovascular death in RA patients with a low BMI and this remained after correction for traditional CV risks (17). This seems counter-intuitive but low BMI in RA patients reflects the rheumatoid cachexia associated with chronic inflammation that is linked to CV risk.

RA patients' general health scores were worse than their OA counterparts. In particular, RA patients on steroids and with elevated CRP had worse general health.

The Short Form 36 (SF-36) was summarized into physical and mental component scores. There was no difference in these scores between RA and OA patients. However, RA patients on biologic agents has significantly better mental component scores than those not on a biologic agent.

The Short Form 36 is a generic measure of health status and quality of life and has been validated for use in RA (18). It gives a subjective measure of how the patient feels and not just a measure of their disease. Use of DMARD therapy in RA is associated with long-term beneficial effects on physical function and clinically meaningful improvements in Short Form 36 scores have been documented (19).

We also collected data from a health assessment questionnaire (HAQ), a frequently used tool in RA patients in the clinical setting. Female patients with RA had a significantly worse HAQ than females with OA. Not surprisingly, we found that RA patients on corticosteroids reported a higher HAQ score than those not on corticosteroids. This reflects the fact that disease activity impacts on health quality in general.

Also of interest is the fact that overweight OA patients reported a significantly higher HAQ score than those with a normal BMI.

Both physician and patients' assessment of disease activity and pain were assessed using visual analogue scales and RA patients fared worse in all these measures compared to OA patients.

While the mean DAS28ESR score, a measure of disease activity in RA, was not particularly high at 3.62, when DAS28ESR was analysed as a categorical variable quiet a significant proportion of RA patients had active disease, with 68% of them having a DAS28ESR of greater than 3.2. RA patients with a DAS28ESR of greater than 3.2 had significantly worse general health and quality of life scores.

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) was used to assess patient pain and function in the OA group. OA patients being treated with anti-inflammatory medication did not have better scores than those not on NSAIDs. Not surprisingly, overweight and obese OA patients had worse pain and function scores than those with a normal BMI.

Baseline Phlebotomy:

OA patients had a higher corrected calcium concentration than RA patients, although calcium concentrations for both groups were in the normal reference range. Whether this is of any clinical relevance or not is not known. OA patients may have relatively higher serum calcium concentrations due to increased cartilage and bone turnover.

Glomerular filtration rate was lower in the OA group. Reasons one might expect for this would be an older population or more extensive use of NSAIDs. However, mean age was similar in our RA and OA groups and current NSAID use was actually higher in the RA group. One potential reason for this difference in eGFR is that OA patients may have had a greater cumulative dose of NSAIDs since their diagnosis, however this is not information we had to hand at the time of analysis.

Albumin is a recognised marker of disease activity in RA and other chronic inflammatory states. It is therefore not surprising that levels correlated with ESR, white cell count, pro-inflammatory cytokine concentrations and disease activity scores.

The relationship we found between smoking status and antibodies for RA are in line with numerous other studies (20, 21). Exposure to tobacco smoke is a known risk factor for the development of anti-CCP antibodies. Linn-Rasker et al found that tobacco exposure increased the risk of developing anti-CCP antibodies only in RA patients who tested positive for the shared epitope, suggesting that this relationship is specific for RA patients, as they did not such a relationship with undifferentiated arthritis (20).

Fasting lipid and glucose profiles were similar in RA and OA patients. Concerning though is the number of patients with an elevated serum fasting cholesterol who are not on standard treatment.

It was interesting to note that RA females had a more favourable lipid profile than RA males. As expected, abnormalities of lipid profile tended to cluster with other CV risks. RA smokers had higher concentrations of triglycerides than non-smokers. BMI was also related to triglyceride concentrations.

Triglyceride levels were worse in sero-positive RA patients. RA patients who are anti- CCP antibody positive have a more aggressive form of the disease and it makes

sense that these patients who suffer from more extensive extra-articular manifestations, are also at a higher CVD risk than sero-negative RA patients.

A study of 80 RA patients by Arnab et al reported that anti-CCP antibody was associated with subclinical atherosclerosis, in the form of carotid intima-media thickness and also with left ventricular diastolic dysfunction, while this association was not seen in RA patients who were anti-CCP antibody negative (22).

HLD concentrations in both our RA and OA groups correlated negatively with PAI-1, a marker of thrombosis. PAI-1 concentrations, along with other inhibitors of the fibrinolytic system, are associated with increased CVD risk in the general population (23). Coagulation factors have been found in higher circulating concentrations in RA patients and a diagnosis of RA can predict increased levels of PAI-1 (24).

Our finding of an association between lipid profile and markers of thrombosis support the multifactorial nature of CVD development.

RA patients with evidence of atherosclerotic plaque on ultrasound had a more abnormal lipid profile than those with no plaque. As discussed in more detail in chapter 6 on subclinical atherosclerosis, serum LDL appears to be a marker for subclinical carotid atherosclerosis in RA patients. This finding reinforces the importance of thorough screening for CV risks.

Although the association we found between LDL and carotid disease was just for the presence of plaque, other recent studies have also reported a correlation with carotid intima-media thickness (cIMT) (25, 26).

Fasting glucose also correlated with subclinical atherosclerosis in the RA group. Diabetes has been linked to the presence of carotid disease in RA patients without overt cardiovascular symptoms. Ajeganova et al found that cIMT after a 5 year follow-up period was associated with a diagnosis of diabetes in RA patients (27). In 2005, Del Rincon et al also described an strong association between diabetes and sub-clinical carotid disease in RA patients (28).

The presence of diastolic dysfunction was associated with a less favourable lipid profile in the RA group. Serum LDL concentrations were higher in those with evidence of diastolic dysfunction on transthoracic echocardiography. These findings tie in with research in the general population that has found an association between risk of diastolic heart failure and presence of hyperlipidaemia (29, 30).

Aiming for strict targets with respect to lipid concentrations, may have favourable effects not only atherosclerotic burden but also myocardial function.

We have shown that both RA and OA patients have features of the metabolic syndrome which go unrecognised in the rheumatology clinic. A recent meta-analysis of metabolic syndrome in RA by Zhang J et al identified a significant association between these 2 diseases, with an overall odds ratio of 1.24 (95% CI, 1.03, 1.50) (31).

The US National HANES study reported that metabolic syndrome was more prevalent in patients with OA (32). Also, the Michigan Bone Health and Metabolism Study found that 2 or more cardiometabolic risk factors were associated with persistent knee pain in obese females (33).

ECG:

On electrocardiogram examination, we did not find incident atrial fibrillation (AF) or any other arrhythmia. Lindhardsen et al conducted a large scale population based study and found that of 18,247 patients, who developed RA over a 13 year period, 744 developed atrial fibrillation and 718 developed stroke (34). They reported that the overall incidence of AF in RA patients was 40% higher than in the general population. The absolute risk ranged from 25% to 70%, depending on patient age. However, the strength of the association that Lindhardsen et al found is limited by that fact that information on traditional cardiovascular risk factors, potential confounders, was not always available. It would be interesting in the future to follow-up our RA cohort over time to assess the incidence of AF and other arrhythmias and compare with the above study.

Conclusions:

As described above, RA patients tend to have worse general health and disease activity scores than OA patients. Also, general health measures give us useful information regarding wellbeing and physical functioning. They highlight that DMARD usage has health benefits that extends beyond merely controlling RA disease activity.

We found a high rate of smoking, past and present, in both groups. This is an important modifiable risk factor to concentrate on in general and also in particular in sero-positive RA patients because smoking is linked to seropositive disease, which is the more aggressive form of RA.

The lower BMI in our RA group and the fact that this has been shown to be associated with increased CV risk in other studies, highlights the point that RA patients may not have the typical body habitus of patients we consider to be at increased CV risk. Rheumatologists need to be cognisant of the fact that even though RA patients may not be overweight, they are still at an increased risk of future CV events.

The number of both RA and OA patients who had an elevated fasting serum cholesterol and were not on treatment demonstrates that traditional CV risks are not being targeted appropriately by rheumatologists. There are 2 reasons for this; either rheumatologists are so consumed with controlling disease activity and concentration on monitoring patients on a multitude of DMARD medications that they do not

consider CV screening or patients are screened for CV risks but we are unequipped to deal with actual management of these risks.

The associations we found between anti-CCP antibody positivity and lipid profile in RA patients reinforces the importance of assessing CV risks in RA patients, in particular those who are sero-positive.

Metabolic syndrome exists in conjunction with inflammatory arthritis but is not being recognised during routine clinical assessment. Education of health care staff regarding the features of metabolic syndrome should improve this and hence have a positive impact on overall cardiovascular health in these patients.

References:

1. Panoulas VF, Metsios GS, Pace AV, John H, Treharne GJ, Banks MJ, et al. Hypertension in rheumatoid arthritis. Rheumatology. 2008;47(9):1286-98. Epub 2008/05/10.

2. Boyer JF, Gourraud PA, Cantagrel A, Davignon JL, Constantin A. Traditional cardiovascular risk factors in rheumatoid arthritis: a meta-analysis. Joint, bone, spine : revue du rhumatisme. 2011;78(2):179-83. Epub 2010/09/21.

3. Gonzalez A, Maradit Kremers H, Crowson CS, Ballman KV, Roger VL, Jacobsen SJ, et al. Do cardiovascular risk factors confer the same risk for cardiovascular outcomes in rheumatoid arthritis patients as in non-rheumatoid arthritis patients? Annals of the rheumatic diseases. 2008;67(1):64-9. Epub 2007/05/23.

4. Toms TE, Panoulas VF, Smith JP, Douglas KM, Metsios GS, Stavropoulos-Kalinoglou A, et al. Rheumatoid arthritis susceptibility genes associate with lipid levels in patients with rheumatoid arthritis. Annals of the rheumatic diseases. 2011;70(6):1025-32. Epub 2011/03/15.

5. Pollono EN, Lopez-Olivo MA, Lopez JA, Suarez-Almazor ME. A systematic review of the effect of TNF-alpha antagonists on lipid profiles in patients with rheumatoid arthritis. Clinical rheumatology. 2010;29(9):947-55. Epub 2010/04/13.

6. Jamnitski A, Visman IM, Peters MJ, Dijkmans BA, Voskuyl AE, Nurmohamed MT. Beneficial effect of 1-year etanercept treatment on the lipid

profile in responding patients with rheumatoid arthritis: the ETRA study. Annals of the rheumatic diseases. 2010;69(11):1929-33. Epub 2010/05/26.

7. H-L. R. A study of the effect of tocilizumab on markers of atherogenic risk in patients with moderate to severe rheumatoid arthritis. http://clinicaltrials.gov/show/NCT00535782

8. McCarey DW, McInnes IB, Madhok R, Hampson R, Scherbakov O, Ford I, et al. Trial of Atorvastatin in Rheumatoid Arthritis (TARA): double-blind, randomised placebo-controlled trial. Lancet. 2004;363(9426):2015-21. Epub 2004/06/23.

9. Chung CP, Oeser A, Solus JF, Gebretsadik T, Shintani A, Avalos I, et al. Inflammation-associated insulin resistance: differential effects in rheumatoid arthritis and systemic lupus erythematosus define potential mechanisms. Arthritis and rheumatism. 2008;58(7):2105-12. Epub 2008/06/26.

10. Cheal KL, Abbasi F, Lamendola C, McLaughlin T, Reaven GM, Ford ES. Relationship to insulin resistance of the adult treatment panel III diagnostic criteria for identification of the metabolic syndrome. Diabetes. 2004;53(5):1195-200. Epub 2004/04/28.

11. Solomon DH, Massarotti E, Garg R, Liu J, Canning C, Schneeweiss S. Association between disease-modifying antirheumatic drugs and diabetes risk in patients with rheumatoid arthritis and psoriasis. JAMA : the journal of the American Medical Association. 2011;305(24):2525-31. Epub 2011/06/23.

12. Costenbader KH, Feskanich D, Mandl LA, Karlson EW. Smoking intensity, duration, and cessation, and the risk of rheumatoid arthritis in women. The American journal of medicine. 2006;119(6):503 e1-9. Epub 2006/06/06.

13. Albano SA, Santana-Sahagun E, Weisman MH. Cigarette smoking and rheumatoid arthritis. Seminars in arthritis and rheumatism. 2001;31(3):146-59. Epub 2001/12/12.

14. Lorenzo C, Williams K, Hunt KJ, Haffner SM. The National Cholesterol Education Program - Adult Treatment Panel III, International Diabetes Federation, and World Health Organization definitions of the metabolic syndrome as predictors of incident cardiovascular disease and diabetes. Diabetes care. 2007;30(1):8-13. Epub 2006/12/29.

15. Callahan LF, Pincus T. Formal education level as a significant marker of clinical status in rheumatoid arthritis. Arthritis and rheumatism. 1988;31(11):1346-57. Epub 1988/11/01.

16. Odegard S, Finset A, Kvien TK, Mowinckel P, Uhlig T. Work disability in rheumatoid arthritis is predicted by physical and psychological health status: a 7-year study from the Oslo RA register. Scandinavian journal of rheumatology. 2005;34(6):441-7. Epub 2006/01/06.

17. Gabriel SE. Heart disease and rheumatoid arthritis: understanding the risks. Annals of the rheumatic diseases. 2010;69 Suppl 1:i61-4. Epub 2010/01/09.

18. Jenkinson C, Layte R, Coulter A, Wright L. Evidence for the sensitivity of the SF-36 health status measure to inequalities in health: results from the Oxford

healthy lifestyles survey. Journal of epidemiology and community health. 1996;50(3):377-80. Epub 1996/06/01.

19. Strand V. Longer term benefits of treating rheumatoid arthritis: assessment of radiographic damage and physical function in clinical trials. Clinical and experimental rheumatology. 2004;22(5 Suppl 35):S57-64. Epub 2004/11/24.

20. Linn-Rasker SP, van der Helm-van Mil AH, van Gaalen FA, Kloppenburg M, de Vries RR, le Cessie S, et al. Smoking is a risk factor for anti-CCP antibodies only in rheumatoid arthritis patients who carry HLA-DRB1 shared epitope alleles. Annals of the rheumatic diseases. 2006;65(3):366-71. Epub 2005/07/15.

21. Klareskog L, Stolt P, Lundberg K, Kallberg H, Bengtsson C, Grunewald J, et al. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. Arthritis and rheumatism. 2006;54(1):38-46. Epub 2005/12/31.

22. Arnab B, Biswadip G, Arindam P, Shyamash M, Anirban G, Rajan P. Anti-CCP antibody in patients with established rheumatoid arthritis: Does it predict adverse cardiovascular profile? Journal of cardiovascular disease research. 2013;4(2):102-6. Epub 2013/09/13.

23. Fibrinogen Studies C, Danesh J, Lewington S, Thompson SG, Lowe GD, Collins R, et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. JAMA : the journal of the American Medical Association. 2005;294(14):1799-809. Epub 2005/10/13.

24. Dimitroulas T, Douglas KM, Panoulas VF, Toms T, Smith JP, Treharne GJ, et al. Derangement of hemostasis in rheumatoid arthritis: association with demographic, inflammatory and metabolic factors. Clinical rheumatology. 2013;32(9):1357-64. Epub 2013/05/16.

25. Solomon A, Woodiwiss AJ, Abdool-Carrim AT, Stevens BA, Norton GR, Dessein PH. The carotid artery atherosclerosis burden and its relation to cardiovascular risk factors in black and white Africans with established rheumatoid arthritis: a cross-sectional study. The Journal of rheumatology. 2012;39(9):1798-806. Epub 2012/07/04.

26. Targonska-Stepniak B, Drelich-Zbroja A, Majdan M. The relationship between carotid intima-media thickness and the activity of rheumatoid arthritis. Journal of clinical rheumatology : practical reports on rheumatic & musculoskeletal diseases. 2011;17(5):249-55. Epub 2011/07/23.

27. Ajeganova S, Ehrnfelt C, Alizadeh R, Rohani M, Jogestrand T, Hafstrom I, et al. Longitudinal levels of apolipoproteins and antibodies against phosphorylcholine are independently associated with carotid artery atherosclerosis 5 years after rheumatoid arthritis onset--a prospective cohort study. Rheumatology. 2011;50(10):1785-93. Epub 2011/07/12.

28. del Rincon I, Freeman GL, Haas RW, O'Leary DH, Escalante A. Relative contribution of cardiovascular risk factors and rheumatoid arthritis clinical manifestations to atherosclerosis. Arthritis and rheumatism. 2005;52(11):3413-23. Epub 2005/10/29.

29. Gianotti G, Cenni A, Bianchi G, Masetti M, Zappoli P, Muscari A, et al. Diastolic dysfunction and cardiovascular risk in old subjects: Possible association with NAFLD? Archives of gerontology and geriatrics. 2014;58(2):188-95. Epub 2013/11/26.

30. de las Fuentes L, Brown AL, Mathews SJ, Waggoner AD, Soto PF, Gropler RJ, et al. Metabolic syndrome is associated with abnormal left ventricular diastolic function independent of left ventricular mass. European heart journal. 2007;28(5):553-9. Epub 2007/02/22.

31. Zhang J, Fu L, Shi J, Chen X, Li Y, Ma B, et al. The risk of metabolic syndrome in patients with rheumatoid arthritis: a meta-analysis of observational studies. PloS one. 2013;8(10):e78151. Epub 2013/11/10.

32. Singh G, Miller JD, Lee FH, Pettitt D, Russell MW. Prevalence of cardiovascular disease risk factors among US adults with self-reported osteoarthritis: data from the Third National Health and Nutrition Examination Survey. The American journal of managed care. 2002;8(15 Suppl):S383-91. Epub 2002/11/06.

33. Sowers M, Karvonen-Gutierrez CA, Palmieri-Smith R, Jacobson JA, Jiang Y, Ashton-Miller JA. Knee osteoarthritis in obese women with cardiometabolic clustering. Arthritis and rheumatism. 2009;61(10):1328-36. Epub 2009/10/01.

34. Lindhardsen J, Ahlehoff O, Gislason GH, Madsen OR, Olesen JB, Svendsen JH, et al. Risk of atrial fibrillation and stroke in rheumatoid arthritis: Danish nationwide cohort study. Bmj. 2012;344:e1257. Epub 2012/03/10.

Chapter 4

Serum, Plasma and Urinary Markers of Inflammation, Endothelial Dysfunction and Thrombosis

Introduction:

Atherosclerotic plaques have characteristics similar to the inflamed joint in RA. They both display enhanced expression of adhesion molecules and pro-inflammatory cytokine-secreting cells (1). Endothelial dysfunction, the earliest stage of atherosclerosis, has been reported in RA patients, independent of their age, duration of disease and disease activity (2).

It is now widely accepted that systemic markers of inflammation are independent predictors of coronary heart disease in adults with or without pre-existing heart disease (3).

There is a wide range of biomarkers currently available for the assessment of inflammation, endothelial function and altered coagulation. Some, including PAI-1 and CRP are used in the clinical setting to assess CVD risk while others, such as pro-inflammatory cytokines and adhesion molecules tend to be utilised in the research setting.

Traditional Inflammatory markers in CVD:

ESR and CRP are non-specific inflammatory markers, but when elevated predict future cardiovascular events and mortality in RA patients (4, 5), and in the general population (6). Gabriel et al, in a prospective study of 603 RA patients, reported a two-fold higher hazard of cardiovascular death in RA patients with 3 or more ESR measurements of $\geq$ 60mm/hr (7).

CRP is often considered merely as a marker of inflammation; however there is evidence to support its role as a strong inflammatory mediator. In inflamed tissues, CRP activates immune cells and is involved in triggering a cascade of events, including chemokine release from monocytes and up-regulation of adhesion molecules (8). Multiple prospective studies of healthy subjects have shown that CRP predicts myocardial infarction mortality (9) and stroke (10). Danesh J et al previously showed that after correction for age, smoking and other cardiovascular risk factors, CRP was still strongly associated with CVD(11).

Hyperuricaemia is associated with coronary heart disease, stroke and hypertension, however it is still unclear whether serum uric acid level is an independent risk factor for cardiovascular disease (12). Kim et al, in a recent meta-analysis, found a modest association between elevated uric acid levels and CHD events. This was independent of traditional CVD risk factors and was more pronounced in females (12).

Cardiovascular risk appears not only to be associated with elevated levels of uric acid but also with levels in the accepted normal reference ranges (13).

Feig et al report that the association between uric acid and CVD remains controversial, with Framingham Heart Study experts arguing that it should not be relied on as a risk factor (14). The general consensus currently is that asymptomatic hyperuricaemia should not be treated with allopurinol and futher research is required to gain understanding of the biologic function of uric acid.

Adhesion molecules in RA and CVD:

Measurement of adhesion molecule levels is one method of assessing endothelial function. Adhesion molecules are biomarkers of endothelial activation and activated endothelial cells express increased amounts of V-CAM and I-CAM and selectins. These molecules are involved in monocyte adherence and rolling to the endothelial surface (15). Selectins mediate the initial 'tethering' of leucocytes to the endothelial layer, while integrins and members of the immunoglobulin superfamily, V-CAM and I-CAM are responsible for firm adhesion and transendothelial migration of leucocytes (16).

All selectins and members of the immunoglobulin superfamily are also abundantly expressed in arthritic synovial tissues (17). Cell to cell and cell to extracellular matrix interactions via adhesion molecules are important in the role of synovial

inflammation. However, further studies in arthritis are required to define their role and whether they may be potential therapeutic targets.

The clinical usefulness of adhesion molecules is debatable at present. There is evidence that they may not yield any additional information in CV risk above and beyond that which traditional risk factors already provide (18).

Cytokines in RA and CVD:

A number of pro-inflammatory cytokines are particularly involved in RA pathogenesis, these include TNFα, IL1 and IL6. They are all found in abundance in the synovium and serum of RA patients and correlate with disease activity. They are in a position to alter function at distant tissues, including the vascular endothelium and as a result they are capable of generating a variety of pro-atherogenic changes including endothelial dysfunction, prothrombotic effects, pro-oxidative stress and dyslipidaemia (19).

Over the last 2 decades, multiple therapies for RA have been developed to target these cytokines and their effects have revolutionised the treatment and prognosis of RA (20).

TNFα also plays a pivotal role in the pathogenesis of CV disease. Advanced glycation end-products (AGEs), receptor for AGEs (RAGE) and NF-κB signalling play key roles in increasing both circulating and local vascular TNFα production. The resulting increase in TNFα expression induces the production of reactive oxygen species and this triggers the onset of endothelial dysfunction, now recognized as the

earliest stage of atherosclerosis (21). In animal studies, TNFα gene knockout mice have less endothelial activation (22) and a decrease in the extent of atherosclerosis (23).

Coupled with TNFα's effect on inflammatory and endothelial cells, it also induces insulin resistance, dyslipidaemia and a prothrombotic state (24).

Investigation of IL-6 associations with CVD has been less extensive, as its effects may be more difficult to determine. This is due to its short half-life of 2 hours and large within-person variability. It is a proximal mediator in the inflammatory cascade compared to the further downstream CRP and fibrinogen. Jenny et al reported that increases in IL-6 were associated with increased risk of cardiovascular events in healthy elderly patients (25). In 2008 Danesh et al published a population based long term study (part of the Reykjavik and British Regional Heart Studies) which found that long-term IL-6 concentrations were associated with CHD, with risk comparable to some major established risk factors but causality was not proven (26). They reported an adjusted odds ratio for CHD in the combined studies of 1.46 (CI95%), (1.29, 1.65) for each 2 standard deviation increase in baseline IL-6 levels.

Interleukin-6 has also been shown to correlate with markers of sub-clinical atherosclerosis in the general population. Szklo M et al found higher IL-6 concentrations in healthy controls with carotid atherosclerosis. However they did not find the same association in RA patients (27).

In a large follow-up study of 3035 adults in the Framingham Heart Study, Benjamin EJ et al found that both TNFα and IL-6 concentrations were related to cardiovascular mortality (28).

Interleulin-1 is one of the primary pro-inflammatory cytokines in RA and stimulates synovial cell synthesis of IL-6 (29). It is also one of the main cytokines involved in the pathogenesis of atherosclerosis and has many atherogenic effects. These include enhanced expression of cell surface adhesion molecules and selectins on endothelial cells, smooth muscle cells and marcophages. It is also involved in the production of reactive oxygen species and inducing cell proliferation (30)..

Van Tassel et al recently reported that the expression of IL-1α and IL-1β appear to correlate with the progression of atherosclerotic plaques, with minimal expression in healthy coronary arteries and high expression in complicated plaques (31). IL-1β is difficult to measure in plasma so there are few studies showing an increased level in patients with a greater atherosclerotic burden (32, 33).

Anti-IL-1 therapy in cardiovascular disease is still in its infancy but preliminary data suggests a beneficial role in atherosclerosis and atherothrombosis (34, 35).

Markers of thrombosis in RA and CVD:

The plasminogen activator / plasmin system is an endogenous defence mechanism against intravascular thrombosis (36). The most important inhibitor of this system is plasminogen activator inhibitor-1 (37). It has been classified as an acute phase reactant and inflammatory cytokines such as TNFα have been shown to trigger its production (38). Inflammation can initiate clotting mechanisms and reduce the body's natural anticoagulant mechanisms (39).

There is a significant amount of evidence that inhibitors of the fibrinolytic system, including PAI-1 (40-42) are associated with an increased CVD risk in the general population. PAI-1 activity is heightened in people post-myocardial infarction who go on to develop a further MI (43).

More recently, there is also evidence of abnormalities in the coagulation system correlating with CVD in RA populations. George Kitas et al examined a panel of coagulation markers in 141 RA patients and 50 controls and found that coagulation factors were higher in RA patients and having a diagnosis of RA predicting increased levels of tPA, PAI-1 and fibrinogen, independent of age and sex (44). Some earlier studies of coagulation in RA found similar results (45, 46).

This link between abnormal coagulation and inflammation may be one of the mechanisms by which CVD risk is increased in RA.

Urinary protein as a marker of vascular disease in general and RA:

Endothelial dysfunction is involved in the development of microalbuminuria. Structural changes in the glomerulus over time results in microalbuminuria transitioning from a reversible to a fixed entity (47). It is a marker of microvasculopathy and has been shown to be associated with an increased risk of stroke and cardiovascular outcomes (48).

The connection between markers of inflammation, endothelial function and coagulation in RA has not been studied in detail in an Irish population. Therefore, we examined our RA group with respect to the above and tried to identify if RA patients were more prone to disorders of endothelial function and coagulation than OA controls.

Aims:

- To compare ESR, CRP, serum urate, as markers of inflammation and cardiovascular disease in RA and OA.
- To compare serum concentrations of adhesion molecules and inflammatory cytokines in RA and OA patients.
- To examine both groups for any association between concentrations of adhesion molecules and cytokines and cardiovascular disease risks and pre-clinical markers of atherosclerosis.
- To investigate if markers of thrombosis; plasminogen activator -1 and fibrinogen were different in RA and OA patients or were associated with cardiovascular risks in either group.
- To investigate urinary measures of protein in RA and OA patients and to assess their use as potential CV markers.

Methods:

Blood was drawn for ESR, CRP, urate, fibrinogen, PAI-1 activity, PAI-antigen, serum cytokine and adhesion molecule concentrations.

ESR and fibrinogen samples were analysed by the haematology laboratory in CUH in the usual manner. CRP and serum urate were analysed in the usual manner by the biochemistry department in CUH.

Cytokine and Adhesion Molecule Analysis:

The cytokine profile of each serum sample was analysed quantitatively using a multiplex chemi-luminescent array (Randox), a high fidelity system capable of determining the concentration of IL-1α, -1β, -2, -4, -6, -8, -10, TNFα, IFNγ, VEGF, EGF and MCP-1. The protocol followed the manufacturer's instructions and entailed the incubation of 100 µl of un-diluted sample, calibrator or control on a biochip pre-coated with immobilized capture antibody, to which 200 µl of assay diluents had been added. Following a 1 hour incubation at 37°C on a thermoshaker (370 rpm) each well was washed twice with wash buffer (Tris buffered saline) and 300 µl of conjugate (HRP-labelled detection antibodies) was added. This was incubated for a further hour at 37 °C on the thermoshaker (370 rpm) prior to 2 further washes. At this time 250 µl of a 1:1 combination of luminal and peroxide was added and incubated in the dark for 2 minutes. Samples were then analysed using the EVIDENCE Investigator platform. EVIDENCE software was then used to determine

the intercalated x-values from the resultant standard curve, the results expressed as pg/ml.

The same multiplex chemi-luminescent immunoassay (Randox) was used to analyse the adhesion molecule profile. The Randox adhesion molecule biochip array simultaneously and quantitatively measured E-selectin, L-selectin, P-selectin, intracellular adhesion molecue-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1).

PAI-1 analysis:

A blood sample was taken and collected in a plastic tube containing sodium citrate to measure PAI-1 activity and PAI-1 antigen levels. Blood samples were centrifuged at 5000 rpm for 20 minutes at 4 degrees Celsius. The upper 2/3's of plasma was extracted from the sample and this was re-centrifuged for a further 20 minutes to remove any platelets. Again the upper 2/3's was removed and divided into aliquots. Three 500 µl aliquots were collected for each patient. Aliquots were transferred on ice for storage in a minus 80 degree Celsius freezer and stored for analysis at a later stage.

When samples were collected for all the study recruits, the aliquots of frozen plasma were transported on dry ice to Professor Nuala Booth, Department of Molecular and Cell Biology, University of Aberdeen, Institute of Medical Sciences. PAI-1 antigen level was measured by quantitative ELISA and a specific activity assay was utilized to measure PAI-1 activity levels according to the manufacturer's instructions.

Urine for albuminuria measurement:

An early morning urine sample of at least 20mls was collected from each patient for measurement of urinary protein-creatinine ratio and microalbuminuria by standardised methods in the biochemistry department of CUH.

Statistical Methods:

Means and standard deviations were calculated for normally distributed data and compared using a student t-test. Medians and interquartile ranges were calculated for non-normally distributed data and compared using a Mann Whitney U test. Bivariate correlations were calculated for continuous variables. Logistic regression analysis was performed to analyse the relationship between the presence of subclinical carotid atherosclerosis, peripheral vascular disease, ultrasound evidence of endothelial dysfunction and diastolic dysfunction and haematological markers of inflammation, endothelial dysfunction and thrombosis in Rheumatoid Arthritis and osteoarthritis. Odds ratios and 95% confidence intervals were computed. A p-value of <0.05 was considered significant.

Results for Routine Inflammatory Markers:

Comparison of ESR, CRP and Urate in RA and OA:

Neither ESR nor CRP was normally distributed in the study group so median values are quoted and nonparametric tests used for comparisons. Median ESR in the RA group was 18 mm/hr (7 – 25) compared with 7 mm/hr (5 – 11) in the OA group, p <0.000. CRP was significantly higher in patients with RA compared to those with OA, 6.2 mg/l (5 – 16.05) compared with 5 mg/l (2 – 6), p=0.000.

Serum urate was similar in RA and OA patients, 281 Umol/l (SD 79) compared with 278 Umol/l (SD 61), p=0.782. Males had a significantly higher serum urate than females in both groups, p=0.000. Six RA patients and 3 OA patients had hyperuricaemia, this difference was not significant, p = 0.728, Fisher's exact test.

ESR, CRP and Urate Levels and RA Serological Markers and Anti-Rheumatic Medications:

Median ESR was significantly higher in RA patients not on a DMARD at the time of the study, p=0.010 (table 4.1). CRP was significantly lower in RA patients on a biologic treatment compared to those not taking a biologic, 5 mg/l (5 – 11.6) versus 11.6 mg/l (5.3 – 18.9), p=0.007. RA patients on steroids had a significantly higher CRP than those not on steroids, 11.6 mg/l (5 – 25.7) compared with 5 mg/l (5 –

11.6), p=0.041. RA patients on DMARDS, biologic agents, steroids or NSAIDs had similar serum urate levels to those not taking the above medications.

ESR, CRP and serum urate levels were similar in sero-positive and sero-negative RA patients.

		ESR, mm/hr (IQR)	p
DMARD	Yes	13 (7 – 23)	0.010*
	No	25 (20 – 35)	
Biologic agent	Yes	16 (5 -26)	0.798
	No	18 (8 – 25)	
NSAID	Yes	18 (7 – 25)	0.720
	No	14 (6 – 28)	
Steroid	Yes	18 (8 – 32)	0.326
	No	16 (5 – 25)	

Table 4.1: comparison of ESR level across the different treatment categories in the RA group

ESR, CRP and Urate levels and Markers of Cardiovascular Disease in RA:

Serum urate concentrations were significantly higher in overweight RA patients compared with their normal weight counterparts (table 4.2). There was a significant correlation between waist circumference and serum urate in the RA group, r =0.603, p=0.000 (figure 4.1). RA patients with abdominal obesity, as defined by an elevated waist hip ratio, had a significantly higher serum urate than RA patients with a normal waist hip ratio (table 4.2). We found no associations between ESR, CRP and anthropometric variables.

Fasting glucose and lipid profile correlated significantly with serum urate in the RA group (table 4.3). There was no significant correlation between ESR, CRP and fasting blood profile.

ESR and CRP correlated significantly with fibrinogen levels in the RA group, r = 0.454, p < 0.000 and r = 0.455, p < 0.000. RA patients with an elevated PAI-1 antigen level had a significantly higher CRP and serum urate than those with a PAI-1 in the normal reference range, 14 mg/l (12 – 22.45) compared with 5 mg/l (5 – 13.15), p=0.007 for CRP and 329 Umol/l (SD 41) compared with 266 Umol/l (SD 84), p = 0.002 for urate.

RA patients with endothelial dysfunction, abnormal carotid intima media thickness or diastolic dysfunction did not have significantly different ESR or CRP readings to those RA patients without evidence of endothelial dysfunction, carotid atherosclerosis or diastolic dysfunction, p=0.867, p=0.480, p=0.495 for ESR and p =0.440, p=0.263, p=0.622 for CRP.

Using logistic regression analysis, the presence of diastolic dysfunction, subclinical carotid atherosclerosis, abnormal ankle brachial index and endothelial dysfunction in the RA group, were not found to be associated with ESR, CRP or serum urate (table 4.4).

		Serum Urate Umol/l (SD)	p
RA	High BMI	307 (81)	0.005*
	Normal BMI	250 (64)	
RA	High waist hip ratio	315 (62)	0.001*
	Normal waist hip ratio	245 (80)	

Table 4.2: Comparison of serum urate in RA patients with normal and high BMI and normal and high waist hip ratio. SD, standard deviation; p, significance level; BMI, body mass index (kg/m^2)

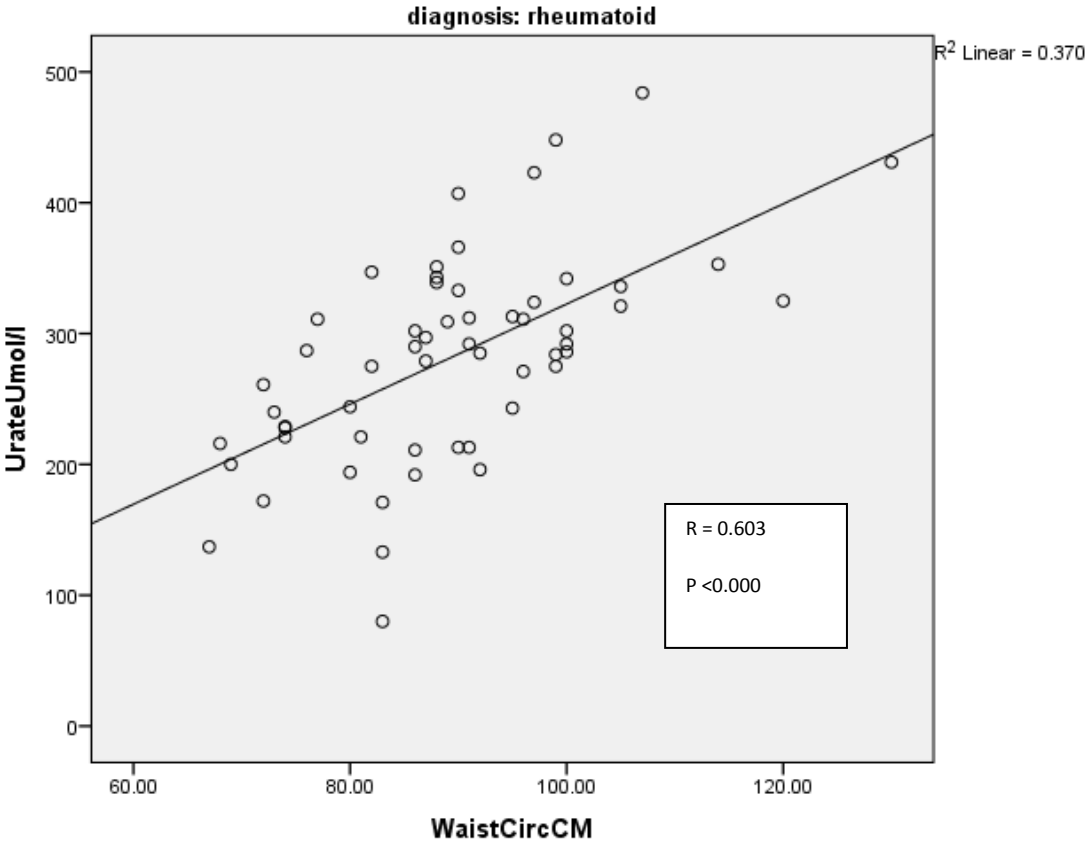


Figure 4.1: Correlation between waist circumference and serum urate in RA patients

	Correlation with serum urate, r	P
Fasting glucose	0.294	0.027*
Fasting cholesterol	0.138	0.306
Serum LDL	0.295	0.034*
Serum HDL	-0.337	0.014*
Serum triglycerides	0.377	0.005*

Table 4.3: Association between serum urate and fasting glucose and lipid profile in RA patients

	ESR OR (95%CI)	p	CRP OR (95%CI)	p	Urate OR (95%CI)	p
Diastolic Dysfunction	1.01 (0.97, 1.06)	0.647	0.99 (0.95, 1.05)	0.925	1.01 (0.99, 1.02)	0.084
Subclinical carotid atherosclerosis	0.93 (0.86, 1.02)	0.136	1.07 (0.99, 1.14)	0.056	1.00 (0.99, 1.01)	0.648
Peripheral vascular disease	1.03 (0.99, 1.06)	0.155	1.01 (0.96, 1.07)	0.653	0.99 (0.98, 1.0)	0.145
Endothelial dysfunction	1.00 (0.96, 1.05)	0.687	1.00 (0.96, 1.05)	0.858	1.00 (0.99, 1.01)	0.520

Table 4.4: Association of cardiovascular disease markers with ESR , CRP and serum urate in the RA group, using logistic regression; OR, odds ratio; CI, confidence interval

ESR, CRP and Urate in OA:

Using logistic regression analysis, the presence of diastolic dysfunction, subclinical carotid atherosclerosis, abnormal ankle brachial index and endothelial dysfunction in the OA group, were not found to be associated with ESR, CRP or serum urate (table 4.5).

	ESR OR (95%CI)	p	CRP OR (95%CI)	p	Urate OR (95%CI)	p
Diastolic Dysfunction	0.96 (0.86, 1.07)	0.478	1.01 (0.82, 1.25)	0.923	1.01 (0.99, 1.02)	0.197
Subclinical carotid atherosclerosis	0.82 (0.63, 1.07)	0.150	1.08 (0.82, 1.43)	0.590	1.01 (0.99, 1.02)	0.403
Peripheral vascular disease	0.91 (0.67, 1.24)	0.547	0.89 (0.49, 1.59)	0.683	0.97 (0.94, 1.01)	0.151
Endothelial dysfunction	0.93 (0.80, 1.02)	0.344	0.98 (0.74, 1.30)	0.882	1.00 (0.99, 1.02)	0.531

Table 4.5: Association of cardiovascular disease markers with ESR ,CRP and urate in the OA group, using logistic regression; OR, odds ratio; CI, confidence interval.

Adhesion molecules Results:

Comparison of Adhesion Molecule Concentrations in RA and OA:

Serum samples were collected for 5 adhesion molecules; VCAM, ICAM, E-selectin, P-selectin, L-selectin. Mean values in the RA and OA groups were compared using an independent t-test (table 4.6). RA patients had significantly higher concentrations of VCAM and P- selectin compared to OA patients. RA patients had significantly lower concentrations of L-selectin than OA patients. The concentrations of adhesion molecules were similar in males and females.

Adhesion molecules	RA	OA	P value
Mean VCAM, ng/ml (SD)	704.74 (329.98)	559.77 (182.10)	0.004 *
Mean ICAM, ng/ml (SD)	292.26 (155.84)	268.19 (91.79)	0.325
E – selectin, ng/ml (SD)	17.97 (7.47)	15.82 (5.82)	0.267
P – selectin, ng/ml (SD)	172.71 (79.15)	144.75 (52.40)	0.029 *
L – selectin, ng/ml (SD)	1089.54 (365.08)	1213.89 (280.25)	0.047*

Table 4.6: Comparison of adhesion molecule concentrations in RA and OA patients

Adhesion Molecule Concentrations and RA Serological Markers and Anti-Rheumatic Medications:

RA patients on biologic agents, DMARDS, NSAIDS and steroids were compared to those not on the above treatments and no difference was found with respect to serum levels of VCAM or ICAM. Current dose of prednisolone did correlate with serum VCAM levels, r = -0.286, p=0.024. RA patients who were on steroids at the time of the study also had a significantly lower serum L-selectin than RA patients not on steroids, 974.41 ng/ml (SD 310.60) versus 1184.36 ng/ml (SD 383.31), p = 0.020. Current steroid dose correlated significantly with serum L-selectin level, r = -0.361, p = 0.004. Otherwise, we found no difference in serum levels of the selectins with respect to anti-rheumatic medication.

Serum VCAM was found to be significantly higher in RA patients who were anti-CCP antibody and rheumatoid factor positive compared to those were antibody negative and serum ICAM was significantly higher in patients with a positive rheumatoid factor (table 4.7). Rheumatoid factor level correlated significantly with serum levels of VCAM and ICAM, r=0.358, p=0.004 and r = 0.382, p = 0.003, respectively. Serum E-selectin significantly correlated with CRP and rheumatoid factor, r = 0.253, p=0.047 and r =0.341, p=0.007, respectively.

		Mean VCAM Ng/ml (SD)	p	Mean ICAM Ng/ml (SD)	P
RA	Anti-CCP positive	748.82 (351.58)	0.017*	299.60 (111.92)	0.666
	Anti-CCP negative	566.63 (203.33)		268.68 (102.22)	
RA	Rheumatoid factor positive	739.46 (346.07)	0.027*	312.31 (164.33)	0.001*
	Rheumatoid factor negative	560.10 (205.02)		204.78 (59.91)	

Table 4.7: Comparison of serum VCAM and ICAM levels in sero-negative and sero-positive RA patients.

Adhesion Molecule Concentrations and Cardiovascular Markers in RA:

ICAM concentration in the RA group correlated significantly with smoking pack years, r = 0.307, p = 0.018. P-selectin was found to be elevated in RA smokers compared with RA non-smokers, 189.97ng/ml (SD89.64) versus 151.76ng/ml (SD59.19), p=0.049 and levels also correlated with smoking pack years, r=0.264, p=0.038.

Both E- and P- selectin were associated with systolic blood pressure (table 4.8). Body mass index and waist circumference were significantly associated with serum L-selectin level in the RA group, r = - 0.382, p = 0.002 and r = -0.332, p = 0.008. This association was not seen in the OA group.

Total cholesterol and serum urate were also associated with low levels of L-selectin in the RA group, r = - 0.276, p = 0.030 and r = -0.311 p = 0.018 (table 4.9).

Correlation with systolic BP		Correlation coefficient, r	p
RA	E-selectin	0.292	0.037*
	P-selectin	0.290	0.039*

Table 4.8: Association between systolic blood pressure and E- and P- selectin in RA patients

Correlation with L-selectin	Correlation coefficient, r	p
Body mass index (kg/m^2)	- 0.382	0.002*
Waist circumference (cm)	- 0.332	0.008*
Total cholesterol (mmol/l)	- 0.276	0.030*
Serum urate (umol/l)	- 0.311	0.018*

Table 4.9: Association between serum L-selectin and markers of obesity, total cholesterol and serum urate in RA patients

L-selectin was significantly higher in RA patients with no evidence of endothelial dysfunction on FMD and in those with a normal common carotid IMT, compared to those with an abnormal FMD or abnormal cIMT, p=0.017 and p=0.014, respectively (table 4.10).

Urinary protein creatine ratio correlated significantly with serum VCAM in the RA group, r = 0.284, p = 0.042.

On transthoracic echocardiogram, e', a marker of diastolic dysfunction, was associated with serum ICAM levels in RA, r =-0.359, p=0.014. E-selectin was significantly associated with a number of markers of diastolic dysfunction in the RA group (table 4.11). Those RA patients with a BNP level above 125pg/ml had a significantly lower L-selectin than RA patients with BNP in the normal range, p = 0.026.

Using logistic regression analysis, the presence of endothelial dysfunction, subclinical atherosclerosis or diastolic dysfunction were not found to be associated with serum levels of VCAM. ICAM, E-, P- or L-selectin.

		L-selectin Ng/ml (SD)	P
RA	FMD < 5% (ie endothelial dysfunction)	917.95 (168.93)	0.017*
	FMD >5%	1100.11 (353.94)	
RA	cIMT >0.9mm (ie subclinical atherosclerosis)	927.39 (137.56)	0.014*
	cIMT <0.9mm	1127.38 (403.11)	
RA	BNP > 125pg/ml	832.50 (75.66)	0.026*
	BNP <125pg/ml	1103.24 (381.91)	

Table 4.10: Comparison of L-selectin levels in RA patients with and without markers of endothelial dysfunction, subclinical atherosclerosis and diastolic dysfunction

	E-selectin correlation with diastolic dysfunction Correlation coefficient = r	p
E wave velocity	-0.329	0.011*
A wave velocity	-0.289	0.026*
E/A ratio	-0.402	0.002*
e' lateral	-0.484	0.000*
e'septal	-0.415	0.003

Table 4.11: Correlation between E-selectin concentration and markers of diastolic dysfunction in RA

Osteoarthritis and Adhesion Molecule Concentrations:

We did not find any significant associations between adhesion molecule concentrations and cardiovascular markers in the OA group.

However, serum VCAM levels were significantly associated with a number of general health status measures, including HAQ (health assessment questionnaire), WOMAC total score and physical function score and both the physician and patient visual analogue scale for pain and disease activity (table 4.12).

VCAM	Correlation coefficient, r	p
HAQ	0.445	0.002*
WOMAC total	0.382	0.008*
WOMAC physical	0.453	0.001*
Physician VAS of disease activity	0.305	0.037*
Patient VAS for pain	0.288	0.050*
Patient VAS of disease activity	0.445	0.002*

Table 4.12: Correlation between serum VCAM and measures of general health in OA group.

Cytokine Results:

Comparison of Cytokine Concentrations in RA and OA:

12 different cytokines were measured in both groups. These included interleukin – 1a and 1b, Il 2, 4, 6, 8, 10, interferon gamma, TNFα, MCP1, EGF and VEGF. Serum levels of cytokines were not normally distributed so median values are reported and nonparametric tests used for analysis. All cytokine concentrations, other than VEGF and MCP-1 were significantly higher in our RA group (table 4.13). Serum concentrations of cytokines were similar in males and females.

	RA	OA	P value
Cytokines			
Il 1α, pg/ml (IQR)	1.41 (0.93 – 4.13)	0.56 (0.48 – 0.88)	0.008*
Il 1β, pg/ml (IQR)	4.23 (2.01 – 12.51)	0.00 (0 – 0)	0.000*
Il 2, pg/ml (IQR)	22.39 (15.82 -61.89)	4.99 (0 - 15.26)	0.000 *
Il 4, pg/ml (IQR)	5.65 (4.30 - 12.57)	3.47 (2.76 – 4.63)	0.000*
Il 6, pg/ml (IQR)	12.62 (4.20 – 27.44)	2.88 (1.79 – 5.22)	0.000 *
Il 8, pg/ml (IQR)	21.98 (12.00 – 47.17)	13.69 (8.33 – 27.39)	0.014*
Il 10, pg/ml (IQR)	5.10 (2.98 – 18.60)	0.00 (0 – 0)	0.000 *
VEGF, pg/ml (IQR)	180.42 (87.62 – 219.25)	119.88 (68.47 – 216.33)	0.200
IFNγ, pg/ml (IQR)	5.76 (2.98 – 18.60)	1.55 (0 – 3.44)	0.000 *
TNFα, pg/ml (IQR)	13.29 (6.77 – 24.98)	4.83 (3.03 – 6.70)	0.000 *
MCP1, pg/ml (IQR)	228.27 (154.87 – 355.90)	215.33 (175.81 – 359.80)	0.910
EGF, pg/ml (IQR)	38.00 (12.98 – 89.42)	4.20 (0 – 39.13)	0.000*

Table 4.13: comparison of cytokine concentrations between RA and OA patients

Cytokine Concentrations and RA Serological Markers and Anti-Rheumatic Medications:

RA patients on synthetic or biologic DMARDS or NSAIDS had similar serum concentrations of cytokines to those not taking DMARDS or NSAIDS. Monocyte chemotactic protein type 1 was significantly higher in RA patients not on a biologic agent, 277.10 pg/ml (191.43 – 415.33) compared with 215.12 pg/ml (153.32 – 271.40) in RA patients taking a biologic agent, $p = 0.045$.

RA patients who were on steroid treatment at the time of the study had a significantly higher Il-6 serum concentration than RA patients not on steroids, 20.82 pg/ml (7.13 – 40.18) versus 8.72 pg/ml (3.21-16.22), $p = 0.018$. There was a significant correlation between serum Il6 concentration and current steroid dose, $r = 0.319$, $p = 0.015$.

Anti-CCP antibody positive RA patients had significantly higher serum concentrations of the majority of cytokines tested, than anti-CCP negative patients (table 4.14). This was also the case for RA patients who tested positive for rheumatoid factor (table 4.15). Serum concentrations of cytokines correlated with anti-CCP antibody and rheumatoid factor levels (table 4.16 and figure 4.2).

RA patients with low disease activity, as characterised by a DAS28ESR less than 3.2, had significantly lower concentrations of VEGF and TNFα than RA patients with moderate or high disease activity (table 4.17). Those with a low DAS28CRP of less than 3.2, also had a lower serum concentration of TNFα than RA patients with a DAS28CRP greater than 3.2, p=0.042. DAS28ESR and DAS28CRP correlated significantly with a number of different cytokines (table 4.18). The numbers of tender and swollen joint counts were significantly associated with serum Il1α and Il8 concentrations (table 4.19).

RA patients with a family history of RA had higher concentrations of a number of pro-inflammatory cytokines compared to those with no family history of RA (table 4.20).

Cytokines, pg/ml	Anti-CCP antibody positive	Anti-CCP antibody negative	p
Il1α (IQR)	1.95 (1.13 – 5.75)	0.66 (0.49 – 1.04)	0.000
Il1β (IQR)	5.86 3.36 – 14.75)	0.00 (0.00 – 0.00)	0.000
Il2 (IQR)	29.16 (17.78 – 73.86)	17.76 (6.02 – 22.39)	0.024*
Il4 (IQR)	6.95 (4.63 – 16.56)	4.47 (3.47 – 5.14)	0.003*
Il6 (IQR)	14.62 (6.34 – 33.99)	3.63 (1.05 – 13.19)	0.003*
Il8 (IQR)	26.27 (18.36 – 52.90)	12.12 (8.35 – 15.86)	0.006*
Il10 (IQR)	7.03 (3.67 – 19.20)	0.00 (0.00 – 0.00)	0.004*
VEGF (IQR)	188.95 (101.15 – 261.18)	111.14 (48.57 – 192.35)	0.028*
TNFα (IQR)	13.99 (7.74 – 26.10)	8.30 (5.52 – 18.44)	0.114
MCP1 (IQR)	240.76 (170.77 – 374.04)	174.71 (153.44 – 254.86)	0.173
EGF (IQR)	37.99 (16.64 – 88.21)	46.98 (4.40 – 94.96)	0.586
IFN (IQR)	6.89 (3.34 – 19.24)	0.00 (0.00 – 9.99)	0.012*

Table 4.14: Comparison of serum concentrations of cytokines in anti-CCP positive and negative RA patients

Cytokines, pg/ml	Rheumatoid factor positive	Rheumatoid factor negative	P
Il1α (IQR)	1.96(1.13 – 5.75)	0.67 (0.52 – 1.02)	0.000
Il1β (IQR)	5.93(3.36 – 14.75)	0.00 (0.00 – 1.81)	0.000
Il2 (IQR)	29.16(17.78 – 73.86)	15.82 (10.92 – 19.97)	0.007*
Il4 (IQR)	6.86 (4.55 – 15.53)	4.63 (4.13 – 5.14)	0.015*
Il6 (IQR)	14.35(5.90 – 33.42)	3.25 (0.53 – 9.62)	0.006*
Il8 (IQR)	28.52(18.36 – 52.90)	12.23 (11.09 – 15.30)	0.005*
Il10 (IQR)	6.70 (3.67 – 19.51)	0.00 (0.00 – 0.34)	0.001*
VEGF (IQR)	188.95 (101.15 – 232.48)	96.98 (56.08 – 163.67)	0.037*
TNFα (IQR)	13.71 (7.74 – 26.10)	8.57 (5.11 – 14.89)	0.110
MCP1 (IQR)	235.84(160.20 – 374.04)	192.80 (155.52 – 276.76)	0.445
EGF (IQR)	34.83 (15.59 – 86.86)	63.82 (5.99 – 114.21)	0.820
IFN (IQR)	7.26 (3.41 – 19.24)	0.00 (0.00 – 1.49)	0.002*

Table 4.15: Comparison of serum cytokine concentrations rheumatoid factor positive and negative RA patients

	Correlation with anti-CCP antibody level, r	p	Correlation with rheumatoid factor level, r	p
Il1α	0.431	0.001*	0.627	0.000*
Il1β	0.515	0.000*	0.698	0.000*
Il2	0.346	0.008*	0.570	0.000*
Il4	0.468	0.000*	0.560	0.000*
Il6	0.408	0.001*	0.476	0.000*
Il8	0.355	0.006*	0.528	0.000*
Il10	0.511	0.000*	0.626	0.000*
VEGF	0.321	0.014*	0.336	0.010*
TNFα	0.255	0.053	0.485	0.000*
IFNγ	0.396	0.002*	0.602	0.000*
MCP	0.164	0.219	0.121	0.364
EGF	0.067	0.618	-0.183	0.170

Table 4.16: Association between serum cytokine concentrations and RA antibodies

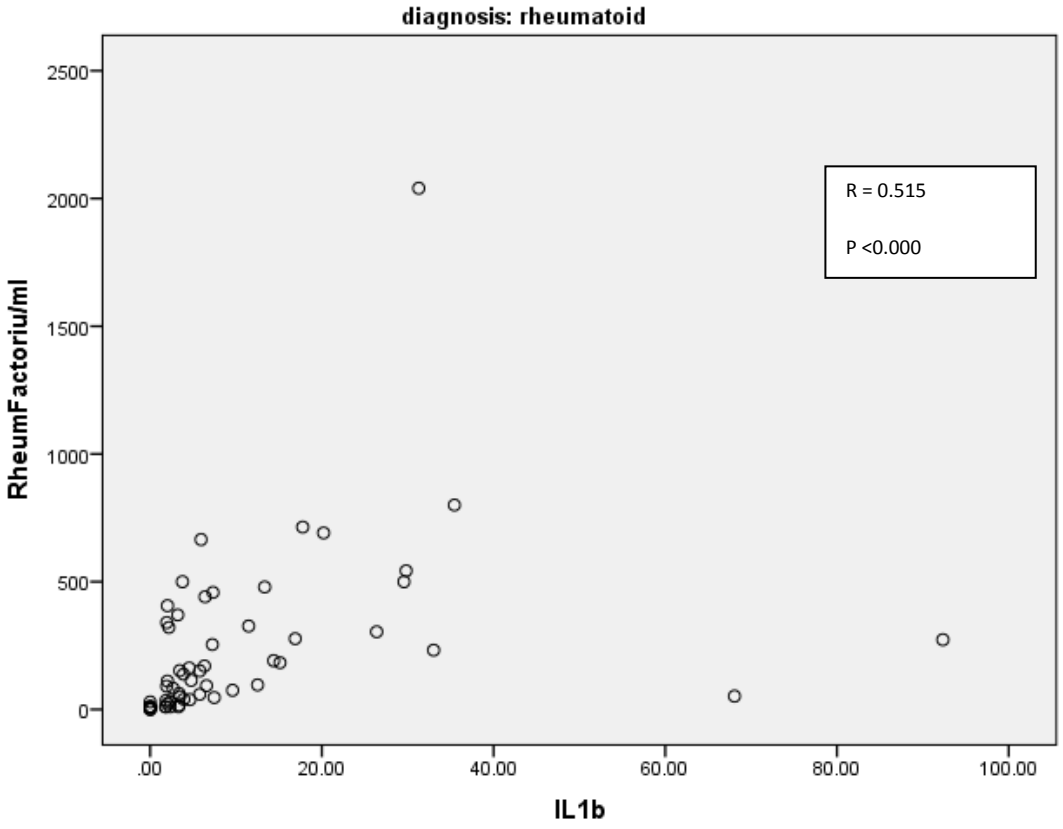


Figure 4.2: Correlation between serum Il1β concentration and rheumatoid factor level in RA patients

	DAS28ESR <3.2	DAS28ESR >3.2	p
VEGF pg/ml (IQR)	101.61 (55.64 – 195.56)	190.65 (110.34 – 237.05)	0.024*
TNFα pg/ml (IQR)	7.74 (5.73 – 14.62)	14.40 (9.41 – 26.10)	0.027*

Table 4.17: Comparison of serum VEGF and TNFα concentrations in RA patients with low and high disease activity

	Correlation with DAS28ESR, r	p	Correlation with DAS28CRP, r	p
Il1α	0.408	0.001*	0.337	0.010*
Il1β	0.331	0.011*	0.257	0.051
Il2	0.183	0.169	0.161	0.226
Il4	0.280	0.033*	0.217	0.103
Il6	0.317	0.015*	0.253	0.055
Il8	0.377	0.003*	0.281	0.033*
Il10	0.286	0.029*	0.208	0.117
VEGF	0.321	0.014*	0.268	0.042*
TNFα	0.361	0.005*	0.276	0.036*
IFNγ	0.292	0.026*	0.194	0.144
MCP	0.228	0.085	0.142	0.288
EGF	-0.031	0.819	0.060	0.652

Table 4.18: correlation between disease activity scores and serum cytokine concentrations in RA patients

	Correlation with tender joint count	p	Correlation with swollen joint count	p
Il1α	R = 0.355	0.006*	R = 0.300	0.022*
Il1β	R = 0.253	0.055	R = 0.259	0.049*
Il2	R = 0.207	0.118	R = 0.123	0.359
Il4	R = 0.256	0.053	R = 0.235	0.075
Il6	R = 0.178	0.181	R = 0.202	0.128
Il8	R = 0.316	0.016*	R = 0.327	0.012*
Il10	R = 0.234	0.077	R = 0.271	0.040*

Table 4.19: Correlations between tender and swollen joint counts and serum cytokine concentrations in RA patients

	Positive family history of RA	No family history of RA	p
Il2, pg/ml (IQR)	34.19 (20.24 – 105.17)	20.78 (15.82 – 34.69)	0.039*
Il1α, pg/ml (IQR)	2.89 (1.35 – 7.10)	1.13 (0.88 – 2.88)	0.035*
Il1β, pg/ml (IQR)	11.47 (3.21 – 26.38)	3.44 (1.93 – 6.55)	0.027*

Table 4.20: Comparison of cytokine concentrations in RA patients with and without a family history of RA

Cytokine Concentrations and Cardiovascular Markers in RA:

11 of the 12 cytokines were significantly higher in RA patients who smoked compared with RA non-smokers (table 4.21). Furthermore, smoking pack years was significantly associated with serum cytokine concentrations (table 4.22).

Serum cytokine concentrations did not correlate with fasting glucose or lipid profile in RA patients.

RA patients with evidence of abnormal common carotid IMT, low ABI, abnormal FMD and diastolic dysfunction were compared to RA patients with cIMT, ABI, FMD and diastolic marker values in the normal ranges. No differences in serum cytokine concentrations were found between the different groups, except for serum IL1α concentrations in the group of RA patients with evidence of endothelial dysfunction, as documented by abnormal FMD. Those with ultrasound proven endothelial dysfunction had a significantly higher concentration of IL1α, $p=0.031$.

Using logistic regression analysis, the presence of diastolic dysfunction, subclinical carotid atherosclerosis, abnormal ankle brachial index and endothelial dysfunction in the RA group, were not found to be associated with serum cytokine concentrations, OR (95%), 1.02 (0.95, 1.08), 1.09 (0.97, 1.23), 1.03 (0.92, 1.15) and 1.02 (0.98, 1.05), $p=0.602$, $p=0.159$, $p=0.601$, $p=0.411$, respectively.

Cytokines, pg/ml	Smoker	Non-Smoker	P
Il1α (IQR)	4.62 (1.50 – 13.37)	1.13 (0.66 – 2.98)	0.001*
Il1β (IQR)	14.38 (3.68 – 29.69)	3.21 (1.89 – 6.82)	0.001*
Il2 (IQR)	61.89 (22.39 – 96.40)	20.24 (15.82 – 30.94)	0.004*
Il4 (IQR)	13.86 (7.22 – 27.40)	4.63 (4.13 – 6.86)	0.000*
Il6 (IQR)	61.89 (12.69 – 41.57)	6.54 (3.30 – 17.73)	0.004*
Il8 (IQR)	46.50 (23.33 – 70.03)	18.36 (10.75 – 29.08)	0.005*
Il10 (IQR)	18.70 (7.43 – 31.32)	3.60 (0.00 – 5.90)	0.000*
VEGF (IQR)	207.54 (171.29 – 349.77)	125.29 (68.96 - -191.93)	0.001*
TNFα (IQR)	20.39 (12.04 – 49.41)	9.68 (6.28 – 16.98)	0.004*
MCP1 (IQR)	254.31 (221.11 – 414.57)	215.52 (149.98 – 306.87)	0.031*
EGF (IQR)	33.08 (24.48 – 83.56)	55.71 (11.48 – 97.71)	0.980
IFNγ (IQR)	18.60 (5.76 – 48.08)	3.41 (0.00 – 8.32)	0.000*

Table 4.21: Comparison of serum cytokine concentrations in RA smokers and non-smokers

Correlation between smoking pack years and serum cytokine concentrations (pg/ml)	Correlation coefficient, r	p
Il1α	0.427	0.001*
Il1β	0.402	0.002*
Il2	0.307	0.019*
Il4	0.390	0.002*
Il6	0.351	0.007*
Il8	0.267	0.043*
Il10	0.505	0.000*
VEGF	0.512	0.000*
TNFα	0.317	0.015*
Interferon γ	0.430	0.001*
MCP-1	0.325	0.013*
EGF	0/081	0.547

Table 4.22: Correlation between smoking pack years and serum cytokine concentrations in RA patients

Osteoarthritis and Cytokine Concentrations:

Within the OA group, we found significant correlations between disease duration and multiple serum cytokine concentrations (table 4.23). OA patients with a family history of MI had a significantly higher serum Il8 concentration than OA patients with no family history of MI, 20.17pg/ml (12 – 42.46) versus 9.65pg/ml (6.93 – 18.52), p=0.005.

Serum concentrations of Il10 in the OA group were found to have a negative correlation with mean common carotid IMT, r = -0.394, p=0.008. E/A ratio in the OA group correlated negatively with serum Il2 and Il6 concentrations, r = -0.416, p=0.004 and r = -0.304, p=0.038, respectively.

Using logistic regression analysis, the presence of diastolic dysfunction, subclinical carotid atherosclerosis, abnormal ankle brachial index and endothelial dysfunction in the OA group, were not found to be associated with serum cytokine concentrations, OR(95%), 1.09 (0.99, 1.19). 1.04 (0.92, 1.17), 1.09 (0.80, 1.49), 0.98 (0.88, 1.09), p=0.071, p=0.530, p=0.588, p=0.699, respectively.

Correlation between disease duration and serum cytokine concentrations (pg/ml)	Correlation coefficient, r	p
Il1β	0.384	0.008*
Il4	0.387	0.007*
Il6	0.301	0.040*
Il8	0.295	0.044*

Table 4.23: Association between serum cytokine concentrations and length of diagnosis in OA patients

Results for Markers of Thrombosis:

Comparison of Thrombotic Markers in RA and OA:

Fibrinogen levels where compared in RA and OA patients, mean values were higher in the RA group and this approached significance, 3.29g/l (SD 0.69) compared with 3.05g/l (SD0.49) in the OA group, p=0.051.

Plasminogen activator inhibitor-1 (PAI-1) antigen and activity levels were measured. PAI -1 was not normally distributed in the study population so medians and nonparametric tests were used for analysis. There was no significant difference in median PAI-1 antigen and activity levels between RA and OA patients (table 4.24). In both the OA and RA groups, males had higher PAI-1 activity and PAI-1 antigen levels than females (table 4.25). This difference was significant in the RA group.

Using logistic regression analysis, a diagnosis of RA was not found to be associated with either PAI-1 activity or antigen level more so than a diagnosis of OA, OR (95% CI), 0.413 (0.036, 4.716), p=0.477 and 3.58 (0.93, 13.75), p=0.063.

	RA	OA	p
Median PAI-1 antigen (IQR)	24.48ng/ml (15.78-35.51)	23.52ng/ml (13.43-36.12)	0.446
Median PAI-1 activity (IQR)	21.48U/ml (17.80-27.31)	23.60U/ml (19.63-30.72)	0.195

Table 4.24: comparison of PAI-1 antigen and activity levels in RA and OA patients

		Median PAI-1 activity U/ml (IQR)	p	Median PAI-1 antigen Ng.ml (IQR)	p
RA	Males	24.46 (20.34-31.10)	0.006*	34.98 (26.62-62.77)	0.000*
	Females	20.01 (15.51-24.09)		21.02 (11.38-27.96)	
OA	Males	32.12 (20.94-34.59)	0.128	28.89 (22.04- 33.62)	0.298
	Females	22.67 (18.13-26.92)		22.60 (11.82-36.96)	

Table 4.25: Comparison of PAI-1 activity and antigen levels in male and female patients with RA and OA.

Markers of Thrombosis and RA Serological Markers and Anti-Rheumatic Medications:

Within the RA group, fibrinogen levels were significantly higher in patients not on a biologic DMARD, p=0.007. Otherwise, we found no difference in fibrinogen levels when different medications were compared.

RA patients who were taking a biologic agent at the time of the study, had a significantly lower PAI-1 activity level compared to those not on a biologic agent, 19.81 U/ml (17.36-22.51) versus 24.15 U/ml (19.28-30.80), p = 0.05. PAI-1 antigen levels were similar in the 2 groups. Those patients on DMARDs, NSAIDs or steroids did not have a significantly different PAI-1 activity level to those patients not on the above treatments.

There was no significant difference in PAI-1 activity and antigen levels when RA patients with active disease were compared to those with low disease activity or remission, p = 0.365 and p = 0.177. RA patients with a low disease activity had a significantly lower fibrinogen level compared with those with more active disease, 2.99 g/l (SD 0.79) versus 3.43 g/l (SD 0.61), p = 0.048.

Within the RA group, PAI-1 activity correlated significantly with CRP, anti -CCP antibody, rheumatoid factor, fibrinogen and urate levels (table 4.26). PAI-1 antigen also correlated with urate in the RA group, r=0.546, p=0.000. There was no association found between PAI-1 and urate in the OA group.

PAI-1 activity	Correlation with PAI-1 activity, R = correlation coefficient	p
CRP	0.359	0.008*
Anti-CCP antibody	0.335	0.013*
Rheumatoid factor	0.281	0.040*
Fibrinogen	0.370	0.007*
Urate	0.303	0.035*

Table 4.26: Correlation between PAI-1 activity and markers of inflammation and seropositivity in RA patients, p, significance level; r, correlation coefficient.

Markers of Thrombosis and Cardiovascular Markers in RA:

PAI-1 activity level was higher in RA smokers compared to non-smokers but this did not achieve statistical significance. RA patients who drank more than 14 units of alcohol per week has a significantly higher PAI-1 activity level compared with those who consumed less than 14 units per week, 27.95U/ml (24.69-43.95) compared with 21.11U/ml (17.45-26.04), p=0.036.

There was no significant difference in PAI-1 antigen levels between smokers and non-smokers or between those who consumed greater than 14 units of alcohol per week and those who consumed less.

RA patients who were overweight or obese had a significantly higher PAI-1 activity level and PAI-1 antigen compared to those RA patients with a normal BMI, p=0.016 and p=0.040, respectively. This difference was not seen across the categories of BMI in the OA group. RA patients with evidence of abdominal obesity, as defined by the WHO, as a waist-hip ratio of greater than 0.85 in females and greater than 0.9 in males, had significantly higher levels of PAI-1 activity and antigen than RA patients with normal waist-hip ratio, p=0.014 and p=0.001 (table 4.27). PAI-1 activity and antigen level correlated positively with waist hip ratio in RA patients, r=0.482, p=0.000 and r=0.629, p=0.000, respectively (figure 4.3).

Systolic and diastolic blood pressure were significantly associated with both PAI-1 activity and antigen in the RA group, but not in the OA group, r =0.417 p=0.003 and r=0.329, p=0.022, respectively.

We examined the RA group to see if there was a difference in PAI-1 activity or antigen levels in those with and without evidence of preclinical atherosclerosis or diastolic dysfunction. There was no difference in PAI-1 concentrations when those with abnormal IMT, abnormal ABI, low FMD or evidence of diastolic dysfunction were compared to those RA patients who had no evidence of preclinical atherosclerosis or diastolic dysfunction. However, mean common carotid IMT was found to correlate significantly with PAI-1 activity in the RA group, $r = 0.294$, $p = 0.013$.

RA patients with abnormal cIMT, ABI, FMD or evidence of diastolic dysfunction did not have a higher fibrinogen than those with normal IMT, ABI, FMD and diastolic function.

		Median PAI-1 activity U/ml (IQR)	p	Median PAI-1 antigen Ng/ml (IQR)	p
RA	Normal BMI	20.37 (15.13-22.49)	0.016*	22.38 (11.67-29.78)	0.04*
	High BMI	24.01 (18.48-31.23)		26.62 (19.04-53.90)	
RA	Normal waist-hip ratio	18.70 (15.09-24.62)	0.014*	16.79 (10.72026.78)	0.001*
	Abdominal obesity	23.24 (19.38-30.80)		33.03 (22.60-48.03)	

Table 4.27: Comparison of PAI-1 activity and antigen levels in RA patients with normal and high BMI and also in RA patients with normal or high waist hip ratios.

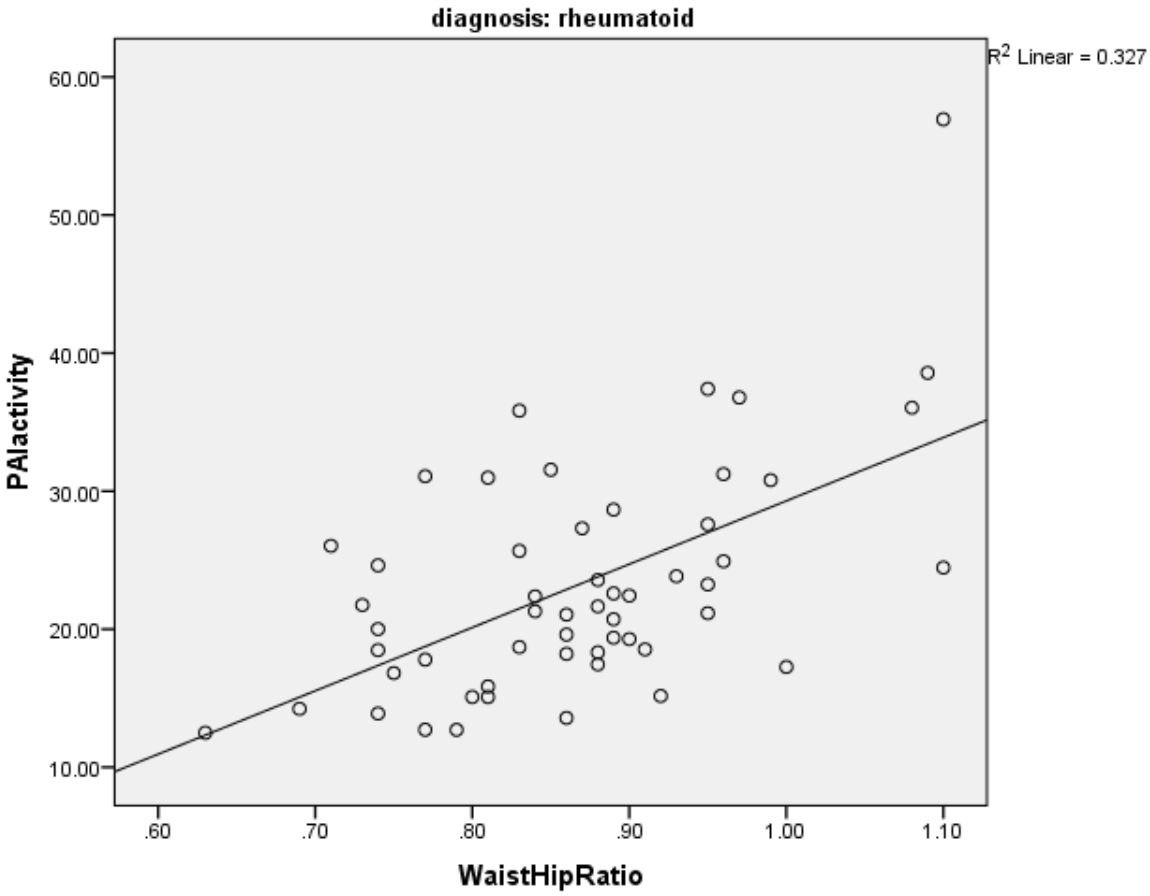


Figure 4.3: Correlation between PAI activity and waist hip ratio in RA

Osteoarthritis and Markers of Thrombosis:

Within the OA group, fibrinogen level correlated with weight, waist circumference and BMI, r=0.322, r=0.454, r=0.468, p=0.046, p=0.004, p=0.003, respectively.

OA patients who were current or former smokers had a higher PAI-1 activity level than OA non-smokers, 25.92U/ml (22.87-32.81) compared with 20.68U/ml (17.45-22.67), p=0.008. Smoking pack years correlated significantly with PAI-1 activity, r=0.431, p=0.003.

Results for Urinary Protein-Creatinine Ratio and Microalbuminuria:

Median values are quoted as these variables were not normally distributed. Median protein-creatinine ratio (PCR) was significantly higher in the RA group (table 4.28). Levels of microalbuminuria were similar between the 2 groups.

Urinary PCR was significantly lower in RA patients currently taking a DMARD, compared to RA patients not on a DMARD at the time of the study, 5.54 (4.29 – 7.21) versus 7.61 (5.50 – 10.07), p=0.027. There was no significant difference in PCR between RA patients on a biologic agent currently and those not on one, p=0.733.

PCR was associated with serum levels of VCAM and L-selectin in the RA group, r = 0.284, p =0.042 and r =0.281, p=0.043.

We found a significant correlation between microalbuminuria and E/e', a marker of diastolic dysfunction, r = 0.402, p =0.009.

	RA	OA	p
Median PCR (IQR)	5.85 (4.41 – 7.96)	4.86 (2.89 – 7.03)	0.023*
Median microalbuminuria, mg/mmol (IQR)	0.55 (0.34 – 0.92)	0.42 (0.33 – 0.57)	0.094

Table 4.28: Protein creatinine ratio and microalbuminuria in RA and OA patients. IQR, interquartile range; p, significance level.

Discussion:

Comparison of ESR, CRP and Urate in RA and OA:

As expected, both ESR and CRP were significantly higher in the RA group. Also, not surprisingly, RA patients on synthetic and biologic DMARDS had significantly lower inflammatory markers. We found similar levels of inflammatory markers in sero- positive and sero-negative RA patients.

CRP was found to be significantly associated with markers of thrombosis; fibrinogen and PAI-1 antigen in RA patients. Kitas et al, also found significant correlations between CRP and coagulation factors in RA patients (44). Whether these associations are due to inflammation or are markers of cardiovascular dysfunction has not yet been fully elucidated.

A large prospective study of incident CVD events in RA was published in 2013 and reported that CRP level was associated with higher CVD risk in RA patients under 65 years and reductions in CRP were associated with a decreased CVD risk in RA patients over 65 years (49). This validates the importance of early control of inflammation in RA to reduce CVD risk.

Serum urate was similar in RA and OA patients. We did find significant associations between serum urate levels and a number of anthropometric variables in our RA group. There is mounting evidence that serum uric acid plays a role in the development of cardiovascular diseases (50). Levels of serum urate have been shown to correlate significantly with blood pressure and carotid intima-media thickness

(51). Earlier studies have reported a strong association between obesity and hyperuricaemia (52). A recent large study by Ohya et al, found a significantly higher prevalence of metabolic syndrome in patients with baseline hyperuricaemia and that hyperuricaemia was an independent predictor of metabolic syndrome (53).

A recent review by van Holten et al, of multiple meta-analyses of biomarkers that predict cardiovascular disease risk, found that the highest risk for cardiovascular disease is reported for elevated CRP (RR 2.43 (95%CI), (2.10-2.83)), followed by fibrinogen and lipids. Uric acid, together with fibrinogen, were the strongest risk markers for primary stroke (54).

Comparison of Adhesion Molecules in RA and OA:

The serum concentrations of 2 pro-inflammatory adhesion molecules, VCAM and P-selectin were significantly higher in our RA group compared to our OA group. These adhesion molecules are biomarkers of endothelial dysfunction. Increased expression and upregulation of adhesion molecules in conjunction with pro-inflammatory cytokines, is one of the earliest processes in damage to the endothelial cell layer (55). Leucocyte adhesion to endothelial cells is mediated by the above adhesion molecules. During this process selectins facilitate leucocyte "tethering" and "rolling", while members of the immunoglobulin superfamily: VCAM and ICAM, are responsible for migration and adhesion of leucocytes (56).

Dessein et al also reported significantly higher concentrations of circulating adhesion molecules in 74 RA patients compared to controls (57) and found that VCAM concentrations were associated with common carotid atherosclerosis in their RA group. These findings have also been reported in earlier studies (45).

These findings are important because elevated levels of adhesion molecules have been shown to be associated with CV risk factors and future CV events (58, 59). There is also evidence that in RA patients, these biomarkers may be more important than traditional CV risk factors in cardiovascular disease (60). Also of interest, is a study in 2006 by Gonzalez-Gay et al that reported a reduction in multiple adhesion molecules post-infliximab infusion (61). Foster et al also found a reduction in VCAM and E-selectin concentrations in RA patients measured 1 week after anti-TNF therapy (62).

L-selectin concentrations were lower in the RA group. There is very little recent literature on the concentration of L-selectin in RA patients and the results from earlier studies are controversial. In 2004, Ates et al, reported a lower L-selectin level in RA patients compared to controls (63). Sfikakis et al found no difference in L-selectin concentrations between RA patients and controls (64). In contrast, Littler et al found significantly higher L-selectin concentrations in RA patients compared to controls (65).

L-selectin is expressed on leucocytes and is rapidly shed during leucocyte activation and transmigration across the endothelial cell lining, therefore one would expect increased levels of L-selectin in activated leucocytes (66). However, one theory for

the lower concentrations of L-selectin in RA, is a downregulation of L-selectin expression as a protective mechanism to stop further leucocyte migration (67) and use of some anti-rheumatic medications, in particular, methotrexate and corticosteroids have also been shown to reduce L-selectin expression (68).

Adhesion Molecule Concentrations and RA Serological Markers and Anti-Rheumatic Medications:

Within our RA group, serum concentrations of adhesion molecules were similar for those on synthetic and biologic DMARDs to those RA patients not on a DMARD at the time of the study. We did find a correlation between glucocorticoid dose and concentrations of both VCAM and L-selectin. Glucocorticoids have previously been shown to suppress serum concentrations of VCAM and ICAM in RA patients (69). Although glucocorticoids have may negative effects on metabolic and cardiovascular risks, they may, via their effect on pro-inflammatory cytokines and adhesion molecules, attenuate atherogenesis in RA.

Seropositivity in our RA group was associated with higher concentrations of VCAM, ICAM and E-selectin. E-selectin also correlated with CRP. A review of 10 studies by J Giles in 2006 found that sero-positivity and CRP together with a host of other RA markers, were implicated in the development of RA-associated accelerated atherosclerosis (70). In 2011, Agewall S et al found that the presence of anti-CCP antibodies and rheumatoid factor IgM in RA patients was associated with endothelial dysfunction independent of other CV risk factors (71). Szekanecz et al also reported

that sero-positive RA patients had more pronounced endothelial dysfunction and carotid atherosclerosis than RA patients who were anti-CCP antibody and rheumatoid factor negative (72). Our findings are consistent with published work.

Adhesion Molecule Concentrations and Cardiovascular Markers in RA:

With regard to traditional CV risks, smoking in our RA group was significantly associated with serum ICAM and P-selectin concentrations. Smoking has been shown to be associated with elevated serum ICAM and smoking cessation improves ICAM concentrations (73). This may be one of the mechanisms by which smoking increases cardiovascular risk.

Systolic blood pressure in our RA group was also associated with E- and P- selectin levels. A study by Sanada H et al found that patients with hypertension had elevated levels of the selectins and that treatment of the hypertension resulted in improvement of selectin concentrations (74).

With regard to markers of subclinical vascular disease, we found that RA patients with evidence of an abnormal carotid IMT and those with endothelial dysfunction on brachial artery ultrasound had significantly lower concentrations of L-selectin. Also, lower concentrations of L-selectin were found to correlate with total cholesterol and serum urate levels RA patients. L-selectin was also negatively correlated with body mass index and waist circumference.

We have found that L-selectin appears to have significant negative associations with a number of markers of pre-clinical atherosclerosis and also with elements of the metabolic syndrome. L-selectin is down regulated in chronic inflammation. A lower level is associated with CVD in the general population (75).

It appears from our dataset, that elevated levels of L-selectin may be linked to anti-atherogenic properties in RA patients and perhaps has a protective role, but this needs to be examined further with larger study numbers and in a prospective manner. Sodergren et al had similar findings with respect to L-selectin and markers of atherosclerosis in RA (76). They reported that reduced levels of L-selectin were strongly related to low FMD and increased IMT.

A number of markers of diastolic dysfunction, including e', E/A ratio and NT-proBNP, were associated with higher concentrations of pro-inflammatory adhesion molecules but lower concentrations of L-selectin. In particular, E-selectin, a serum biomarker of endothelial dysfunction, had a negative association with markers of diastolic dysfunction. Masiha S et al recently reported similar associations between E-selectin and diastolic dysfunction in a population study of elderly patients. This was the first study demonstrating the association. The mechanisms are still unclear but it does appear from their research that biomarkers of endothelial dysfunction play a role in myocardial remodelling (77).

Finally, urinary protein-creatinine ratio, a marker of vascular disease, was significantly correlated with serum VCAM concentrations in our RA patients.

This is a significant and novel finding and has not been previously reported in the RA literature. Lin J et al previously reported that serum VCAM concentrations were significantly higher in type II diabetic patients with low GFR. They concluded that elevation of serum biomarkers of endothelial dysfunction may be the link between renal insufficiency and cardiovascular risk in diabetic patients (78).

This simple urine measurement appears to be a surrogate marker of early microvasculopathy in RA and can easily be checked in clinic.

.

Osteoarthritis and Adhesion Molecule Concentrations:

There was no association between adhesion molecule concentrations and markers of cardiovascular disease in our OA group. A recent study by Lia Pulsatelli et al found significantly higher concentrations of serum VCAM in patients with erosive hand OA compared to healthy controls (79). VCAM has been found to be expressed in OA joints and has been suggested as a marker of OA disease severity (80). None of our OA patients had obvious clinical features of synovitis and only 1 had evidence of OA changes in the hands. Perhaps our OA group were of a different phenotype and this may explain the difference in results of adhesion molecules.

Serum VCAM concentrations correlated significantly with measures of general health, disability and disease activity scores in OA patients. This is interesting because health related quality of life scores for other chronic diseases have been shown to be associated with serum VCAM concentrations. In a study of CHF outpatients, a poor score on the Kansas City Cardiomyopathy Questionnaire, that assesses physical function and quality of life in heart failure, was associated with high concentrations of serum VCAM and was also associated with a shorter event-free survival (81). This emphasises the usefulness of self-administered questionnaires.

Comparison of Cytokine Concentrations in RA and OA:

As expected, the majority of serum cytokine concentrations were higher in RA than OA patients. IL4 and IL10, both of which have anti-inflammatory properties, were also higher in RA patients. Although serum concentrations of VEGF and MCP1 were not significantly different between the 2 groups, both were still higher in the RA group. This is in keeping with the inflammatory nature of RA, which is driven in particular by TNFα, IL6 and IL1. However, there is emerging evidence that these pro-inflammatory cytokines also play a role in articular cartilage degradation and OA development and OA associated pain (82).

Cytokine Concentrations and RA Serological Markers and Anti-Rheumatic Medications:

Patients on biologic or synthetic DMARDS or NSAIDS had similar concentrations of serum cytokines to those not on DMARDS or NSAIDS.

One would expect to demonstrate lower levels of circulating cytokines in patients whose disease was controlled with biologic DMARDs. Perhaps our RA treatment subgroups were too small to detect a significant difference.

MCP1 was the only cytokine which was lower in RA patients on a biologic agent compared to those not on one at the time of the study. MCP-1 is a potent atherosclerotic and RA factor. Levels have been associated with the incidence of

coronary artery disease in the general population and with the clinical symptoms of RA (83). It is interesting that this chemokine, which plays a role in the pathogenesis of both conditions, was suppressed in patients taking biologic DMARDs and this finding supports the evidence that treatment with biologic DMARDs not only improves RA disease scores and function but also has beneficial effects on CV risk. Barnabe et al reported that TNF suppression in RA patients was associated with reduced risk of all heart disease events (84).

Naredo et al reported a lower level of carotid sub-clinical atherosclerosis in RA patients on a biologic DMARD compared to those on a synthetic DMARD (85). These findings of better CV risk profile in patients treated with TNF inhibitors substantiate the link in cytokine pathways between the 2 diseases.

Patients with a flare of disease activity are often treated with short courses of corticosteroids to control synovitis and relieve symptoms. IL6 is one of the main pro-inflammatory cytokines responsible for driving the inflammatory burden of RA. It is therefore not surprising that we found higher IL6 concentrations in RA patients on corticosteroids and the steroid dose was found to correlate significantly with IL6 concentration.

With regard to disease activity, as expected, the serum concentrations of TNFα were significantly lower in those RA patients who had low disease activity or were in remission compared to those with moderate or high disease activity.

Sero-positive RA patients had significantly higher concentrations of serum cytokines than RA patients who tested negative for anti-CCP antibody and Rheumatoid factor

IgM. The titres of anti-bodies also correlated with cytokine concentrations. This reflects the fact that those with anti-CCP antibody and Rheumatoid factor IgM positivity have more aggressive disease. This is well documented in the literature. Cortina et al reported that sero-positive patients had higher DAS scores and more radiological damage than sero-negative RA patients (86). In 2010, a study of RA patients with active disease found that anti-citrullinated peptide antibodies were markers independently associated with impaired left ventricular relaxation (87). They may exert a pro-atherogenic effect via common inflammatory cytokines but this remains to be investigated.

Cytokine levels were also higher in those with a first degree relative of RA in our study. There appears be a familial clustering of serum cytokine levels within families with RA. Fritzler et al found that people with a first degree relative with RA had higher serum concentrations of cytokines than those with no such family history (88).

Cytokine Concentrations and Cardiovascular Markers in RA:

A history of smoking was associated with increased levels of serum cytokines in our RA group. Smoking has been shown to stimulate a low-grade inflammatory response and elevated levels of CRP, IL6 and TNFα have been reported in smokers. It is felt that these abnormalities, in conjunction with effects on the coagulation and fibrinolytic systems, may be some of the mechanisms by which smoking increases cardiovascular risk (89). Smoking is the main environmental risk factor for the development of RA and it predisposes to seropositive RA. The risk of developing RA is twice as high in male smokers compared to males who do not smoke and for female smokers the risk is 1.3 times greater than non-smokers (90).

The molecular mechanisms connecting smoking and the development of RA have not been identified in detail yet, however, they are most likely connected to the effect of smoking on inflammatory cytokines.

Cytokine concentrations were similar in patients with and without evidence of peripheral vascular disease, subclinical carotid atherosclerosis, endothelial dysfunction and diastolic dysfunction. However, serum IL1α concentrations were higher in RA patients with endothelial dysfunction.

IL-1 is one of the primary pro-inflammatory cytokines in RA and one of its many functions is to induce adhesion molecule expression, V-CAM and I-CAM on endothelial cells (91) which explains why we found the above association.

OA and Cytokine Concentrations:

Disease duration was associated with multiple serum cytokines in our OA group (table 23).

Synovial inflammation is now known to play a role in the pathogenesis of OA and levels of inflammation correlate with the degree of cartilage pathology (92). It is plausible that as cartilage disease progresses over time, synovitis, as reflected by increased inflammation cytokines would also be associated with disease duration.

E/A ratio, a marker of diastolic dysfunction was associated with levels of IL2 and IL6 in our OA patients. Collier et al reported increased levels of Il6 and other pro-inflammatory cytokines in heart failure patients with preserved ejection fraction (93). Based on this report, it makes sense that we might see an association between pre-clinical diastolic heart failure and inflammatory cytokines in our cohort, particularly as there is emerging evidence of a causal role for IL6 in coronary heart disease (94).

Comparison of Thrombotic Markers in RA and OA:

In our study, RA patients had a higher mean fibrinogen than OA controls.

For more than half a century, fibrinogen has been found to be associated with acute thrombosis (95). In 1984, the Gothenburg Heart Study was the first prospective study to report an association between fibrinogen levels and future cardiovascular events (96).

Fibrinogen is an acute phase reactant and may be elevated along with CRP and ESR in active RA. It is also an important marker for predicting cardiovascular disease and stroke in populations without pre-existing cardiovascular disease (54). However, whether elevated fibrinogen levels are just an association or have an actual causal role in cardiovascular disease is still unclear. Danesh J et al reported that after adjustment for traditional cardiovascular risks, the hazard ratio for CHD in patients with no prior history of cardiovascular disease was 1.8 per 1g/L increase in fibrinogen level (40).

Given that the risk of cerebrovascular disease in RA is double that of the general population (97), fibrinogen may be a useful screening tool to identify those at risk RA patients, particularly those with low disease activity whose elevated fibrinogen level would be less likely to be related to RA disease activity.

We did not find a difference in PAI-1 activity or antigen levels between RA and OA patients. This is in contrast to a recent study of 141 RA patients and 50 controls by Kitas et al, where the RA group had significantly higher PAI-1 levels. The differences between the studies with respect to PAI-1 levels may be explained by the fact that in the study by Kitas, the RA patients were significantly older than controls and the control group were healthy volunteers. Also of note, in the above study 20% of the RA group were on an anti-platelet agent and over 40% were taking anti-hypertensives which suggest they had a greater baseline CV risk profile than our RA group.

In contrast to Kitas's work, a study comparing 125 RA patients and 132 controls found a lower PAI-1 in the RA group compared to controls (98).

Markers of Thrombosis and RA Serological Markers and Anti-Rheumatic Medications:

We reported a significantly higher fibrinogen level in RA patients not on a biologic DMARD compared to those on a biologic DMARD. This finding is in keeping with a study by Sandoo et al, which reported a significant improvement in fibrinogen levels in RA patients started on anti-TNF therapy (99).

We found that RA patients on a biologic DMARD had a significantly lower PAI-1 activity than those not on a biologic agent. In 2006, Agirbasli et al reported a reduction in inflammatory markers and PAI-1 in RA patients treated with infliximab (100).

We detected a significant association between PAI-1 activity and CRP, urate, anti-CCP antibody and rheumatoid factor IgM. A number of small studies have reported abnormalities in coagulation in RA patients and correlation of these abnormalities with disease activity and inflammation (45, 101).

Similar to our findings, Kitas et al demonstrated several coagulation abnormalities in RA patients, including derangement of fibrinogen, PAI-1 and tissue plasminogen activator and also reported an association with inflammation factors including ESR,

CRP and DAS scores (44). These results provide further evidence for the relationship between inflammation and fibrinolytic abnormalities in RA.

Markers of Thrombosis and Cardiovascular Markers in RA:

Our RA patients with high body mass index and abdominal obesity had significantly higher levels of PAI-1 activity and antigen.

Increased risk of CVD in the general population has been shown to be associated with elevated levels of PAI-1. An increase in PAI-1 and other coagulation biomarkers leads to a pro-thrombotic state. An early study by Wallbery-Jonsson et al, found that baseline levels of PAI-1 in RA patients were significantly elevated in those who developed a cardiovascular event in the 2-year follow-up period (102).

This evidence, combined with the fact that our RA patients with a more pro-atherogenic body habitus had higher levels of PAI-1 reinforces the connection between RA and CV risk.

Blood pressure and carotid subclinical atherosclerosis were also associated with PAI-1 activity in the RA group. Wallberg-Jonsson et al, found a significant association between cIMT and tissue plasminogen activator and they also found an association between carotid atherosclerotic plaque and PAI-1 in RA patients (103).

In 2010, Sodergren et al also found significant associations between PAI-1 and systolic blood pressure and between tPA mass and carotid IMT in RA patients

(76).Therefore, PAI-1 appears to be linked to increased CV risk and pre-clinical markers of atherosclerosis in RA patients.

RA patients with evidence of subclinical atherosclerosis or diastolic dysfunction were not found to have higher fibrinogen levels than those with no evidence of subclinical atherosclerosis or diastolic dysfunction.

Osteoarthritis and Markers of Thrombosis:

We reported a significant correlation between a number of anthropometric measurements associated with the metabolic syndrome and fibrinogen level in OA patients. Fibrinogen levels have been shown to correlate with components of the metabolic syndrome (104). Given the recent research into the links between OA and the metabolic syndrome, our findings are not that surprising. OA is now known to be associated with hypertension, dyslipidaemia, hyperglycaemia and obesity (105) and has been linked to the metabolic syndrome as a whole. The NHANES III study, analysing more than 7000 adults, found that the metabolic syndrome was more prevalent in people with OA (106).

Smoking status was associated with PAI- activity in OA patients. This is in keeping with other research showing that smokers in general have higher levels of PAI-1 (89, 107).

Urinary Protein Measures in RA and OA:

We found that protein-creatinine ratio (PCR) was higher in RA patients compared to OA patients and that RA patients on a DMARD had a better PCR than those not on a DMARD. PCR correlated significantly with serum biomarkers of endothelial dysfunction and microalbuminuria correlated with diastolic dysfunction.

Microalbuminuria is a recognised index of vascular disease and excess cardiovascular mortality (108). There are also reports that it may be useful in IHD, stroke and peripheral arterial disease prognosis (109). It is a marker of endothelial dysfunction and is thought to be due to endothelial dysfunction of the glomerulus (110).

A higher prevalence of microalbuminuria has been reported in RA patients compared to controls and one early study found rates of 27.7% in RA patients compared with 7.8% in controls (111).

Albuminuria is increased in patients with heart failure and elevated levels have been shown to predict future hospitalization for heart failure in patients without heart failure (112).

Our findings of an association with diastolic dysfunction are supported by recent research by Katz et al. They studied the relationship between urine albumin-creatinine ratio (UACR) to cardiac mechanics in 1894 adults in the general population and reported an independent association between UACR and E/e' ratio, a marker of increased LV filling pressures (113).

Conclusions:

CRP is a useful and readily available marker in RA. Its functions appear to be two-fold; persistently elevated levels identify RA patients with on-going inflammation that needs to be addressed to reduce future CV risk and elevated CRP levels correlate with other cardiovascular markers such as PAI-1 and fibrinogen and may help to identify patients who should be evaluated for CV risk more meticulously.

Serum urate is emerging as another potential marker of CVD. However, in RA patients with active inflammation it may be difficult to tease out whether elevated levels are due to an acute phase reaction or predict those at increased CV risk.

Our findings of an association between serum urate and BMI, waist hip ratio and waist circumference are in keeping with previous research that reports an association with the metabolic syndrome. If serum urate is in fact a marker of CVD in RA, targeting weight control in these patients may reduce its concentration and subsequent CV risk.

Similar to other research in this area, we found that serum concentrations of adhesion molecules were significantly higher in our RA group. Because both of our study groups had very similar rates of traditional risk factors, this supports the growing body of evidence that factors other than traditional CV risks are driving the increased rate of CV disease in RA patients.

Based on our findings of higher concentrations of adhesion molecules in RA patients who were anti-CCP antibody and rheumatoid factor IgM positive and on the

association between CV risk and RA sero-positivity in multiple other studies, rheumatologist should be extra vigilant in screening for CV risks and disease in these patients.

The fact that smoking is quite prevalent in rheumatoid populations, with a possible causal link to the development of RA, combined with our findings of an association with endothelial biomarkers of atherosclerosis, emphasises the importance of targeting traditional CV risks aggressively in these patients.

Our findings in relation to serum biomarkers and diastolic dysfunction in RA demonstrate a link between endothelial dysfunction and cardiac remodelling in this group.

Of interest, from the analysis of our OA group, self-administered health related questionnaires correlated with a marker of endothelial dysfunction and this displays how relevant these questionnaires may be in the general assessment of RA patients.

As we expected, serum cytokine concentrations were higher in RA patients.

Interestingly, MCP-1, a common chemokine in both RA and CVD pathways was lower in those on a biologic DMARD, suggesting an anti-atherogenic effect of biologic agents in addition to their role in RA disease. This may be a useful future therapeutic target to treat both diseases simultaneously.

The association between smoking and inflammatory cytokines, fits with the known effects of smoking on TNFα, IL6 and CRP. Given that smoking is a significant common environmental risk factor for both RA and CVD and is preventable,

emphasis should be placed on smoking cessation at the rheumatology clinic and not left to the cardiologist.

Although previously thought to be a degenerative condition, there is now evidence that OA also has an inflammatory component at the level of the synovium and we have found that the degree of inflammatory burden in these patients correlated with disease duration.

In our RA patients, fibrinogen levels are higher than in OA. Fibrinogen measurements may be useful in screening for future cardiovascular and cerebrovascular events in RA patients. However, due to the multitude of assays available to measure fibrinogen and the great variation in results between laboratories, standardization of this assay is required before using it in the clinical setting.

Higher fibrinogen levels in our OA patients with an elevated BMI and the known link between fibrinogen and the metabolic syndrome, adds weight to growing evidence that OA is closely associated with the metabolic syndrome.

The association between PAI-1 activity and multiple markers of disease activity in our RA group supports previous research identifying a link between inflammation and coagulation abnormalities. PAI-1 was also linked to subclinical atherosclerosis in our RA patients, which suggests that abnormal coagulation may be one of the mechanisms by which CVD is increased in RA.

Urinary protein-creatinine ratio is higher in RA patients and correlates with diastolic dysfunction. Our findings suggest a pathophysiological link between endothelial dysfunction and diastolic dysfunction in RA that needs to be investigated further. This suggests that measurement of albuminuria in RA patients may help to detect those at risk of diastolic dysfunction who would benefit from transthoracic echocardiogram. This is the first known study showing such a correlation in RA.

References:

1. Sherer Y, Shoenfeld Y. Mechanisms of disease: atherosclerosis in autoimmune diseases. Nature clinical practice Rheumatology. 2006;2(2):99-106. Epub 2006/08/26.

2. Del Rincon I, Williams K, Stern MP, Freeman GL, O'Leary DH, Escalante A. Association between carotid atherosclerosis and markers of inflammation in rheumatoid arthritis patients and healthy subjects. Arthritis and rheumatism. 2003;48(7):1833-40. Epub 2003/07/09.

3. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation. 2002;105(9):1135-43. Epub 2002/03/06.

4. Book C, Saxne T, Jacobsson LT. Prediction of mortality in rheumatoid arthritis based on disease activity markers. The Journal of rheumatology. 2005;32(3):430-4. Epub 2005/03/03.

5. Goodson N, Marks J, Lunt M, Symmons D. Cardiovascular admissions and mortality in an inception cohort of patients with rheumatoid arthritis with onset in the 1980s and 1990s. Annals of the rheumatic diseases. 2005;64(11):1595-601. Epub 2005/04/22.

6. Ridker PM, Wilson PW, Grundy SM. Should C-reactive protein be added to metabolic syndrome and to assessment of global cardiovascular risk? Circulation. 2004;109(23):2818-25. Epub 2004/06/16.

7. Maradit-Kremers H, Nicola PJ, Crowson CS, Ballman KV, Gabriel SE. Cardiovascular death in rheumatoid arthritis: a population-based study. Arthritis and rheumatism. 2005;52(3):722-32. Epub 2005/03/08.

8. Montecucco F, Steffens S, Burger F, Pelli G, Monaco C, Mach F. C-reactive protein (CRP) induces chemokine secretion via CD11b/ICAM-1 interaction in human adherent monocytes. Journal of leukocyte biology. 2008;84(4):1109-19. Epub 2008/07/02.

9. Gonzalez-Gay MA, Gonzalez-Juanatey C, Pineiro A, Garcia-Porrua C, Testa A, Llorca J. High-grade C-reactive protein elevation correlates with accelerated atherogenesis in patients with rheumatoid arthritis. The Journal of rheumatology. 2005;32(7):1219-23. Epub 2005/07/05.

10. Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. Circulation. 1998;98(8):731-3. Epub 1998/09/04.

11. Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P, et al. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. Bmj. 2000;321(7255):199-204. Epub 2000/07/21.

12. Kim SY, Guevara JP, Kim KM, Choi HK, Heitjan DF, Albert DA. Hyperuricemia and coronary heart disease: a systematic review and meta-analysis. Arthritis care & research. 2010;62(2):170-80. Epub 2010/03/02.

13. Niskanen LK, Laaksonen DE, Nyyssonen K, Alfthan G, Lakka HM, Lakka TA, et al. Uric acid level as a risk factor for cardiovascular and all-cause mortality in

middle-aged men: a prospective cohort study. Archives of internal medicine. 2004;164(14):1546-51. Epub 2004/07/28.

14. Feig DI, Kang DH, Johnson RJ. Uric acid and cardiovascular risk. The New England journal of medicine. 2008;359(17):1811-21. Epub 2008/10/24.

15. Michiels C. Endothelial cell functions. Journal of cellular physiology. 2003;196(3):430-43. Epub 2003/08/02.

16. Szekanecz Z, Koch AE. Vascular involvement in rheumatic diseases: 'vascular rheumatology'. Arthritis research & therapy. 2008;10(5):224. Epub 2008/10/25.

17. Agarwal SK, Brenner MB. Role of adhesion molecules in synovial inflammation. Current opinion in rheumatology. 2006;18(3):268-76. Epub 2006/04/04.

18. Constans J, Conri C. Circulating markers of endothelial function in cardiovascular disease. Clinica chimica acta; international journal of clinical chemistry. 2006;368(1-2):33-47. Epub 2006/03/15.

19. Sattar N, McCarey DW, Capell H, McInnes IB. Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. Circulation. 2003;108(24):2957-63. Epub 2003/12/17.

20. Ulfgren AK, Andersson U, Engstrom M, Klareskog L, Maini RN, Taylor PC. Systemic anti-tumor necrosis factor alpha therapy in rheumatoid arthritis down-regulates synovial tumor necrosis factor alpha synthesis. Arthritis and rheumatism. 2000;43(11):2391-6. Epub 2000/11/18.

21. Zhang H, Park Y, Wu J, Chen X, Lee S, Yang J, et al. Role of TNF-alpha in vascular dysfunction. Clinical science. 2009;116(3):219-30. Epub 2009/01/03.

22. Ohta H, Wada H, Niwa T, Kirii H, Iwamoto N, Fujii H, et al. Disruption of tumor necrosis factor-alpha gene diminishes the development of atherosclerosis in ApoE-deficient mice. Atherosclerosis. 2005;180(1):11-7. Epub 2005/04/13.

23. Branen L, Hovgaard L, Nitulescu M, Bengtsson E, Nilsson J, Jovinge S. Inhibition of tumor necrosis factor-alpha reduces atherosclerosis in apolipoprotein E knockout mice. Arteriosclerosis, thrombosis, and vascular biology. 2004;24(11):2137-42. Epub 2004/09/04.

24. Dixon WG, Symmons DP. What effects might anti-TNFalpha treatment be expected to have on cardiovascular morbidity and mortality in rheumatoid arthritis? A review of the role of TNFalpha in cardiovascular pathophysiology. Annals of the rheumatic diseases. 2007;66(9):1132-6. Epub 2007/01/26.

25. Jenny NS, French B, Arnold AM, Strotmeyer ES, Cushman M, Chaves PH, et al. Long-term assessment of inflammation and healthy aging in late life: the Cardiovascular Health Study All Stars. The journals of gerontology Series A, Biological sciences and medical sciences. 2012;67(9):970-6. Epub 2012/03/01.

26. Danesh J, Kaptoge S, Mann AG, Sarwar N, Wood A, Angleman SB, et al. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. PLoS medicine. 2008;5(4):e78. Epub 2008/04/11.

27. Kobayashi H, Giles JT, Polak JF, Blumenthal RS, Leffell MS, Szklo M, et al. Increased prevalence of carotid artery atherosclerosis in rheumatoid arthritis is artery-specific. The Journal of rheumatology. 2010;37(4):730-9. Epub 2010/01/30.

28. Schnabel RB, Yin X, Larson MG, Yamamoto JF, Fontes JD, Kathiresan S, et al. Multiple inflammatory biomarkers in relation to cardiovascular events and mortality in the community. Arteriosclerosis, thrombosis, and vascular biology. 2013;33(7):1728-33. Epub 2013/05/04.

29. Koch AE, Kunkel SL, Harlow LA, Mazarakis DD, Haines GK, Burdick MD, et al. Epithelial neutrophil activating peptide-78: a novel chemotactic cytokine for neutrophils in arthritis. The Journal of clinical investigation. 1994;94(3):1012-8. Epub 1994/09/01.

30. Young JL, Libby P, Schonbeck U. Cytokines in the pathogenesis of atherosclerosis. Thrombosis and haemostasis. 2002;88(4):554-67. Epub 2002/10/04.

31. Van Tassell BW, Toldo S, Mezzaroma E, Abbate A. Targeting interleukin-1 in heart disease. Circulation. 2013;128(17):1910-23. Epub 2013/10/23.

32. Ikonomidis I, Lekakis J, Revela I, Andreotti F, Nihoyannopoulos P. Increased circulating C-reactive protein and macrophage-colony stimulating factor are complementary predictors of long-term outcome in patients with chronic coronary artery disease. European heart journal. 2005;26(16):1618-24. Epub 2005/04/01.

33. Saitoh T, Kishida H, Tsukada Y, Fukuma Y, Sano J, Yasutake M, et al. Clinical significance of increased plasma concentration of macrophage colony-

stimulating factor in patients with angina pectoris. Journal of the American College of Cardiology. 2000;35(3):655-65. Epub 2000/03/15.

34. Abbate A, Kontos MC, Grizzard JD, Biondi-Zoccai GG, Van Tassell BW, Robati R, et al. Interleukin-1 blockade with anakinra to prevent adverse cardiac remodeling after acute myocardial infarction (Virginia Commonwealth University Anakinra Remodeling Trial [VCU-ART] Pilot study). The American journal of cardiology. 2010;105(10):1371-7 e1. Epub 2010/05/11.

35. Abbate A, Van Tassell BW, Biondi-Zoccai G, Kontos MC, Grizzard JD, Spillman DW, et al. Effects of interleukin-1 blockade with anakinra on adverse cardiac remodeling and heart failure after acute myocardial infarction [from the Virginia Commonwealth University-Anakinra Remodeling Trial (2) (VCU-ART2) pilot study]. The American journal of cardiology. 2013;111(10):1394-400. Epub 2013/03/05.

36. Vaughan DE. PAI-1 and atherothrombosis. Journal of thrombosis and haemostasis : JTH. 2005;3(8):1879-83. Epub 2005/08/17.

37. Sprengers ED, Kluft C. Plasminogen activator inhibitors. Blood. 1987;69(2):381-7. Epub 1987/02/01.

38. Sawdey M, Podor TJ, Loskutoff DJ. Regulation of type 1 plasminogen activator inhibitor gene expression in cultured bovine aortic endothelial cells. Induction by transforming growth factor-beta, lipopolysaccharide, and tumor necrosis factor-alpha. The Journal of biological chemistry. 1989;264(18):10396-401. Epub 1989/06/25.

39. Esmon CT. The interactions between inflammation and coagulation. British journal of haematology. 2005;131(4):417-30. Epub 2005/11/12.

40. Fibrinogen Studies C, Danesh J, Lewington S, Thompson SG, Lowe GD, Collins R, et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. JAMA : the journal of the American Medical Association. 2005;294(14):1799-809. Epub 2005/10/13.

41. Yarnell J, McCrum E, Rumley A, Patterson C, Salomaa V, Lowe G, et al. Association of European population levels of thrombotic and inflammatory factors with risk of coronary heart disease: the MONICA Optional Haemostasis Study. European heart journal. 2005;26(4):332-42; discussion 17-8. Epub 2004/12/25.

42. Pahor M, Elam MB, Garrison RJ, Kritchevsky SB, Applegate WB. Emerging noninvasive biochemical measures to predict cardiovascular risk. Archives of internal medicine. 1999;159(3):237-45. Epub 1999/02/16.

43. Hamsten A, de Faire U, Walldius G, Dahlen G, Szamosi A, Landou C, et al. Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. Lancet. 1987;2(8549):3-9. Epub 1987/07/04.

44. Dimitroulas T, Douglas KM, Panoulas VF, Toms T, Smith JP, Treharne GJ, et al. Derangement of hemostasis in rheumatoid arthritis: association with demographic, inflammatory and metabolic factors. Clinical rheumatology. 2013;32(9):1357-64. Epub 2013/05/16.

45. Wallberg-Jonsson S, Cvetkovic JT, Sundqvist KG, Lefvert AK, Rantapaa-Dahlqvist S. Activation of the immune system and inflammatory activity in relation

to markers of atherothrombotic disease and atherosclerosis in rheumatoid arthritis. The Journal of rheumatology. 2002;29(5):875-82. Epub 2002/05/23.

46. Kamper EF, Kopeikina LT, Trontzas P, Potamianou A, Tsiroglou E, Stavridis JC. The effect of disease activity related cytokines on the fibrinolytic potential and cICAM-1 expression in rheumatoid arthritis. The Journal of rheumatology. 2000;27(11):2545-50. Epub 2000/11/28.

47. Abdelhafiz AH, Ahmed S, El Nahas M. Microalbuminuria: marker or maker of cardiovascular disease. Nephron Experimental nephrology. 2011;119 Suppl 1:e6-10. Epub 2011/08/17.

48. Jassal SK, Langenberg C, von Muhlen D, Bergstrom J, Barrett-Connor E. Usefulness of microalbuminuria versus the metabolic syndrome as a predictor of cardiovascular disease in women and men>40 years of age (from the Rancho Bernardo Study). The American journal of cardiology. 2008;101(9):1275-80. Epub 2008/04/26.

49. Ajeganova S, Andersson ML, Frostegard J, Hafstrom I. Disease Factors in Early Rheumatoid Arthritis Are Associated with Differential Risks for Cardiovascular Events and Mortality Depending on Age at Onset: A 10-year Observational Cohort Study. The Journal of rheumatology. 2013;40(12):1958-66. Epub 2013/08/21.

50. Edwards NL. The role of hyperuricemia in vascular disorders. Current opinion in rheumatology. 2009;21(2):132-7. Epub 2009/04/03.

51. Cicero AF, Salvi P, D'Addato S, Rosticci M, Borghi C, Brisighella Heart Study g. Association between serum uric acid, hypertension, vascular stiffness and

subclinical atherosclerosis: data from the Brisighella Heart Study. Journal of hypertension. 2014;32(1):57-64. Epub 2013/12/07.

52. Takahashi S, Yamamoto T, Tsutsumi Z, Moriwaki Y, Yamakita J, Higashino K. Close correlation between visceral fat accumulation and uric acid metabolism in healthy men. Metabolism: clinical and experimental. 1997;46(10):1162-5. Epub 1997/10/10.

53. Nagahama K, Inoue T, Kohagura K, Ishihara A, Kinjo K, Ohya Y. Hyperuricemia predicts future metabolic syndrome: a 4-year follow-up study of a large screened cohort in Okinawa, Japan. Hypertension research : official journal of the Japanese Society of Hypertension. 2013. Epub 2013/11/01.

54. van Holten TC, Waanders LF, de Groot PG, Vissers J, Hoefer IE, Pasterkamp G, et al. Circulating biomarkers for predicting cardiovascular disease risk; a systematic review and comprehensive overview of meta-analyses. PloS one. 2013;8(4):e62080. Epub 2013/05/01.

55. Murdaca G, Colombo BM, Cagnati P, Gulli R, Spano F, Puppo F. Endothelial dysfunction in rheumatic autoimmune diseases. Atherosclerosis. 2012;224(2):309-17. Epub 2012/06/08.

56. Imhof BA, Aurrand-Lions M. Adhesion mechanisms regulating the migration of monocytes. Nature reviews Immunology. 2004;4(6):432-44. Epub 2004/06/03.

57. Dessein PH, Joffe BI, Singh S. Biomarkers of endothelial dysfunction, cardiovascular risk factors and atherosclerosis in rheumatoid arthritis. Arthritis research & therapy. 2005;7(3):R634-43. Epub 2005/05/19.

58. Ponthieux A, Herbeth B, Droesch S, Haddy N, Lambert D, Visvikis S. Biological determinants of serum ICAM-1, E-selectin, P-selectin and L-selectin levels in healthy subjects: the Stanislas study. Atherosclerosis. 2004;172(2):299-308. Epub 2004/03/17.

59. O'Malley T, Ludlam CA, Riemermsa RA, Fox KA. Early increase in levels of soluble inter-cellular adhesion molecule-1 (sICAM-1); potential risk factor for the acute coronary syndromes. European heart journal. 2001;22(14):1226-34. Epub 2001/07/07.

60. Solomon DH, Curhan GC, Rimm EB, Cannuscio CC, Karlson EW. Cardiovascular risk factors in women with and without rheumatoid arthritis. Arthritis and rheumatism. 2004;50(11):3444-9. Epub 2004/11/06.

61. Gonzalez-Gay MA, Garcia-Unzueta MT, De Matias JM, Gonzalez-Juanatey C, Garcia-Porrua C, Sanchez-Andrade A, et al. Influence of anti-TNF-alpha infliximab therapy on adhesion molecules associated with atherogenesis in patients with rheumatoid arthritis. Clinical and experimental rheumatology. 2006;24(4):373-9. Epub 2006/09/08.

62. Foster W, Carruthers D, Lip GY, Blann AD. Inflammatory cytokines, endothelial markers and adhesion molecules in rheumatoid arthritis: effect of intensive anti-inflammatory treatment. Journal of thrombosis and thrombolysis. 2010;29(4):437-42. Epub 2009/07/07.

63. Ates A, Kinikli G, Turgay M, Duman M. Serum-soluble selectin levels in patients with rheumatoid arthritis and systemic sclerosis. Scandinavian journal of immunology. 2004;59(3):315-20. Epub 2004/03/20.

64. Sfikakis PP, Charalambopoulos D, Vaiopoulos G, Mavrikakis M. Circulating P- and L-selectin and T-lymphocyte activation and patients with autoimmune rheumatic diseases. Clinical rheumatology. 1999;18(1):28-32. Epub 1999/03/24.

65. Littler AJ, Buckley CD, Wordsworth P, Collins I, Martinson J, Simmons DL. A distinct profile of six soluble adhesion molecules (ICAM-1, ICAM-3, VCAM-1, E-selectin, L-selectin and P-selectin) in rheumatoid arthritis. British journal of rheumatology. 1997;36(2):164-9. Epub 1997/02/01.

66. Hogg N, Berlin C. Structure and function of adhesion receptors in leukocyte trafficking. Immunology today. 1995;16(7):327-30. Epub 1995/07/01.

67. Bond A, Hay FC. L-selectin expression on the surface of peripheral blood leucocytes from rheumatoid arthritis patients is linked to disease activity. Scandinavian journal of immunology. 1997;46(3):312-6. Epub 1997/10/07.

68. Mojcik CF, Shevach EM. Adhesion molecules: a rheumatologic perspective. Arthritis and rheumatism. 1997;40(6):991-1004. Epub 1997/06/01.

69. Dessein PH, Joffe BI. Suppression of circulating interleukin-6 concentrations is associated with decreased endothelial activation in rheumatoid arthritis. Clinical and experimental rheumatology. 2006;24(2):161-7. Epub 2006/06/10.

70. Giles JT, Post W, Blumenthal RS, Bathon JM. Therapy Insight: managing cardiovascular risk in patients with rheumatoid arthritis. Nature clinical practice Rheumatology. 2006;2(6):320-9. Epub 2006/08/26.

71. Hjeltnes G, Hollan I, Forre O, Wiik A, Mikkelsen K, Agewall S. Anti-CCP and RF IgM: predictors of impaired endothelial function in rheumatoid arthritis

patients. Scandinavian journal of rheumatology. 2011;40(6):422-7. Epub 2011/12/14.

72. Szekanecz Z, Kerekes G, Der H, Sandor Z, Szabo Z, Vegvari A, et al. Accelerated atherosclerosis in rheumatoid arthritis. Annals of the New York Academy of Sciences. 2007;1108:349-58. Epub 2007/09/26.

73. Halvorsen B, Lund Sagen E, Ueland T, Aukrust P, Tonstad S. Effect of smoking cessation on markers of inflammation and endothelial cell activation among individuals with high risk for cardiovascular disease. Scandinavian journal of clinical and laboratory investigation. 2007;67(6):604-11. Epub 2007/09/14.

74. Sanada H, Midorikawa S, Yatabe J, Yatabe MS, Katoh T, Baba T, et al. Elevation of serum soluble E- and P-selectin in patients with hypertension is reversed by benidipine, a long-acting calcium channel blocker. Hypertension research : official journal of the Japanese Society of Hypertension. 2005;28(11):871-8. Epub 2006/03/25.

75. Rainer TH. L-selectin in health and disease. Resuscitation. 2002;52(2):127-41. Epub 2002/02/14.

76. Sodergren A, Karp K, Boman K, Eriksson C, Lundstrom E, Smedby T, et al. Atherosclerosis in early rheumatoid arthritis: very early endothelial activation and rapid progression of intima media thickness. Arthritis research & therapy. 2010;12(4):R158. Epub 2010/08/18.

77. Masiha S, Sundstrom J, Lind L. Inflammatory markers are associated with left ventricular hypertrophy and diastolic dysfunction in a population-based sample

of elderly men and women. Journal of human hypertension. 2013;27(1):13-7. Epub 2012/01/13.

78. Lin J, Hu FB, Rimm EB, Rifai N, Curhan GC. The association of serum lipids and inflammatory biomarkers with renal function in men with type II diabetes mellitus. Kidney international. 2006;69(2):336-42. Epub 2006/01/13.

79. Pulsatelli L, Assirelli E, Dolzani P, Addimanda O, Mancarella L, Pavloska B, et al. Increased serum vascular cell adhesion molecule (VCAM)-1 levels in patients with erosive hand osteoarthritis. Rheumatology. 2013;52(2):400-2. Epub 2012/11/23.

80. Schett G, Kiechl S, Bonora E, Zwerina J, Mayr A, Axmann R, et al. Vascular cell adhesion molecule 1 as a predictor of severe osteoarthritis of the hip and knee joints. Arthritis and rheumatism. 2009;60(8):2381-9. Epub 2009/08/01.

81. Parissis JT, Nikolaou M, Farmakis D, Paraskevaidis IA, Bistola V, Venetsanou K, et al. Self-assessment of health status is associated with inflammatory activation and predicts long-term outcomes in chronic heart failure. European journal of heart failure. 2009;11(2):163-9. Epub 2009/01/27.

82. Lee AS, Ellman MB, Yan D, Kroin JS, Cole BJ, van Wijnen AJ, et al. A current review of molecular mechanisms regarding osteoarthritis and pain. Gene. 2013;527(2):440-7. Epub 2013/07/09.

83. Martinovic I, Abegunewardene N, Seul M, Vosseler M, Horstick G, Buerke M, et al. Elevated monocyte chemoattractant protein-1 serum levels in patients at risk for coronary artery disease. Circulation journal : official journal of the Japanese Circulation Society. 2005;69(12):1484-9. Epub 2005/11/26.

84. Barnabe C, Martin BJ, Ghali WA. Systematic review and meta-analysis: anti-tumor necrosis factor alpha therapy and cardiovascular events in rheumatoid arthritis. Arthritis care & research. 2011;63(4):522-9. Epub 2010/10/20.

85. Naredo E, Moller I, Corrales A, Bong DA, Cobo-Ibanez T, Corominas H, et al. Automated radiofrequency-based US measurement of common carotid intima-media thickness in RA patients treated with synthetic vs synthetic and biologic DMARDs. Rheumatology. 2013;52(2):376-81. Epub 2012/10/16.

86. del Val del Amo N, Ibanez Bosch R, Fito Manteca C, Gutierrez Polo R, Loza Cortina E. Anti-cyclic citrullinated peptide antibody in rheumatoid arthritis: relation with disease aggressiveness. Clinical and experimental rheumatology. 2006;24(3):281-6. Epub 2006/07/28.

87. Marasovic-Krstulovic D, Martinovic-Kaliterna D, Fabijanic D, Morovic-Vergles J. Are the anti-cyclic citrullinated peptide antibodies independent predictors of myocardial involvement in patients with active rheumatoid arthritis? Rheumatology. 2011;50(8):1505-12. Epub 2011/04/13.

88. El-Gabalawy HS, Robinson DB, Smolik I, Hart D, Elias B, Wong K, et al. Familial clustering of the serum cytokine profile in the relatives of rheumatoid arthritis patients. Arthritis and rheumatism. 2012;64(6):1720-9. Epub 2012/02/23.

89. Yano Y, Hoshide S, Shimada K, Kario K. The impact of cigarette smoking on 24-hour blood pressure, inflammatory and hemostatic activity, and cardiovascular risk in Japanese hypertensive patients. Journal of clinical hypertension. 2013;15(4):234-40. Epub 2013/04/05.

90. Sugiyama D, Nishimura K, Tamaki K, Tsuji G, Nakazawa T, Morinobu A, et al. Impact of smoking as a risk factor for developing rheumatoid arthritis: a meta-analysis of observational studies. Annals of the rheumatic diseases. 2010;69(1):70-81. Epub 2009/01/29.

91. Morales-Ducret J, Wayner E, Elices MJ, Alvaro-Gracia JM, Zvaifler NJ, Firestein GS. Alpha 4/beta 1 integrin (VLA-4) ligands in arthritis. Vascular cell adhesion molecule-1 expression in synovium and on fibroblast-like synoviocytes. Journal of immunology. 1992;149(4):1424-31. Epub 1992/08/15.

92. Scanzello CR, Goldring SR. The role of synovitis in osteoarthritis pathogenesis. Bone. 2012;51(2):249-57. Epub 2012/03/06.

93. Collier P, Watson CJ, Voon V, Phelan D, Jan A, Mak G, et al. Can emerging biomarkers of myocardial remodelling identify asymptomatic hypertensive patients at risk for diastolic dysfunction and diastolic heart failure? European journal of heart failure. 2011;13(10):1087-95. Epub 2011/07/02.

94. Collaboration IRGCERF, Sarwar N, Butterworth AS, Freitag DF, Gregson J, Willeit P, et al. Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. Lancet. 2012;379(9822):1205-13. Epub 2012/03/17.

95. Eidelman RS, Hennekens CH. Fibrinogen: a predictor of stroke and marker of atherosclerosis. European heart journal. 2003;24(6):499-500. Epub 2003/03/20.

96. Wilhelmsen L, Svardsudd K, Korsan-Bengtsen K, Larsson B, Welin L, Tibblin G. Fibrinogen as a risk factor for stroke and myocardial infarction. The New England journal of medicine. 1984;311(8):501-5. Epub 1984/08/23.

97. Zoller B, Li X, Sundquist J, Sundquist K. Risk of subsequent ischemic and hemorrhagic stroke in patients hospitalized for immune-mediated diseases: a nationwide follow-up study from Sweden. BMC neurology. 2012;12:41. Epub 2012/06/20.

98. Torres-Carrillo N, Torres-Carrillo NM, Martinez-Bonilla GE, Vazquez-Del Mercado M, Palafox-Sanchez CA, Oregon-Romero E, et al. Plasminogen activator inhibitor-1 C/G polymorphism in relation to plasma levels in rheumatoid arthritis. Clinical and experimental medicine. 2009;9(3):223-8. Epub 2009/02/25.

99. Sandoo A, van Zanten JJ, Toms TE, Carroll D, Kitas GD. Anti-TNFalpha therapy transiently improves high density lipoprotein cholesterol levels and microvascular endothelial function in patients with rheumatoid arthritis: a pilot study. BMC musculoskeletal disorders. 2012;13:127. Epub 2012/07/25.

100. Agirbasli M, Inanc N, Baykan OA, Direskeneli H. The effects of TNF alpha inhibition on plasma fibrinolytic balance in patients with chronic inflammatory rheumatical disorders. Clinical and experimental rheumatology. 2006;24(5):580-3. Epub 2006/12/22.

101. Lau CS, McLaren M, Hanslip J, Kerr M, Belch JJ. Abnormal plasma fibrinolysis in patients with rheumatoid arthritis and impaired endothelial fibrinolytic response in those complicated by vasculitis. Annals of the rheumatic diseases. 1993;52(9):643-9. Epub 1993/09/01.

102. Wallberg-Jonsson S, Dahlen GH, Nilsson TK, Ranby M, Rantapaa-Dahlqvist S. Tissue plasminogen activator, plasminogen activator inhibitor-1 and von

Willebrand factor in rheumatoid arthritis. Clinical rheumatology. 1993;12(3):318-24. Epub 1993/09/01.

103. Wallberg-Jonsson S, Ohman M, Rantapaa-Dahlqvist S. Which factors are related to the presence of atherosclerosis in rheumatoid arthritis? Scandinavian journal of rheumatology. 2004;33(6):373-9. Epub 2005/03/30.

104. Kostapanos M, Florentin M, Elisaf MS, Mikhailidis DP. Hemostatic Factors and the Metabolic Syndrome. Current vascular pharmacology. 2013. Epub 2013/10/31.

105. Zhuo Q, Yang W, Chen J, Wang Y. Metabolic syndrome meets osteoarthritis. Nature reviews Rheumatology. 2012;8(12):729-37. Epub 2012/08/22.

106. Singh G, Miller JD, Lee FH, Pettitt D, Russell MW. Prevalence of cardiovascular disease risk factors among US adults with self-reported osteoarthritis: data from the Third National Health and Nutrition Examination Survey. The American journal of managed care. 2002;8(15 Suppl):S383-91. Epub 2002/11/06.

107. Ozaki K, Hori T, Ishibashi T, Nishio M, Aizawa Y. Effects of chronic cigarette smoking on endothelial function in young men. Journal of cardiology. 2010;56(3):307-13. Epub 2010/10/15.

108. Mykkanen L, Zaccaro DJ, Wagenknecht LE, Robbins DC, Gabriel M, Haffner SM. Microalbuminuria is associated with insulin resistance in nondiabetic subjects: the insulin resistance atherosclerosis study. Diabetes. 1998;47(5):793-800. Epub 1998/05/20.

109. Mpofu S, Kaushik VV, Grundy G, Moots RJ. Microalbuminuria: is it a predictor of ischaemic heart disease in rheumatoid arthritis? Rheumatology. 2004;43(4):537-8; author reply 8. Epub 2004/03/17.

110. Jensen JS, Borch-Johnsen K, Jensen G, Feldt-Rasmussen B. Microalbuminuria reflects a generalized transvascular albumin leakiness in clinically healthy subjects. Clinical science. 1995;88(6):629-33. Epub 1995/06/01.

111. Pedersen LM, Nordin H, Svensson B, Bliddal H. Microalbuminuria in patients with rheumatoid arthritis. Annals of the rheumatic diseases. 1995;54(3):189-92. Epub 1995/03/01.

112. Gerstein HC, Mann JF, Yi Q, Zinman B, Dinneen SF, Hoogwerf B, et al. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. JAMA : the journal of the American Medical Association. 2001;286(4):421-6. Epub 2001/07/31.

113. Katz DH, Selvaraj S, Aguilar FG, Martinez EE, Beussink L, Kim KY, et al. Association of Low-Grade Albuminuria with Adverse Cardiac Mechanics: Findings from the HyperGEN Study. Circulation. 2013. Epub 2013/10/01.

Chapter 5

Macrovascular Endothelial Function in RA

Background:

Endothelial cells are a single lining layer of all blood vessels and traditionally were considered to primarily provide a barrier to diffusion of molecules from the vessel lumen into the interstitial space (1). It is now known that the endothelial lining is much less inert than previously thought. It is involved in platelet activation, leukocyte adhesion, modulation of thrombosis and regulation of vasomotor tone by nitric oxide, endothelin, prostacyclin and angiotensinogen (2). Endothelial dysfunction is accepted as one of the earliest stages of atherosclerosis and has been identified even in early childhood (3). It can predict cardiovascular events even in patients with normal appearing coronary arteries on angiography (4). The Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension define endothelial dysfunction as an imbalance between vasodilating and vasocontricting substances produced by or acting on endothelial cells (5). Reactive oxygen species are felt to contribute considerably to this phenomenon.

The gold standard for diagnosis of endothelial dysfunction is via intracoronary infusion of acetylcholine. This is a highly invasive and costly procedure. Therefore, a number of less invasive methods of assessing endothelial function have been developed. These include macrovascular assessment via flow-mediated dilatation of

the brachial artery, forearm plethysmography, finger-pulse plethysmography and pulse curve analysis and microvascular assessment via laser Doppler skin flowmetry.

Flow-mediated dilatation is the dilatation of a blood vessel in response to "shear stress". The precise mechanism for a blood vessels ability to detect shear forces and modulate vasomotor tone in response are not fully understood.

Non-invasive ultrasound FMD of the brachial artery is the most widely used method in research studies (6). The brachial artery is measured before and after an increase in shear stress that is induced by reactive hyperaemia.

FMD is usually reported as the change in post-ischaemic diameter as a percentage of the baseline diameter (7). It is important to be aware that the baseline brachial artery diameter influences the percentage FMD in 2 ways. First, a larger baseline diameter results in a smaller percentage change, because the absolute change in diameter will be less, and secondly, smaller arteries appear to dilate more than larger arteries do. Therefore it is important to report the absolute change in diameter from baseline to post-ischaemic diameter in addition to the percentage change. There have been a number of recommendations from 3 different working groups with regard to what FMD variables should be reported as markers of endothelial function. The International Brachial Artery Reactivity Task Force published guidelines for reporting of FMD and recommended that the artery diameter 60 seconds post-cuff deflation be used to calculate FMD (8). The American Society of Echocardiography Noninvasive Vascular Ultrasound Report recommended also measuring the arterial diameter 60 seconds post–cuff deflation to calculate FMD (9). However, the European Society of Hypertension Working Group on Endothelin and Endothelial

Factors advised using the maximum diameter post-cuff deflation rather than FMD at a particular time post-deflation. They reported that using only the diameter at 60seconds to calculate FMD will miss significant dilatation in up to 70% of cases (5)

There is no consensus on what the normal range for flow mediated dilatation is, however an FMD of less than 5% is considered as impaired endothelial function (10). There is a wide variation in mean FMD values across populations and between gender and race. Females have a higher brachial FMD% than males and Caucasians have higher values than blacks (11). Bots et al reported that some of the variability in absolute values is due to the technical aspects of FMD measurement, including length of time of cuff inflation and cuff position (12).

The effect of endothelium-independent vasodilatation is assessed by administration of sublingual glyceryl trinitrate (GTN). This is typically performed after FMD. Nitroglycerin mediated dilatation (NMD) is a measure of smooth muscle function and not a marker of endothelial function (8). The degree of brachial artery dilatation and its duration are typically greater post GTN administration compared to what occurs with FMD. The maximum obtainable vasodilator response is seen after GTN is administered. It is typically quoted as 15-20%, however there is no normal reference range (13). If NMD is impaired, it indicates the probability of smooth muscle cell dysfunction of the arterial wall owing to structural changes in vascular tissues (14). It has been postulated that reduced vasodilator response may be due to changes in baseline vasomotor tone in the presence of atherosclerosis (15).

Flow mediated dilatation has demonstrated endothelial dysfunction early in the course of rheumatoid arthritis and before any symptoms of cardiovascular disease are present (16). FMD has also been found to be associated with markers of

inflammation in RA patients (17-19). A number of trials have shown a benefit of statin therapy on endothelial function in RA patients (20, 21). There is also evidence that anti-rheumatic medications, used to control RA disease activity, have a beneficial effect on FMD but it is still a matter of debate whether these effects are transient or prolonged (22, 23). In 2012, Sandoo et al found that cardiovascular risks in RA patients more so than RA disease activity markers were associated with endothelial dysfunction (24). Endothelial dysfunction in RA, as assessed by FMD has not been compared to controls in an Irish population and there is no current data on the effects of RA disease activity and treatment and Irish RA patients.

Aims:

Our primary aim was to compare flow mediated dilatation and nitroglycerin mediated dilatation as markers of macrovascular endothelial dysfunction in RA and OA patients without clinical evidence of cardiovascular disease. Secondary aims were to investigate if markers of inflammation, anti-rheumatic medications and cardiovascular risks were associated with macrovascular endothelial dysfunction in either group.

Methods:

Brachial artery ultrasound to assess flow mediated and nitroglycerin mediated dilatation are discussed in detail in chapter 2.

Briefly, the brachial artery was imaged above the antecubital fossa in the longitudinal plane and a blood pressure cuff was placed below the antecubital fossa on the mid-forearm. A baseline, resting 2D ultrasound image is then acquired for 2-5 minutes. Following this, the blood pressure cuff is inflated to 50mm Hg above the recorded stable systolic blood pressure for complete occlusion of arterial forearm inflow for 5 minutes. This forearm ischaemia produces dilation of downstream resistance vessels via auto-regulatory mechanisms. On release of the cuff, a brief hyper-perfusion state through the brachial artery is induced to accommodate the dilated resistance vessels. The resulting increase in shear stress in the brachial artery

causes the brachial artery to dilate and the longitudinal image of the artery is recorded continuously for the next 2 minutes.

The FMD, defined as the percentage increase in mean diameter over a 10 second interval, 60 seconds after tourniquet deflation, is automatically averaged and calculated by the software from diastolic vessel diameters, using the following formula:

$$\frac{\text{Maximum diameter} - \text{baseline diameter}}{\text{baseline diameter}} \times 100\%$$

Endothelium-Independent vasodilatation with nitroglycerin was then assessed after a rest period of 10 minutes to allow return of brachial artery diameter to resting, steady state perfusion. An exogenous NO donor is then administered in the form of a single dose of sublingual nitroglycerin spray (0.4 mg). This is given to determine the maximum obtainable vasodilator response, and to serve as a measure of endothelium-*independent* vasodilation which indicates vascular smooth muscle function. Peak vasodilation occurs 3-4 minutes after nitroglycerin administration and the brachial artery image was continuously captured during this time period.

Statistical Methods:

Means and standard deviations were calculated for normally distributed data and compared using a student t-test. Medians and interquartile ranges were calculated for non-normally distributed data and compared using a Mann Whitney U test. Bivariate correlations were calculated for continuous variables. Logistic regression analysis was performed to analyse the relationship between the presence of endothelial dysfunction, as defined by an FMD of less than 5% and a diagnosis of Rheumatoid Arthritis. Odds ratios and 95% confidence intervals were computed. A p-value of <0.05 was considered significant. SPSS 20 was used for all statistical analysis.

Results:

Comparison of FMD and NMD in RA and OA patients:

Forty-one patients with OA and 51 with RA attended for the investigation. The mean brachial artery diameter at baseline for the total group was 3.85mm (SD+/- 0.82). In the RA group, mean baseline diameter was 3.91mm (SD+/- 0.84), compared with 3.78mm (SD+/- 0.79) in the OA group, p = 0.442. Mean values for FMD % and absolute FMD were similar in RA and OA patients. Peak FMD values were also similar in the 2 groups. Nitroglycerin mediated dilatation was also assessed and mean values were similar between the RA and OA groups (table 5.1). Age was not found to correlate with FMD or NMD in either the RA or OA group.

For the purpose of logistic regression analysis, an FMD value of less than 5% at 60 seconds post cuff deflation was taken as a marker of abnormal endothelial function. We also examined peak FMD post cuff deflation as a second marker of endothelial function. Using logistic regression analysis, neither FMD at 60 seconds nor peak FMD were found to be associated with a diagnosis of RA more so than OA; OR (95%CI), 1.69 (0.677, 4.223), p = 0.261 and OR (95%CI), 1.60 (0.538, 4.787), p = 0.397, respectively. Using logistic regression analysis, nitroglycerin mediated dilatation was not found to be associated with a diagnosis of RA more so than a diagnosis of OA; OR (95%), 0.826 (0.329, 2.076), p=0.685. Nitroglycerin mediated dilatation was found to be associated with baseline brachial artery diameter in both

RA and OA patients; OR (95%CI), 2.46 (1.028, 5.867), p =0.043 and 5.65 (1.578, 20.194), p =0.008.

	RA Mean (SD)	OA Mean (SD)	p
Diameter at 60 seconds (mm)	4.20 (0.89)	4.08 (0.83)	0.534
FMD % at 60 seconds	7.33 (3.28)	8.14 (3.49)	0.262
Absolute FMD at 60 seconds(mm)	0.28 (0.14)	0.30 (0.12)	0.481
Peak FMD %	8.47 (3.59)	8.94 (3.55)	0.531
Absolute peak FMD (mm)	0.33 (0.15)	0.33 (0.12)	0.930
NMD% at 4 minutes	15.60 (6.66)	15.72 (7.35)	0.941
Absolute NMD at 4 minutes (mm)	0.59 (0.23)	0.56 (0.22)	0.605
Peak NMD %	18.12 (7.54)	16.37 (7.19)	0.315
Absolute peak NMD (mm)	0.68 (0.26)	0.59 (0.22)	0.100

Table 5.1: Comparison of FMD and NMD in RA and OA groups. RA, rheumatoid arthritis; OA, osteoarthritis; SD, standard deviation; mm, millimetres; FMD, flow mediated dilatation; NMD, nitroglycerin mediated dilatation.

Ten OA patients (24%) had an abnormal FMD compared with 18 RA patients (35.3%). Using a chi2 test to compare the groups, no difference was found, p=0.259. Twenty-one (51%) of the OA group had a NMD of less than 15% compared with 19 (37%) of the RA group, p=0.684.

When we examined the RA group with an abnormal FMD more closely, we found 2/3 of them were overweight or obese and 2/3 had moderate or high disease activity, as defined by a DAS28ESR and DAS28CRP of greater than 3.2 (table 5.2).

	RA patients with abnormal FMD N (%)
Males	6/18 (33.3%)
Smokers	7/18 (39%)
Overweight	12/18 (67%)
DAS28ESR > 3.2	12/18 (67%)
High CRP	6/18 (33.3%)
High ESR	9/18 (50%)
On synthetic DMARD	13/18 (72%)
On Biologic DMARD	12/18 (67%)
On NSAID	10/18 (56%)
On glucocorticoid	9/18 (50%)
On statin	5/18 (28%)

Table 5.2: Summary of baseline parameters for RA patients with an abnormal FMD

Comparison of FMD and NMD in Males and Females:

Using an independent t-test, we found that males had a significantly larger baseline diameter then females, 4.72mm (SD+/- 0.53) versus 3.48mm (SD+/- 0.60), $p < 0.001$. There was no significant difference between males with OA and RA, $p = 0.545$. The same was true when females with RA and OA were compared, $p = 0.807$. When males and females were compared, males with OA had a significantly lower FMD and NMD than females with OA. However, the opposite was found in the RA group with respect to FMD, both at 60 seconds and peak FMD (table 5.3).

	RA males (SD)	RA females (SD)	p	OA males (SD)	OA females (SD)	p
FMD% at 60 seconds	8.02 (3.72)	6.93 (2.98)	0.284	6.38 (2.47)	8.63 (3.61)	0.044*
Absolute FMD at 60 seconds (mm)	0.37 (0.16)	0.23 (0.09)	0.002*	0.31 (0.12)	0.30 (0.12)	0.931
Peak FMD %	8.61 (4.11)	8.38 (3.33)	0.839	6.54 (2.41`)	9.61 (3.55)	0.007*
Absolute peak FMD (mm)	0.40 (0.18)	0.28 (0.10)	0.019*	0.31 (0.13)	0.33 (0.12)	0.706
NMD% at 4 minutes	14.27 (6.74)	16.50 (6.60)	0.295	11.11 (4.65)	17.01 (7.51)	0.022*
Absolute NMD at 4 minutes (mm)	0.64 (0.26)	0.55 (0.21)	0.257	0.52 (0.21)	0.57 (0.23)	0.624
Peak NMD%	16.86 (8.67)	18.93 (6.78)	0.425	12.06 (4.47)	17.58 (7.41)	0.026*
Absolute peak NMD (mm)	0.76 (0.31)	0.63 (0.21)	0.149	0.57 (0.21)	0.59 (0.23)	0.840

Table 5.3: Comparison of FMD and NMD percentage and absolute values between males and females in both the OA and RA groups. RA, rheumatoid arthritis; OA, osteoarthritis, SD, standard deviation; mm, millimetres; FMD, flow mediated dilatation; NMD, nitroglycerin mediated dilatation; p significance level.

RA Disease Activity and Macrovascular Endothelial Function:

We examined the effect of treatment and disease activity in the RA group on FMD. Within the RA group, mean baseline brachial artery diameter was similar regardless of whether patients were, or were not, currently on NSAIDS, steroids, DMARDS or biologic agents. RA patients on DMARDS, biologic agents, NSAIDs or steroids, had a similar degree of FMD to RA patients not on the above treatments. However, RA patients on a biologic agent were found to have a larger NMD response than those not on a biologic agent at the time of scanning, p =0.037*.

The diameter of the brachial artery after cuff deflation was significantly correlated with ESR in the RA group, r = -0.324, p=0.021. Peak diameter post ischaemia was also associated with ESR, r=-0.281, p=0.046. Actual FMD was significantly associated with the number of swollen joints in RA patients, r = -0.339, p = 0.015. Actual peak FMD was associated with number of swollen joints in the RA group, r = - 0.280, p = 0.047. Anti-CCP antibody and rheumatoid factor status did not have an effect on FMD.

Assessment of Relationship Between Macrovascular Endothelial Function and Markers of Cardiovascular Disease in RA:

When we explored the cardiovascular risk factors and subclinical markers of atherosclerosis in the RA group, we found a number of correlations with FMD.

RA patients with a family history of stroke had a significantly worse FMD than those with no family history of stroke. FMD% was 5.37 (SD 1.79) in the patients with a first degree relative with a history of stroke, compared with 7.70 (SD 3.38) in those without a family history, p=0.01. Absolute values for FMD were also lower in the RA patients with family history of stroke, 0.20 mm (SD 0.07) versus 0.30 mm (SD 0.14), p = 0.011.

Within the RA group current smoking status did not have an effect on FMD. There was no difference in baseline artery diameter when smokers and non-smokers were compared, p = 0.385.

NMD % at 4 minutes correlated with BMI, waist circumference, waist-hip ratio, r = -0.382, p=0.013, r = -0.411, p=0.007, r =-0.399, p=0.009. The actual NMD at 4 minutes correlated with BMI, r = - 0.320, p=0.039. The peak NMD% correlated significantly with weight, waist circumference, waist hip ratio, r= -0.356, p=0.022, r =-0.38, p=0.014, r = -0.375, p=0.016.

Statin use at the time of the study was not associated with a difference in baseline diameter in either the RA or OA groups, p = 0.802 and p = 0.394. RA patients on a statin had a significantly lower FMD, both at 60 seconds post cuff deflation and peak dilatation, compared with those RA patients not taking a statin at the time of

scanning (table 5.5). This difference with respect to use of statin therapy was not seen in the OA group. Within the RA group, the presence of endothelial dysfunction as determined by peak FMD, was significantly associated with current use of statin therapy, OR (95%CI), 10.28 (1.932, 54.662), p=0.006. This remained the case after simultaneous adjustment for current serum total cholesterol and LDL levels, OR (CI), 9.33 (1.459, 59.611), p=0.018. This association was not seen in OA patients on statins.

There was no significant difference in FMD between RA patients with an elevated serum cholesterol level at the time of scanning compared to those with a total cholesterol level in the normal reference range, p = 0.344. The diameter of the brachial artery after cuff deflation was significantly correlated with total cholesterol in the RA group, r = -0.287, p = 0.041. Peak diameter post ischaemia was also associated with total cholesterol, r = -0.288, p = 0.040. Total cholesterol levels correlated with the brachial artery diameter post GTN, r = - 0.32, p=0.039. The peak NMD% correlated significantly with fasting glucose, r = -0.446, p=0.011.

	Current statin	No current statin	p
FMD% at 60 seconds	5.25 (2.39)	7.72 (3.30)	0.027*
Absolute FMD at 60 seconds (mm)	0.19 (0.08)	0.29 (0.14)	0.008*
Peak FMD%	6.02 (3.47)	8.93 (3.47)	0.055*
Absolute peak FMD (mm)	0.22 (0.10)	0.34 (0.15)	0.015*

Table 5.5: FMD in RA patients on a statin compared with those not taking the medication. RA, rheumatoid arthritis; OA, osteoarthritis, SD, standard deviation; mm, millimetres; FMD, flow mediated dilatation; NMD, nitroglycerin mediated dilatation; p significance level.

RA patients with abnormal ABI and cIMT had similar FMD to those with ABI and cIMT in the normal ranges, p=0.137 and p=0.399, respectively. Baseline brachial artery diameter in the RA group correlated significantly with mean common carotid IMT, r = 0.307, p=0.040. NMD % at 4 minutes correlated with mean ABI, r =0.344, p=0.026.

Baseline brachial artery diameter in the RA group also had significant correlations with some of the echocardiographic markers of diastolic dysfunction, including left ventricular mass and posterior wall size (table 5.6). Absolute FMD values were worse in RA patients with an elevated BNP, 0.14mm (SD 0.04), compared with 0.29mm (SD 0.14), p=0.019. FMD percentage and absolute FMD at 60 seconds post deflation were significantly correlated with serum BNP level, r = - 0.295, p = 0.046 and r = -0.336, p=0.023.

.

	LA size (cm)	LVDD (cm)	PW thickness (cm)	LV mass (g)
Baseline brachial artery diameter (mm)	R=0.388	R=0.492	R= 0.350	R= 0.496
P value	0.005*	0.000*	0.012*	0.000*

Table 5.6: Correlation between baseline brachial artery diameter and markers of diastolic function, mm millimetres; LA left atrial; LVDD left ventricular diastolic diameter; PW posterior wall; LV left ventricular; cm centimetres; g grams R correlation coefficient, p significance level.

Assessment of FMD and NMD in OA patients:

Within the OA group, the number of years since diagnosis correlated significantly with size of brachial artery after cuff deflation, r = 0.346, p = 0.027. Peak diameter post ischaemia and diameter post sublingual GTN were both associated with numbers of years of diagnosis, r = -0.352, p=0.024 and r =-0.417, p=0.018.

OA patients who smoked had a significantly worse FMD% than OA non-smokers, 6.08% (SD 3.02) compared with 8.72% (SD 3.44), p=0.044. Patients with a prior diagnosis of hypertension or hypercholesterolaemia had similar baseline diameters to those without such diagnoses. NMD % and absolute NMD at 4 minutes were found to correlate with fasting glucose, r = -0.469, p=0.007 and r =-0.429, p=0.014.

Within the OA group, the brachial artery diameter after cuff deflation and peak diameter post ischaemia both correlated significantly with a number of inflammatory cytokines: IL4, r = -0.476, p = 0.002 and r = -0.352, p = 0.024; IL8, r =-0.349, p= 0.025 and r = -0.520, p=0.000; TNFα, r = -0.382, p = 0.014 and r = -0.376, p=0.015.

FMD% and peak FMD% in the OA group were both significantly correlated with serum IL10 levels, r =-0.400, p = 0.010, r = -0.376, p = 0.015. Absolute FMD was associated with serum IL4 levels, r = -0.319, p = 0.042. The brachial artery diameter post-GTN correlated with serum IL4 and IL8 levels, r = -0.47, p=0.007, r = -0.437, p=0.012.

We did not find any associations between inflammatory cytokine and FMD in our RA group.

Discussion:

Macrovascular function in RA and OA patients:

Our primary aim in this study was to compare RA patients and OA patients with respect to measures of flow mediated (FMD) and nitroglycerin mediated dilatation (NMD). We did not find a significant difference in percentage or absolute values for FMD or NMD between the 2 groups. However, we did discover that 35.3 % of the RA group and 24% of the OA group had evidence of endothelial dysfunction, as documented with abnormal FMD. Also, a significant proportion of both groups demonstrated lower NMD than normal.

A number of studies support our findings. In 2010, Foster et al found no difference in FMD measurements between RA patients and community controls (25). The same researchers studied FMD in patients with early inflammatory arthritis in 2011 and found that FMD was again similar to controls (26). Van Doornum et al also report similar results for macrovascular function as assessed by FMD in 25 RA patients and 25 controls. Both groups were free of cardiovascular risk factors (27). Södergren A et al studied 79 patients with early RA and found no significant difference in FMD compared to controls, but did find that FMD in the RA group was related to serum biomarkers of endothelial dysfunction to a greater extent than FMD in the control group (28).

Contrary to our results, a study of 20 RA patients in 2009 found significantly worse FMD and NMD measurements compared to controls. However, their control group

were made up of normal healthy individuals, as opposed to OA patients and their study numbers were significantly smaller than ours (16). Kerekes et al, reported a lower FMD but a similar NMD in RA patients compared to healthy controls (18).

Vaudo et al, compared RA and OA patients with respect to endothelial dysfunction and reported a significantly worse FMD in the RA group (17). The patients with RA in this study had a longer disease duration than our RA patients and had a significantly higher total cholesterol than the OA control group. There was no significant difference in traditional CV risks between our OA and RA group. Vaudo et al also excluded patients with a family history of CAD, whereas we did not. Also, as mentioned in chapter 3, our OA group had a significantly higher rate of MI compared with our RA group. These differences may explain why we did not find a better FMD in our OA group.

Comparison of FMA and NMD in males and females:

We compared FMD and NMD variables in males and females in our study because previous research has shown had females tend to have a higher FMD than males (11). In keeping with this, our male OA group had a significantly lower FMD than female OA patients, but interestingly, RA females had a worse FMD than males with RA.

Baseline brachial artery diameter tends to be larger in males in general and it has been postulated that this larger baseline diameter is the reason for a smaller percentage FMD that is often reported in males compared to females and some

researchers have suggested than FMD% can only be compared objectively between patients with the same brachial artery size (29). In our RA group, baseline brachial artery diameter was larger in males than in females, as expected. Therefore, one would expect that RA males would have a worse FMD than RA females, based on the theory above. We examined a number of other parameters that could have potentially been linked to a worse FMD in females; including age, disease duration, disease activity markers, smoking status and serum lipid levels (described in chapter 3). However, these were all similar in males and females. We also compared pre- and post- menopausal females with respect to brachial artery diameter and FMD, in case the higher levels of circulating oestrogens associated with a pre-menopausal state were driving the difference between the sexes, but no difference was found between pre- and post-menopausal females.

RA Disease Measures and Endothelial Function:

Flow mediated dilatation in RA patients on DMARDS (synthetic and biologic), NSAIDS and/ or glucocorticoids did not differ from RA patients not taking the above anti-rheumatic medications. However, nitroglycerin mediated dilatation was better in RA patients on a biologic DMARD compared to those not prescribed a biologic DMARD.

A number of studies have found that endothelial function improves with biologic DMARD use, in particular anti-TNF therapy (30, 31). Mazzaccoli et al reported a significant improvement in FMD immediately after first dose of etanercept and

infliximab, however they did not find a difference in FMD at 12 weeks when compared with pre- TNF inhibitor FMD measurements. In 2010, Tikiz et al reported a significant improvement in FMD after 12 weeks of Etanercept therapy. In contrast to our results, neither of the above studies found a better NMD in those treated with biologic DMARDS. The reasons for this may be differences in the study designs. Our cross-sectional study gives a snapshot of NMD in patients on a biologic agent at a particular point in time whereas the other 2 studies were prospective but had smaller numbers.

With respect to RA disease activity in our study, ESR correlated negatively with the post-ischaemic artery diameter and swollen joint count correlated negatively with absolute FMD. When we examined the RA patients with evidence of abnormal FMD, we found that 2/3's of them had either moderate or high disease activity.

We did not find an association between endothelial dysfunction and RA auto-antibody positivity.

In 2009, Hannawi et al, reported results similar to ours, when they found a significant correlation between markers of RA disease activity and both FMD and NMD (16). This suggests that the inflammatory burden of RA is linked to both dysfunction at the level of the endothelium and to vascular smooth muscle damage.

In contrast to our study, a cross-sectional study of 99 RA patients in 2012, did not find an association between markers of RA activity and macrovascular endothelium-dependent function. ESR measurements were similar in the 2 studies; however, mean disease duration was shorter in our study, 7 yrs, compared with 11 years in the above study. Research has previously shown that the inflammatory burden of RA is often highest earlier in the course of the disease (32) and perhaps this may explain why we found significant correlations between ESR and endothelial dysfunction.

Interestingly, the above group did find that global cardiovascular risk, as assessed by Framingham and Reynolds risk scores, correlated significantly with nitroglycerin-mediated dilatation. (24). They hypothesised from this study that CVD risk more so than RA disease related markers, may influence endothelial function.

From the above studies, we can conclude that treatment with a biologic agent improves endothelial function, although it may be short-lived. We have also seen that inflammatory markers in RA are associated with the presence of both endothelial and vascular smooth muscle dysfunction.

CVD risks and subclinical markers in RA patients and macrovascular endothelial function:

We examined the RA group separately for associations between macrovascular endothelial function and CV traditional risks and subclinical markers of atherosclerosis. With regard to family history, RA patients with a first degree relative with a history of CVA had a significantly worse FMD than those with no family history of CVA. Smoking status did not appear to have an effect on FMD.

Anthropometric measurements were associated with nitroglycerin mediated dilatation. In particular, BMI and waist-hip ratio were found to correlate negatively with peak NMD% and NMD% at 4 minutes post-GTN. Metsios et al recently reported the effects of an individualised exercise programme on endothelial function in RA (33). They have shown that nitroglycerin mediated dilatation significantly improved with a 6 month individualised resistance and aerobic training programme.

We can postulate from these results and the fact that exercise has a beneficial effect on anthropometric measurements in general, that improvements in BMI and waist-hip ratio could be associated with improvements in endothelial function in RA.

Using both bivariate correlation and logistic regression, we found that statin use at the time of brachial artery ultrasound was associated with a lower FMD in RA patients. However, FMD was similar in RA patients with an elevated serum total cholesterol and those with a total cholesterol in the normal range. Total cholesterol levels correlated with brachial artery diameter post-ischaemia.

Two studies have shown that treatment with statin therapy improves both disease activity and endothelial dysfunction in RA patients. El-Barbary et al found that atorvastatin in combination with methotrexate significantly improved FMD and disease activity in 30 RA patients after 6 months of treatment (21). Wilkinson IB et al reported a significant improvement in FMD and disease activity scores in RA patients treated with either simvastatin or ezitimibe for 6 weeks (33). The fact that we found a worse FMD in RA patients on statin therapy compared with those not on statin therapy, may be explained by the fact that those RA patients on a statin could have had a higher inflammatory burden or greater cardiovascular risk and by the cross-sectional nature of our work.

With regard to markers of subclinical atherosclerosis, FMD did not differ between RA patients with abnormal ABI and cIMT and those with ABI and cIMT measurements in the normal range. NMD was found to correlate with mean ABI.

We did not find any other study in the literature comparing ABI and FMD in RA patients, however, a prospective study of rosuvastatin therapy in systemic sclerosis, did report that, although FMD improved significantly after 6 months of therapy, no

change was seen in ABI measurements. This finding is perhaps due to differing effects of statin therapy on various vascular beds.

Similar to our findings for cIMT and FMD, Sandoo et al did not find an association between FMD and cIMT in 200 RA patients. They postulated that vascular function as assessed by FMD and vascular morphology as assessed by carotid IMT reflect distinct phases and mechanisms in the process of atherosclerosis and are therefore not comparable (24).

Both absolute and percentage FMD were negatively associated with serum BNP levels in RA patients. We have previously discussed in chapter 6, that serum NT-proBNP levels were not associated with other markers of diastolic function in our RA group and this has been supported in other research (34). Our findings of an association between BNP and endothelial dysfunction may suggest that NT-proBNP is a marker of inflammation and disease activity rather than a marker of diastolic function in RA. Bruce IN et al reported that levels of NT-proBNP correlated with HAQ and CRP in inflammatory polyarthritis and predicted CVD mortality (35).

Macrovascular Endothelial Function in Osteoarthritis:

We also examined the OA group separately and found that duration of disease was significantly associated with brachial artery diameter post ischaemic and post-GTN. Although we did not find a link between disease duration in RA and endothelial dysfunction, there are a number of studies that have reported an association (19, 22). As far as we are aware, there are no studies for comparison, examining the relationship between disease duration in OA and endothelial function.

Fasting glucose in the OA group correlated with absolute and percentage NMD values. This is not surprising given the mounting evidence identifying OA as a metabolic disease with close association with the metabolic syndrome. It is now understood that obesity-related OA is one aspect of a group known as metabolic osteoarthritis, which includes OA related to hypertension, dyslipidaemia and diabetes mellitus (36).

A number of pro- inflammatory cytokines, including IL8 and TNFα, were found to be significantly associated with a low FMD and NMD in OA patients. This finding was not seen in our RA group. Our RA group had significantly higher IL8 and TNF concentrations than the OA group. Perhaps there is a low grade level of inflammation in the OA group, which we did not detect with routine inflammatory markers that may explain the association between NMD and pro-inflammatory cytokines. There is emerging evidence that OA has a significant inflammatory component (37). In OA, pro-inflammatory cytokines stimulate release of cartilage-

degrading proteinases by chondrocytes (38). Future research on the inflammatory burden of OA and its effect on vascular disease may change previous beliefs that OA was primarily a degenerative disease.

Conclusions:

No significant difference in flow-mediated and nitroglycerin-mediated dilatation was found between our RA and OAgroups. There seems to be conflicting evidence regarding the rate of macrovascular dysfunction in RA compared to the general population, as described above. This is no doubt in part due to the small sample sizes in the majority of studies so far. Large scale prospective studies are required to tease out further whether RA itself is a risk factor for endothelial dysfunction.

Our finding of a significant correlation between markers of RA disease activity and endothelial dysfunction, demonstrate that RA remission induction would have beneficial effect not only on disability reduction in the long term but also on vascular function and supports the growing body of evidence that aggressive anti-rheumatic treatment will improve cardiovascular outcomes as well as RA disease specific outcomes.

Associations between anthropometric measurements and NMD, together with knowledge that exercise improves NMD in RA patients, demonstrate the importance of exercise prescription in RA patients.

Lack of an association between markers of subclinical atherosclerosis and endothelial dysfunction suggests that both are 2 distinct processes, perhaps occurring

at different time points in the progression of atherosclerosis in RA and are therefore not equivalent.

Associations between endothelial dysfunction and glucose and pro-inflammatory cytokines in our OA group, support other recent research that OA is an inflammatory disease and is closely linked to the metabolic syndrome.

References:

1. Munzel T, Sinning C, Post F, Warnholtz A, Schulz E. Pathophysiology, diagnosis and prognostic implications of endothelial dysfunction. Annals of medicine. 2008;40(3):180-96. Epub 2008/04/03.

2. Pohl U, Holtz J, Busse R, Bassenge E. Crucial role of endothelium in the vasodilator response to increased flow in vivo. Hypertension. 1986;8(1):37-44. Epub 1986/01/01.

3. Khan N, Pumphrey C, Clarke J, Jahangiri M. Aortic root replacement in pregnancy. Journal of the Royal Society of Medicine. 2003;96(11):551-2. Epub 2003/11/05.

4. Halcox JP, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA, et al. Prognostic value of coronary vascular endothelial dysfunction. Circulation. 2002;106(6):653-8. Epub 2002/08/07.

5. Deanfield J, Donald A, Ferri C, Giannattasio C, Halcox J, Halligan S, et al. Endothelial function and dysfunction. Part I: Methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. Journal of hypertension. 2005;23(1):7-17. Epub 2005/01/12.

6. Donald AE, Halcox JP, Charakida M, Storry C, Wallace SM, Cole TJ, et al. Methodological approaches to optimize reproducibility and power in clinical studies of flow-mediated dilation. Journal of the American College of Cardiology. 2008;51(20):1959-64. Epub 2008/05/17.

7. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet. 1992;340(8828):1111-5. Epub 1992/11/07.

8. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. Journal of the American College of Cardiology. 2002;39(2):257-65. Epub 2002/01/15.

9. Roman MJ, Naqvi TZ, Gardin JM, Gerhard-Herman M, Jaff M, Mohler E, et al. Clinical application of noninvasive vascular ultrasound in cardiovascular risk stratification: a report from the American Society of Echocardiography and the Society of Vascular Medicine and Biology. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography. 2006;19(8):943-54. Epub 2006/08/02.

10. Soltesz P, Der H, Kerekes G, Szodoray P, Szucs G, Danko K, et al. A comparative study of arterial stiffness, flow-mediated vasodilation of the brachial artery, and the thickness of the carotid artery intima-media in patients with systemic autoimmune diseases. Clinical rheumatology. 2009;28(6):655-62. Epub 2009/02/19.

11. Gonzalez-Gay MA, Gonzalez-Juanatey C, Vazquez-Rodriguez TR, Martin J, Llorca J. Endothelial dysfunction, carotid intima-media thickness, and accelerated atherosclerosis in rheumatoid arthritis. Seminars in arthritis and rheumatism. 2008;38(2):67-70. Epub 2008/04/09.

12. Bots ML, Westerink J, Rabelink TJ, de Koning EJ. Assessment of flow-mediated vasodilatation (FMD) of the brachial artery: effects of technical aspects of the FMD measurement on the FMD response. European heart journal. 2005;26(4):363-8. Epub 2004/12/25.

13. Kelm M. Flow-mediated dilatation in human circulation: diagnostic and therapeutic aspects. American journal of physiology Heart and circulatory physiology. 2002;282(1):H1-5. Epub 2001/12/19.

14. Adams MR, Robinson J, McCredie R, Seale JP, Sorensen KE, Deanfield JE, et al. Smooth muscle dysfunction occurs independently of impaired endothelium-dependent dilation in adults at risk of atherosclerosis. Journal of the American College of Cardiology. 1998;32(1):123-7. Epub 1998/07/21.

15. Schachinger V, Zeiher AM. Quantitative assessment of coronary vasoreactivity in humans in vivo. Importance of baseline vasomotor tone in atherosclerosis. Circulation. 1995;92(8):2087-94. Epub 1995/10/15.

16. Hannawi S, Marwick TH, Thomas R. Inflammation predicts accelerated brachial arterial wall changes in patients with recent-onset rheumatoid arthritis. Arthritis research & therapy. 2009;11(2):R51. Epub 2009/04/07.

17. Vaudo G, Marchesi S, Gerli R, Allegrucci R, Giordano A, Siepi D, et al. Endothelial dysfunction in young patients with rheumatoid arthritis and low disease activity. Annals of the rheumatic diseases. 2004;63(1):31-5. Epub 2003/12/16.

18. Kerekes G, Szekanecz Z, Der H, Sandor Z, Lakos G, Muszbek L, et al. Endothelial dysfunction and atherosclerosis in rheumatoid arthritis: a multiparametric analysis using imaging techniques and laboratory markers of

inflammation and autoimmunity. The Journal of rheumatology. 2008;35(3):398-406. Epub 2008/01/19.

19. Sarli B, Baktir AO, Cebicci M, Dogan Y, Demirbas M, Kurtul S, et al. Predictors of Endothelial Dysfunction in Patients With Rheumatoid Arthritis. Angiology. 2013. Epub 2013/10/01.

20. Hall FC. Does treatment with DMARDs decrease the risk of cardiovascular disease in patients with RA? Nature clinical practice Rheumatology. 2007;3(5):254-5. Epub 2007/04/05.

21. El-Barbary AM, Hussein MS, Rageh EM, Hamouda HE, Wagih AA, Ismail RG. Effect of atorvastatin on inflammation and modification of vascular risk factors in rheumatoid arthritis. The Journal of rheumatology. 2011;38(2):229-35. Epub 2010/11/03.

22. Gonzalez-Juanatey C, Testa A, Garcia-Castelo A, Garcia-Porrua C, Llorca J, Gonzalez-Gay MA. Active but transient improvement of endothelial function in rheumatoid arthritis patients undergoing long-term treatment with anti-tumor necrosis factor alpha antibody. Arthritis and rheumatism. 2004;51(3):447-50. Epub 2004/06/10.

23. Bosello S, Santoliquido A, Zoli A, Di Campli C, Flore R, Tondi P, et al. TNF-alpha blockade induces a reversible but transient effect on endothelial dysfunction in patients with long-standing severe rheumatoid arthritis. Clinical rheumatology. 2008;27(7):833-9. Epub 2007/12/14.

24. Sandoo A, Kitas GD, Carroll D, Veldhuijzen van Zanten JJ. The role of inflammation and cardiovascular disease risk on microvascular and macrovascular

endothelial function in patients with rheumatoid arthritis: a cross-sectional and longitudinal study. Arthritis research & therapy. 2012;14(3):R117. Epub 2012/05/19.

25. Foster W, Carruthers D, Lip GY, Blann AD. Inflammation and microvascular and macrovascular endothelial dysfunction in rheumatoid arthritis: effect of treatment. The Journal of rheumatology. 2010;37(4):711-6. Epub 2010/02/17.

26. Foster W, Lip GY, Raza K, Carruthers D, Blann AD. An observational study of endothelial function in early arthritis. European journal of clinical investigation. 2012;42(5):510-6. Epub 2011/10/12.

27. Van Doornum S, McColl G, Jenkins A, Green DJ, Wicks IP. Screening for atherosclerosis in patients with rheumatoid arthritis: comparison of two in vivo tests of vascular function. Arthritis and rheumatism. 2003;48(1):72-80. Epub 2003/01/16.

28. Sodergren A, Karp K, Boman K, Eriksson C, Lundstrom E, Smedby T, et al. Atherosclerosis in early rheumatoid arthritis: very early endothelial activation and rapid progression of intima media thickness. Arthritis research & therapy. 2010;12(4):R158. Epub 2010/08/18.

29. Mizia-Stec K, Gasior Z, Mizia M, Haberka M, Holecki M, Zwolinska W, et al. Flow-mediated dilation and gender in patients with coronary artery disease: arterial size influences gender differences in flow-mediated dilation. Echocardiography. 2007;24(10):1051-7. Epub 2007/11/16.

30. Mazzoccoli G, Notarsanto I, de Pinto GD, Dagostino MP, De Cata A, D'Alessandro G, et al. Anti-tumor necrosis factor-alpha therapy and changes of flow-mediated vasodilatation in psoriatic and rheumatoid arthritis patients. Internal and emergency medicine. 2010;5(6):495-500. Epub 2010/09/17.

31. Tikiz H, Arslan O, Pirildar T, Tikiz C, Bayindir P. The effect of anti-tumor necrosis factor (TNF)-alpha therapy with etanercept on endothelial functions in patients with rheumatoid arthritis. Anadolu kardiyoloji dergisi : AKD = the Anatolian journal of cardiology. 2010;10(2):98-103. Epub 2010/04/13.

32. Quinn MA, Conaghan PG, Emery P. The therapeutic approach of early intervention for rheumatoid arthritis: what is the evidence? Rheumatology. 2001;40(11):1211-20. Epub 2001/11/16.

33. Metsios GS, Stavropoulos-Kalinoglou A, Veldhuijzen van Zanten JJ, Nightingale P, Sandoo A, Dimitroulas T, et al. Individualised exercise improves endothelial function in patients with rheumatoid arthritis. Annals of the rheumatic diseases. 2013. Epub 2013/08/02.

34. Crowson CS, Myasoedova E, Davis JM, 3rd, Roger VL, Karon BL, Borgeson D, et al. Use of B-type natriuretic peptide as a screening tool for left ventricular diastolic dysfunction in rheumatoid arthritis patients without clinical cardiovascular disease. Arthritis care & research. 2011;63(5):729-34. Epub 2011/01/13.

35. Mirjafari H, Welsh P, Verstappen SM, Wilson P, Marshall T, Edlin H, et al. N-terminal pro-brain-type natriuretic peptide (NT-pro-BNP) and mortality risk in early inflammatory polyarthritis: results from the Norfolk Arthritis Registry (NOAR). Annals of the rheumatic diseases. 2013. Epub 2013/03/21.

36. Sellam J, Berenbaum F. Is osteoarthritis a metabolic disease? Joint, bone, spine : revue du rhumatisme. 2013;80(6):568-73. Epub 2013/11/02.

37. Berenbaum F. Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society. 2013;21(1):16-21. Epub 2012/12/01.

38. Goldring SR, Goldring MB. The role of cytokines in cartilage matrix degeneration in osteoarthritis. Clinical orthopaedics and related research. 2004(427 Suppl):S27-36. Epub 2004/10/14.

Chapter 6

Sub-Clinical Carotid Atherosclerosis in RA

Introduction:

Carotid intimal medial thickness reflects early atherosclerosis and is frequently used to assess excess cardiovascular risk. It is non-invasive for the patient and the carotid artery is easily accessible for ultrasound interrogation. It is a strong predictor for future vascular events in the general population (1).

In 2001, the National Cholesterol Education Program Adult Treatment Panel III agreed that cIMT could be used as an adjunct in CHD risk assessment. It is recognized by the American Heart Association as a surrogate marker for coronary artery disease. The American Society of Echocardiography Carotid Intimal Medial Thickness Task Force recommend that measuring cIMT and identifying presence of carotid plaque can be useful for refining CVD risk assessment in patients at intermediate CVD risk (2).

Carotid IMT has been found to be increased in RA patients with longstanding disease, but recently it has been shown that increased cIMT is evident in RA patients within 1 year of symptom onset (3). There have also been reports that cIMT progresses more rapidly in RA patients compared to controls and that it correlates with RA disease activity (4). Plaque, as well as IMT, is also more common in RA patients than controls (5).

Recently Corrales et al identified that carotid ultrasound is a more sensitive marker of subclinical atherosclerosis in RA patients than coronary artery calcification score (6). They categorised the CV risk of 95 RA patients using the EULAR modified Systemic Coronary Risk Evaluation tool (mSCORE) and found that carotid ultrasound examination had a higher sensitivity than coronary artery calcification scores for identifying patients with high mSCOREs. They also found that over 50% of RA patients with a coronary artery calcification score of 0 had evidence of carotid plaque on ultrasound. This study highlights the usefulness of carotid ultrasound for stratifying CV risk in RA patients.

This evidence is further supported by a study of over 300 RA patients that found the addition of carotid ultrasound assessment to the EULAR modified SCORE tool increased the sensitivity of the mSCORE tool, particularly in patients with high CV risk. Severe carotid ultrasound abnormalities were found in 63% of RA patients with a moderate mSCORE (7).

There is also research supporting this in the general population. The Multi-Ethnic Study of Atherosclerosis has found that increased cIMT readings are predictive of CVD events in adults with no evidence of coronary calcification (8).

The assessment of cIMT and presence of plaque as markers of pre-clinical atherosclerosis in RA has not been extensively investigated in an Irish population.

Aims:

Our primary aim was to compare carotid intima-medial thickness and degree of carotid plaque, as markers of sub-clinical atherosclerosis, in RA and OA patients without clinical evidence of cardiovascular disease. Secondary aims were to investigate if markers of inflammation, anti-rheumatic medications and cardiovascular risks were associated with sub-clinical atherosclerosis in either group.

Methods:

Patients attended the radiology department for carotid ultrasound examination. B mode ultrasound using a Toshiba Xario system and linear array 7mHz probe was used and scans were performed by a trained radiographer. IMT (intima-media thickness) values were obtained at specific points for the right and left common carotid artery and for both the right and left internal carotid artery (RCCA, RICA, LCCA, LICA). An average of three readings was taken for each variable and a composite value, expressed in mm was recorded. Each of the 4 vessels was assessed for presence of plaque. Plaque was defined as a distinct protrusion > 1.5mm into the vessel lumen. This was recorded as either present or absent. If present, plaque size was recorded and expressed in mm. Both the radiographer performing the examination and the radiologist reading and interpreting the scans, were blinded to the identity of the study subjects and are unaware of whether they formed part of RA or OA group.

Based on recommendations from the American Society of Echocardiography (2) and the Mannheim Consensus document (9), carotid intimal medial thickness (cIMT) values were reported as a mean of the cIMT from the left and right common carotid arteries. A mean cIMT of greater than 0.9mm was considered abnormal (6).

Statistical Methods:

Means and standard deviations were calculated for normally distributed data and compared using a student t-test. Medians and interquartile ranges were calculated for non-normally distributed data and compared using a Mann Whitney U test. Bivariate correlations were calculated for continuous variables. Logistic regression analysis was performed to analyse the relationship between the presence of subclinical atherosclerosis, as defined by presence of carotid plaque and / or cIMT greater than 0.9mm, and a diagnosis of Rheumatoid Arthritis. Odds ratios and 95% confidence intervals were computed. A p-value of < 0.05 was considered significant. SPSS 20 was used for all statistical analysis.

Results:

Comparison of Carotid Intima-Media Thickness in RA and OA groups:

99 subjects from the study group attended for carotid ultrasound examination. 54 of these had a diagnosis of RA and 45 were patients with OA. A mean common carotid intimal medial thickness (cIMT) of 0.71mm (+/-SD 0.32) in the RA group and 0.73mm (+/-SD 0.23) in the OA group was found. Using an independent t-test, no significant difference between the 2 groups was seen, p 0.614.

Eight patients (14.8%) in the RA group had an abnormal cIMT, ie mean cIMT greater than 0.9mm. Seven patients (15.6%) in the OA group had abnormal cIMT readings.

Carotid IMT and Characteristics of RA patients:

In patients with RA, there was a significant positive correlation between age and mean common cIMT, Pearsons correlation, r= 0.425, p=0.001 (figure 6.1).

Within the RA group, disease duration was longer in those with an abnormal cIMT compared to those RA patients whose cIMT fell within the normal range, 12.75 yrs (+/- SD 10.85yrs) versus 8.37 yrs (+/- SD 6.13yrs). However, this difference was not statistically significant, p=0.3.

We also investigated whether there was a difference in cIMT between those on DMARDS, biologic agents, steroids and NSAIDS and those RA patients not on the above therapies (table 6.1). It is noteworthy that the mean cIMT was higher in RA patients not currently on a biologic agent compared with those on a biologic at time of ultrasound, 0.807 mm(SD 0.443 mm) versus 0.632 mm (SD 0.163 mm), p=0.048.

We investigated this further to see if there was a higher rate of steroid and NSAID use in the biologic DMARD group, which may have been contributing to reduction of inflammation. There was no significant difference in current steroid dose between RA patients on a biologic agent and those not currently taking a biologic agent. NSAID use was higher in RA patients not currently on a biologic, p=0.008.

		Mean cIMT (mm)	SD (+/-)	P value
Sex	Male	0.740	0.248	0.525
	Female	0.687	0.361	
Current synthetic DMARD	Yes	0.717	0.362	0.633
	No	0.680	0.192	
Current Biologic DMARD	Yes	0.632	0.163	0.048*
	No	0.807	0.443	
Current steroid use	Yes	0.746	0.438	0.465
	No	0.675	0.188	
Current NSAID use	Yes	0.736	0.441	0.562
	No	0.681	0.168	
Current smoker	Yes	0.629	0.149	0.137
	No	0.734	0.362	
Family history of any CVD	Yes	0.714	0.416	0.903
	No	0.702	0.245	

Table 6.1: Mean cIMT in RA patients, comparing sex, current smoking status and current RA therapies, cIMT, carotid intimal medial thickness; mm, millimetres; SD, standard deviation; CVD, cardiovascular disease; p, significance at 0.05 level

Markers of CV disease and Carotid IMT measurements in RA:

Within the RA group, we investigated whether there was a difference in cIMT measurements between those with and without CV risks. Bivariate correlations were used to look for associations between cIMT and CV risks (table 6.1 and table 6.2).

Systolic blood pressure was found to have a clinically significant association with cIMT, r = 0.419, p = 0.004. Fasting glucose also correlated with cIMT measurements, r = 0.351, p = 0.012. On logistic regression analysis, fasting glucose was associated with an abnormal cIMT in patients with rheumatoid arthritis, OR (95%), 3.224 (1.161-8.954), p=0.025.

A prior diagnosis of hypercholesterolaemia was found to be associated with an abnormal cIMT in the RA group, OR (95%CI), 6.667 (1.306-34.027), p=0.023. This association remained significant after simultaneous adjustment for traditional cardiovascular risk factors (table 6.3). To further investigate the significant association between a previous diagnosis of hypercholesterolaemia and presence of abnormal cIMT on ultrasound, logistic regression analysis was performed to assess for an association between current serum fasting cholesterol level and abnormal cIMT. This did not reveal any significant association, OR (95%CI), 0.524 (0.108-2.552), p=0.424. This lack of association can be explained by the fact that all but one of the patients with a prior diagnosis of hypercholesterolaemia were taking a statin at the time that blood was drawn for fasting cholesterol level.

Within the RA group, alcohol consumption was associated with the presence of an abnormal cIMT, OR (95%), 13.2 (1.761-98.926), p=0.012. This was still significant after adjustment for age, smoking status, presence of hypertension and hypercholesterolaemia, OR (95%CI), 14.523 (1.342-157.126), p=0.028. However, our wide confidence interval reflects the small sample size.

Carotid IMT was not associated with smoking in the RA group, r = 0.024, p =0.836.

Two measures from transthoracic echocardiogram; LV posterior wall thickness, a marker of diastolic dysfunction and LV mass, a marker of hypertension, were both found to significantly correlate with cIMT measurements in the RA group, r = 0.358, p = 0.009 and r = 0.359, p = 0.009, respectively.

Also, PAI-1, a marker of thrombosis, was found to correlate significantly with cIMT in the RA group, an association which was not seen in the OA group, r = 0.294, p =0.045.

Correlation between mean cIMT and markers of CVD and metabolic syndrome	Correlation coefficient, r	p
Age, yrs	0.425	0.001*
Smoking Pack years	0.024	0.863
Systolic Blood Pressure	0.419	0.004*
Body Mass Index (kg/m^2)	0.252	0.066
Waist Hip Ratio	0.188	0.174
LV posterior wall thickness	0.358	0.009*
LV mass (g)	0.359	0.009*
Total cholesterol (mmol/l)	0.056	0.687
Fasting Glucose (mmol/l)	0.351	0.012*
PAI activity	0.294	0.045*
Fibrinogen	0.001	0.997

Table 6.2: Correlations between cIMT and markers of CVD and the metabolic syndrome in the RA group. RA, rheumatoid arthritis; cIMT, carotid intimal medial thickness; r, correlation co-efficient; p, significance level 0.05

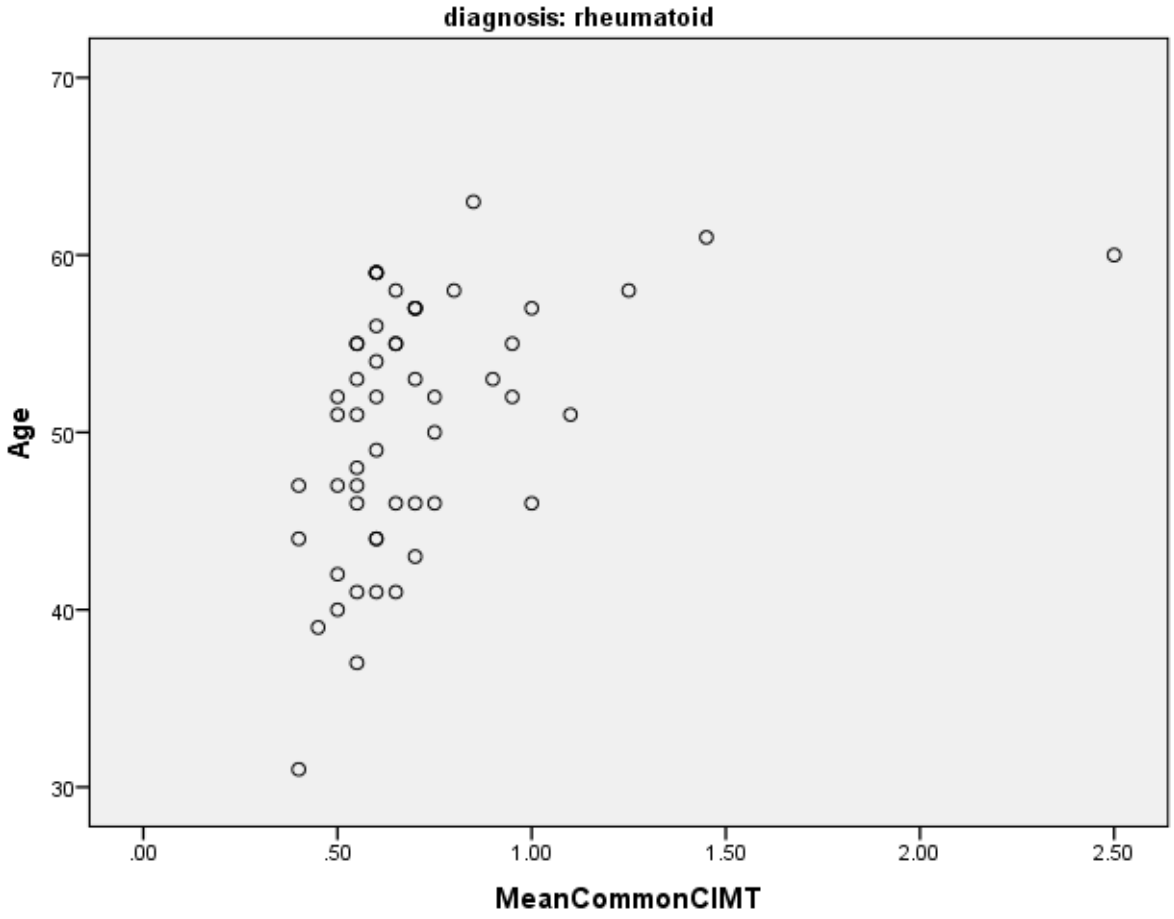


Figure 6.1: Scatter plot demonstrating the positive correlation between age and mean common carotid IMT in RA patients

cIMT in RA group	OR	95%CI	p	Adjusted OR (a)	Adjusted 95%CI	p
Age	1.146	0.991-1.326	0.067	1.115	0.920-1.351	0.269
Sex	1.875	0.413-8.512	0.415	1.786	0.213-14.983	0.593
Family history of CVD	0.433	0.079-2.380	0.336	0.127	0.010-1.630	0.113
Prior diagnosis of Hypercholesterolaemia	6.667	1.306-34.027	0.023*	14.467	1.461-143.26	0.022*
Fasting glucose (mmol/l)	3.224	1.161-8.954	0.025*	6.104	0.723-32.169	0.050*
Total cholesterol (mmol/l)	0.524	0.108-2.552	0.424	1.241	0.426-3.612	0.692
LDL (mmol/l)	0.590	0.140-2.457	0.472	0.267	0.042-1.697	0.162
Smoking status	1.818	0.388-8.514	0.448	3.160	0.374-26.713	0.291
Alcohol consumption	13.2	1.761-98.926	0.012*	14.523	1.342-157.126	0.028*

a = OR after simultaneous adjustment for age, sex, smoking status, diagnosis of hypertension and family history of CVD

Table 6.3: Summary of association between cIMT and CVD risks in the RA group analysed using logistic regression. RA, rheumatoid arthritis; OR, odds ratio; CI, confidence interval; p, 0.05 significance level

Results for Presence of Carotid Plaque:

Carotid plaque was present in 12 of the 99 subjects who had a carotid ultrasound. Seven of the 12 were patients with RA. Using Chi squared testing, no significant difference in the number of RA patients and OA patients who had plaque visible on ultrasound was found, p=0.779.

Of the 12 patients with evidence of plaque on ultrasound, 6 were smokers, 3 were already on a statin, 8 had a higher than normal BMI and waist-hip ratio.

Carotid Plaque and Lipid Profile:

Serum HDL was significantly higher in RA patients without presence of plaque compared to those with plaque burden on ultrasound, 1.65mmml/l (SD 0.46mmol/l) versus 1.33mmol/l (SD 0.31 mmol/l), p=0.04. LDL levels were higher in the RA patients who had evidence of plaque on ultrasound compared to RA patients with no plaque, 3.67 mmol/l (SD 0.49 mmol/l) versus 3.15 mmol/l (SD 0.54 mmol/l), p = 0.033.

Interestingly, serum LDL level was significantly associated with the presence of carotid plaque in RA patients on logistic regression, OR (CI 95%), 9.058 (1.178-69.635*)* p = 0.034. This remained significant after simultaneous adjustment for traditional cardiovascular risk factors, p = 0.046. LDL level was not found to be

associated with the presence of carotid plaque in the OA group, OR (95%CI), 1.988, (0.619-6.383), p = 0.248 (table 4).

Carotid Plaque and Diastolic Dysfunction:

E/e' lateral, a marker of diastolic dysfunction, was associated with presence of plaque in both the RA and OA groups, p=0.017 and p=0.007 respectively. E/e' (average lateral and septal combined) was also associated with carotid plaque in both the RA and OA groups, p =0.031 and p=0.019 respectively (table 6.4). After simultaneous adjustment for age and hypertension, these associations were no longer significant in the RA group, p = 0.093. Significance remained in the OA group, but confidence intervals were very wide, p = 0.012.

Carotid Plaque in RA group	Odds ratio	95% CI	p
Male sex	1.320	(0.30, 9.25)	0.733
Current biologic therapy	2.019	(0.42, 18.14)	0.428
Current steroid use	1.800	(0.38, 10.99)	0.473
Family History RA	2.455	(0.58, 20.01)	0.284
Dx of HTN	4.286	(0.78, 23.43)	0.093
Rheumatoid factor positive	2.057	(0.22, 18.87)	0.524
Fasting Cholesterol	1.469	(0.50, 4.36)	0.488
LDL	9.058	(1.18, 69.65)	0.034*
E wave velocity	1.131	(1.03, 1.25)	0.014*
E/A ratio <1	1.706	(0.31, 9.42)	0.540
E/e'lateral	2.616	(1.19, 5.75)	0.017*
E/e'average	2.189	(1.08, 4.46)	0.031*
Diastolic dysfunction	8.700	(0.81, 93.49)	0.074

Table 6.4: Summary of results for variables associated with carotid plaque in the RA group, using logistic regression analysis. HTN, hypertension; ABI, ankle brachial index; E wave, early diastolic filling velocity; E/A ratio, ratio of early to late diastolic filling velocity; e', early diastolic mitral annular velocity; p, significance level

Carotid Disease in the OA group:

As with the RA group, age also correlated with cIMT in the OA group, r = 0.487, p=0.001. A prior diagnosis of hypercholesterolaemia was found to be associated with an abnormal cIMT in the OA group, 9.375 (1.525-57.621), p=0.016.

Discussion:

Comparison of Carotid Intima-Media Thickness in RA and OA groups:

Carotid IMT did not differ between rheumatoid and osteoarthritis patients in our study. A number of studies have found that cIMT is increased in RA patients compared with healthy controls and they have postulated that this difference is due to the inflammatory burden of the disease (5, 10-12). The majority of these studies used healthy adults as their control group and this is one reason why we may have found conflicting results.

However, similar to our findings, del Rincon et al did not find a difference in cIMT when they compared 204 RA patients with 102 healthy controls (13).

Our control group were patients attending a rheumatology department with a diagnosis of osteoarthritis. There are a number of potential reasons why we did not find a difference in cIMT; OA patients attending a rheumatology clinic are at the more severe end of the disease spectrum and may be more likely to be exposed to NSAID use over time. Also, some traditional cardiovascular risk factors were significantly higher in the OA group, as previously discussed in chapter 3, a family history of myocardial infarction was significantly higher in the OA group (60% compared with 25% in the RA group), p= 0.001. Mean BMI was also significantly higher in the OA group, p=0.017. However, we have corrected for these differences in traditional risk factors, using logistic regression analysis.

Another factor to consider is that females with OA are more likely to have an inflammatory element to their disease during the perimenopausal period and this may be associated with an increased cardiovascular risk. There is also emerging evidence that OA is associated with an increased risk of CVD, especially IHD and CHF. Goldsmith et al found that older males and adult females with OA had a higher risk of CVD compared with non-OA controls (14). In the Rotterdam study, Hoeven et al conducted a large prospective study to investigate if atherosclerosis was associated with the presence and progression of OA. They found that atherosclerosis, as assessed by carotid IMT and presence of plaque was independently associated with hand and knee OA in women (15).

Carotid IMT and Characteristics of RA patients:

As expected, we found that increasing age correlated with mean cIMT. Age related increases in cIMT of about 0.010 mm per year in healthy males and 0.014 mm per year in healthy females have been reported (16). The progression rates of cIMT in patients with known coronary artery disease are up to 3 times that of healthy individuals (17).

Although not statistically significant, we did find that RA patients with an abnormal cIMT had longer disease duration than those with cIMT in the normal range. A number of studies have found similar results (18-20). However, the literature appears to be contradictory in this area; numerous studies have found evidence of

atherosclerotic disease, documented as abnormal cIMT, in RA patients with disease duration of 12 months or less (3, 11, 21).

These conflicting results suggest that the degree of carotid atherosclerosis in RA may be linked to RA disease activity rather than disease duration.

In this study, 29 of the 63 RA patients were on a biologic DMARD at the time of carotid ultrasound and they had a significantly more favourable cIMT than those not currently on a biologic DMARD. Concomitant medications such as steroid and NSAID use were not driving this difference. This is in keeping with results from other studies which have found anti-TNF agents to have a beneficial effect on cIMT over time (22-24). Giles et al followed 158 RA patients over 3 years and found that those taking tumour necrosis factor (TNF) inhibitors at baseline had a 37% lower adjusted rate of progression in cIMT compared with those not on anti-TNF therapy at baseline (25).

These findings support evidence that TNF inhibitors appear to be associated with reduced risk of all heart disease events (26).

Markers of CV disease and Carotid IMT measurements in RA:

A prior diagnosis of hypercholesterolaemia was associated with the presence of abnormal cIMT in the RA group. Our findings for this association are in keeping with multiple other studies that have found a link between serum levels of lipids and both cIMT and carotid plaque (20, 27, 28). Dessein et al recently reported that serum cholesterol / HDL ratio was significantly associated with cIMT and carotid plaque in RA patients (28). In 2011, Majdan M et al studied 74 RA patients without evidence of CVD and found that serum cholesterol was positively associated with cIMT (20). Oxidized LDL antibodies have also been shown to be independently associated with cIMT in RA patients (27).

Our findings and those listed above indicate that abnormal lipid profiles are independently associated with subclinical atherosclerosis in patients with RA.

We also found that fasting glucose correlated significantly with cIMT in RA patients. Del Rincon et al had similar findings (29). They looked at numbers of RA patients with carotid plaque who had diabetes mellitus, as opposed to a single fasting glucose measurement. They described a significant association between cIMT and presence of diabetes mellitus. Their RA patients with evidence of carotid plaque also had a significantly higher rate of diabetes mellitus than those with no plaque on ultrasound. A prospective study of 114 RA patients in Sweden found that a diagnosis of diabetes was significantly associated with carotid IMT at a 5 year follow-up (30).

With regard to the results of the transthoracic echocardiography, LV mass in the RA group was significantly correlated with carotid IMT. Left ventricular mass reflects hypertension and we also found that systolic blood pressure correlated significantly with cIMT in the RA group. These correlations were not seen with the OA control group. Patients with RA are at a higher risk of developing hypertension than the general population, due to frequent NSAID use over time and also steroid use, often in quiet high doses. The above association with a surrogate marker of atherosclerosis highlights the importance of close monitoring of blood pressure in RA and early intervention if required. Puato et al recently reported a higher cIMT in patients with psoriatic arthritis (PsA) and hypertension when compared to normotensive patients with PsA (31). Del Rincon et al found a significant association between hypertension and presence of carotid plaque in a large cohort of RA patients (29). In their study of 631 RA patients they reported that those with presence of plaque on ultrasound had a significantly higher rate of hypertension. They also found that a diagnosis of hypertension was associated with cIMT.

PAI-1, a marker of thrombosis, correlated significantly with cIMT in our RA patient group. This indicates that RA patients with abnormal cIMT also have altered haemostasis and links between these pathways may be responsible for the excess CVD seen in RA.

Sodergren et al found that disease activity markers in RA patients were associated with higher concentrations of PAI-1 and other markers of thrombosis. They also reported a significant association between cIMT and some thrombotic markers (32). In an earlier study of 39 RA patients, Wallberg-Jonsson et al also described a

significant association between cIMT and PAI-1, along with other thrombotic markers and adhesion molecules (33).

Although not a traditional marker of CV disease, it is interesting to note that alcohol consumption was associated with carotid IMT in RA patients, even after adjustment for traditional CV risk factors. As reflected in our wide confidence intervals, the numbers of RA patients consuming more than 14 units of alcohol per week was small and based on this it is not possible to conclude whether this lifestyle choice is a risk factor for cIMT in RA patients. We did not find any evidence of this in the literature. Studying cIMT and perhaps progression of cIMT over time in a larger cohort of RA patients who drink more than the recommended amount of alcohol would be interesting to investigate further.

Carotid Plaque and Lipid Profile:

In our RA group, serum HLD was better in patients without evidence of carotid plaque. Also, serum LDL level was significantly associated with presence of plaque on carotid ultrasound in the RA group but not in the OA control group. This highlights the importance of screening for traditional cardiovascular risk factors in RA patients, as those with elevated LDL levels may be more prone to developing carotid disease. Identifying these patients early and treating promptly with statin therapy may help to reduce their atherosclerotic disease burden in the longer-term.

Carotid Plaque and Diastolic Dysfunction:

E/e', a marker of diastolic dysfunction on tissue Doppler imaging, was significantly associated with presence of plaque in both the RA and OA group. However, after adjustment for age, this finding was no longer significant. As both of these parameters are known to deteriorate with increasing age, it makes sense that age, rather than diagnosis is the reason for this association.

Ciftci et al examined diastolic function and carotid IMT in 30 RA patients with no evidence of CVD and found similar results. They examined coronary flow reserve and found that it correlated positively with E/A ratio and negatively with IMT (34).

Conclusions:

Contrary to previous research, we did not find that RA patients had a greater degree of carotid IMT compared with OA controls. However, our OA control group are not comparable to normal healthy controls as there is emerging evidence that OA itself has an inflammatory component and is associated with increased CV risk.

Whether or not RA disease duration is a risk factor for abnormal cIMT is a matter for debate. As mentioned above, abnormal cIMT has been reported in longstanding disease but also in patients who have a diagnosis of RA for less than 1 year. This demonstrates the importance of screening for CVD early in the course of RA, when the burden of inflammation is often high prior to control with anti-rheumatic medications. Traditionally, when rheumatologists think of CVD risk in RA patients, it tends to be those patients with a long disease duration and their focus early in the course of RA is often solely on disease activity control rather than CV risk assessment.

Our findings of an association of serum lipid measurements with both cIMT and carotid plaque indicate that RA patients with an abnormal lipid profile would appear to be at a greater risk of developing future CV events. The fact that we have shown a link between lipid profile and subclinical atherosclerosis reinforces the importance of CV risk screening in this population. RA patients who have an abnormal lipid profile may benefit from carotid artery ultrasound to further stratify their CV risk and rheumatologists should have a low threshold for treatment with statin therapy.

PAI-1, a measure of haemostasis, correlates with sub-clinical atherosclerosis in RA patients. This highlights the fact that a number of mechanisms including altered coagulation and atheroma formation are involved in atherosclerosis and CV disease in this group.

References:

1. Cao JJ, Arnold AM, Manolio TA, Polak JF, Psaty BM, Hirsch CH, et al. Association of carotid artery intima-media thickness, plaques, and C-reactive protein with future cardiovascular disease and all-cause mortality: the Cardiovascular Health Study. Circulation. 2007;116(1):32-8. Epub 2007/06/20.

2. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography. 2008;21(2):93-111; quiz 89-90. Epub 2008/02/12.

3. Hannawi S, Haluska B, Marwick TH, Thomas R. Atherosclerotic disease is increased in recent-onset rheumatoid arthritis: a critical role for inflammation. Arthritis research & therapy. 2007;9(6):R116. Epub 2007/11/08.

4. Nagata-Sakurai M, Inaba M, Goto H, Kumeda Y, Furumitsu Y, Inui K, et al. Inflammation and bone resorption as independent factors of accelerated arterial wall thickening in patients with rheumatoid arthritis. Arthritis and rheumatism. 2003;48(11):3061-7. Epub 2003/11/13.

5. Roman MJ, Moeller E, Davis A, Paget SA, Crow MK, Lockshin MD, et al. Preclinical carotid atherosclerosis in patients with rheumatoid arthritis. Annals of internal medicine. 2006;144(4):249-56. Epub 2006/02/24.

6. Corrales A, Parra JA, Gonzalez-Juanatey C, Rueda-Gotor J, Blanco R, Llorca J, et al. Cardiovascular risk stratification in rheumatic diseases: carotid ultrasound is more sensitive than Coronary Artery Calcification Score to detect subclinical atherosclerosis in patients with rheumatoid arthritis. Annals of the rheumatic diseases. 2013;72(11):1764-70. Epub 2013/07/16.

7. Corrales A, Gonzalez-Juanatey C, Peiro ME, Blanco R, Llorca J, Gonzalez-Gay MA. Carotid ultrasound is useful for the cardiovascular risk stratification of patients with rheumatoid arthritis: results of a population-based study. Annals of the rheumatic diseases. 2013. Epub 2013/03/19.

8. Folsom AR, Kronmal RA, Detrano RC, O'Leary DH, Bild DE, Bluemke DA, et al. Coronary artery calcification compared with carotid intima-media thickness in the prediction of cardiovascular disease incidence: the Multi-Ethnic Study of Atherosclerosis (MESA). Archives of internal medicine. 2008;168(12):1333-9. Epub 2008/06/25.

9. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Desvarieux M, et al. Mannheim intima-media thickness consensus. Cerebrovascular diseases. 2004;18(4):346-9. Epub 2004/11/04.

10. van Sijl AM, Peters MJ, Knol DK, de Vet HC, Gonzalez-Gay MA, Smulders YM, et al. Carotid intima media thickness in rheumatoid arthritis as compared to control subjects: a meta-analysis. Seminars in arthritis and rheumatism. 2011;40(5):389-97. Epub 2010/10/05.

11. Chatterjee Adhikari M, Guin A, Chakraborty S, Sinhamahapatra P, Ghosh A. Subclinical atherosclerosis and endothelial dysfunction in patients with early

rheumatoid arthritis as evidenced by measurement of carotid intima-media thickness and flow-mediated vasodilatation: an observational study. Seminars in arthritis and rheumatism. 2012;41(5):669-75. Epub 2011/11/01.

12. Pieringer H, Schumacher S, Stuby U, Biesenbach G. Augmentation index and large-artery remodeling in patients with longstanding rheumatoid arthritis compared with healthy controls. Seminars in arthritis and rheumatism. 2009;39(3):163-9. Epub 2008/10/01.

13. Del Rincon I, Williams K, Stern MP, Freeman GL, O'Leary DH, Escalante A. Association between carotid atherosclerosis and markers of inflammation in rheumatoid arthritis patients and healthy subjects. Arthritis and rheumatism. 2003;48(7):1833-40. Epub 2003/07/09.

14. Rahman MM, Kopec JA, Anis AH, Cibere J, Goldsmith CH. Risk of cardiovascular disease in patients with osteoarthritis: a prospective longitudinal study. Arthritis care & research. 2013;65(12):1951-8. Epub 2013/08/09.

15. Hoeven TA, Kavousi M, Clockaerts S, Kerkhof HJ, van Meurs JB, Franco O, et al. Association of atherosclerosis with presence and progression of osteoarthritis: the Rotterdam Study. Annals of the rheumatic diseases. 2013;72(5):646-51. Epub 2012/05/09.

16. Howard G, Sharrett AR, Heiss G, Evans GW, Chambless LE, Riley WA, et al. Carotid artery intimal-medial thickness distribution in general populations as evaluated by B-mode ultrasound. ARIC Investigators. Stroke; a journal of cerebral circulation. 1993;24(9):1297-304. Epub 1993/09/01.

17. Crouse JR, 3rd, Tang R, Espeland MA, Terry JG, Morgan T, Mercuri M. Associations of extracranial carotid atherosclerosis progression with coronary status and risk factors in patients with and without coronary artery disease. Circulation. 2002;106(16):2061-6. Epub 2002/10/16.

18. Gonzalez-Juanatey C, Llorca J, Gonzalez-Gay MA. Correlation between endothelial function and carotid atherosclerosis in rheumatoid arthritis patients with long-standing disease. Arthritis research & therapy. 2011;13(3):R101. Epub 2011/06/24.

19. Kumeda Y, Inaba M, Goto H, Nagata M, Henmi Y, Furumitsu Y, et al. Increased thickness of the arterial intima-media detected by ultrasonography in patients with rheumatoid arthritis. Arthritis and rheumatism. 2002;46(6):1489-97. Epub 2002/07/13.

20. Targonska-Stepniak B, Drelich-Zbroja A, Majdan M. The relationship between carotid intima-media thickness and the activity of rheumatoid arthritis. Journal of clinical rheumatology : practical reports on rheumatic & musculoskeletal diseases. 2011;17(5):249-55. Epub 2011/07/23.

21. Turiel M, Tomasoni L, Sitia S, Cicala S, Gianturco L, Ricci C, et al. Effects of long-term disease-modifying antirheumatic drugs on endothelial function in patients with early rheumatoid arthritis. Cardiovascular therapeutics. 2010;28(5):e53-64. Epub 2010/03/27.

22. Angel K, Provan SA, Fagerhol MK, Mowinckel P, Kvien TK, Atar D. Effect of 1-year anti-TNF-alpha therapy on aortic stiffness, carotid atherosclerosis, and

calprotectin in inflammatory arthropathies: a controlled study. American journal of hypertension. 2012;25(6):644-50. Epub 2012/03/02.

23. Gonzalez-Juanatey C, Vazquez-Rodriguez TR, Miranda-Filloy JA, Gomez-Acebo I, Testa A, Garcia-Porrua C, et al. Anti-TNF-alpha-adalimumab therapy is associated with persistent improvement of endothelial function without progression of carotid intima-media wall thickness in patients with rheumatoid arthritis refractory to conventional therapy. Mediators of inflammation. 2012;2012:674265. Epub 2012/08/18.

24. Ferrante A, Giardina AR, Ciccia F, Parrinello G, Licata G, Avellone G, et al. Long-term anti-tumour necrosis factor therapy reverses the progression of carotid intima-media thickness in female patients with active rheumatoid arthritis. Rheumatology international. 2009;30(2):193-8. Epub 2009/04/24.

25. Giles JT, Post WS, Blumenthal RS, Polak J, Petri M, Gelber AC, et al. Longitudinal predictors of progression of carotid atherosclerosis in rheumatoid arthritis. Arthritis and rheumatism. 2011;63(11):3216-25. Epub 2011/10/04.

26. Barnabe C, Martin BJ, Ghali WA. Systematic review and meta-analysis: anti-tumor necrosis factor alpha therapy and cardiovascular events in rheumatoid arthritis. Arthritis care & research. 2011;63(4):522-9. Epub 2010/10/20.

27. Peters MJ, van Halm VP, Nurmohamed MT, Damoiseaux J, Tervaert JW, Twisk JW, et al. Relations between autoantibodies against oxidized low-density lipoprotein, inflammation, subclinical atherosclerosis, and cardiovascular disease in rheumatoid arthritis. The Journal of rheumatology. 2008;35(8):1495-9. Epub 2008/07/04.

28. Solomon A, Woodiwiss AJ, Abdool-Carrim AT, Stevens BA, Norton GR, Dessein PH. The carotid artery atherosclerosis burden and its relation to cardiovascular risk factors in black and white Africans with established rheumatoid arthritis: a cross-sectional study. The Journal of rheumatology. 2012;39(9):1798-806. Epub 2012/07/04.

29. del Rincon I, Freeman GL, Haas RW, O'Leary DH, Escalante A. Relative contribution of cardiovascular risk factors and rheumatoid arthritis clinical manifestations to atherosclerosis. Arthritis and rheumatism. 2005;52(11):3413-23. Epub 2005/10/29.

30. Ajeganova S, Ehrnfelt C, Alizadeh R, Rohani M, Jogestrand T, Hafstrom I, et al. Longitudinal levels of apolipoproteins and antibodies against phosphorylcholine are independently associated with carotid artery atherosclerosis 5 years after rheumatoid arthritis onset--a prospective cohort study. Rheumatology. 2011;50(10):1785-93. Epub 2011/07/12.

31. Puato M, Ramonda R, Doria A, Rattazzi M, Faggin E, Balbi G, et al. Impact of hypertension on vascular remodeling in patients with psoriatic arthritis. Journal of human hypertension. 2013. Epub 2013/07/12.

32. Sodergren A, Karp K, Boman K, Eriksson C, Lundstrom E, Smedby T, et al. Atherosclerosis in early rheumatoid arthritis: very early endothelial activation and rapid progression of intima media thickness. Arthritis research & therapy. 2010;12(4):R158. Epub 2010/08/18.

33. Wallberg-Jonsson S, Ohman M, Rantapaa-Dahlqvist S. Which factors are related to the presence of atherosclerosis in rheumatoid arthritis? Scandinavian journal of rheumatology. 2004;33(6):373-9. Epub 2005/03/30.

34. Ciftci O, Yilmaz S, Topcu S, Caliskan M, Gullu H, Erdogan D, et al. Impaired coronary microvascular function and increased intima-media thickness in rheumatoid arthritis. Atherosclerosis. 2008;198(2):332-7. Epub 2008/01/01.

Chapter 7

Peripheral Vascular Disease in RA

Introduction:

Peripheral vascular disease is a chronic occlusive disease of the arteries in the lower extremities caused by atherosclerosis. Prevalence of PVD increases with age and worldwide figures range from 3 to 12 percent (1). It is more prevalent in older individuals and those with risk factors for cardiovascular disease. The risk of developing PVD is increased with smoking, hypertension, diabetes, hyperlipidaemia and the metabolic syndrome (2).

PVD often coexists with other atherosclerotic diseases. Aronow et al studied 1,886 people with a mean age of 81 years and found that 58% of the 468 people with PVD also had coexistent coronary artery disease (CAD) and 34% had a prior ischaemic stoke (3). People who suffer from PVD have an increased risk for cardiovascular and all-cause mortality (4).

Coronary artery disease has been the primary focus of cardiovascular risk assessment in patients with rheumatoid arthritis (RA). Much less is known about the risk factors for PVD (5) in RA. One prospective study of 813 RA patients found no difference in rates of peripheral arterial events compared to non-RA controls (6). In 2004, del Rincon et al, found that glucocorticoid use in RA patients was significantly associated with arterial incompressibility, as defined by an elevated ABI > 1.4. This

incompressibility is due to calcified arterial walls and it results in increased vascular stiffness. They did not report an association between steroid use and lower-limb artery obstruction.

The prevalence of PVD in other inflammatory arthropathies has not been investigated thoroughly. One cross-sectional study in 2006, reported an overall risk of PVD in patients with psoriatic arthritis (PsA) of 1.6 (1.2-2.0) compared to non-PsA controls (7).

Ankle brachial index is the standard non-invasive tool for the assessment of peripheral arterial disease. It is not only a marker of peripheral artery disease but also of generalized atherosclerosis. Lower measurements of the ABI have been associated with the presence of cardiovascular risk factors and with higher rates of coronary and cerebrovascular disease (8, 9). In 2008, a meta-analysis performed by the Ankle Brachial Index Collaboration, reported that ABI provided independent risk information compared with the Framingham risk score (FRS) and also, an ABI < 0.9 in combination with FRS, approximately doubled the risk of all cause and cardiovascular mortality and major coronary events across all Framingham risk groups (10).

Multiple studies, including the Strong Heart Study (11, 12) have found that the relationship between CVD and ABI is non-linear. High ABI values (>1.40) may be due to increased arterial stiffness resulting in poor compressibility, occuring in particular in diabetic patients. This may help to explain why those with a high ABI are at an increased CV risk.

In 2007, the Fourth Joint Task Force of the European Society of Cardiology suggested that ABI be considered for the purposes of cardiovascular risk assessment (13).

PVD has not been studied in RA patients in an Irish population.

Aims:

To compare ABI measurements, as a marker of PVD, in RA and OA patients

To investigate if there was a relationship between ABI and other markers of subclinical atherosclerosis in RA.

Methods:

Ankle brachial index was performed in the usual manner. Blood pressure was recorded in both arms after a period of at least 15 minutes rest. The highest systolic reading of the 2 was used in the index calculation. For ankle pressure measurement, the cuff was placed around the calf and it was then inflated 20 mmHg above the upper limb systolic pressure.

A Nicolet Elite handheld Doppler ultrasound with an 8 MHz probe was used to measure ABI. The ultrasound probe was placed over the dorsalis pedis artery and the blood pressure cuff slowly deflated. If the dorsalis pedis artery was not easily palpable, the posterior tibial artery was used instead. The pressure at which arterial pulsation was first heard, was recorded and used in the calculation. ABI for each leg was calculated as a ratio of ankle pressure to upper limb systolic pressure. A mean of both lower limb ABIs was then calculated. A value of 0.96 or greater was considered as a normal ankle brachial index measurement (14).

$$\frac{\text{Ankle Pressure (mmHg)}}{\text{Highest Arm Pressure (mmHg)}}$$

Statistical Methods:

Means and standard deviations were calculated for normally distributed data and compared using a student t-test. Medians and interquartile ranges were calculated for non-normally distributed data and compared using a Mann Whitney U test. Bivariate correlations were calculated for continuous variables. Logistic regression analysis was performed to analyse the relationship between the presence of peripheral vascular disease and a diagnosis of Rheumatoid Arthritis. Odds ratios and 95% confidence intervals were computed. A p-value of <0.05 was considered significant. SPSS 20 was used for all statistical analysis.

Results:

108 recruits had an ankle brachial index (ABI) measured as part of the study. Mean ABI for the total group was 1.035 (+/- 0.071). The mean ABI in the RA group was 1.013 (+/- 0.073) compared with a mean ABI of 1.059 (+/- 0.063) in the OA group, see table 7.1. Using an independent t-test, this difference was found to be significant, $p < 0.001$, see figure 7.1.

Both male and female patients with rheumatoid arthritis had a significantly worse ABI than their OA counterparts, p=0.012 and p=0.043, respectively. Nine patients with rheumatoid arthritis had an abnormal ABI, while 3 of the OA group had an abnormal ABI. Using a Chi^2 test, this was not a significant difference between the groups, p=0.150. No one in the study had a non-compressible ABI.

Using logistic regression analysis, PVD was not found to be associated with markers of inflammation or disease activity scores in the RA group, OR (CI 95%), 1.03 (0.99, 1.07) p=0.211 and OR (CI 95%), 2.18 (0.19, 25.23), p = 0.532, respectively.

ABI was better in RA patients currently on a biologic DMARD, however this difference was not significant, p=0.076 (see table 7.2). E –selectin levels were also associated with ABI in the RA group, r= -0.265, p=0.043. There was no significant correlation between other adhesion molecules (ICAM, VCAM, L-selectin, P-selectin) and ABI, or between inflammatory cytokines and ABI.

ABI was lower in those RA patients with a family history of rheumatoid arthritis, compared to RA patients who had no family member with RA, 0.988 (+/-0.059) versus 1.023 (+/- 0.076), p=0.063.

Four of the RA patients with abnormal ABI measurements were smokers. Four were less than 50 years old and 3 of these had never smoked. RA patients with a family history of peripheral vascular disease (PVD) had a lower ABI than RA patients with a negative family history for PVD, 0.997 (+/-0.063) versus 1.023 (+/- 0.078), p=0.051.

Within the RA group, those who were taking a statin had a significantly lower ABI compared with those RA patients not taking a statin, 0.986 (+/-0.036) versus 1.019(+/-0.078), p=0.046. RA patients with a prior diagnosis of hypercholesterolaemia had a worse ABI than those RA patients with no such prior diagnosis, 0.987 (+/-0.034) versus 1.019 (+/-0.079), p=0.039. Fasting glucose was associated with ABI in the RA group, r= -0.301, p=0.023.

PAI activity levels were associated with ABI in the RA group, r= -0.280, p=0.04. GTN FMD at 4 and 6 minutes was significantly correlated with ABI in RA patients, r = 0.344, p = 0.026 and r = 0.324, p = 0.036 respectively. Common carotid IMT in the RA group was associated with ABI, r = - 0.297, p=0.033. E/E prime, a marker of diastolic dysfunction, also correlated with ABI in the RA group, r = -0.322, p=0.024.

Using logistic regression analysis, the presence of carotid subclinical atherosclerosis, endothelial dysfunction or diastolic dysfunction in the RA and OA groups were not found to be associated with abnormal ankle brachial index (table 7.3).

No associations were found between ankle brachial index and any of the pro-inflammatory cytokines on linear correlation or logistic regression.

In the OA group, there was a significant correlation between smoking pack years and ABI, r= -0.305, p=0.035. This was not seen in the RA group. NT-proBNP level in the OA group also significantly correlated with ABI, r=-0.349, p=0.037.

		Mean ABI	SD	P value
Diagnosis	RA	1.013	0.073	0.001
	OA	1.059	0.063	
Males	RA	0.992	0.075	0.012
	OA	1.064	0.061	
Females	RA	1.026	0.070	0.043
	OA	1.060	0.065	

Table 7.1: Comparison of mean ABI in RA and OA males and females

		Mean ABI (SD)	P
Synthetic DMARD	Yes	1.018 (0.07)	0.436
	No	0.999 (0.09)	
Biologic DMARD	Yes	1.028 (0.09)	0.076
	No	0.996 (0.04)	
Steroid use	Yes	1.010 (0.07)	0.782
	No	1.016 (0.08)	
Statin use	Yes	0.986 (0.04)	0.046*
	No	1.019 (0.08)	
Hypertension	Yes	1.001 (0.09)	0.667
	No	1.015 (0.07)	
Hypercholesterolaemia	Yes	0.987 (0.03)	0.039*
	No	1.019 (0.08)	
Smoker	Yes	0.997 (0.07)	0.061
	No	1.033 (0.08)	
Family history of PVD	Yes	0.997 (0.06)	0.051
	No	1.023 (0.08)	
Family history of RA	Yes	0.988 (0.06)	0.063
	No	1.023 (0.07)	

Table 7.2: Comparison of mean ABI in RA patients with respect to medications used, CVD risks and family history. PVD, peripheral vascular disease; p, significance level; SD, standard deviation.

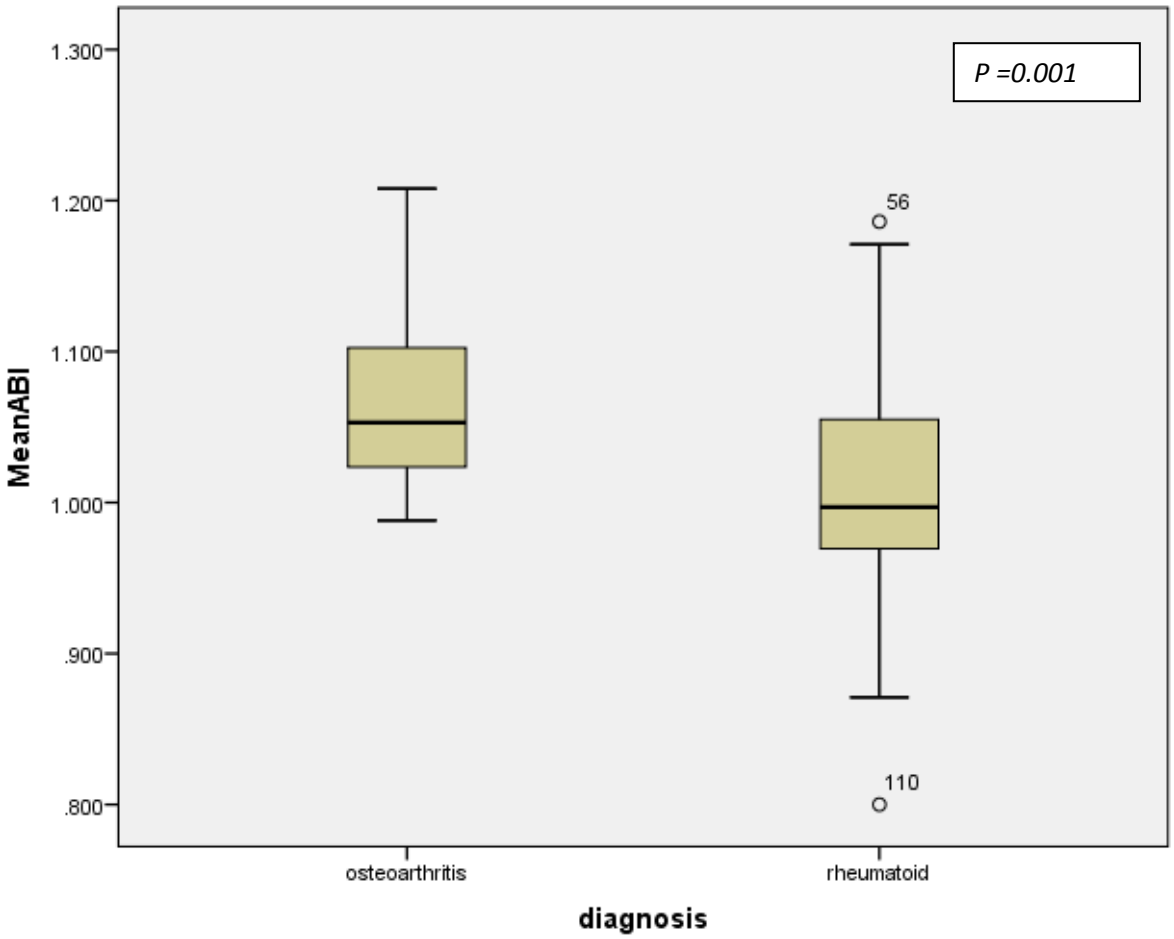


Figure 7.1: Boxplot comparing mean ABI measurements in RA and OA patients, ABI, ankle brachial index.

		Abnormal ABI OR (CI 95%)	p
RA	Endothelial dysfunction	0.329 (0.04, 3.06)	0.327
	Carotid atherosclerosis	1.76 (0.29, 10.58)	0.536
	Diastolic dysfunction	1.62 (0.32, 8.17)	0.567
OA	Endothelial dysfunction	7.5 (0.60, 93.59)	0.118
	Carotid atherosclerosis	1.30 (0.91, 8.02)	0.523
	Diastolic dysfunction	4.14 (0.35, 49.66)	0.262

Table 7.3: Association between ABI and markers of CVD in RA and OA patients. OR, odds ratio, CI, confidence interval; p, significance level.

Discussion:

Ankle brachial index was found to be significantly lower in the RA group compared to the OA group of patients. No association was seen between ABI and inflammatory markers or disease activity scores in RA patients on logistic regression analysis. Sixty-six percent of our RA patients were smokers compared with 33% of the OA group, which may explain the lower mean ABI in the RA group.

In a similar study to ours, Belch et al investigated subclinical markers of atherosclerosis in RA patients and also found that RA patients had a significantly lower ABI than controls (15). In 2005, a study of lower limb arterial blood flow in 234 RA patients found a greater frequency of arterial obstruction and incompressibility compared with controls and this was independent of age, sex, and cardiovascular risk factors (16). However, in contrast to our findings, Gabriel et al in 2012, reported no significant difference in the cumulative incidence of peripheral vascular disease over 10 years in RA and non-RA subjects (6), 1.6 (SE 0.8) versus 1.7 (SE 0.9), p=0.60. Smoking rates in the 2 groups were similar, which may explain the difference from our results.

Abnormal ABI measurement was seen in 15% of the RA group and 6.25% of the OA group, none of whom has symptoms of peripheral vascular disease. The fact that our patients with evidence of PVD, as documented by a low ABI, did not report typical symptoms of PVD is not surprising. Fifty percent of elderly people with documented PVD have no symptoms. Often these people are unable to walk far or fast enough to

reproduce symptoms of intermittent claudication due to arthritis, pulmonary disease and other comorbidities (17). Ahmad et al recently compared ABI in 100 RA patients and 100 controls, all of whom were asymptomatic from a peripheral vascular point of view. They found a rate of 7% for abnormal ABI in the RA group and 5% in the control group (18). The higher rate of PVD in our RA group compared to the above study can be explained by the fact that the mean age in our patients was higher and an abnormal ABI was defined as < 0.96, compared with an ABI value of < 0.9 in Ahmad's group.

In 2013, Guellec et al reported a prevalence of 10% of PVD in RA patients, as defined by an ABI < 0.9. They found that those with a low ABI were older and more often had a history of coronary artery disease compared to those RA patients with a normal ABI. They also reported that 5% of the RA study group had incompressible arteries, as defined by an ABI > 1.3 and diabetes was more common in this group (19).

Subclinical atherosclerosis of the femoral artery, as defined by intima-media thickness and presence of plaque, has been reported as being more advanced in RA patients compared with controls. The same group also found that femoral plaques were less frequent than carotid plaques in RA patients, but were independent of age in contrast to carotid plaque (5)

Overall, the evidence would seem to suggest that RA patients have a higher rate of PVD compared with controls, however, large scale follow-up studies are required to delineate this further.

Inflammation in RA and ABI:

The RA patients were studied with respect to treatment. A lower ABI was seen in those not on a biologic DMARD. This approached statistical significance. TNF inhibitors, the most commonly prescribed biologic DMARD in our study, have previously been shown to have beneficial effects on the progression of subclinical atherosclerosis in RA (20). Choice of anti-rheumatic treatment is an important consideration in RA patients with evidence of cardiovascular disease, as aggressive control of inflammation early in the disease process will also have a positive impact on future cardiovascular events (21).

Levels of E-selectin in the RA group, a pro-inflammatory adhesion molecule, correlated significantly with ABI measurements. There was no significant correlation between other adhesion molecules (ICAM, VCAM, L-selectin, P-selectin) and ABI, or between inflammatory cytokines and ABI.

E-selectin is a known inflammatory mediator in atherosclerosis and RA, so it is not surprising that it is elevated in our RA patients with low ABI.

ABI and Markers of subclinical CVD and traditional cardiovascular risks in RA patients:

Four of our 9 RA patients with an abnormal ABI were non-smokers. Although smoking is the most well-known associated risk factor with PVD, non-smokers are not entirely protected. Lu L et al found that 3.2% of 4231 non-smokers had intermittent claudication and that second-hand smoke exposure was independently associated with intermittent claudication (22).

In this study RA patients with a family history of peripheral vascular disease had a lower ABI than those with no PVD in their family ($p=0.051$). Valentine et al, found that a positive family history of PVD was a major determinant of occult PVD and that it was at least as important as other traditional vascular risks (23).

RA patients in this study, with a prior diagnosis of hypercholesterolaemia had a lower ABI than those with a normal lipid profile. ABI was also found to correlate with fasting glucose levels in RA patients. Dyslipidaemia and diabetes are known risk factors that predispose to peripheral artery disease (24). Given the association of ABI with the above traditional CV risks in our study, rheumatologists need to be vigilant with respect to targeting traditional cardiovascular risks in RA patients to protect the arterial bed.

A number of markers of subclinical atherosclerosis and diastolic dysfunction; nitroglycerin mediated dilatation (NMD) of the brachial artery, common carotid artery intima-media thickness (cIMT) and mitral annular velocity on transthoracic echocardiography, were all found to correlate with ABI in RA patients.

On examination of the brachial artery, no association was found between endothelial dysfunction, as measured by flow mediated dilatation and ABI. However, abnormal nitroglycerin mediated dilatation; a measure of vascular smooth muscle dysfunction was significantly correlated with ABI. Exogenous nitroglycerin causes vasodilatation by direct action on the smooth muscle. Its effect is independent of the endothelium. Early studies of endothelial function have found that smooth muscle dysfunction occurs independently of endothelial –dependent dilatation in adults at risk of atherosclerosis (25). If NMD is impaired, it indicates the probability of smooth muscle cell dysfunction of the arterial wall owing to structural changes in vascular tissues. This phenomenon occurs in systemic sclerosis (26), however it is not typically reported in studies of endothelial dysfunction in RA patients. One small study of 18 patients with early arthritis did find a poorer nitroglycerin mediated response in disease subjects compared to controls (27).

Our findings suggest that damage to the vasculature in RA patients may not be confined to just the endothelium and this warrants further studies to investigate the association further.

Miszalski-Jamka et al recently found an association between cIMT and low ABI and also reported that together they were associated with more extensive coronary artery disease and a higher coronary plaque burden , as seen on CT coronary angiography, than in patients with normal ABI and cIMT measurements (28). As far as we are aware, our study is the first to show an association between abnormal ABI and cIMT in RA patients.

Yamasaki et al, examined 120 patients with an ABI <0.9 and found an elevated BNP in 30% and also reported a strong association with E/E prime, a marker of diastolic dysfunction (29). This is one of the first studies to identify a high prevalence of diastolic dysfunction in PVD.

Further, activity levels of plasminogen activator inhibitor 1 (PAI-1), a potent inhibitor of fibrinolysis, correlated significantly with ABI in RA patients. Markers of a hypercoagulable state, including PAI-1 are known to be associated with the presence of PVD and increased levels of PAI-1 may be linked to peripheral arterial disease severity (30). Kitas et al have found that having RA predicts increased levels of PAI-1 and other markers of fibrinolysis, including fibrinogen, thrombomodulin and protein C. They also reported a link between metabolic factors such as hypertriglyceridaemia and insulin resistance and PAI-1 levels in RA (31).

The association between PVD and coagulation in our RA patients suggests that factors such as an imbalance in coagulation and inflammation, in addition to atherosclerosis, are involved in the pathogenesis of cardiovascular disease in RA patients.

Interestingly, we did not find correlations between ABI and the above markers of subclinical atherosclerosis in the OA group.

Conclusions:

PVD is less well studied than coronary artery disease in RA patients. The limited data available suggests than there is an increased prevalence of peripheral arterial disease compared to controls. However, whether this difference is driven by the inflammatory burden of RA or excess traditional risk factors has not been fully identified.

We have shown that ABI measurement would be useful to identify silent PVD in RA patients, highlighted by the fact that 15% of our RA group have abnormally low ABI. This cheap, easily performed screening tool for systemic and well as peripheral artery disease may be particularly helpful in RA patients who do not complain of the classical claudication symptoms due to their primary disease limiting exercise capacity.

A link has been shown between ABI and other cardiovascular disease measures, such as carotid IMT and diastolic function, indicating that abnormal ABI in RA patients coexists with abnormalities in other vascular beds.

Finally, although we cannot attribute a treatment benefit to biologic agents with respect to ABI in our group due to the cross-sectional nature of our study, it does support multiple other published studies, that have found a CV protective effect from aggressive anti-inflammatory therapy.

References:

1. Writing Group M, Writing Committee M, Accf/Aha Task Force M. 2011 ACCF/AHA Focused Update of the Guideline for the Management of patients with peripheral artery disease (Updating the 2005 Guideline): a report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. Circulation. 2011;124(18):2020-45. Epub 2011/10/01.

2. Smith SC, Jr., Milani RV, Arnett DK, Crouse JR, 3rd, McDermott MM, Ridker PM, et al. Atherosclerotic Vascular Disease Conference: Writing Group II: risk factors. Circulation. 2004;109(21):2613-6. Epub 2004/06/03.

3. Aronow WS, Ahn C. Prevalence of coexistence of coronary artery disease, peripheral arterial disease, and atherothrombotic brain infarction in men and women > or = 62 years of age. The American journal of cardiology. 1994;74(1):64-5. Epub 1994/07/01.

4. Criqui MH, McClelland RL, McDermott MM, Allison MA, Blumenthal RS, Aboyans V, et al. The ankle-brachial index and incident cardiovascular events in the MESA (Multi-Ethnic Study of Atherosclerosis). Journal of the American College of Cardiology. 2010;56(18):1506-12. Epub 2010/10/19.

5. Stamatelopoulos KS, Kitas GD, Papamichael CM, Kyrkou K, Zampeli E, Fragiadaki K, et al. Subclinical peripheral arterial disease in rheumatoid arthritis. Atherosclerosis. 2010;212(1):305-9. Epub 2010/06/18.

6. Bacani AK, Gabriel SE, Crowson CS, Heit JA, Matteson EL. Noncardiac vascular disease in rheumatoid arthritis: increase in venous thromboembolic events? Arthritis and rheumatism. 2012;64(1):53-61. Epub 2011/09/10.

7. Han C, Robinson DW, Jr., Hackett MV, Paramore LC, Fraeman KH, Bala MV. Cardiovascular disease and risk factors in patients with rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis. The Journal of rheumatology. 2006;33(11):2167-72. Epub 2006/09/19.

8. Newman AB, Siscovick DS, Manolio TA, Polak J, Fried LP, Borhani NO, et al. Ankle-arm index as a marker of atherosclerosis in the Cardiovascular Health Study. Cardiovascular Heart Study (CHS) Collaborative Research Group. Circulation. 1993;88(3):837-45. Epub 1993/09/01.

9. van der Meer IM, Bots ML, Hofman A, del Sol AI, van der Kuip DA, Witteman JC. Predictive value of noninvasive measures of atherosclerosis for incident myocardial infarction: the Rotterdam Study. Circulation. 2004;109(9):1089-94. Epub 2004/03/03.

10. Ankle Brachial Index C, Fowkes FG, Murray GD, Butcher I, Heald CL, Lee RJ, et al. Ankle brachial index combined with Framingham Risk Score to predict cardiovascular events and mortality: a meta-analysis. JAMA : the journal of the American Medical Association. 2008;300(2):197-208. Epub 2008/07/10.

11. Resnick HE, Lindsay RS, McDermott MM, Devereux RB, Jones KL, Fabsitz RR, et al. Relationship of high and low ankle brachial index to all-cause and cardiovascular disease mortality: the Strong Heart Study. Circulation. 2004;109(6):733-9. Epub 2004/02/19.

12. McDermott MM, Liu K, Criqui MH, Ruth K, Goff D, Saad MF, et al. Ankle-brachial index and subclinical cardiac and carotid disease: the multi-ethnic study of

atherosclerosis. American journal of epidemiology. 2005;162(1):33-41. Epub 2005/06/18.

13. Graham I, Atar D, Borch-Johnsen K, Boysen G, Burell G, Cifkova R, et al. European guidelines on cardiovascular disease prevention in clinical practice: full text. Fourth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). European journal of cardiovascular prevention and rehabilitation : official journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology. 2007;14 Suppl 2:S1-113. Epub 2007/11/06.

14. Daigle RJ. Techniques in non-invasive vascular diagnosis.

15. Alkaabi JK, Ho M, Levison R, Pullar T, Belch JJ. Rheumatoid arthritis and macrovascular disease. Rheumatology. 2003;42(2):292-7. Epub 2003/02/22.

16. del Rincon I, Haas RW, Pogosian S, Escalante A. Lower limb arterial incompressibility and obstruction in rheumatoid arthritis. Annals of the rheumatic diseases. 2005;64(3):425-32. Epub 2004/07/24.

17. McDermott MM, Greenland P, Liu K, Guralnik JM, Criqui MH, Dolan NC, et al. Leg symptoms in peripheral arterial disease: associated clinical characteristics and functional impairment. JAMA : the journal of the American Medical Association. 2001;286(13):1599-606. Epub 2001/10/05.

18. Ahmad S, Garg S, Dhar M, Srivastava S, Biswas D, Barthwal SP, et al. Predictors of atherosclerosis in rheumatoid arthritis. VASA Zeitschrift fur Gefasskrankheiten. 2012;41(5):353-9. Epub 2012/08/24.

19. Guellec D, Bressollette L, Gueguen F, Jousse-Joulin S, Marhadour T, Devauchelle-Pensec V, et al. Is routine ankle-brachial pressure index evaluation useful in rheumatoid arthritis? Joint, bone, spine : revue du rhumatisme. 2013;80(1):111-3. Epub 2012/07/31.

20. Gonzalez-Juanatey C, Vazquez-Rodriguez TR, Miranda-Filloy JA, Gomez-Acebo I, Testa A, Garcia-Porrua C, et al. Anti-TNF-alpha-adalimumab therapy is associated with persistent improvement of endothelial function without progression of carotid intima-media wall thickness in patients with rheumatoid arthritis refractory to conventional therapy. Mediators of inflammation. 2012;2012:674265. Epub 2012/08/18.

21. Dixon WG, Watson KD, Lunt M, Hyrich KL, British Society for Rheumatology Biologics Register Control Centre C, Silman AJ, et al. Reduction in the incidence of myocardial infarction in patients with rheumatoid arthritis who respond to anti-tumor necrosis factor alpha therapy: results from the British Society for Rheumatology Biologics Register. Arthritis and rheumatism. 2007;56(9):2905-12. Epub 2007/09/01.

22. Lu L, Mackay DF, Pell JP. Meta-analysis of the association between cigarette smoking and peripheral arterial disease. Heart. 2013. Epub 2013/08/08.

23. Valentine RJ, Guerra R, Stephan P, Scoggins E, Clagett GP, Cohen J. Family history is a major determinant of subclinical peripheral arterial disease in young adults. Journal of vascular surgery. 2004;39(2):351-6. Epub 2004/01/27.

24. Ness J, Aronow WS, Newkirk E, McDanel D. Prevalence of symptomatic peripheral arterial disease, modifiable risk factors, and appropriate use of drugs in

the treatment of peripheral arterial disease in older persons seen in a university general medicine clinic. The journals of gerontology Series A, Biological sciences and medical sciences. 2005;60(2):255-7. Epub 2005/04/09.

25. Adams MR, Robinson J, McCredie R, Seale JP, Sorensen KE, Deanfield JE, et al. Smooth muscle dysfunction occurs independently of impaired endothelium-dependent dilation in adults at risk of atherosclerosis. Journal of the American College of Cardiology. 1998;32(1):123-7. Epub 1998/07/21.

26. Soltesz P, Der H, Kerekes G, Szodoray P, Szucs G, Danko K, et al. A comparative study of arterial stiffness, flow-mediated vasodilation of the brachial artery, and the thickness of the carotid artery intima-media in patients with systemic autoimmune diseases. Clinical rheumatology. 2009;28(6):655-62. Epub 2009/02/19.

27. Foster W, Lip GY, Raza K, Carruthers D, Blann AD. An observational study of endothelial function in early arthritis. European journal of clinical investigation. 2012;42(5):510-6. Epub 2011/10/12.

28. Miszalski-Jamka T. Comment on "Coronary artery calcium in type 2 diabetes: a nested case-control study". Polskie Archiwum Medycyny Wewnetrznej. 2013;123(3):132. Epub 2013/03/29.

29. Yamasaki S, Izawa A, Shiba Y, Tomita T, Miyashita Y, Koyama J, et al. Presence of diastolic dysfunction in patients with peripheral artery disease. Angiology. 2013;64(7):540-3. Epub 2013/02/13.

30. Mota AP, de Castro Santos ME, Lima e Silva F, de Carvalho Schachnik NC, de Oliveira Sousa M, das Gracas Carvalho M. Hypercoagulability markers in

patients with peripheral arterial disease: association to ankle-brachial index. Angiology. 2009;60(5):529-35. Epub 2008/11/19.

31. Dimitroulas T, Douglas KM, Panoulas VF, Toms T, Smith JP, Treharne GJ, et al. Derangement of hemostasis in rheumatoid arthritis: association with demographic, inflammatory and metabolic factors. Clinical rheumatology. 2013;32(9):1357-64. Epub 2013/05/16.

Chapter 8

Diastolic function in Rheumatoid Arthritis

Introduction:

Diastolic dysfunction, an echocardiographic diagnosis, occurs due to increased stiffness of the left ventricular wall, which impairs diastolic blood flow from the left atrium to the left ventricle. Reports of the prevalence of diastolic dysfunction in the general population vary from 28% (1) to 65% (2). Clinical conditions responsible for primary diastolic dysfunction include hypertension, coronary artery disease, cardiomyopathy and obesity. It occurs frequently without clinical symptoms and is associated with an increase in mortality and incident congestive heart failure in the general population (1, 3).

A number of studies have found an increased prevalence of diastolic dysfunction in RA compared to healthy controls (4-6). Crowson et al reported the prevalence of diastolic dysfunction in RA to be approximately 37% (4). The reasons for this increased rate in RA patients has not been fully elucidated, however; chronic inflammation, disease duration and disease activity have been postulated as potential mechanisms.

The relative risk of congestive heart failure in patients with rheumatoid arthritis has been reported as 1.43 (95% CI 1.24-1.33) compared with osteoarthritis controls (7). In 2008, Gabriel et al compared the clinical presentation and management of heart

failure in RA patients and the general population. They found that RA patients were less likely to present with paroxysmal nocturnal dyspnoea, dyspnoea on exertion or orthopnoea than non-RA patients (8). Interestingly, RA patients were 33% less likely than non-RA patients to undergo echocardiographic assessment for their symptoms, therefore highlighting that RA patients receive different evaluation and management compared to their non-RA counterparts. They postulated that this may have been due to differences in clinical presentations between the 2 groups.

In 2009, the American Society of Echocardiography published recommendations for the evaluation of left ventricular diastolic function in the Journal of the American Society of Echocardiography (9). In these guidelines a practical approach to grading diastolic dysfunction is explained.

Summarized as follows:

Normal diastolic function is defined as: septal e' ≥ 8 cm/sec, lateral e' ≥ 10 cm/sec, LA volume < 34 ml/m^2

Grade I diastolic dysfunction as: septal e' < 8cm/sec, lateral e' < 10cm/sec, LA ≥ 34 ml/m2, E/A < 0.8 and DT > 200 ms, E/e' average < 8.

Grade II diastolic dysfunction as: septal e' < 8cm/sec, lateral e' < 10cm/sec, LA ≥ 34ml/m^2, E/A 0.8-1.5, DT 160-200ms, E/e' average 9-12.

Grade III diastolic dysfunction as: septal e' < 8 cm/sec, lateral e' < 10 cm/sec, LA ≥ 34 ml/m^2, E/A > 2, DT < 160ms, E/e' average > 13.

NT-proBNP and BNP levels are recognised non-invasive markers of LV dysfunction (10). Recent studies have found that levels of NT-proBNP correlate with conventional Doppler and tissue Doppler imaging parameters used for the diagnosis of diastolic dysfunction on echocardiography (11-13). RA patients have been shown to have elevated levels of BNP, however BNP may not be as useful as a screening tool of LV diastolic dysfunction in RA patients as it is in the general population (4).

To date, the presence of diastolic function in RA has not been studied extensively in the Irish population.

Aims:

Our primary aim was to compare echocardiographic markers of diastolic function in RA and OA patients without clinical evidence of cardiovascular disease. Secondary aims were to examine systolic function on transthoracic echocardiogram and to investigate if markers of inflammation, anti-rheumatic medications and cardiovascular risks were associated with diastolic function in either group.

Methods:

Transthoracic Echocardiography:

A transthoracic echocardiogram was performed on 51 RA patients and 46 OA patients, using a Vivid–I GE-Medical portable echocardiography machine with a 2.5Hz probe. Using 2-D and M-mode echo, measurements were taken for ejection fraction, fractional shortening, left ventricular (LV) wall size and LV diameter. LV mass in grams was calculated based on Devereaux's formula, a validated method and recommended by the ASE as part of the assessment of LV function. All measurements were made in accord with ASE guidelines.

Mitral inflow velocities were measured using pulsed wave Doppler to assess LV filling. These included peak early filling velocity, m/s (E wave), late diastolic filling velocity, m/s (A wave), E/A ratio and deceleration time (DT) of early filling velocity

in milliseconds. Tissue Doppler imaging (TDI) was utilised to acquire lateral and septal mitral annular velocities including; systolic velocity (s), early diastolic velocity (e') and late diastolic velocity (a'). E/e' septal and E/e' lateral ratios and their average (E/e' average) were calculated.

NT-proBNP analysis:

At the time of the echocardiogram, blood was drawn for NT-proBNP. Samples were centrifuged and serum pipetted into aliquots. Serum was then stored at minus 80 degrees and samples were analysed in batches. The Roche cobas proBNP II kit was used on an Elecsys 1010 analyzer in the department of biochemistry, in Cork University Hospital, to analyse all serum samples. Samples were analysed according to the manufacturers' standard operating procedures.

Statistical Methods:

Means and standard deviations were calculated for normally distributed data and compared using a student t-test. Medians and interquartile ranges were calculated for non-normally distributed data and compared using a Mann Whitney U test. Logistic regression analysis was performed to analyse the relationship between the presence of diastolic dysfunction and a diagnosis of Rheumatoid Arthritis. Odds ratios and 95% confidence intervals were computed. A p-value of <0.05 was considered significant. SPSS 20 was used for all statistical analysis.

Results:

Systolic Function:

96 of the 111 total study group attended for their outpatient echocardiogram appointment. Systolic function was measured as described in chapter 2. Measures of systolic function are reported below as means (SD) and compared using an independent t-test. Mean aortic outlet size in the RA group was 2.59 cm (+/- 0.36) and 2.61 cm (+/- 0.34) in the OA group. The groups were compared using an independent t-test and no statistically significant difference was found (p= 0.890).

Left atrial diameter was similar in the RA and OA groups, 3.30 cm (+/- 0.47) vs 3.35 cm (+/- 0.42) respectively, p = 0.618. Overall, males had a greater left atrial size than females, 3.58 cm (+/- 0.47) vs 3.21 cm (+/- 0.38), p = 0.000.

The ratio of left atrial diameter to aortic outlet diameter (LA/Ao) was similar in the RA and OA groups, 1.29 (+/- 0.19) vs 1.30 (+/- 0.21), p = 0.707.

Left ventricular systolic diameter (LVSD) was similar in RA and OA patients. Mean LVSD in the RA group was 3.09 cm (+/- 0.49), compared with 3.06 cm (+/- 0.34) in the OA group, p = 0.754. LVSD was significantly larger in males compared with females, 3.25 cm (+/- 0.51) vs 3.01 cm (+/- 0.38), p = 0.018. This was also the case when the RA group was analysed separately, RA male LVSD 3.28 cm (+/-0.54) versus RA females LVSD 2.97 cm (+/- 0.42), p = 0.024. There was no significant difference in LVSD between OA males and OA females, p = 0. 418.

Left ventricular diastolic diameter (LVDD) was not different in RA patients and OA patients, 4.55 cm (+/-0.60) and 4.43 cm (+/- 0.42), p = 0.215. Males had a significantly larger LVDD compared with females, 4.83cm (+/-0.59) vs 4.36cm (+/- 0.43), p = 0.000. Male RA patients had a mean LVDD of 4.94 cm (+/- 0.61) compared with 4.31 cm (+/- 0.45) for female RA patients, p < 0.000 (figure 8.1). There was no significant difference in LVDD between male and female OA patients, p = 0.443. When males were analysed separately, RA males were found to have a significantly larger LVDD compared with male OA patients, 4.94 cm (+/- 0.61) vs 4.53 cm (+/- 0.43), p = 0.044. Female OA patients and female RA patients had similar LVDDs, p = 0.335.

Mean septal wall thickness in the RA group was 0.93 cm (0+/- 0.12) and 0.93 cm (+/-0.13) for the OA group, p = 1.00. In the RA group, mean posterior wall thickness was 0.96cm (+/- 0.12) and it was 0.99 cm (+/-0.14) in the OA group, p = 0.225.

Mean ejection fraction in the RA group was 61% (+/- 8.28) compared with 60% (+/- 6.29) in the OA group, p = 0.426, (table 8.1). Ejection fraction was also similar in males and females. Looking at the RA group separately, there was no significant difference between ejection fractions in males and females, 63% (+/- 8.27) vs 60% (+/- 8.26), p = 0.272. Again using an independent t-test and looking at the OA group alone, there was no significant difference in ejection fractions in males and females, 61% (+/- 7.10) vs 60% (+/- 6.17), p = 0.680.

5 patients (3 with RA and 2 with OA) had an ejection fraction of less than 55%. Nobody had an EF of less than 45%.

Mean fractional shortening (%) in the RA group was 33% (+/- 5.98) compared with 31% (+/- 4.42) in the OA group, $p = 0.155$. Using an independent t-test, the difference in fractional shortening between males and females approached statistical significance, $p = 0.051$.

LV mass in grams was calculated based on Devereaux's formula, a validated method and recommended by the ASE as part of the assessment of LV function.

$$1.04[(LVDd + PW + SW)^3 - (LVDd)^3] \times 0.8 + 0.6g$$

Mean LV mass in the RA group was 147 g (+/- 45.47) compared with 142 g (+/- 30.57) in the OA group, $p = 0.467$. As expected, the mean LV mass was significantly greater in males than females, 169.95 g (+/- 46.33) versus 134.47 g (+/- 30.94) respectively, $p < 0.000$.

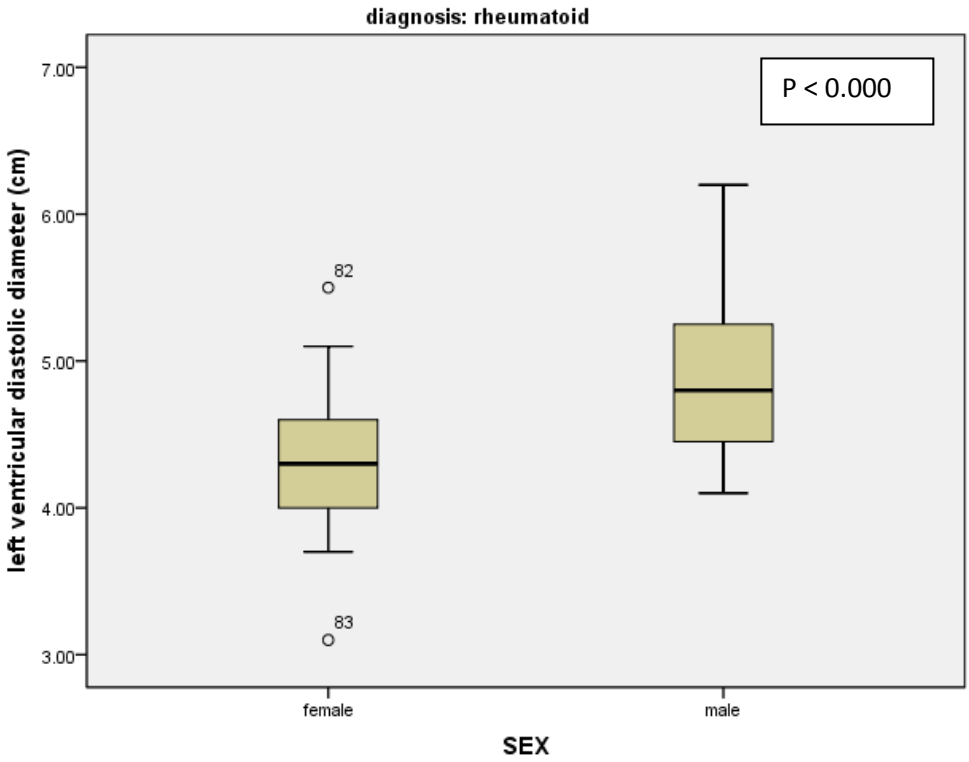


Figure 8.1: Boxplots comparing LVDD (cm) in male and female RA patients

	RA N = 63	OA N = 48	P value
LA (cm), mean (SD)	3.30 (0.47)	3.35 (0.42)	0.618
LVSD (cm), mean (SD)	3.09 (0.49)	3.06 (0.34)	0.754
LVDD (cm), mean (SD)	4.55 (0.60)	4.43 (0.42)	0.215
Posterior Wall (cm)	0.96 (0.12)	0.99 (0.14)	0.225
Septal Wall (cm)	0.93 (0.12)	0.93 (0.13)	1.000
LV mass (grams), mean (SD)	147 (45.47)	142 (30.57)	0.467
Ejection Fraction (%), mean (SD)	61 (8.28)	60 (6.29)	0.426
Fractional Shortening (%), mean (SD)	33 (5.98)	31 (4.42)	0.155

Table 8.1: 2D and M-mode echocardiographic results for RA and OA patients.

Diastolic Function:

The mean E wave velocity was greater in the RA group compared with the OA group, p = 0.043, (table 8.2). E wave velocities were similar in males and females, 0.63 m/sec (+/- 0.98) vs 0.64 m/sec (+/- 0.14), p = 0.601. Within the RA group, E wave velocities were similar for males and females. Similar velocities were also recorded for males and females in the OA group. Comparing females in the RA and OA groups, there was no statistically significant difference in mean E wave velocities, p = 0.121. However, male RA patients had a significantly greater E wave velocity compared with male OA patients, 0.65 m/sec (+/- 0.10) vs 0.58 m/sec (+/- 0.06), p = 0.015.

A wave velocities were similar in the RA and OA group, 0.60 m/sec (+/- 0.11) vs 0.57 m/sec (+/- 0.15), p = 0.226. Males in the RA group had a significantly greater A wave velocity than male OA patients, p = 0.004.

Mean E/A ratio in the RA group was 1.11 (+/- 0.27), compared with 1.11 (+/- 0.19), p = 0.915. E/A values were also very similar in male and females, 1.11 (+/- 0.18) vs 1.11 (+/- 0.26), p = 0.973. This finding held through when the RA and OA groups were analysed separately

Deceleration time was similar for RA and OA patients, 188.67 msec (SD 29.62) compared with 191.19 msec (SD36.71), p=0.701.

Mean values for e' lateral readings were 12.25 cm/sec (+/- 3.45) in the RA group and 11.70 cm/s (+/- 2.32) in the OA group, p = 0.347. Mean E prime septal was significantly higher in the RA group, 8.88 cm/sec (+/- 2.23) versus 7.74 cm/sec (+/- 1.73), p = 0.006, (table 3).

Mean E/e' lateral was similar in RA patients and OA patients, p = 0.264. Mean E/e' septal was 7.71 (+/- 2.19) in the RA group, compared with 8.21 (+/- 2.38) in the OA group, p = 0.293. When the values for E/e' lateral and septal were combined, as recommended by the ASE, the results were similar in the RA and OA groups, p = 0.830.

Age in both the RA and OA groups was significantly correlated with E/e', r= 0.283, p=0.049 and r=0.439, p=0.002.

	RA N = 63	OA N = 48	P value
E wave (cm/sec), mean (SD)	66.33 (11.48)	61.33 (13.37)	0.043*
A wave (cm/sec), mean (SD)	59.82 (10.81)	56.69 (14.93)	0.226
E/A ratio, mean (SD)	1.11 (0.27)	1.11 (0.19)	0.915
DT (msec), mean (SD)	188.67 (29.62)	191.19 (36.71)	0.701
e’ lateral (cm/sec), mean (SD)	12.25 (3.45)	11.70 (2.32)	0.347
e' septal (cm/sec), mean (SD)	8.88 (2.23)	7.74 (1.73)	0.006*
E/e' lateral ratio, mean (SD)	5.56 (1.47)	5.34 (1.25)	0.264
E/e' septal ratio, mean (SD)	7.71 (2.19)	8.21 (2.38)	0.293
E/e' average ratio, mean (SD)	6.47 (1.53)	6.40 (1.46)	0.830

Table 8.2: Mitral inflow velocities and tissue Doppler measurements for RA and OA patients

Two RA patients and 4 OA patients had an elevated BNP as defined as a value of greater than 125pg/ml. Median N-terminal pro-BNP in the RA group was 45.90 pg/ml (33.00- 71.97) compared with 48.75 pg/ml (33.50- 68.48) in the OA group, p = 0.729. Using logistic regression analysis, elevated BNP was not associated with a diagnosis of RA, OR (95%), 0.29 (0.05, 1.68), p=0.167.

Females had a significantly higher NTpro-BNP than males, 55.30 pg/ml (38.94- 76.78) vs 33.62 pg/ml (15.90- 42.97), p < 0.000. When the RA group were analysed separately, the female group still had a significantly higher value for pro-BNP than male RAs, p <0.000.

There was no significant correlation between echocardiographic markers of diastolic dysfunction and serum NT-proBNP in RA patients. However, we did find that ESR correlated with NT-proBNP in the RA group and this approached statistical significance, r = 0.244, p=0.067.

N-terminal pro-BNP was similar in RA patients on DMARDS, biologics, NSAID and steroids compared to RA patients not on the above medications, p=0.885. NTpro-BNP was significantly higher in RA patients with an elevated waist hip ratio, ie abdominal obesity, 51pg/ml (37.00 – 66.15) versus 45.75pg/ml (33.50 – 62.78), p=0.032.

OA patients with an elevated BNP had a significantly lower ejection fraction and fractional shortening than those with BNP of less than 125pg/mg (table 8.3).

	Elevated BNP	Normal range BNP	p
Ejection fraction %, (SD)	57.00 (1.41)	60.28 (6.31)	0.021*
Fractional shortening %, (SD)	29.25 (0.50)	31.69 (4.42)	0.005*

Table 8.3: Comparison of ejection fraction and fractional shortening in OA patients with elevated and normal BNP levels. P, significance level, SD, standard deviation.

Based on the ASE criteria for a diagnosis of diastolic dysfunction, as described in the introduction, we identified 17 (33.3%) RA patients and 16 (34.8%) OA patients with diastolic dysfunction (table 8.4). There was no difference in the frequency of grade I or grade II diastolic dysfunction between the 2 groups. No one in the group had grade III diastolic dysfunction.

Binary logistic regression analysis was used to analyse the relationship between diastolic dysfunction and a diagnosis of rheumatoid arthritis. Using logistic regression analysis and taking an E/A ratio of less than 1 as a marker of diastolic dysfunction, a diagnosis of Rheumatoid arthritis was not found to predict the presence of diastolic dysfunction, more so than a diagnosis of osteoarthritis, OR (95%CI), 1.22 (0.52,2.67), p 0.626. Following simultaneous adjustment for age, gender, smoking history, presence of hypertension or diabetes, level of BNP, TNF, Il6 and CRP, the odds of having diastolic dysfunction with Rheumatoid arthritis was not significantly higher than that for osteoarthritis, OR (95%CI), 1.27 (0.36,4.53), p 0.710. Neither was a diagnosis of RA associated with an abnormal e' lateral, OR (CI 95%), 1.31 (0.47, 3.60), p=0.605 or an abnormal E/e' average, OR (CI 95%), 1.60 (0.48, 5.30), p = 0.442. Using the ASE criteria to diagnose diastolic dysfunction with a combination of echocardiographic measurements, as described in the methods section, a diagnosis of RA was not found to be associated with diastolic dysfunction, OR (CI 95%), 0.94 (0.40, 2.17), p = 0.880.

		RA n=51	OA n=46	Total n=97	P value
Normal Diastolic Function	N	34	30	64	
	%	66.7	65.2	66	
Grade I Diastolic Dysfunction	N	9	10	19	0.612
	%	17.6	21.7	19.6	
Grade 2 Diastolic Dysfunction	N	8	6	13	0.487
	%	15.7	10.9	13.4	
Grade 3 Diastolic Dysfunction	N	0	0	0	N/A
	%	0	0	0	
Presence of any grade Diastolic Dysfunction	N	17	16	33	0.880
	%	33.3	34.8	34	

Table 8.4: Numbers of RA and OA patients with diastolic dysfunction, using ASE diagnostic criteria

Disease activity, anti-rheumatic medications and diastolic function:

RA patients with a high DAS28ESR and DAS28CRP of greater than 3.2 (ie moderate or high disease activity) were compared to those with low disease activity, defined by both a DAS28ESR and DAS28CRP of less than 3.2. There was no significant difference with respect to markers of diastolic function between the 2 groups. However, E/A ratio was lower in RA patients with moderate or high disease activity, 1.06 (SD 0.24), compared with 1.21 (SD 0.30) in the RA group with low disease activity. This approached statistical significance, p=0.06.

RA patients on a DMARD had a significantly higher E/A ratio, e' lateral and e' septal, compared with those RA patients not taking a DMARD, p=0.004, p=0.048, p=0.024, respectively (table 8.5). RA patients on a biologic agent had less features of diastolic dysfunction than those not on a biologic (table 8.6). The E/A was higher in those on a biologic agent, however this did not achieve statistical significance, p=0.668.

Using logistic regression analysis, a diagnosis of diastolic dysfunction was not found to be associated with the use of synthetic or biologic DMARDS, OR (CI 95%), 0.38 (0.09, 1.50), p=0.172 and OR (CI 95%), 0.91 (0.27, 3.10), p=0.885.

RA patients on an NSAID has significantly lower fractional shortening, ejection fraction and E velocity compared to those RA patients not on NSAIDS, p=0.008, p=0.009, p=0.04 (table 8.7). There was no difference in markers of diastolic function between RA patients on steroids and those not taking steroids.

Left atrial size, although within the normal range, was significantly larger in OA patients who were taking an NSAID at the time of the study compared to those not taking NSAIDS, 3.55 cm (SD0.45) versus 3.25 cm (SD0.37), p=0.033.

	DMARD	No DMARD	p
E/A (SD)	1.41 (0.29)	1.02 (0.17)	0.044*
e' lateral, cm/s (SD)	12.74 (3.51)	10.67 (2.84)	0.048*
e' septal, cm/s (SD)	9.21 (2.29)	7.73 (1.62)	0.024*
E/e' (SD)	6.27 (1.44)	7.16 (1.71)	0.135

Table 8.5: Comparison of markers of diastolic function in RA patients on a DMARD with those not on a DMARD, p, significance level; SD, standard deviation

	Current biologic agent	No biologic agent	p
Posterior wall thickness, cm (SD)	0.92 (0.13)	0.99 (0.12)	0.016*
Septal wall thickness, cm (SD)	0.89 (0.11)	0.94 (0.13)	0.035*
E velocity, cm/s (SD)	68.22 (11.78)	62.38 (12.53)	0.024*
E/A ratio (SD)	1.13 (0.29)	1.10 (0.21)	0.668
E/e' (SD)	6.58 (1.67)	6.39 (1.44)	0.640

Table 8.6: comparison of markers of diastolic function between RA patients on a biologic agent and those not on a biologic agent. P, significance level; SD, standard deviation

	Current NSAID	No current NSAID	p
Fractional shortening % (SD)	30.90 (5.31)	34.93 (6.04)	0.008*
Ejection fraction % (SD)	58.35 (7.74)	63.86 (8.00)	0.009*
E velocity, cm/s (SD)	63.39 (10.42)	69.48 (11.89)	0.040*
E/A ratio (SD)	1.08 (0.27)	1.13 (0.26)	0.465
E/e' (SD)	6.28 (1.45)	6.70 (1.63)	0.361

Table 8.7: Comparison of markers of systolic and diastolic function in RA patients on an NSAID with RA patients not currently on an NSAID. P, significance level; SD standard deviation.

Cardiovascular risks and diastolic function:

RA patients with a prior diagnosis of hypertension had a significantly greater E/e' than those with normal blood pressure, 7.79 (SD1.63) versus 6.21 (SD1.39), p=0.029. Using logistic regression analysis, a diagnosis of diastolic dysfunction was associated with hypertension in the RA group, OR (95%), 8.73 (1.53, 49.76), p=0.015. However, following simultaneous adjustment for age, smoking and obesity, this association was no longer statistically significant, OR (95%), 5.70 (0.73, 44.46), p=0.097.

A higher E/e' was also seen in RA patients with an elevated serum cholesterol, 6.71 (SD1.65) compared with 5.79 (0.89), p=0.017. As already discussed in the chapter on fasting lipid profile, RA patients with evidence of diastolic dysfunction on transthoracic echocardiogram had a less favourable lipid profile than RA patients with normal diastolic function (table 8.8). In both RA and OA patients, there was a significant correlation between lipid profile and E/e', as a marker of diastolic dysfunction on echocardiography (table 8.9).

Smoking status did not affect markers of diastolic function in either RA or OA patients. OA patients with a family history of myocardial infarction had a significantly lower ejection fraction than those with no such family history, 58.41% (SD 6.19) versus 62.16% (SD 5.89), p=0.041.

	Evidence of diastolic dysfunction	Normal diastolic function	p
Total cholesterol, mmol/l (SD)	5.79 (0.64)	5.37 (0.79)	0.048*
Serum LDL, mmol/l (SD)	3.55 (0.54)	3.14 (0.59)	0.025*

Table 8.8: Comparison of lipid profile in RA patients with and without evidence of diastolic dysfunction

		Correlation with E/e', r	p
RA	Total cholesterol	0.255	0.077
	LDL	0.508	0.001*
OA	Total cholesterol	0.316	0.034*
	LDL	0.227	0.139

Table 8.9: Correlation between E/e’ and lipid profile in RA and OA patients

Both RA and OA patients with presence of plaque on carotid ultrasound had significantly higher E/e' ratios compared to those with no evidence of carotid plaque (table 8.10, figure 8.2 and 8.3). E/e' correlated significantly with ABI in the RA group, r = -0.322, p=0.024 and also with microalbuminuria in the RA group, r = 0.402, p=0.009.

		E/E prime (SD)	p
RA	Carotid plaque present	8.77 (0.16)	0.000*
	No carotid plaque present	6.31 (1.48)	
OA	Carotid plaque present	9.00 (1.26)	0.005*
	No carotid plaque present	6.09 (1.10)	

Table 8.10: comparison of patients with and without carotid plaque with respect to E/E prime ratio. P, significance level; SD, standard deviation.

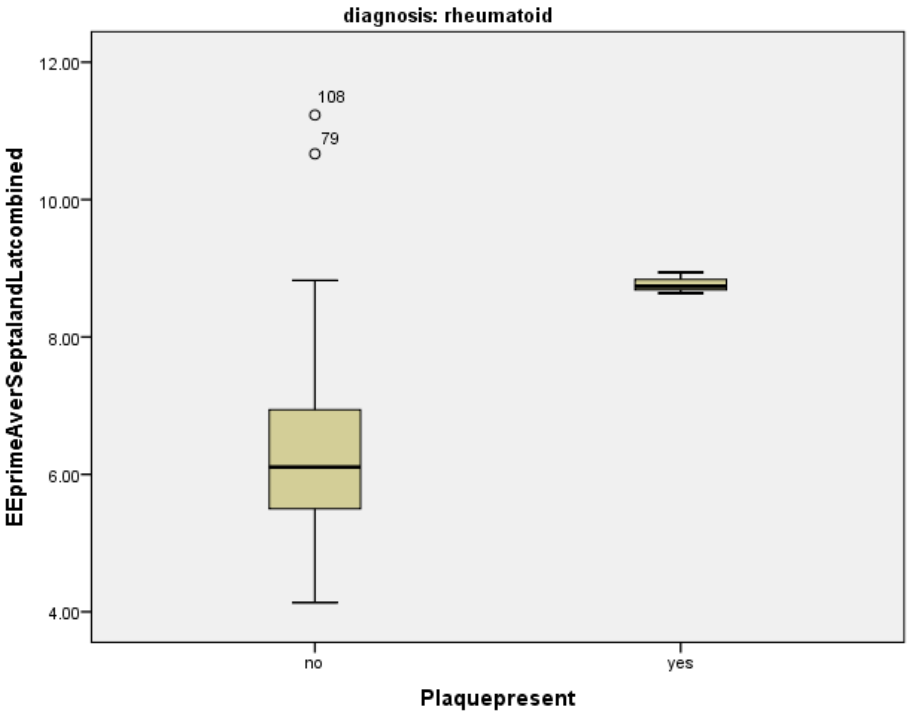


Figure 8.2: Boxplot comparing E/E prime in RA patients with and without carotid plaque

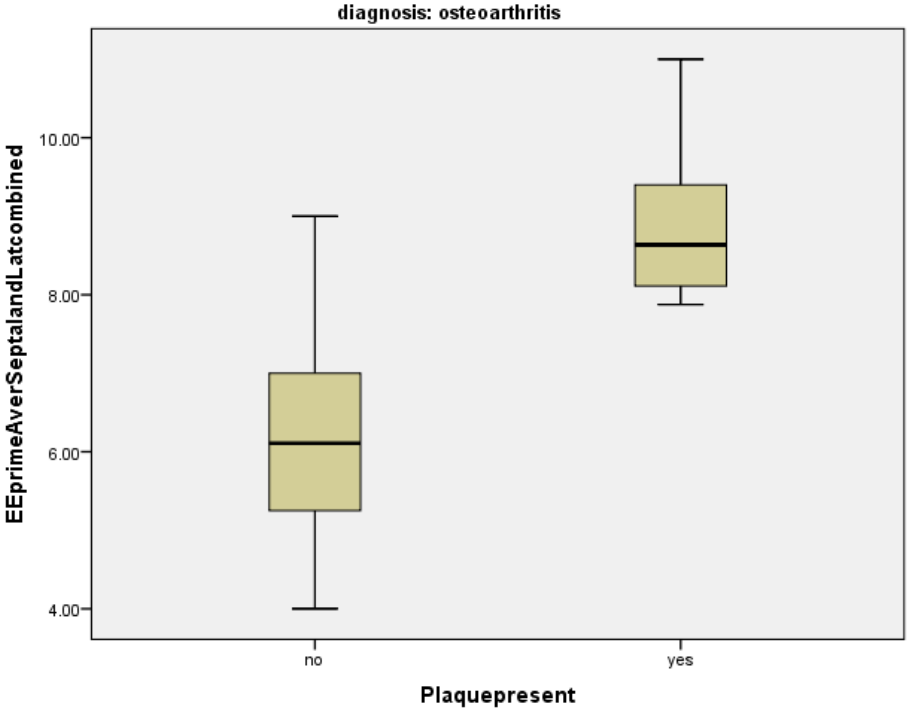


Figure 8.3: Boxplot comparing E/E prime in OA patients with and without carotid plaque

Discussion:

The aims of this chapter were to compare diastolic function in RA and OA patients and also to investigate if an association between diastolic dysfunction and inflammatory markers, cardiovascular risks and anti- rheumatic medications exists in either group.

Left atrial and ventricular sizes were assessed by measuring LA diameter and left ventricular systolic and diastolic diameters. LA size was similar in both groups in our study. A recent meta-analysis by Aslam et al, reported that a number of studies found that RA patients had a significantly larger mean left atrial size compared with controls (14). The left atrium reflects the burden of LV diastolic filling and is a marker of LV diastolic dysfunction. LA enlargement has been shown to be associated with an increased risk of atrial fibrillation (15) and cardiovascular events (16). Size and volume of the left atrium increases as diastolic dysfunction progresses (17). No one in the study group had atrial fibrillation or a history of prior cardiovascular events. Also, of the 33 patients in our group with echocardiographic evidence of diastolic dysfunction, no one had grade III diastolic dysfunction. Therefore, perhaps the patients with evidence of diastolic dysfunction have not had it long enough to cause significant atrial remodeling and enlargement as yet.

Left ventricular diastolic diameter, a marker of systolic function, was significantly larger in males with RA compared to males with OA (table). However, this value was still well within the normal reference range for men, as recommended by the ASE (18). With regard to assessment of left ventricular systolic function, we measured ejection fraction and fractional shortening. These were similar in both

groups, with mean values in the accepted normal range (18). A number of studies have also found similar EF and FS readings between RA patients and controls (6, 19, 20). Gabriel et al, reported a significantly lower EF in a large cohort of RA patients compared to controls, however, the mean age of their RA patients was 10 years older than ours and they also included patients with a prior history of CVD (21).

Patients with diastolic heart failure often have normal ventricular wall thickness; however, they may have evidence of concentric hypertrophy or remodelling and on foot of this the American Society of Echocardiography recommend assessment of LV mass as part of the evaluation of diastolic function (9). We measured septal and posterior wall thickness to calculate LV mass. Both walls were of similar size in RA and OA patients. LV mass was also similar in RA and OA patients (table). A number of studies, whose patients had a mean age similar to ours, support this finding (5, 20) but there is also evidence of both an elevated LV mass in younger RA patients (22) and LV mass significantly lower than in controls, in studies involving older RA patients (21). Crowson and colleagues explain that LV mass in RA may go through sequential changes from myocardial hypertrophy to remodelling and wasting.

As markers of diastolic function, we measured early diastolic LV filling (E wave), late diastolic filling (A wave), deceleration time and E/A ratio was calculated. Tissue Doppler imaging was used to record the mitral annular early diastolic velocity, e', at the lateral and septal walls. E/e' was then calculated. The American Society of echocardiography recommends using a combination of variables for the assessment of diastolic function, rather than relying on individual variables, which may be misleading (9). Based on their recommendations for the grading of diastolic

dysfunction, we used E prime septal and lateral, E/A ratio, deceleration time and E/e' in combination to investigate if any of our study patient has evidence of diastolic dysfunction.

It was found that RA patients had a higher E wave than OA patients and RA males had a higher E and A wave than OA males. Deceleration time, E/A ratio and E/e' ratio were both similar in RA and OA patients (table). 33.3% of RA patients 34.8% of OA patients had evidence of diastolic dysfunction.

Similar to our results, in 2011, Abdul Muizz et al reported no difference in diastolic function, using both pulsed wave Doppler and tissue Doppler imaging techniques, in RA patients and age matched controls (23). They also found no evidence of a correlation between disease activity or duration and markers of diastolic dysfunction.

Tomas L et al recently reported that RA patients in their study had a significantly worse diastolic function compared to controls (24). They had similar patient numbers to our study; however, the mean duration of disease in their RA group was 11.8 years compared to 7 years in our RA group. With regard to the cardiovascular risk profile in their study, the RA group had a significantly higher prevalence of hypertension and impaired glucose tolerance compared to the control group. Hypertension is one of the commonest causes of diastolic dysfunction and this may have been driving the increased rate of diastolic dysfunction that they found in the RA group. In our cohort, there was no difference in prevalence of hypertension or impaired glucose tolerance in RA and OA patients.

It is also important to note that Tomas et al used a low E/A ratio alone to define diastolic dysfunction. All other echocardiographic parameters were similar in their RA and control group and tissue Doppler imaging, which is a more sensitive tool for detecting diastolic dysfunction, was not employed.

Contrary to our findings, Vizzardi E et al reported a significant difference in diastolic dysfunction on tissue Doppler imaging, in RA patients compared to controls (25). In 2010, Sherine Gabriel's group also described a higher prevalence of diastolic dysfunction in RA patients compared with controls, however, as mentioned above, this study enrolled RA patients with a history of CVD and the RA group also had a significantly higher rate of hypertension and significantly higher blood pressure readings during the study, compared with the control group. LV mass is their RA group was also lower than controls which may suggest that myocardial remodelling had already taken effect (21).

It is noteworthy that the majority of other studies investigating diastolic dysfunction compared RA patients to healthy controls, while our control group had a diagnosis of osteoarthritis. There is now increasing evidence that OA has inflammatory components to its pathophysiology (26), especially in postmenopausal females. This may be responsible for diminishing the difference in results between the 2 groups. There is also more recent evidence of an increased risk of CVD in both younger and older females and in older males, compared to their non-OA counterparts (27). Rahman et al reported that females under 65 years had a 66% increased risk of IHD and a 29% increased risk of CHF. The risks were not as high in older females and males but were still statistically significant. If the rates of CHF are higher in OA patients than healthy controls, it seems plausible that a precursor of CHF, namely

diastolic dysfunction, would also be a more frequent finding in OA patients compared to non-OA controls.

The rate of progression of diastolic dysfunction in RA is currently unknown; however a small 5 year follow-up study by Yazici et al, report that cardiac function was conserved without major deterioration when compared with baseline echocardiography (19).

Based on a number of studies in the assessment of left ventricular dysfunction (28), a value of greater than 125 pg/ml was considered as abnormal for our patients N-terminal pro-BNP natriuretic peptide measurements.

The vast majority of RA (96.5%) and OA (89%) patients had a serum NT-proBNP in the normal range and there was no significant difference in values between the 2 groups. Contrary to our results, a study in 2011 by Gabriel et al, found that RA patients had a significantly higher median BNP and that more RA patients had an abnormal BNP level, compared with non-RA controls (4). However, there was a significantly higher proportion of non-white patients in their RA group and more recently, Selim et al have reported that Asian and black patients have higher baseline BNP levels than white and Hispanic patients (29) Our study was made up entirely of Caucasians.

We did find a significantly higher NT-proBNP in RA females compared with RA males. This gender difference is well reported in the literature (30, 31). Higher oestrogen levels in females have been postulated as a potential reason for the higher levels of BNP and NT-proBNP (30) and conversely, testosterone may be responsible for lowering BNP levels (32).

A higher NT-proBNP level was seen in RA patients with abdominal obesity compared to those with a normal waist hip ratio. However, we did not find a difference in NT-proBNP in obese patients compared to non-obese patients. A number of studies have previously found an association between obesity and low BNP levels and there have been suggestions that visceral fat aids clearance of natriuretic peptides via increased expression of clearance receptors on adipocytes (33, 34).

Markers of systolic function, including ejection fraction and fractional shortening, were significantly worse in OA patients with an elevated NT-proBNP. This is in keeping with Luchner's early study of BNP as a marker of left ventricular dysfunction, where they reported that reduced LV ejection fraction was significantly associated with increased circulating concentrations of BNP (35).

No correlation was seen between serum NT-proBNP and echocardiographic markers of diastolic function in either RA or OA patients. Only 2 of the 16 OA patients with evidence of diastolic dysfunction had an elevated NT-proBNP at the time of echocardiography and none of the 17 RA patients with diastolic dysfunction had an

elevated NT-proBNP. Our results are supported by a similar study in 2011 by Sherine Gabriel et al (4). This group found that few of their RA cohort who had elevated BNP actually had evidence of diastolic dysfunction and that BNP in the normal range was less likely to out rule diastolic dysfunction in RA patients compared to non-RA patients. The fact that we did not identify a correlation between NT-proBNP and diastolic dysfunction may in part be due to the fact that the majority of our study group with diastolic dysfunction had grade I changes, with a smaller number having evidence of grade II diastolic dysfunction and no one with grade III diastolic dysfunction.

We did note a correlation between ESR and NT-proBNP levels. In a large population – based inception cohort of RA patients, Crowson et al reported that in those patients who developed heart failure in the 15 year follow-up period, the proportion with an elevated ESR, was highest in the 6 months preceding onset of heart failure. They concluded from this that inflammatory stimuli may be involved in triggering heart failure in this group and that ESR may be a useful evaluation tool for heart failure in RA patients (36).

We further examined the RA group to see if markers of diastolic function were associated with disease activity and anti-rheumatic medications.

RA patients with active disease, as determined by DAS28ESR and DAS28CRP, had a lower E/A ratio than those RA patients with low disease activity and those in remission. A study similar to ours also reported an association between E/A ratio and disease activity scores in RA patients (6). However, in contrast, Vizzzardi et al found no correlation between inflammatory disease parameters and diastolic function (25). These differences are probably in part due to small study sizes and patients with a wide range of disease activities being included.

RA patients who were not taking a synthetic DMARD at the time of the transthoracic echocardiogram were found to have a significantly lower E/A ratio. Lateral and septal mitral annular velocities (e') on tissue Doppler imaging were also significantly lower in the non-DMARD group. These findings demonstrate a poorer diastolic function in RA patients not on a DMARD. However, after correcting for potential confounders on logistic regression, we did not find a significant association between presence of diastolic dysfunction and use of a synthetic DMARD.

Gabriel et al examined 244 RA subjects for evidence of diastolic dysfunction (21) and found that anti- rheumatic medication did not appear to impact on diastolic function. On initial analysis they found that RA patients prescribed methotrexate had a 2.1 odds ratio (CI 95%, 1.01,4.2) for an association with diastolic dysfunction. However, similar to our findings, after correcting for confounders, including CV risk, RA duration and inflammatory cytokine concentrations, this association was reduced and no longer statistically significant.

RA patients on a biologic DMARD had a higher E/A ratio compared with those not on a biologic agent (non-significant). The biologic group also had a significantly better E wave velocity, another marker of diastolic function. Posterior wall and septal wall thickness was greater in the non-biologic group. Again, these findings suggests that RA patients not on a biologic DMARD had evidence of worse diastolic function compared to those taking a biologic DMARD.

There has been a lot of debate with regard to the effects of anti-TNF therapy on cardiac function, in particular with respect to heart failure. Two high profile clinical trials set up in the late 1990s to establish whether TNF inhibitors (etanercept and infliximab) would be of benefit in the treatment of patients with advanced congestive heart failure (CHF), yielded generally unfavourable results. New or worsening CHF was reported in 47 of approximately 300,000 patients treated with either infliximab or etanercept. 81% of these cases had no prior history of CHF (37, 38). Following these adverse events, anti-TNF medications were felt by many clinicians to be more detrimental than beneficial to cardiac function. Contrary to the above, a study published by Bernatsky et al in 2005, reported a 30% reduction in hospitalizations for new-onset CHF in RA patients on DMARDS, including TNF inhibitors (39).

More recently, Senel et al found that etanercept was safe with respect to cardiac function and lipid profile in RA patients followed up for 6 months (40). They reported no significant change in diastolic function and ejection fraction during the follow-up period. Etanercept has also been shown to improve arterial stiffness in RA patients treated and followed up with serial measurements of augmentation index over a 4 month period (41).

These conflicting reports highlight that the direction of the impact of anti-TNF medication on CV risk in RA patients is still largely unknown.

In our study there was no difference in diastolic function in RA patients on steroids and those not taking steroid medication. Patients in both the RA and OA groups who were on an NSAID at the time of the study had more features of systolic and diastolic dysfunction compared to those not taking an NSAID. This is in keeping with a large body of evidence that now exists that both coxibs and NSAIDS are associated with an increased risk of heart failure. A recent meta-analysis of NSAID trials has reported a risk of hospitalization due to heart failure in those treated with NSAIDS to be double that of placebo (42).

RA patients with a prior diagnosis of hypertension had a significantly higher E/e' ratio than normotensive RA patients. We found a significant unadjusted odds ratio of 8.73 (1.53, 49.76) for a diagnosis of diastolic dysfunction in the RA hypertensive group. This ties in with hypertension in the general population being one of the commonest causes of diastolic dysfunction and heart failure with preserved ejection fraction (43).

Also, RA patients in our study with a prior diagnosis of hypercholesterolaemia had a significantly higher E/e' than RA patients without hypercholesterolaemia. The total serum cholesterol and LDL measurements were significantly higher in RA patients with evidence of diastolic dysfunction compared to RA patients with normal

diastolic function. Patients with hyperlipidaemia and other features of the metabolic syndrome have been shown to have evidence of diastolic dysfunction and are at an increased risk of diastolic heart failure (44, 45).

Within the RA group, E/e' was associated with a number of markers of subclinical vascular dysfunction, namely, carotid artery plaque, microalbuminuria and ankle brachial index. Chahal et al have previously reported the close relationship of carotid artery plaque to LV systolic and diastolic dysfunction and they advocate the use of carotid ultrasound as a screening tool for identifying patients who may be at risk of developing heart failure syndromes (46). The fact that we demonstrated a significantly higher E/e' in patients with evidence of carotid plaque, suggests that any LV diastolic function abnormalities may be due to atherosclerosis rather than hypertensive disease.

Microalbuminuria is a marker of endothelial dysfunction and has been shown to be associated with adverse cardiovascular outcomes in diabetes, hypertensives and the general population (47). Elevated urinary albumin-creatinine ratio in patients without evidence of heart failure, predicts future hospitalization for heart failure (48). We found that microalbuminuria correlated positively with E/e', a marker of diastolic dysfunction. This is in keeping with the results of a recent large study examining the relationship between albuminuria and adverse cardiac mechanics (49) and suggests a link between endothelial dysfunction and subclinical diastolic dysfunction.

Ankle brachial index, a measure of peripheral vascular disease (PVD), correlated significantly with E/e' in the RA group. Yamasaki et al have reported prevalence of high BNP and E/e' as markers of diastolic dysfunction in patients with PVD, as defined by an ankle brachial index of less than 0.9 (50). PVD is associated with a 2-fold increase in the prevalence of heart failure (51). Ankle brachial index measurement is cheap and easy to record rapidly in the out-patient setting and provides important information about vascular risk, allowing preventative measures to be introduced in a timely fashion if required.

Conclusion:

We report similar prevalence of diastolic dysfunction in RA and OA patients. The fact that our study group were naïve of any CV events and there was no significant difference in CV risk profile between our groups, may explain why our results are different to some of the recent published work that has found increased rates of diastolic dysfunction in rheumatoid patients compared to controls. Also, as mentioned above, our control group may have inflammatory components making it more similar to our RA group than healthy controls. We have found that a third of both the RA and OA groups have echocardiographic evidence of diastolic dysfunction, despite no symptoms of cardiac disease. This highlights the importance of diastolic function assessment at the time of echocardiography in these groups.

With regard to the direct effect of anti-rheumatic medication on diastolic function, it is difficult to attribute either harm or benefit to these treatments, due to the large number of factors at play, including RA disease activity, traditional CV risks, pro-inflammatory cytokines, and immune dysregulation.

The lack of an association between serum NT-proBNP and markers of diastolic dysfunction in our study group suggests that NT- proBNP may not be a useful screening tool for diastolic dysfunction in RA or OA patients.

Based on our findings of a correlation with E/E prime, microalbuminuria would appear to be a useful, inexpensive screening tool for identifying RA patients who may be at risk of diastolic dysfunction and would benefit from early echocardiographic examination. Carotid ultrasound, to quantify plaque as well as IMT, also appears to provide information about those at risk of diastolic dysfunction. Finally, use of ankle brachial index measurements also appear to provide relevant information regarding diastolic dysfunction, in those with abnormal ABI scores.

References:

1. Redfield MM, Jacobsen SJ, Burnett JC, Jr., Mahoney DW, Bailey KR, Rodeheffer RJ. Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic. JAMA : the journal of the American Medical Association. 2003;289(2):194-202. Epub 2003/01/09.

2. Halley CM, Houghtaling PL, Khalil MK, Thomas JD, Jaber WA. Mortality rate in patients with diastolic dysfunction and normal systolic function. Archives of internal medicine. 2011;171(12):1082-7. Epub 2011/06/29.

3. Kane GC, Karon BL, Mahoney DW, Redfield MM, Roger VL, Burnett JC, Jr., et al. Progression of left ventricular diastolic dysfunction and risk of heart failure. JAMA : the journal of the American Medical Association. 2011;306(8):856-63. Epub 2011/08/25.

4. Crowson CS, Myasoedova E, Davis JM, 3rd, Roger VL, Karon BL, Borgeson D, et al. Use of B-type natriuretic peptide as a screening tool for left ventricular diastolic dysfunction in rheumatoid arthritis patients without clinical cardiovascular disease. Arthritis care & research. 2011;63(5):729-34. Epub 2011/01/13.

5. Gonzalez-Juanatey C, Testa A, Garcia-Castelo A, Garcia-Porrua C, Llorca J, Ollier WE, et al. Echocardiographic and Doppler findings in long-term treated rheumatoid arthritis patients without clinically evident cardiovascular disease. Seminars in arthritis and rheumatism. 2004;33(4):231-8. Epub 2004/02/24.

6. Marasovic-Krstulovic D, Martinovic-Kaliterna D, Fabijanic D, Morovic-Vergles J. Are the anti-cyclic citrullinated peptide antibodies independent predictors

of myocardial involvement in patients with active rheumatoid arthritis? Rheumatology. 2011;50(8):1505-12. Epub 2011/04/13.

7. Wolfe F, Freundlich B, Straus WL. Increase in cardiovascular and cerebrovascular disease prevalence in rheumatoid arthritis. The Journal of rheumatology. 2003;30(1):36-40. Epub 2003/01/01.

8. Davis JM, 3rd, Roger VL, Crowson CS, Kremers HM, Therneau TM, Gabriel SE. The presentation and outcome of heart failure in patients with rheumatoid arthritis differs from that in the general population. Arthritis and rheumatism. 2008;58(9):2603-11. Epub 2008/09/02.

9. Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography. 2009;22(2):107-33. Epub 2009/02/04.

10. Goto T, Ohte N, Wakami K, Asada K, Fukuta H, Mukai S, et al. Usefulness of plasma brain natriuretic peptide measurement and tissue Doppler imaging in identifying isolated left ventricular diastolic dysfunction without heart failure. The American journal of cardiology. 2010;106(1):87-91. Epub 2010/07/09.

11. Mornos C, Ionac A, Cozma D, Rusinaru D, Maximov D, Petrescu L, et al. The relationship between tissue Doppler imaging and seric NTproBNP levels in sinus rhythm patients: a prospective study. The international journal of cardiovascular imaging. 2008;24(4):399-407. Epub 2007/11/01.

12. Sonoda H, Ohte N, Goto T, Wakami K, Fukuta H, Kikuchi S, et al. Plasma N-terminal pro-brain natriuretic peptide levels identifying left ventricular diastolic dysfunction in patients with preserved ejection fraction. Circulation journal : official journal of the Japanese Circulation Society. 2012;76(11):2599-605. Epub 2012/08/11.

13. Grewal J, McKelvie R, Lonn E, Tait P, Carlsson J, Gianni M, et al. BNP and NT-proBNP predict echocardiographic severity of diastolic dysfunction. European journal of heart failure. 2008;10(3):252-9. Epub 2008/03/12.

14. Aslam F, Bandeali SJ, Khan NA, Alam M. Diastolic dysfunction in rheumatoid arthritis: a meta-analysis and systematic review. Arthritis care & research. 2013;65(4):534-43. Epub 2012/09/25.

15. Benjamin EJ, Levy D, Vaziri SM, D'Agostino RB, Belanger AJ, Wolf PA. Independent risk factors for atrial fibrillation in a population-based cohort. The Framingham Heart Study. JAMA : the journal of the American Medical Association. 1994;271(11):840-4. Epub 1994/03/16.

16. Laukkanen JA, Kurl S, Eranen J, Huttunen M, Salonen JT. Left atrium size and the risk of cardiovascular death in middle-aged men. Archives of internal medicine. 2005;165(15):1788-93. Epub 2005/08/10.

17. Oh JK SJ, Jamil Tajik A. The Echo Manual. 3rd ed2006.

18. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European

Association of Echocardiography, a branch of the European Society of Cardiology. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography. 2005;18(12):1440-63. Epub 2005/12/27.

19. Yazici D, Tokay S, Aydin S, Toprak A, Inanc N, Khan SR, et al. Echocardiographic evaluation of cardiac diastolic function in patients with rheumatoid arthritis: 5 years of follow-up. Clinical rheumatology. 2008;27(5):647-50. Epub 2008/01/16.

20. Birdane A, Korkmaz C, Ata N, Cavusoglu Y, Kasifoglu T, Dogan SM, et al. Tissue Doppler imaging in the evaluation of the left and right ventricular diastolic functions in rheumatoid arthritis. Echocardiography. 2007;24(5):485-93. Epub 2007/04/26.

21. Liang KP, Myasoedova E, Crowson CS, Davis JM, Roger VL, Karon BL, et al. Increased prevalence of diastolic dysfunction in rheumatoid arthritis. Annals of the rheumatic diseases. 2010;69(9):1665-70. Epub 2010/05/26.

22. Perna M, Roman MJ, Alpert DR, Crow MK, Lockshin MD, Sammaritano L, et al. Relationship of asymmetric dimethylarginine and homocysteine to vascular aging in systemic lupus erythematosus patients. Arthritis and rheumatism. 2010;62(6):1718-22. Epub 2010/02/16.

23. Abdul Muizz AM, Mohd Shahrir MS, Sazliyana S, Oteh M, Shamsul AS, Hussein H. A cross-sectional study of diastolic dysfunction in rheumatoid arthritis and its association with disease activity. International journal of rheumatic diseases. 2011;14(1):18-30. Epub 2011/02/10.

24. Tomas L, Lazurova I, Oetterova M, Pundova L, Petrasova D, Studencan M. Left ventricular morphology and function in patients with rheumatoid arthritis. Wiener klinische Wochenschrift. 2013;125(9-10):233-8. Epub 2013/04/13.

25. Vizzardi E, Cavazzana I, Bazzani C, Pezzali N, Ceribelli A, Bonadei I, et al. Echocardiographic evaluation of asymptomatic patients affected by rheumatoid arthritis. Journal of investigative medicine : the official publication of the American Federation for Clinical Research. 2012;60(8):1204-8. Epub 2012/11/01.

26. Zhuo Q, Yang W, Chen J, Wang Y. Metabolic syndrome meets osteoarthritis. Nature reviews Rheumatology. 2012;8(12):729-37. Epub 2012/08/22.

27. Rahman MM, Kopec JA, Anis AH, Cibere J, Goldsmith CH. Risk of cardiovascular disease in patients with osteoarthritis: a prospective longitudinal study. Arthritis care & research. 2013;65(12):1951-8. Epub 2013/08/09.

28. Nielsen LS, Svanegaard J, Klitgaard NA, Egeblad H. N-terminal pro-brain natriuretic peptide for discriminating between cardiac and non-cardiac dyspnoea. European journal of heart failure. 2004;6(1):63-70. Epub 2004/03/12.

29. Krim SR, Vivo RP, Krim NR, Qian F, Cox M, Ventura H, et al. Racial/Ethnic Differences in B-Type Natriuretic Peptide Levels and Their Association With Care and Outcomes Among Patients Hospitalized With Heart FailureFindings From Get With The Guidelines–Heart Failure. JACC: Heart Failure. 2013;1(4):345-52.

30. Redfield MM, Rodeheffer RJ, Jacobsen SJ, Mahoney DW, Bailey KR, Burnett JC, Jr. Plasma brain natriuretic peptide concentration: impact of age and

gender. Journal of the American College of Cardiology. 2002;40(5):976-82. Epub 2002/09/13.

31. Wang TJ, Larson MG, Levy D, Leip EP, Benjamin EJ, Wilson PW, et al. Impact of age and sex on plasma natriuretic peptide levels in healthy adults. The American journal of cardiology. 2002;90(3):254-8. Epub 2002/07/20.

32. Chang AY, Abdullah SM, Jain T, Stanek HG, Das SR, McGuire DK, et al. Associations among androgens, estrogens, and natriuretic peptides in young women: observations from the Dallas Heart Study. Journal of the American College of Cardiology. 2007;49(1):109-16. Epub 2007/01/09.

33. Clerico A, Giannoni A, Vittorini S, Emdin M. The paradox of low BNP levels in obesity. Heart failure reviews. 2012;17(1):81-96. Epub 2011/04/28.

34. Mehra MR, Uber PA, Park MH, Scott RL, Ventura HO, Harris BC, et al. Obesity and suppressed B-type natriuretic peptide levels in heart failure. Journal of the American College of Cardiology. 2004;43(9):1590-5. Epub 2004/05/04.

35. Luchner A, Burnett JC, Jr., Jougasaki M, Hense HW, Heid IM, Muders F, et al. Evaluation of brain natriuretic peptide as marker of left ventricular dysfunction and hypertrophy in the population. Journal of hypertension. 2000;18(8):1121-8. Epub 2000/08/23.

36. Maradit-Kremers H, Nicola PJ, Crowson CS, Ballman KV, Jacobsen SJ, Roger VL, et al. Raised erythrocyte sedimentation rate signals heart failure in patients with rheumatoid arthritis. Annals of the rheumatic diseases. 2007;66(1):76-80. Epub 2006/07/05.

37. Chung ES, Packer M, Lo KH, Fasanmade AA, Willerson JT, Anti TNFTACHFI. Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor-alpha, in patients with moderate-to-severe heart failure: results of the anti-TNF Therapy Against Congestive Heart Failure (ATTACH) trial. Circulation. 2003;107(25):3133-40. Epub 2003/06/11.

38. Mann DL, McMurray JJ, Packer M, Swedberg K, Borer JS, Colucci WS, et al. Targeted anticytokine therapy in patients with chronic heart failure: results of the Randomized Etanercept Worldwide Evaluation (RENEWAL). Circulation. 2004;109(13):1594-602. Epub 2004/03/17.

39. Bernatsky S, Hudson M, Suissa S. Anti-rheumatic drug use and risk of hospitalization for congestive heart failure in rheumatoid arthritis. Rheumatology. 2005;44(5):677-80. Epub 2005/03/24.

40. Senel S, Cobankara V, Taskoylu O, Karasu U, Karapinar H, Erdis E, et al. The safety and efficacy of etanercept on cardiac functions and lipid profile in patients with active rheumatoid arthritis. Journal of investigative medicine : the official publication of the American Federation for Clinical Research. 2012;60(1):62-5. Epub 2011/11/09.

41. Galarraga B, Khan F, Kumar P, Pullar T, Belch JJ. Etanercept improves inflammation-associated arterial stiffness in rheumatoid arthritis. Rheumatology. 2009;48(11):1418-23. Epub 2009/09/08.

42. Coxib, traditional NTC, Bhala N, Emberson J, Merhi A, Abramson S, et al. Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs:

meta-analyses of individual participant data from randomised trials. Lancet. 2013;382(9894):769-79. Epub 2013/06/04.

43. Lester SJ, Tajik AJ, Nishimura RA, Oh JK, Khandheria BK, Seward JB. Unlocking the mysteries of diastolic function: deciphering the Rosetta Stone 10 years later. Journal of the American College of Cardiology. 2008;51(7):679-89. Epub 2008/02/19.

44. Gianotti G, Cenni A, Bianchi G, Masetti M, Zappoli P, Muscari A, et al. Diastolic dysfunction and cardiovascular risk in old subjects: Possible association with NAFLD? Archives of gerontology and geriatrics. 2014;58(2):188-95. Epub 2013/11/26.

45. de las Fuentes L, Brown AL, Mathews SJ, Waggoner AD, Soto PF, Gropler RJ, et al. Metabolic syndrome is associated with abnormal left ventricular diastolic function independent of left ventricular mass. European heart journal. 2007;28(5):553-9. Epub 2007/02/22.

46. Chahal NS, Lim TK, Jain P, Chambers JC, Kooner JS, Senior R. The distinct relationships of carotid plaque disease and carotid intima-media thickness with left ventricular function. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography. 2010;23(12):1303-9. Epub 2010/09/25.

47. Givertz MM, Postmus D, Hillege HL, Mansoor GA, Massie BM, Davison BA, et al. Renal Function Trajectories and Clinical Outcomes in Acute Heart Failure. Circulation Heart failure. 2013. Epub 2013/11/28.

48. Gerstein HC, Mann JF, Yi Q, Zinman B, Dinneen SF, Hoogwerf B, et al. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. JAMA : the journal of the American Medical Association. 2001;286(4):421-6. Epub 2001/07/31.

49. Katz DH, Selvaraj S, Aguilar FG, Martinez EE, Beussink L, Kim KY, et al. Association of Low-Grade Albuminuria with Adverse Cardiac Mechanics: Findings from the HyperGEN Study. Circulation. 2013. Epub 2013/10/01.

50. Yamasaki S, Izawa A, Shiba Y, Tomita T, Miyashita Y, Koyama J, et al. Presence of diastolic dysfunction in patients with peripheral artery disease. Angiology. 2013;64(7):540-3. Epub 2013/02/13.

51. Anand RG, Ventura HO, Mehra MR. Is heart failure more prevalent in patients with peripheral arterial disease? A meta-analysis. Congestive heart failure. 2007;13(6):319-22. Epub 2007/11/30.

Chapter 9

Summary and Concluding Remarks

Summary of our findings:

To the best of our knowledge, this is the first study of its kind in RA patients in Ireland. We conducted a cross-sectional examination of markers of pre-clinical atherosclerosis and diastolic dysfunction is RA patients and OA controls. Our hypothesis was that patients with rheumatoid arthritis are more likely to display evidence of pre-clinical atherosclerosis compared to patients with osteoarthritis, due to the inflammatory effects of their disease on vascular function.

Traditional CV risks and Markers of Inflammation:

To investigate the relative contributions of both traditional cardiovascular risk factors and inflammation in RA as a non- traditional risk factor, to the development of CV events, we collected information on traditional CV risk profiles for both groups and also measured a number of markers of inflammation to identify if these were associated with pre-clinical CVD.

We found that the prevalence of traditional CV risk factors were similar in RA and OA patients. We identified that 7 RA and 7 OA patients fitted the criteria for the metabolic syndrome. This diagnosis had not been previously documented. Over 2/3's of both the RA and OA groups had elevated levels of fasting serum cholesterol, however only 20% of these patients were taking a statin.

Markers of inflammation were compared between RA and OA groups. CRP was significantly higher in our RA group, which is not at all surprising. It was also higher in RA patients not on DMARD therapy. Levels correlated with markers of thrombosis in our RA group. Based on these finding and evidence that links CRP levels to future CVD events in RA patients, patients with a persistently elevated CRP should be evaluated appropriately for CV risk.

Given the fact the hyperuricaemia is an independent predictor of the metabolic syndrome and that we found significant associations between serum urate level and BMI and waist-hip ratio in our RA patients, it would appear that measurement of this inflammatory marker may have a predictive value in RA patients with respect to their CV risk.

We measured a number of pro-inflammatory cytokines in both groups and not surprisingly, concentrations were higher in RA patients. RA patients on a biologic DMARD had lower concentrations of MCP-1, a common chemokine in both RA and CVD pathways, suggesting an anti-atherogenic effect of biologic agents in addition to their role in controlling RA disease activity. This may be an important future consideration as a potential therapeutic target to treat both diseases simultaneously.

Smoking status, a risk factor for both CVD and RA, was associated with inflammatory cytokine concentrations in our RA group, supporting the theory that the pathological process of CVD in RA is due to the combined effects of traditional and inflammatory risks.

Fibrinogen, a marker of thrombosis, was significantly higher in the RA group. Markers of thrombosis correlated with measures of disease activity and subclinical atherosclerosis in our RA group, suggesting that abnormal haemostasis is one of the mechanisms linked with CVD development in RA.

In support of the increasing body of evidence that OA has in fact an inflammatory component to it, is the correlation we reported between inflammatory cytokines and OA disease duration. This finding goes against the traditional theory that OA is merely a degenerative condition and suggests that our control group also had a degree of inflammation driving their condition.

Subclinical Atherosclerosis:

We did not find a difference in the prevalence of subclinical atherosclerosis between RA patients and OA controls. However, our OA control group are not comparable to normal healthy controls due to the inflammatory component to their disease and recent evidence of increased CV risk in OA patients.

The importance of screening for traditional CV risks in RA patients, in particular, abnormal lipid profiles, was highlighted in our findings of an association of serum lipid measurements with both cIMT and carotid plaque. The fact that we have shown a link between the 2 reinforces the importance of CV risk screening in this

population. Carotid artery ultrasound may help to stratify RA patients according to CV risk. Rheumatologists should have a low threshold for treatment with statin therapy in this group.

PAI-1 levels also correlated with sub-clinical atherosclerosis in RA patients. This highlights the fact that a number of mechanisms including altered haemostasis and atheroma formation are involved in the pathogenesis of CVD in this group.

Ankle brachial index, a measure of peripheral vascular disease (PVD), was significantly lower in our RA group compared with the OA group. 15% of the RA group had abnormal ABI measurements, despite no symptoms of PVD. This demonstrates that ABI is a useful tool for identifying silent disease in patients who may not be able to exercise sufficiently to generate symptoms of claudication, due to mobility restrictions. Also of interest is our finding that evidence of PVD in RA patients was associated with vascular disease at other sites, demonstrating the systemic nature of CVD in RA patients.

Endothelial Dysfunction:

Serum concentrations of adhesion molecules, which are known biomarkers of endothelial dysfunction, were significantly higher in RA patients. They were also found in higher concentrations in those RA patients who tested positive for Rheumatoid factor and anti-CCP antibody compared to sero-negative RA patients. These results demonstrate that factors other than traditional CV risks are also responsible for the increased CVD risk in RA.

A number of adhesion molecules had significantly higher concentrations in RA smokers than non-smokers, emphasising the important role that smoking plays in both atherosclerosis and RA. Given the causal link between smoking and RA and smoking and atherosclerosis, this preventable risk factor should to be top of the agenda for rheumatologists to target aggressively.

Adhesion molecule concentrations also correlated with markers of diastolic dysfunction in RA patients, suggesting a link between endothelial dysfunction and cardiac function in these patients. Diastolic dysfunction also correlated with, urinary protein-creatinine ratio (PCR), another marker of endothelial dysfunction. Urinary PCR was higher in RA than OA patients.

Our findings suggest a pathophysiological link between endothelial dysfunction and diastolic dysfunction in RA that needs to be investigated further. Measurement of albuminuria in RA patients may help to detect those at risk of diastolic dysfunction who would benefit from transthoracic echocardiogram.

Endothelial dysfunction was also assessed using flow mediated dilatation of the brachial artery. We did not identify a significant difference in flow-mediated and nitroglycerin-mediated dilatation between our RA and OA patients. However, our finding of a significant correlation between markers of RA disease activity and endothelial dysfunction, demonstrate that RA remission induction would have beneficial effects on vascular function and supports the evidence for aggressive anti-rheumatic treatment to improve cardiovascular outcomes in RA.

Associations between endothelial dysfunction and glucose and pro-inflammatory cytokines in our OA group, support recent research that OA is an inflammatory disease and is closely linked to the metabolic syndrome.

Diastolic dysfunction:

RA and OA patients had similar prevalence of diastolic dysfunction.

One third of both the RA and OA groups had echocardiographic evidence of diastolic dysfunction, despite no symptoms of cardiac disease. Diastolic dysfunction is silent until symptoms of heart failure appear. Assessment of diastolic function at the time of echocardiography in RA and OA patients should be incorporated into the examination.

The lack of an association between serum NT-proBNP and markers of diastolic dysfunction in our study group suggests that NT- proBNP may not be a useful screening tool for diastolic dysfunction in RA or OA patients.

Salient points for the practicing clinician:

1) Recognition and management of traditional cardiovascular risks by rheumatologists need to improve. This is evident from our findings that a large number of patients with elevated fasting serum cholesterol were not prescribed a statin and that a proportion of both RA and OA patients have the metabolic syndrome that has been unrecognised.

2) Smoking, a modifiable CV and RA risk, was associated with increased expression of adhesion molecules in our RA group and correlated with serum cytokine concentrations. Its prevention and cessation should be targeted aggressively in the out-patient setting.

3) A novel association between urinary protein creatinine ratio (PCR) and diastolic dysfunction in RA was found. Urinary PCR is a cheap and readily available marker of endothelial dysfunction. It should be used in the rheumatology clinic to identify RA patients at potential risk of diastolic dysfunction.

4) Silent peripheral vascular disease is prevalent in RA patients and is detectable by ankle brachial index, a minimally invasive investigation. Identification of PVD should alert clinicians to the fact that atherosclerosis may be present in other vascular beds, as was the case in our RA patients and prompt further investigation.

5) Osteoarthritis is associated with CV disease and markers of the metabolic syndrome. Traditional CV risks should be addressed in this group also. If inflammation is clinically apparent it should be treated as OA is no longer considered a natural aging process.

Recommendations for the Future:

Coordination of care among rheumatologists, cardiologists and general practioners is crucial to improve detection and management of CV disease in RA patients. Increasing education and physician awareness that RA had a risk profile for developing CVD similar to the magnitude of the risk associated with diabetes mellitus will no doubt improve survival.

A recent study of UK primary care practices by Monk et al, found that rates of screening for CVD were similar in RA and non-RA patients, but diabetic patients were 12 times more likely to receive screening (1).

The COMORA study of comorbidities in RA also recently reported that 30-50% of RA patients do not have their CV risk factors optimally monitored or treated (2).

Traditional cardiovascular risk factors need to be actively screened for in the rheumatology clinic. We advocate the use of modified cardiovascular risk screening tools, such as the mSCORE, as recommended by EULAR (3), as these tools may put the CV risk of RA patients in perspective for non-cardiologists. As described in this score, NSAIDs should be used with caution, the lowest possible does of corticosteroids should be prescribed and control of RA disease activity with anti-rheumatic medications should be assessed regularly.

It is difficult to disentangle the relationship between CV risk, inflammation and treatment effects in RA. Medication used to control RA has beneficial and detrimental effects on CV risk profile. Biologic and synthetic DMARDS,

particularly TNF inhibitiors and methotrexate are powerful agents for suppressing inflammation and also reduce CV risk. However, glucocorticoids are associated with increased CV risk due to their negative metabolic effects (4).

One exciting clinical trial, The Cardiovascular Inflammation Trial (CIRT) is currently enrolling patients post- MI without a history of RA to receive either placebo or methotrexate, to assess if reducing inflammation will decrease future CV events (5).

A second such trial, the Canakinumab anti-inflammatory thrombosis outcomes study (CANTOS), will address the effects of anti- Il1β inhibition on recurrent MI, stroke and rates of CV death in patients with stable coronary artery disease (5). Targeting common cytokine pathways, such as Il6 and Il1 as treatment options for both diseases may prove useful in controlling both diseases with a single agent.

Communication between the specialities of cardiology and rheumatology is of paramount importance because control of CV risk and reduction of future CV events can be achieved not only by cardiac medications and interventions but also by RA disease control with anti-rheumatic medications, in particular biologic.

Study Limitations:

Some limitations of our study merit mention. Our sample was drawn from a subspecialty referral centre, which makes our results most generalizable to the type of RA patient seen by rheumatologists rather than general practitioners. The cross-sectional design of our study is a limitation. It was difficult to recruit large numbers of OA patients with a clean CV profile attending a tertiary referral centre. A larger sample size would allow for more detailed analysis with respect to causation.

It is also important to mention that our OA group are different from "healthy" controls for a number of reasons. Because they attend a hospital based rheumatology clinic, they are more likely to have more severe OA than those attending their general practitioner. Also, increasing evidence of an inflammatory component to OA and its association with features of metabolic syndrome and increased cardiovascular risk, make this control group less representative of "healthy" adults than previously thought.

Future plans:

In this cohort of RA and OA patients we are currently repeating the carotid ultrasound assessment of IMT and presence of plaque to assess the degree of progression, if any, of pre-clinical atherosclerosis over time. Ultrasound scanning is being performed 2 years after the baseline scan. In the preliminary analysis, comparison of interval scans in a subgroup of patients has not identified any progression of IMT over time. However, this will be re-analysed when the repeat carotid ultrasounds are completed for the whole cohort. Disease activity assessments 2 years from baseline will also be analysed to examine for correlations with IMT.

We are also following up both the RA and OA groups to identify patients who have developed cardiovascular events or died since recruitment. In 2012, 1 RA patient had suffered a cerebrovascular event since recruitment and 2 RA patients had died both from malignancy. There were no documented CV events or deaths in the OA group.

Given the accumulating collection of evidence that OA is associated with increased CV risk, it would be interesting to compare our OA group to healthy controls in the community.

References:

1. Monk HL, Muller S, Mallen CD, Hider SL. Cardiovascular screening in rheumatoid arthritis: a cross-sectional primary care database study. BMC family practice. 2013;14:150. Epub 2013/10/11.

2. Dougados M, Soubrier M, Antunez A, Balint P, Balsa A, Buch MH, et al. Prevalence of comorbidities in rheumatoid arthritis and evaluation of their monitoring: results of an international, cross-sectional study (COMORA). Annals of the rheumatic diseases. 2014;73(1):62-8. Epub 2013/10/08.

3. Peters MJ, Symmons DP, McCarey D, Dijkmans BA, Nicola P, Kvien TK, et al. EULAR evidence-based recommendations for cardiovascular risk management in patients with rheumatoid arthritis and other forms of inflammatory arthritis. Annals of the rheumatic diseases. 2010;69(2):325-31. Epub 2009/09/24.

4. Crowson CS, Liao KP, Davis JM, 3rd, Solomon DH, Matteson EL, Knutson KL, et al. Rheumatoid arthritis and cardiovascular disease. American heart journal. 2013;166(4):622-8 e1. Epub 2013/10/08.

5. Ridker PM. Closing the loop on inflammation and atherothrombosis: why perform the cirt and cantos trials? Transactions of the American Clinical and Climatological Association. 2013;124:174-90. Epub 2013/07/23.

Appendix A

Study Questionnaire

COMBINED QUESTIONNAIRE AND CHART REVIEW FOR STUDY OF CARDIOVASCULAS DISEASE IN PATIENTS WITH RHEUMATOID ARHTRITIS

Study number: MRN:

Name: DOB:

Address:

Telephone: Sex: M/ F

Date of diagnosis of RA: / /

Diagnosed by whom: Consultant_____
GP_____

1987 ACR criteria: (need 4)

- EMS> 1 HOUR > 6/52
- Arthritis of at least 3 joints (soft tissue swelling lasting .> 6/52)
- Arthritis of hand joints (wrists, MCPs , PIPs lasting >6/52)
- Symmetrical arthritis (at least one area, lasting > 6/52)
- Nodules
- RF positive
- Radiographic changes (erosions)

Treatment history of RA:

DMARD THERAPY

DMARD	START DATE	STOP DATE	MEAN DOSE	REASON FOR STOPPING

USE OF BIOLOGICS

DRUG	START DATE	FINISH DATE	RESPONSE	REASON FOR STOPPING

Response? Is there a stanardised grading system available for this?

Combination therapy with DMARD and biologic?

Use of steroids since diagnosis? Y/N If yes: Duration:

Current use? Y/N Max. dose:
Previous use? Y/N Mean dose:

Use of NSAIDS:

- Current Y/N
- Previous Y/N

List: Y/N Duration Dose

Difene / Voltarol (Diclofenac)

Brufen (Ibuprofen)

Mobic (Meloxicam)

Feldene (Piroxicam)

Celebrex (Celecoxib)

Arcoxia (Etoricoxib)

Vioxx

Bextra

Aulin (Nimesulid)

Naprosyn
Mefac (Mefanamic acid)

Statins:

Atorvastatin: Y/N Dose:

Pravastatin: Y/N Dose:

Simvastatin: Y/N Dose:

Fluvastatin Y/N Dose:

Other:

Fibrates Y/N

Ezetimibe: Y/N

Aspirin Y/N Dose:

ACEI Y/N Dose:

ARB Y/N

Loop Diuretics Y/N

Thiazides Y/N

B-blocker Y/N

Ca blocker Y/N

PPI Y/N

Bisphosphonate Y/N

Allergies:

Family history of RA: Y/N Who? Age at presentation:

Family history of stroke Y/N Who? Age at presentation:

Family history of heart attack Y/N Who? Age at presentation:

Family history of cardiacstent Y/N Who? Age at presentation:

Family history of CABG Y/N Who? Age at presentation:

Family history of PVD ie. Claudication/ bypass/ angioplasty Y/N Who?
Age at presentation:

Social history:

- Occupation:_____
- Currently working? Y/N If not, is this due to disease? Y/N
- Level of education? Junior Cert Y/N
  Leaving Cert Y/N
  University Y/N

Smoker Y/N Exsmoker Y/N Pack years: _____

Units of C2H5OH/ wk:
Past medical history:

DM Y/N When D(x) ?
Diet / Tabs / Insulin?
Hypertension Y/N

Vitiligo Y/N

Hyperchol Y/N Alopecia Y/N

Raynauds Y/N Autoimmune Hep. Y/N

Thyroid Dis. Y/N (Goitre, hypo/hyperthyroid/ I 131) SLE Y/N

Coeliac Y/N Felty's syn Y/N

Sjogrens Y/N (ask for dry eyes/ dry mouth) Pulm. Fibrosis Y/N

Systemic sclerosis Y/N Osteoporosis Y/N
Atlanto-axial sublux. Y/N Addison's Y/N
Erosions on X-Ray Y/N

Other:

Any first degree relative with any of the above? Y/N If yes, who?

Cardoivascular Symptoms:

- SOB at rest Y/N
- SOB on exertion Y/N
- SOB lying flat/ how many pillows? Y/N
- Cough Y/N
- Chest pain/ discomfort/ tightness on exertion Y/N
- Chest pain/discomfort/tightness at rest Y/N
- Leg swelling/ Ankle swelling(?bilat, ? worse at end of day) Y/N
- Palpitations Y/N
- Jaw pain/ Arm pain Y/N

Examination:

Weight(kg): Height(cm): BMI: Abdom circ:

BP HR RR

Nodules Y/N Joint Effusions: Y/N Where?

??Tinels

Chest:

CVS: S1 S2 ADDED MURMURS

Liver / Spleen enlarged? Y/N Size (cm):

L.nodes: Y/N Location

Peripheral oedema Y/N To what level?

Ankle brachial Index:

Skin rashes Y/N livedo, vitiligo etc

VAS: physician global assessment of disease activity

Not active----------Very active

Patient global assessment of disease activity

Not active----------Very active

Patient global assessment of pain

Not active----------Very active

Appendix B

Short form Questionnaire (SF-36)

1. In general, would you say your health is (circle one)
   Excellent …… 1
   Very Good …… 2
   Good ……. 3
   Fair ……. 4
   Poor ……. 5

2. Compared to one year ago, how would you rate your health in general <u>now</u>?
   Much better now than one year ago …… 1
   Somewhat better now than one year ago …… 2
   About the same as one year ago …… 3
   Somewhat worse now than one year ago ……... 4
   Much worse now than one year ago …… 5

3. The following questions are about activities you might do during a typical day. <u>Does your health now limit you</u> in these activities? If so, how much?

ACTIVITIES	Yes, Limited A Lot	Yes, Limited A Little	No, Not Limited At All
Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	1	2	3
Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	1	2	3
Lifting or carrying groceries	1	2	3
Climbing severa**l** flights of stairs	1	2	3
Climbing one flight of stairs	1	2	3
Bending, kneeling or stooping	1	2	3
Walking more than **onemile**	1	2	3
Walking **half of mile**	1	2	3
Walking one hundreds	1	2	3

yards			
Bathing or dressing yourself	1	2	3

4. During the past 4 weeks, have you had any of the following problems with you work or other regular daily activities as a result of your physical health? (Circle one number on each line)

	YES	NO
Cut down on the **amount of time** you spent on work or other activities	1	2
Accomplished less than you would like	1	2
Were limited in the **kind** of work or other activities	1	2
Had difficulty performing the work or other activities (for example, it took extra effort)	1	2

5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

(Circle one number on each line)

	YES	NO
Cut down on the **amount of time** you spent on work or other activities	1	2
Accomplished less than you would like	1	2
Did not do work or other activities as **carefully** as usual	1	2

6. During the past 4 weeks, to what extend has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours or groups?

Not at all	……	1
Slightly	……	2
Moderately	……	3
Quite a bit	……	4
Extremely	……	5

7. How much bodily pain have you had during the past 4 weeks?

None	……	1
Very mild	……	2
Mild	……	3
Moderate	……	4
Severe	……	5
Very Severe	……	6

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and homework)?

Not at all	……	1
A little bit	……	2
Moderately	……	3
Quite a bit	……	4
Extremely	……	5

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks?

(Circle one number on each line)

ACTIVITIES	All of the Time	Most of the Time	A Good bit of the Time	Some of the Time	A Little of the Time	None of the Time
Did you feel full of life?	1	2	3	4	5	6
Have you been a very nervous person?	1	2	3	4	5	6
Have you felt so down in the dumps	1	2	3	4	5	6

that nothing could cheer you up?						
Have you felt calm and peaceful?	1	2	3	4	5	6
Did you have a lot of energy	1	2	3	4	5	6
Have you felt downhearted and low?	1	2	3	4	5	6
Did you feel worn out?	1	2	3	4	5	6
Have you been a happy person?	1	2	3	4	5	6
Did you feel tired?	1	2	3	4	5	6

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting your friends, relatives, etc)?

All of the time …….. 1
Most of the time ……2
A good bit of the time …… 3
Some of the time ……4
A little of the time …… 5
None of the time …… 6

11. How TRUE or FALSE is each of the following statements for you?
(Circle one number on each line)

ACTIVITIES	Definitey	Mostly True	Don't Know	Mostly False	Definitely False
I seem to get ill more easily than other people	1	2	3	4	5
I am as healthy as anybody I know	1	2	3	4	5
I expect my health to get worse	1	2	3	4	5
My health is excellent	1	2	3	4	5

Appendix C

FMD PROTOCOL

- Patient consent, questionnaire and exam
- Height, weight, hip and waist circumference
- Collect urine sample
- Position patient and explain procedure
- Check BP
- Take bloods and put PAI sample in cooler bag

- Turn on Echo machine
- Press scan head button and select 'Arterial'
- Place BP cuff below right elbow and inflate to check the position is stable, then deflate
- Start scan and locate max diameter of brachial artery above elbow by moving probe from side to side
- Clamp probe in position and remind patient to stay very still
- Adjust gain and colour to improve vessel image
- Turn on computer and click VIA
- Select 'new' and enter patient details
- A reference image is required. Select the region of interest (ROI) on the artery on computer screen and press 'Start'
- Optimise the image by adjusting gain controls on Echo machine and adjust ROI size and position with ROI controls on computer
- Check that Images/sec is at least 25
- When image is optimised, press 'Stop' and then 'Start' to start new session, using only optimised images. Now the 'Study Proper' has begun and gain and controls should be kept constant

- Record a baseline scan for 2 minutes and note times
- Press 'Inflate' button (green button will automatically inserted in the results database) and immediately inflate tourniquet to 50mmHg above systolic
- Check BP
- Monitor sphygmomanometer pressure and check regularly that the image box on the computer screen bisects the artery at its maximum diameter and adjust the probe from side to side if necessary
- Tourniquet should be kept inflated for 4.5 to 5minutes

- A red signal will flash on the computer screen for 'Doppler On' just prior to the time for deflation, no artery diameter recordings occur during Doppler mode
- At the prompt for tourniquet deflation, immediately deflate the tourniquet and note the time and monitor the image for any patient movement (as this causes erroneous results) and correct the image plane as necessary
- Check BP
- Continue to scan for 2 minutes
- Rest for 10 minutes to allow diameter to return to baseline

- Press 'Stop' and then press 'Start' to record new session for GTN part of the study and note which session number for GTN
- Record baseline scan for 2 minutes
- Check BP
- Give 2 puffs of GTN sublingually and immediately click the GTN button (a blue line is automatically inserted into the data analysis) and note the time
- Continue to scan for 5- 6 minutes after time of GTN (max dilatation occurs at approx 3-4 mins post GTN)
- Check BP
- Press 'Stop' to end study

- Select 'File: Open: Data'
- A graph of results is generated. Click 'Next' to get to study session required and the click 'Calculate'
- Dilatation is calculated as a percentage change in diameter from baseline
- Peak dilatation post GTN may need to be marker manually on the graph (roughly 3-4 mins after the blue line)

- Perform ABI while patient still lying down after the FMD study

Appendix D

Abstracts

1) Carotid Intima-media Thickness as a marker of pre-clinical atherosclerosis in rheumatoid arthritis.

   Miriam OSullivan, Cathy Dewhurst, Michael Molloy, Michael Maher, David Kerins, Sinead Harney, Fergus Shanahan. UCC Research Day 2008

2) Rheumatoid arthritis is associated with an increased risk of cardiovascular events, even when traditional risk factors are corrected for. Carotid ultrasound, assessing intimal medial thickness and plaque, is a validated surrogate marker of coronary disease.

   Miriam O Sullivan, Cathy Dewhurst, Michael Molloy, Michael Maher, David Kerins, Sinead Harney, Fergus Shanahan. ISR 2008

3) Endothelial Dysfunction in SLE: a rationale for the use of statin therapy to assess reversibility.

   Miriam O'Sullivan, Patrick Barry, Sinead Harney, Denis O'Mahony.

   ACR 2008

4) Endothelial dysfunction as a marker of pre-clinical cardiovascular disease in patients with rheumatoid arthritis compared to patients with osteoarthritis.

   Miriam O Sullivan, Cathy Dewhurst, Michael Maher, Sinead Harney, David Kerins. ISR 2009

5) Correlation between diastolic function, carotid intimal medial thickness and ankle brachial index, as markers of pre-clinical cardiovascular disease in rheumatoid arthritis patients

   Miriam O Sullivan, Orla Murray, Mary Daly, Cathy Dewhurst, Michael Maher, David Kerins3, Sinead Harney. Eular 2010.

6) Comparison of ankle brachial index, as a marker of subclinical atherosclerosis, in patients with rheumatoid arthritis versus osteoarthritis patients.

   Miriam O'Sullivan, Mary Daly, MGM Molloy, David Kerins, Sinead Harney. ISR 2010

7) Diastolic dysfunction in rheumatoid arthritis patients without cardiovascular symptoms is associated with presence of microalbuminuria and subclinical peripheral vascular disease.

   Miriam O Sullivan, Orla Murray, Mary Daly, Sinead Harney, David M Kerins. ESC 2013.