Rheumatoid Arthritis, Cardiovascular Disease and Inflammation…..

And the Effect of Rheumatoid Arthritis Drug Therapies on this Musketeer Trio

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This thesis is being submitted in total fulfilment of the requirements for the degree of Doctor of Medicine/Doctor of Medical Science

November 2010

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Abstract:

Rheumatoid arthritis (RA) is associated with a significantly higher rate of cardiovascular (CV) disease and mortality (1). The higher CV risk appears to be independent of traditional CV risk factors (2). Atherosclerosis is now recognised as an inflammatory disease, and the relationship between RA and CV disease may partly be explained by chronic systemic inflammation (3). Inflammation plays a role at all stages of the atherosclerotic process from the earliest stages of endothelial dysfunction to plaque development and eventually to plaque complications such as rupture and thrombosis which lead to clinical events such as heart attack and stroke (4). Medications commonly taken by RA patients may exacerbate or improve CV risk. Methotrexate improves RA disease activity, inflammation and CV mortality in RA (5). Non-steroidal anti-inflammatory medications (NSAIDs), cyclo-oxygenase inhibitors (COX-2 inhibitors) and TNFα-inhibitors are commonly used in RA with varying degrees of inflammation suppression. Their effect on CV risk in RA might therefore assist in our understanding of the complex relationship which we hypothesise exists between RA, CV disease and inflammation.

The aim of this thesis was to investigate vascular dysfunction in RA and the relationship to markers of inflammation, and to assess whether intervention with NSAIDs, COX-2 inhibitors and TNFα-inhibitors has an effect on vascular function in RA.

In a cross sectional study of 106 RA and control subjects pulse wave analysis (PWA) was performed. A subgroup of RA patients and controls had known coronary artery disease (CAD). Vascular measures were correlated with markers of inflammation (6). In a double blind placebo controlled trial, 37 RA patients were randomised to a two week course of COX-2 inhibitor, rofecoxib, NSAID, indomethacin or placebo. Flow mediated dilatation (FMD) and PWA were assessed before and after the two week treatment course (7).

In a further intervention study 26 RA patients were randomised to TNFα-inhibitor Infliximab or placebo infusions. Vascular assessments included pulse wave velocity (PWV), PWA, carotid intima media-thickness (CIMT) and Carotid artery plaque (CAP). Follow up was to 56 weeks (8).
Results from the cross sectional study confirmed that vascular function was impaired in RA and in the subgroup of patients with CAD when measured with PWA. Vascular function correlated with acute phase proteins when analysing the study group as a whole. In terms of short term intervention, COX inhibition with either rofecoxib or indomethacin did not significantly improve vascular function at two weeks as measured by FMD and PWA. Markers of inflammation also did not improve with these treatments. Post-hoc analysis of the infliximab intervention study using multivariate ANOVA modelling showed significant reduction in PWV over 56 weeks. RA disease activity and markers of inflammation also improved with this treatment. There was no change in PWA, CIMT or carotid plaques.

In conclusion, patients with RA have impaired vascular function. This was independent of traditional CV risk factors but correlated with markers of inflammation. A short term intervention with a COX-2 inhibitor or NSAID did not improve vascular function at two weeks when measured with PWA in RA patients. Intervention with TNFα inhibitor improved PWV in RA patients at 56 weeks. These findings support the concept that chronic inflammation is associated with vascular dysfunction and this may relate at least in part to the increased CV risk in RA. Therapies effective in suppressing inflammation not only improve RA disease activity but also vascular function.
Declaration:

This is to certify that

1. the thesis comprises only my original work towards the Doctor of Medicine/Doctor of Medical Science except where indicated in the Preface*
2. due acknowledgement has been made in the text to all other material used
3. the thesis is less than 100,000 word in length, exclusive of tables, maps, bibliographies and appendices.

Signature…………………………………………………………………………..
*Preface:*

The papers which make up the body of results of this thesis could not have been accomplished without the work, expertise and commitment of others. However, I can confidently say that I was involved in every aspect of each paper from study design, data collection, data analysis and writing of the papers. Collaborations and level of contribution of others according to paper is listed below:

**Papers:**


   - This was collaborative work between St. Vincent’s Hospital and Royal Melbourne Hospital and between departments of Rheumatology and Medicine at St. Vincent’s hospital.
   - Ling Toh assisted in study design, collation of data and writing of the paper.
   - Andrew Wilson assisted in collation of data and writing of the paper.
   - Kevin Rowley assisted in statistical analysis.
   - Connie Karschimkus assisted in laboratory work.
   - David Prior performed the stress echocardiograms.
   - George Dragicevic assisted in laboratory work.
   - Harry Harianto assisted in laboratory work.
   - Alicia Jenkins assisted with study design and writing of the paper.

• This was collaborative work between departments of Rheumatology and Clinical Pharmacology at Guy’s and St. Thomas’ hospital and King’s College London.
• Ben Yu Jiang performed the FMD measurements.
• Steve Farish assisted with statistical analysis.
• Bruce Kirkham assisted with study design.
• Phil Chowienczyk assisted with study design.


• This was collaborative work between departments of Rheumatology and Clinical Pharmacology at Guy’s and St. Thomas’ hospital and King’s College London.
• Stephen Oakley assisted with statistical analysis and writing of the paper.
• Ben Yu Jiang performed the FMD, CIMT and carotid plaque measurements.
• Bruce Kirkham assisted with study design.
Acknowledgments:

I would like to thank my dear family Matthew, Christopher and Emily for their unconditional love and support. I am also forever grateful to my parents who have provided me with opportunities throughout my life.

I will cherish my experiences in London and the friends and colleagues which made my time in London so enjoyable. I am grateful in particular to Bruce Kirkham and Gabriel Panayi for having faith in me and providing the opportunity for research and clinical pursuits at Guy’s and St. Thomas’ Hospital. This work could not have been completed without the assistance of many people, most of whom I have acknowledged in the preface. There are also many others who have not been mentioned but who also deserve acknowledgement including nurses, scientists and secretarial staff. My thanks go to the people who volunteered to participate in the studies and to the doctors involved in patient care and management.

Finally I would like to thank those who I view as my mentors. They have each taken time out of their busy schedules to help a naïve clinician navigate her way through the world or research. Laurie Clemens, Andrew Wilson, Alicia Jenkins, Bruce Kirkham and Valerie Corrigall (at King’s College London laboratory) have advised, inspired and passed on their invaluable knowledge and experience.

Thank you.
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<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ACE inhibitors</td>
<td>Angiotensin converting enzyme inhibitors</td>
</tr>
<tr>
<td>AIx</td>
<td>Augmentation index</td>
</tr>
<tr>
<td>ANCA</td>
<td>Antineutrophil cytoplasmic antibody</td>
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<tr>
<td>Anti-CCP</td>
<td>Anti-cyclic citrullinated peptide</td>
</tr>
<tr>
<td>AP</td>
<td>Augmented pressure</td>
</tr>
<tr>
<td>Apo A-1</td>
<td>Apolipoprotein A-I</td>
</tr>
<tr>
<td>APPROVe</td>
<td>Adenomatous Polyp Prevention On Vioxx</td>
</tr>
<tr>
<td>AT-1 receptor blockers</td>
<td>Antigiotensin-1 receptor blockers</td>
</tr>
<tr>
<td>bDMARDs</td>
<td>biologic DMARDs</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BSRBR</td>
<td>British Society of Rheumatology biologics registry</td>
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<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>CAP</td>
<td>Carotid artery plaque</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CIMT</td>
<td>Carotid intima media-thickness</td>
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<tr>
<td>CLASS</td>
<td>Celecoxib Long-term Arthritis Safety Study</td>
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<tr>
<td>COX-2 inhibitors</td>
<td>Cyclo-oxygenase inhibitors</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CV</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>DAS 28</td>
<td>Disease Activity Score 28</td>
</tr>
<tr>
<td>DMARD</td>
<td>Disease Modifying Antirheumatic Drug</td>
</tr>
<tr>
<td>DIVERT</td>
<td>Defining the use of Infliximab on Vascular Endothelium in Rheumatoid arthritis Trial</td>
</tr>
<tr>
<td>ESR</td>
<td>Erythrocyte sedimentation rate</td>
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<tr>
<td>EULAR</td>
<td>European League Against Rheumatism</td>
</tr>
<tr>
<td>FMD</td>
<td>Flow mediated dilatation</td>
</tr>
<tr>
<td>GTN</td>
<td>Glyceryl trinitrate</td>
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<tr>
<td>HAQ</td>
<td>Health assessment questionnaire</td>
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<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
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<tr>
<td>HMG-CoA</td>
<td>3-hydroxy-3-methylglutarylcoenzyme A</td>
</tr>
<tr>
<td>HOMA</td>
<td>Homeostatic Model Assessment</td>
</tr>
<tr>
<td>hsCRP</td>
<td>High sensitivity CRP</td>
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</table>
HSP  Heat shock proteins
ICAM-1  Intercellular adhesion molecules
IL  Interleukin
LAE  Large artery elasticity
LDL  Low-density lipoprotein
Lp-PLA2  Lipoprotein associated phospholipase A2
MI  Myocardial infarction
NADPH  Nicotinamide-adenine dinucleotide phosphate
NO  Nitric oxide
NOS  Nitric oxide synthase
NSAIDs  Non-steroidal anti-inflammatory medications
OR  Odds Ratio
Ox-LDL  Oxidised LDL
PGI-2  Prostacyclin
PON  Paraxonase
PWA  Pulse wave analysis
PWV  Pulse wave velocity
RA  Rheumatoid arthritis
RCT  Reverse cholesterol transport
RF  Rheumatoid factor
RR  Relative risk
SAA  Serum amyloid A
SAE  Small artery elasticity
SCORE  Systematic Coronary Risk Evaluation
SMR  Standardised mortality rate
Statin  HMG-CoA (3-hydroxy-3-methylglutarylcoenzyme A) reductase inhibitor
sVCAM-1  Soluble vascular cell adhesion molecules
SVR  Systemic vascular resistance
TC  Total cholesterol
TG  Triglycerides
TNFα  Tumour necrosis factor
TXA-2  Thromboxane A-2
VIGOR  Vioxx Gastrointestinal Outcomes Research
WHO  World Health Organisation
1. Introduction:

1.1 Rheumatoid arthritis and cardiovascular disease

Rheumatoid arthritis (RA) is a chronic, inflammatory, systemic disease. Polyarthritis and synovitis are the most common features, but extra-articular involvement of the skin, ocular, respiratory, neurological, and cardiovascular (CV) systems also commonly occur. Traditionally, CV manifestations were thought mainly to comprise pericarditis, pericardial effusion, myocarditis, and aortitis. More recently, however, premature atherosclerosis, myocardial infarction (MI) and ischaemic stroke are recognised as important CV manifestations (1, 3). Van Doornum et al have suggested that accelerated atherosclerosis be considered an extra-articular manifestation of RA (3). CV disease may be a larger problem than is currently recognised. RA patients are less likely to report chest pain which may in fact be angina. There is also a high incidence of ‘silent’ myocardial infarct and sudden cardiac death (9).

This newly recognised extra-articular manifestation is the main contributor to death in patients with RA (1, 10). Meta-analysis of 24 observational studies found CV disease mortality to be increased by approximately 50% in RA patients compared with the general population (meta-SMR 1.50, 95% CI 1.39-1.61) (11). Life-span is shortened by as much as 3-18 years (3). The excess CV events seen in RA patients are not explained by traditional CV risk factors such as dyslipidaemia, hypertension, smoking, family history, male gender, diabetes and body mass index (2). In fact, studies have found independent predictors of CV death relate to RA disease severity. Increased mortality is associated with high self report variables such as the health assessment questionnaire (HAQ) (12), erythrocyte sedimentation rate (ESR), rheumatoid vasculitis (10), increased number of swollen joints (13) and higher age at disease onset (14). Decreased mortality is associated with low disease activity and response to disease modifying anti-rheumatic drugs (DMARDs) (14, 15). Hence, mortality risk increases with variables which indicate higher levels of inflammation, and decreases with variables which reduce levels of inflammation.

1.2 The role of inflammation in RA and CV disease

The link between RA and atherosclerosis appears to be inflammation. Like RA, atherosclerosis is an inflammatory disease involving both adaptive and innate immunity (16). Both RA and atherosclerosis activate inflammatory cells, cytokines,
and increase expression of adhesion molecules (17). Factors such as interleukin (IL) - 6, IL-18, tumour necrosis α (TNFα), activated T-cells, adhesion molecules such as soluble vascular cell adhesion molecules (sVCAM-1), intercellular adhesion molecules (ICAM-1), E-selectin, P-selectin, oxidised LDL, lipoprotein associated phopholipase A2 (Lp-PLA2), lipoprotein-a, heat shock proteins (HSP), serum amyloid A (SAA), CD-40 ligand, adiponectin and leptin have drawn such interest. Pasceri et al illustrated the similarities between RA and atherosclerosis in 1999 (17), the table is reproduced below.

**Similarities Between Atherosclerosis and Rheumatoid Arthritis**

<table>
<thead>
<tr>
<th></th>
<th>Atherosclerosis</th>
<th>Rheumatoid arthritis</th>
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<tbody>
<tr>
<td>Macrophage activation</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>TNFα</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Metalloproteinase expression</td>
<td>↑ (*UA)</td>
<td>↑</td>
</tr>
<tr>
<td>Interleukin -6</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Mast-cell activation</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>T-cell activation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble IL-2 receptor</td>
<td>↑ (UA)</td>
<td>↑</td>
</tr>
<tr>
<td>CD3+DR+</td>
<td>↑ (UA)</td>
<td>↑</td>
</tr>
<tr>
<td>CD4+CD28-</td>
<td>↑ (UA)</td>
<td>↑</td>
</tr>
<tr>
<td>CD4+IFNγ+</td>
<td>↑ (UA)</td>
<td>↑</td>
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<tr>
<td>Th1/Th2 balance</td>
<td>↑ Th1</td>
<td>↑ Th1</td>
</tr>
<tr>
<td>B-cell activation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autoantibodies (oxLDL, HSP)</td>
<td>0 or ↑</td>
<td>0 or ↑</td>
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<tr>
<td>Rheumatoid factor</td>
<td>0</td>
<td>↑</td>
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<tr>
<td>C-reactive protein</td>
<td>↑ (UA)</td>
<td>↑↑</td>
</tr>
<tr>
<td>Adhesion molecules (VCAM-1, ICAM-1, E-selectin, P-selectin)</td>
<td>↑</td>
<td>↑</td>
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<tr>
<td>Endothelin</td>
<td>↑</td>
<td>↑</td>
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<tr>
<td>Neoangiogenesis</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Possible antigens</td>
<td>HSP, Ox-LDL, infectious agents</td>
<td>Collagen II, Cartilage antigens, HSP, infectious agents</td>
</tr>
</tbody>
</table>
**HSP**, heat shock protein

*UA* indicates systemic markers found increased in patients with unstable angina.

Other factors are expressed in atherosclerotic plaques.

Prospective studies in healthy men and women have demonstrated the acute phase reactant C-reactive protein (CRP) to be a powerful predictor of future myocardial infarct and ischaemic stroke (18, 19) when measured with a high sensitivity assay (hsCRP). CRP has been localized in vessel walls and can bind neutrophils, interact with adhesion molecules, activate complement and enhance tissue factor expression (4). Inflammatory cells and CRP are localized in complex plaques which are prone to rupture. Ridker et al found CRP to be the strongest non-lipid predictor of cardiovascular events (RR for the highest vs. lowest quartile, 2.8; 95% CI, 1.3 to 5.9) and addition of CRP to standard lipid screening significantly improved risk prediction (20).

This has been further demonstrated by the Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) (21). Subjects with low CV risk with LDL cholesterol below current treatment thresholds (< 3.4 mmol per liter) but with elevated hsCRP levels (≥ 2.0 mg per liter) were randomized to rosuvastatin 20 mg daily or placebo. The median hsCRP was 4.3 mg/L, interquartile range 2.8 to 7.2, levels which is generally lower than that found in RA patients. This study demonstrated that rosuvastatin, a HMG-CoA (3-hydroxy-3-methylglutarylcoenzyme A) reductase inhibitor (statin) reduced the levels of low density lipoprotein (LDL) cholesterol and hsCRP at a median follow up of 1.9 years. More importantly, it was found to reduce the risk of stroke, MI, revascularization or unstable angina and death from CV cause in apparently healthy people. This indicates that statins can reduce the risk of CV events even further in people who do not have raised LDL cholesterol but who do have an elevated CRP. Statins appear to have additional anti-inflammatory effects, possibly by reducing induction of MHC class II molecules which decreases immune activation in atherosclerotic plaques (16). The link between CRP and CV events in the general population is fairly well established, but its role in RA where CRP levels are commonly above the ‘normal’ range and fluctuate over time depending on RA disease activity is less clear.
1.3 Increased traditional CV risk factors in RA

The prevalence of metabolic syndrome has been found to be increased in RA compared to controls in one study (42% compared to 11%) and the presence of coronary artery calcification was higher in RA and highest in patients with both RA and metabolic syndrome (22). The metabolic syndrome is a clustering of traditional CV risk factors which is a strong independent risk factor for CV disease, more than the sum of its individual components. The World Health Organisation (WHO) classification of metabolic syndrome (1999) requires diabetes mellitus, impaired glucose tolerance, impaired fasting glucose or insulin resistance, and two of the following:

- Blood pressure: ≥ 140/90 mmHg
- Dyslipidaemia: triglycerides (TG): ≥ 1.695 mmol/L and high-density lipoprotein cholesterol (HDL) ≤ 0.9 mmol/L (male), ≤ 1.0 mmol/L (female)
- Central obesity: waist:hip ratio > 0.90 (male); > 0.85 (female), and/or body mass index > 30 kg/m²
- Microalbuminaemia: urinary albumin excretion ratio ≥ 20 mg/min or albumin:creatinine ratio ≥ 30 mg/g.

Inflammation is associated with a reduction in lipid levels including low-density lipoprotein (LDL), HDL and TG levels (23). This phenomenon has been observed in other inflammatory states including sepsis, post myocardial infarction and post surgery (23). However, changes in lipid parameters during inflammation appear to be more complex. By inducing subacute endotoxaemia in rat and human models, multiple steps of the reverse cholesterol transport (RCT) pathway were found to be impaired. RCT has a major atheroprotective function of HDL. These findings were independent of HDL cholesterol levels, suggesting that irrespective of HDL cholesterol levels, HDL cholesterol function is compromised during chronic inflammation (24). Apolipoprotein A-I (Apo A-1) in HDL promotes RCT processes and reduces formation of oxidised LDL. Pro-inflammatory HDL results in accumulation of oxidants in HDL which inactivates Apo A-1 (25). Furthermore, paraxonase (PON) is reduced in patients with RA, regardless of ESR and CRP levels.
PON is the major enzyme on HDL that allows HDL to protect LDL from oxidation. Lower levels of PON lead to more oxidised LDL (26).

Similarly, inflammation has been found to induce insulin resistance following modulation of specific adipose inflammatory and insulin signaling pathways using an endotoxaemia model (27). Homeostatic Model Assessment (HOMA) index is used as a measure of insulin sensitivity and its calculation is based on fasting insulin and glucose levels. HOMA has been found to be increased in RA patients and to be positively correlated with IL-6, TNFα, CRP, ESR, coronary calcification and DAS 28 (28). Increasing levels of TNFα reduces insulin signaled glucose uptake probably by inhibition of auto-phosphorylation of the insulin receptor (29).

Increased obesity in patients with RA is one factor in insulin resistance. RA patients are prone to obesity not only because of reduced physical activity due to joint pain and deformity, but also because of chronic inflammation and glucocorticoid use. RA patients have a higher visceral fat area which is associated with increased joint deformity, CRP levels, RF seropositivity and lack of current treatment with DMARDs (30).

1.4 Endothelial dysfunction in CV disease
The interaction between these inflammatory molecules and the vascular endothelium may be integral to the development of atherosclerosis and its complications. Vascular endothelium is important for vascular homeostasis. It interacts with the vascular micro-environment by regulating vasodilatation, thrombosis, coagulation, inflammation and smooth muscle cell proliferation and migration (31). Endothelial dysfunction is one of the key early events in the development of atherosclerosis.

Multiple factors are involved with endothelial dysfunction but abnormal nitric oxide (NO) production and metabolism is thought to be a key element. Impaired NO bioavailability predisposes to vasoconstriction, platelet activation, thrombosis, attraction and adherence of leukocytes, oxidant stress, mitogenesis and increased permeability (32). Endothelial cells continually produce a basal amount of constitutive NO synthase (NOS) and also an inducible form of NOS which is stimulated by shear stress and inflammation. Conventional risk factors for CV
disease such as aging, obesity, hypertension, smoking, dyslipidaemia and diabetes inactivate endothelial NOS. These conventional risk factors also increase NO inactivation by activating nicotinamide-adenine dinucleotide phosphate (NADPH). NADPH produces reactive oxygen species which inactivates NO (33).

Subsequent events in the evolution of atherosclerosis and CV events include altered flow (shear stress), lipoprotein modification and lipid accumulation (fatty streak formation), endothelial and plaque inflammation, foam cell formation, smooth muscle cell migration and proliferation, remodelling and plaque complications (34). Endothelial dysfunction is therefore closely related to stiffening of the arteries both functionally and structurally.

1.4.b Measurement of vascular function
The ability to measure the degree of vascular function is therefore regarded as helpful in assessing early atherosclerosis and identifying groups at risk of future CV events. There has been much interest in non-invasive measures which are acceptable to the patient, easy to use not only in research but also in everyday clinical practice and reproducible (35). There is no measure that gives a complete and definitive assessment of vascular health. Different techniques focus on different aspects of the arterial tree and examine it in different regions (36). Morphological assessments of the vascular tree probably best reflect the current severity of vascular damage and functional assessments of vascular function may provide the risk of progression of organ/vascular damage (33).

1. Morphological evaluation of vascular damage
   a. Carotid Intimal MedialThickness (CIMT)
   b. CT and MRI
2. Functional assessment of vascular damage
   a. Flow Mediated Dilatation (FMD)
   b. Pulse Wave Analysis (PWA)
   c. Pulse Wave Velocity (PWV)

There is evidence that some of these measurements correlate with one another, reflecting the pathophysiological link between endothelial dysfunction and arterial...
stiffness. Wilson et al found that some measures of PWA correlated with FMD in healthy subjects and in older diabetics (37). However, the relationships between arterial function measures were different in these groups, suggesting that some measures of vascular function may be more useful in high CV risk groups compared to low CV risk groups. It is unclear which method or combination of methods is best to use for assessment of different aspects of vascular health and which to use in low and high risk subjects.

How well these non-invasive measures of vascular function reflect atherosclerotic load and predict future CV events is also an important consideration. These techniques have been used in subjects with known coronary artery disease with high atherosclerotic burden as well as in subjects with traditional CV risk factors who are at a higher risk for CV disease. Impairment of FMD and increased arterial stiffness has been found in most published studies to correlate with subjects with higher atherosclerotic burden and higher risk of developing CV disease (38-46). Many of these techniques also demonstrate improvement after therapies which have been documented to improve CV risk such as antihypertensive treatments or statins. Some have even shown the ability to predict future CV events in certain patient groups (47-50). This suggests that these measures reflect to a degree the level of vascular health and endothelial dysfunction.

This collation of papers describes the use of non-invasive measures of CV disease in patients with RA using FMD, PWA, PWV and CIMT.

1.5 Flow Mediated Dilatation (FMD)
The best validated test of assessing vascular endothelial function non-invasively is FMD (51). This measures reactive hyperaemia at the brachial artery using ultrasound. The use of glyceryl trinitrate (GTN) as a non-endothelium dependent source of NO is used as the control. A blood pressure cuff is placed at the forearm of a supine subject and inflated to above systolic blood pressure for 5 minutes in order to create vascular ‘shear stress’ proximal to the cuff. Ultrasound measurement at the brachial artery is used to determine the percentage change from baseline to 60 seconds after deflation. FMD relies on NO bioavailability and therefore is an indicator of endothelial function.
FMD has been found to be impaired in patients with known coronary artery disease (CAD) (38) and in groups at risk of CV disease including hypertension, diabetes, smoking and higher BMI (39, 40). Impaired FMD has also been found to predict future CV events (50). Furthermore, various treatments have been found to improve FMD including statins (52), fibrates (53), folic acid (54), ACE inhibitors (55) and AT-1 receptor blockers (56).

1.6 Measures of arterial stiffness
PWA and pulse wave velocity PWV are measures of arterial stiffness or compliance. Techniques used to measure these parameters are somewhat simpler than FMD in the clinical setting. Arterial stiffness is determined by several factors including arterial wall mechanics and vascular wall tone. In the vessel wall, imbalance between collagen and elastin production and degradation contribute to structural causes of arterial stiffening. Thinning and splitting of elastic fibres occurs with aging and hypertension. Changes in deposition of glycoproteins and proteoglycans on the vessel wall also contribute to structural stiffening. Similarly, there is increased accumulation of advance glycation endproducts on collagen and elastin in diabetic patients (57). Vascular smooth muscle cells contribute to wall tone and are regulated by neural influences and vasoactive agents including NO (58). Healthy endothelium with low atherosclerotic burden and good NO bioavailability have better compliance or low arterial stiffness. It appears therefore that arterial stiffness is due to both long term structural changes to the vessel wall as well as changes in vascular wall tone which may vary from instant to instant depending on the endothelial microenvironment (36).

Arterial stiffening leads to increased transmitted pulse wave velocity and pulse wave propagation (59). Arterial stiffening contributes to systolic hypertension and its complications which include increased myocardial oxygen consumption, left ventricular hypertrophy, reduced myocardial perfusion, atrial fibrillation and diastolic heart failure.

1.7 Pulse wave analysis
In this thesis, PWA was measured using two different devices. PWA using a HDI CR-2000 pulsewave analyser (Hypertension Diagnostics, Eagan MN) uses a radial
arterial transducer. It assesses the arterial waveform and calculates arterial elasticity based on the modified Windkessel model. It measures capacitive arterial compliance/Large artery elasticity (LAE), oscillatory/reflective compliance/small artery elasticity (SAE) and systemic vascular resistance (SVR) (60). PWA using this system is abnormal in patients with increased vascular risk including type 1 diabetes (61), type 2 DM (42), hypertension (43), long-term cigarette smoking (44), and is predictive of vascular events (62).

The SphygmoCor (PWA system, SphygmoCor, PWA Medical, Sydney, Australia) uses applanation tonometry at the radial artery. It measures the magnitude of central aortic pressure augmentation. The augmented pressure (AP) is defined as the difference between the second and the first systolic peak, and augmentation index (AIx) is AP expressed as a percentage of the pulse pressure. PWA using this system has been shown to prospectively predict CV risk as independent variables (48). It has also been recently used to demonstrate improvement in vascular stiffness after statin therapy in patients with RA (63).

1.8 Pulse wave velocity

PWV was determined as the speed at which the pulse wave travels between the carotid and femoral arteries, using the SphygmoCor PWA system. These methods have been used to demonstrate worse vascular function in groups at risk of and with CV disease (45, 46) and in patients with systemic vasculitis (64). PWV has been shown also to be a predictor of future CV events (47, 65) and has been recommended as the ‘gold standard’ for estimating arterial elasticity, mainly because it requires little technical expertise and is supported by the greatest amount of epidemiological evidence (66).

1.9 Carotid intima media-thickness (CIMT)

CIMT is a measure of arterial structure or atherosclerotic burden and is measured by high resolution ultrasound (67). CIMT appears not just to estimate atherosclerotic burden but also correlates with risk of future myocardial infarction and stroke independent of traditional CV risk factors (49). It is the only noninvasive imaging test recommended by the American Heart Association for inclusion in the evaluation of risk.
Therapies which have been shown to reduce the progression of CIMT include beta-blockers (68), calcium channel blockers (69) and statins (70). Beneficial effects of these therapies are usually seen with CIMT only after long term treatment, most studies requiring 1 to 3 years of follow up. Recent studies of intensive statin therapy have even shown regression of CIMT (71) and reduced progression or even regression of coronary artery intimal thickening as measured by intravascular ultrasound (72).

1.10 Cardiovascular effects of RA therapies

Some therapies frequently used in RA have drawn particular interest with regard to their CV effects. These include corticosteroids, methotrexate, TNFα-inhibitors, non-steroidal anti-inflammatory medications (NSAIDs) and cyclo-oxygenase inhibitors (COX-2 inhibitors) and statins.

1.10.a Glucocorticoids

Glucocorticoids are commonly used by patients with RA, either intermittently during disease flares or continuously to achieve disease control often in combination with DMARDs. They are effective by reducing inflammation. If atherosclerosis is indeed an inflammatory condition, then it should follow that glucocorticoids improve CV risk. However, glucocorticoids have multiple adverse systemic effects which include obesity, hypertension, and insulin resistance. These factors may predispose to CV disease. Cohort studies have in fact found glucocorticoids to be a risk factor for CV events including MI, stroke, transient ischaemic attacks and heart failure. The relative risk for a CV event in patients receiving glucocorticoids at a dose ≥ 7.5mg was 2.56 (CI, 2.18 to 2.99) in one study (73). The risk for CV events was stronger for patients who were taking glucocorticoids at higher average daily doses, as continuous rather than intermittent doses and for patients who were current users rather than recent or past users (74). It appears therefore that glucocorticoids despite having an anti-inflammatory role, has a net negative effect on CV health in patients with RA.

1.10.b NSAIDs and COX-2 inhibitors

NSAIDs and COX-2 inhibitors also have an anti-inflammatory effect and are used commonly for analgaesia in patients with RA. These drugs may also have a negative CV effect by predisposing to renal impairment, hypertension, peripheral oedema and
congestive heart failure (75). COX-2 inhibitors in particular could theoretically predispose to a pro-thrombotic vascular environment via their selective inhibition of COX-2. Cyclo-oxygenase-1 (COX-1) and COX-2 isoenzymes catalyse the conversion of arachidonic acid to prostaglandins. COX-1 is the main source of production of thromboxane A-2 (TXA-2) which mediates platelet aggregation and vasoconstriction. COX-2 is the main source of prostacyclin (PGI-2) which has vasodilating anti-aggregatory and anti-proliferative effects. Therefore, selective inhibition of COX-2 causes suppression of PGI-2 without affecting TXA-2 and theoretically could predispose to hypertension and thromboembolic events (76).

CV safety of COX-2 inhibitors has been debated since the Vioxx Gastrointestinal Outcomes Research (VIGOR) study (77). This found a fourfold increase in risk of MI in patients taking rofecoxib (50mg/day) compared to patients taking naproxen (incidence of MI, 0.4% vs. 0.1%). The Celecoxib Long-term Arthritis Safety Study (CLASS) did not show a higher incidence of MI in patients taking diclofenac or ibuprofen (78). In the CLASS study use of aspirin was allowed, RA patients were included and naproxen was subsequently thought to have a cardio-protective effect. These were some of the reasons put forward to explain the difference in CV risk between the studies. However, rofecoxib was subsequently withdrawn from the market in 2004 following findings of the Adenomatous Polyp Prevention On Vioxx (APPROVe) study (79). This study was designed to determine whether or not rofecoxib prevented recurrence of colorectal polyps in patients with a history of colorectal adenomas. The study was terminated early when the risk of CV event in the rofecoxib group was double that of the placebo group (1.50 vs. 0.78 events per 100 patient years).

Traditional NSAIDs have been reviewed more closely following the controversy regarding COX-2 inhibitors and CV risk. A nested case controlled study in the United Kingdom found an increased risk of myocardial infarction associated not only with current use of rofecoxib, but also with diclofenac, and ibuprofen. No evidence was found to support a reduction in risk of myocardial infarction associated with current use of naproxen (80).

There is some evidence to support the hypothesis that anti-inflammatory medications may have beneficial effects in atherosclerosis. One study found improved FMD with
the use of COX-2 inhibitor celecoxib in patients with CAD (81). This improved vascular endothelial function correlated to improvement in measures of inflammation including high sensitivity CRP (hsCRP) and oxidised LDL.

1.10.c Methotrexate

Methotrexate is the first line DMARD therapy and widely prescribed for patients with RA. Because methotrexate can increase plasma homocysteine levels it could in theory contribute to the increased CV risk in RA patients (82). The concomitant use of folic acid with methotrexate which is almost in universal use has been shown to reverse methotrexate-induced changes in homocysteine levels. Most evidence suggests that methotrexate reduces mortality risk in RA and that in fact much of the survival benefit is attributable to reduced CV risk (5). In one prospective observational study, patients who did not respond to methotrexate treatment after 1 year had a SMR of 4.11 compared with a SMR of 1.47 in patients who had a >50% response to methotrexate. Those who discontinued methotrexate treatment within the first year had a SMR of 5.56 (15). The use of methotrexate in RA patients has also been shown to be associated with a reduced chance of having the Metabolic syndrome (OR = 0.517, CI 0.33-0.81, P = 0.004) (83). The mechanism by which methotrexate might improve CV risk in RA may be its role in suppressing inflammation, and therefore improving the components of the metabolic syndrome. There may however be a drug-specific effect rather than purely its suppression of inflammation as other DMARDs and glucocorticoids have not been found to be associated with a reduction of metabolic syndrome in RA (83).

1.10.e TNFα inhibitors and other biologicDMARD agents

The biologic DMARDs (bDMARDs) are more powerful suppressers of inflammation and have revolutionised the treatment of RA. These agents are bio-engineered therapies with specific immune targets for therapeutic manipulation in RA. As both RA and atherosclerosis are inflammatory diseases which share immunological processes, it is possible that these targeted therapies may also provide effective selective intervention in atherosclerosis. T_{H1} cells and TNFα are important in RA and atherosclerosis progression. Activated T cells in the shoulder or growing region of atheromatous plaques produce TNFα. TNFα promotes pro-inflammatory mediators,
activates endothelial cells and increases adhesion-molecule expression, thereby promoting thrombus formation (16).

TNFα inhibitors were the first bDMARDs to be used widely and have the most data available regarding CV and mortality outcomes. Infliximab was the first TNFα inhibitor to be introduced and is a chimeric mouse/human antibody administered intravenously. Other TNFα inhibitors subsequently made available for the treatment of RA are etanercept, adalimumab, and golimumab each administered by subcutaneous route. Other bDMARD agents available include rituximab which depletes B cells by targeting CD 20, abatacept which is a T cell co-stimulation modulator, tocilizumab which inhibits IL-6 and anakinra which inhibits IL-1. If the use of methotrexate in RA has a survival benefit, then it should follow in theory that the newer and stronger immuno-suppressant therapies may have an equivalent if not better survival benefit.

The wealth of data so far available supports the hypothesis that the use of TNFα inhibitors in patients with RA reduces the CV and mortality risk (84, 85). Most of this information has been made available from registry data in Britain (84) Europe (86) and North America. Interestingly, the British registry (BSRBR) did not find a significant difference in the incidence of MI when comparing RA patients treated with TNFα inhibitors compared with patients who did not receive TNFα inhibitors but were treated with regular DMARDs. However, multivariate sub-analysis found that the incidence rate ratio of MI in responders compare to non-responders to TNFα inhibitors within 6 months was 0.36 (0.19-0.69) (84). These findings further support the role of inflammation in the promotion of joint disease and atherosclerosis in RA. It also suggests that suppression of inflammation improves not only joint symptoms and damage, but also CV risk and mortality.

The effect of TNFα inhibitor treatment on surrogate markers of CV disease in RA patients has been variable. Beneficial effect in endothelial function (87), active but transient improvement in endothelial function (88), or no improvement (89). Study design, techniques, length of follow up and patient characteristics may contribute to these different outcomes.
Other effects of TNFα inhibitors include reduction in homocysteine levels (90) and enhanced insulin sensitivity (91). These changes should improve CV risk. Lipid levels however rise with bDMARD treatment, particularly evident with the use of tocilizumab (92). This apparent negative effect on CV risk has may be viewed simply as a correction of lipid levels back to baseline by causing resolution of the inflammatory state. In general LDL, HDL and triglyceride levels decrease during states of inflammation (23).

There has, however, been debate as to whether TNFα inhibitor treatment worsens or improves heart failure. It was initially contraindicated in patients with New York Heart Association class III and IV heart failure. This recommendation followed findings from studies in patients with heart failure who were treated with infliximab or etanercept. Anti-TNFα treatment was investigated in this group of patients as it was thought it might improve heart failure. However, the study was ceased prematurely because of increased hospitalisation from heart failure and deaths in patients with heart failure treated with infliximab or etanercept (93). Subsequent investigation has suggested that TNFα inhibitor treatment that effectively reduces the inflammatory activity of RA is more likely to be beneficial rather than harmful to the risk of heart failure, especially if there is no concomitant therapy with glucocorticoids or COX-2 inhibitors (94). Epidemiological studies have found the incidence of heart failure in RA to be higher than in osteoarthritis patients, with RF, ESR, scleritis, RA lung and vasculitis being predictors of heart failure. Heart failure was less common in TNFα inhibitor treated patients (95). The effect of TNFα inhibitor treatment in RA is therefore likely to be different to the effect seen in heart failure patients without RA.

1.10.f Statins
In healthy subjects without hyperlipidemia but with elevated hsCRP levels, statin therapy has been found to reduced the incidence of major CV events (21). Statins therefore appear to have an additional anti-inflammatory effect and may therefore improve CV risk in RA patients. In a randomised double-blind placebo-controlled trial (TARA study), 116 RA patients received 40 mg atorvastatin or placebo in addition to existing DMARD therapy. At 6 months cholesterol, LDL-cholesterol, triglycerides, DAS28 and swollen joint count improved in the group on atorvastatin.
Furthermore, CRP and ESR declined by 50% and 28% respectively in the atorvastatin group (96).

Atorvastatin has also been demonstrated to reduce arterial stiffness in RA patients as measured by PWA augmentation index. In this study of 29 RA patients serum inflammatory markers were unchanged after the 12 weeks of therapy, but total and LDL cholesterol were reduced significantly (63).

1.11 Summary
This thesis is a collection of studies using the described measures of vascular function to assess endothelial function and arterial stiffness in patients with RA. Given the higher mortality and CV event rates in patients with RA, I sought firstly to establish whether patients with RA have worse vascular function compared with the general population. Secondly, I endeavoured to investigate the relationship between markers of inflammation in RA and vascular function. Finally, I investigated whether various drug treatments used commonly in patients with RA have any impact on vascular function.

The first cross sectional study was performed in Melbourne at St. Vincent’s and Royal Melbourne hospitals. The two subsequent randomised placebo-controlled double-blind therapy studies were performed at Guy’s and St. Thomas’ NHS trust, London, UK. PWA was measured using the devices which were available to me at each of the institutions where the studies were performed. The HDI CR-2000 pulsewave analyser was used in Melbourne and the SphygmorCor was used in London.

2. Overview:
2.1 Vascular function in patients with RA compared to control subjects
We performed a cross sectional study in 53 patients with RA and 53 control subjects who did not have RA. Within the RA group, 15 patients had known CAD and 38 were not known to have CAD. The control group matched the RA group with 15 patients with CAD (from the cardiology clinic) and 38 healthy subjects (from the community and hospital staff). PWA was performed in all subjects using the HDI CR-2000 pulsewave analyser (Hypertension Diagnostics, Eagan MN). These vascular
measures were correlated with markers of inflammation (6). We found that small artery elasticity (SAE) and large artery elasticity (LAE) was lower in RA than in control subjects and systemic vascular resistance (SVR) higher. Among the RA patients, the presence of CAD was associated with higher SVR. SAE and LAE were lower but did not reach statistical significance. Among the control subjects, SAE was significantly lower in the group with CAD. LAE was lower and SVR higher but did not reach statistical significance. These results support the hypothesis that vascular function is impaired in patients with CAD and in patients with RA. It is most impaired in patients with both these conditions.

2.2 Relationship between vascular function and traditional vascular risk factors in patients with RA

In the cross sectional study we found that reduced arterial elasticity and increased SVR in RA were not correlated with traditional vascular risk factors (BMI, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, hypertension and smoking).

2.3 Relationship between vascular function and markers of inflammation in patients with RA

In the cross sectional study we found that vascular function correlated with the acute phase proteins when analysing the study group as a whole, but not within the RA group alone. The range of values within the RA and control groups considered in isolation may not have been great enough to expose a relationship. Combining the groups provided a wider range of values for analysis, but did not allow us to make definitive conclusions. We found that the SAE and LAE correlated inversely with hsCRP and SAA. SVR correlated positively with sVCAM-1 (6).

In regression analysis, the observed difference in SAE between RA patients and controls was accounted for by differences in hsCRP values, suggesting that inflammation contributes to the impaired vascular health observed in patients with RA. Even though we found a relationship between arterial elasticity and SAA and sVCAM-1, these acute phase markers did not provide any further information than that given by hsCRP.
2.4 Drug therapies and vascular function in patients with RA:
In our cross sectional study we found that patients with RA had impaired vascular function. The impaired vascular function was found not to be related to traditional vascular risk factors, but was found to be related to markers of inflammation. We then set out to investigate whether drugs commonly used in RA which have some effect on inflammation might improve vascular function. In two separate randomised placebo-controlled double-blind studies we aimed to determine whether drugs which selectively and non-selectively inhibit cyclo-oxygenase (COX), and drugs which inhibit TNFα would improve vascular function in patients with RA.

2.4.a NSAID and COX-2 inhibitors
The first intervention study investigated a two week course of rofecoxib vs. indomethacin vs. placebo in 37 patients with RA. We hypothesised that both the COX-2 inhibitor (rofecoxib) and the non-selective NSAID (indomethacin) would improve vascular function as measured by FMD and PWA. This was based on previous studies using aspirin and celecoxib in patients with CAD (81, 97). The improved vascular function was thought to be due to the anti-inflammatory or anti-oxidant properties of celecoxib. We chose rofecoxib in our study as the COX-2 inhibitor under investigation because of the disparity between CV events seen in the CLASS and VIGOR studies. Indomethacin was chosen as the non-selective NSAID because it was thought to be one of the most potent in its class of drug.

Patients were randomised to one of the three therapy arms. FMD, PWA, RA disease activity and CV risk factors including lipids and hsCRP were assessed before and after the two week treatment course. PWA was measured using the PWA system, SphygmoCor, PWA Medical, Sydney, Australia. FMD and PWA were chosen as the vascular outcome measures because these are able to change in a short time period, unlike measures such as CIMT which may take years.

Contrary to our hypothesis, we found that COX inhibition with either rofecoxib or indomethacin did not improve vascular function as measured by FMD and PWA. Furthermore, we found no significant differences in changes in BP, serum creatinine, ESR or hsCRP between groups. Possible explanations for finding no significant differences may be small numbers, inadequate drug dosage, short treatment period or
confounding by other medications taken by RA patients. However, our findings may indicate that the inflammation in RA is much greater than in CAD (our hypothesis was based on a study in CAD patients). Selective and non-selective NSAIDs may not be powerful enough suppressors of inflammation to impact upon vascular function in this setting.

2.4.b *Anti-TNFα therapies*

Anti-TNFα therapies were the first biologic agents available for the treatment of RA. They are much more powerful suppressors of inflammation than selective and non-selective NSAIDs.

The second intervention study was named DIVERT: Defining the use of Infliximab on Vascular Endothelium in Rheumatoid arthritis Trial. In this study 26 RA patients were randomised 2:1 to either Infliximab (17 patients) or placebo saline infusions (9 patients). Patients randomised to placebo infusions were to change to open label infliximab at 24 weeks. However, placebo-group patients with worsening RA were permitted to enter an escape arm at 16 weeks. All patients in the placebo arm entered this escape arm and changed over to infliximab infusions at week 16.

Vascular assessments included PWV, PWA, CIMT and Carotid artery plaque (CAP). PWV and PWA were measured using the SphygmoCor PWA system at baseline, 24 and 56 weeks. CIMT and CAP were measured at baseline and 56 weeks.

CV risk factors assessed included hsCRP, lipid profile, oxidised LDL, insulin resistance measured by log homeostasis model assessment (HOMA) and adiponectin. Adiponectin is an adipocyte derived peptide involved in the regulation of insulin sensitivity and lipid oxidation and has been found to be protective against CV disease events (98).

In the placebo-controlled double-blind phase of the study, there was significant improvement in tender and swollen joint counts, patient global assessments and disease activity 28 (DAS28) scores at weeks 8 and 16. Arterial stiffness, CIMT, lipid profiles, HOMA and adiponectin were not assessed during the placebo controlled phase of the study.
Post-hoc analysis using multivariate ANOVA modelling showed significant reduction in PWV over 56 weeks. There was no change in AIx as measured by PWA, CIMT and carotid plaques. RA disease activity (DAS28, tender and swollen joint counts, patient global scores and ESR) improved significantly at 24 and 56 weeks. There were no significant changes in BP, triglycerides or adiponectin levels, although HOMA showed a non-significant decrease and serum HDL showed a non-significant increase over the 56 week period. PWV correlated negatively with duration of infliximab therapy (increased arterial stiffness correlated with less duration of infliximab therapy) and positively with patient global assessment (increasing arterial stiffness correlated with increasing patient global assessment of disease activity). Regression modelling found that duration of infliximab therapy (negative correlation) and heart rate (positive correlation) were the only significant influences upon PWV.

This was the first study to demonstrate long-term improvement in RA vascular stiffness with TNFα-blocking therapy. Unfortunately the placebo-controlled part of the study was unable to assess vascular effects of infliximab due to early drop-out of the placebo group. Modifiable CV risk factors including HOMA and adiponectin did not improve significantly over time, despite significant improvements in disease activity and PWV. This suggests that vascular stiffness is somehow linked to chronic active RA but the mechanisms of this link are unclear.

3. Conclusion:
This compilation of research papers provides evidence of vascular dysfunction in patients with RA. Vascular dysfunction did not correlate with traditional CV risk factors, but did correlate with inflammation as measured by CRP, SAA and sVCAM-1. This suggests that the increased CV mortality and morbidity observed in patients with RA is not explained by traditional CV risk factors.

Chronic inflammation in RA is hypothesised to be the link with increased CV risk. Patients with RA may therefore be suffering not only from inflammation of joints which if left uncontrolled may lead to joint damage, but also from inflammation of the vascular endothelium which if left uncontrolled may lead to CV damage. Other
inflammatory rheumatological conditions found to have a greater atherosclerotic burden include systemic lupus erythematosus (99), psoriatic arthritis (100), ankylosing spondylitis (101) and antineutrophil cytoplasmic antibody (ANCA)-associated systemic vasculitis (64).

We did not find any influence of indomethacin or rofecoxib on arterial elasticity in RA over a 2 week treatment period when measured using PWA and FMD. It is not known whether these results reflect the fact that these medications have a very mild effect on inflammation and therefore do not have an impact on vascular dysfunction. Their overall effect on CV risk is more complex because of their potential pro-thrombotic effects.

However, we did find that more intense suppression of inflammation using the TNFα blocking agent infliximab had a beneficial effect on arterial stiffness when measured using PWV in RA over a 56 week period. Duration of infliximab treatment correlated negatively with PWV, but there was no change over time with CV risk factors including HOMA and adiponectin.

Whether other therapies used in RA have an effect on vascular function would contribute to our understanding of inflammation in atherosclerosis. Both methotrexate and prednisolone improve inflammation and disease activity in RA. However, methotrexate has a beneficial effect on mortality and CV risk (5) whilst prednisolone has a net negative impact on CV risk (73). The effect of other biologic agents on vascular function and CV risk would also be of interest. Other TNFα inhibitors including etanercept and adalimumab have mostly demonstrated similar findings with improved CV outcomes and measures. B cell depletion with rituximab has been found to improve FMD and possibly CIMT in a small study of five patients with RA 16 weeks after infusion (102). There is so far no published data on the effect of co-stimulatory blockade with abatacept or IL-6 blockade with tocilizumab on CV function.

There has been much interest in the importance of early intervention in RA. Studies have found that early and aggressive suppression of inflammation in RA not only improves disease activity but also prevents radiological progression of joint damage.
This was demonstrated in the combination of methotrexate and etanercept in active early moderate to severe rheumatoid arthritis (COMET) trial which found that this combination therapy was more effective at achieving clinical remission and suppression of radiological joint damage than methotrexate monotherapy (103). Similarly, the BeSt study found that initial combination therapy with either methotrexate, sulphasalazine and prednisolone or with methotrexate and infliximab were more effective than sequential monotherapy or step up to combination therapy (both starting with methotrexate) (104).

An important question is whether there is a window period in which we must achieve suppression of inflammation and disease activity in order to prevent joint damage and clinically significant atherosclerosis? In a Swedish study of early RA (symptoms present for less than 12 months) no significant difference in FMD or CIMT was found compared to controls (105). Other smaller studies have demonstrated impaired endothelium-dependent vasodilatation in early RA (106, 107). In the Swedish study, CIMT had increased significantly after 18 months in RA patients but not in controls. This increase was significant although all patients with RA were treated with DMARDs and had a low inflammatory activity. There was no change in FMD after 18 months. Further studies in early RA are required to help understand the time of onset of vascular dysfunction and whether there is a critical level of disease activity/inflammation for development of vascular dysfunction.

The clinician should therefore add CV risk factor assessment to routine management of patients with RA. This is particularly important given that CV disease may be ‘silent’ in RA and may precede joint disease (10). RA-specific strategies have been identified by Van Doornum et al (108). These strategies include avoiding or minimising the use of medications commonly used in RA which may worsen traditional risk factors such as diabetes, hypertension and dyslipidaemia. These medications include glucocorticoids, NSAIDs, leflunomide and cyclosporine. Data also supports the CV benefits of effective and sustained control of joint and systemic inflammation in RA. More recently, EULAR evidence-based recommendations for CV risk management in RA and other forms of inflammatory arthritis (psoriatic arthritis and ankylosing spondylitis) have been published (109). The table below summarises the 10 recommendations made:
<table>
<thead>
<tr>
<th>Recommendations</th>
<th>Level of evidence</th>
<th>Strength of recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. RA should be regarded as a condition with higher risk for CV disease, due to both increased prevalence of traditional risk factors and the inflammatory burden.</td>
<td>2b-3</td>
<td>B</td>
</tr>
<tr>
<td>2. Adequate control of disease activity is necessary to lower the CV risk.</td>
<td>2B-3</td>
<td>B</td>
</tr>
<tr>
<td>3. CV risk assessment using national guidelines is recommended annually and should be repeated when antirheumatic treatment has been changed.</td>
<td>3-4</td>
<td>C</td>
</tr>
<tr>
<td>4. Risk score models should be adapted for patients with RA by introducing a 1.5 multiplication factor, particularly in patients who meet 2 of the following 3 criteria:</td>
<td>3-4</td>
<td>C</td>
</tr>
<tr>
<td>- Disease duration of more than 10 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- RF of anti-CCP positivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Presence of extra-articular manifestations</td>
<td></td>
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</tr>
<tr>
<td>5. TC/HDL cholesterol ratio should be used when the SCORE model is used.</td>
<td>3</td>
<td>C</td>
</tr>
<tr>
<td>6. Intervention should be carried out according to national guidelines.</td>
<td>3</td>
<td>C</td>
</tr>
<tr>
<td>7. Statins, ACE inhibitors and/or AT-II blockers are preferred treatment options as these possess potential anti-inflammatory properties.</td>
<td>2a-3</td>
<td>C-D</td>
</tr>
<tr>
<td>8. The role of COX-2 inhibitors and most NSAIDs in CV risk is not well established and needs further investigation. Hence, we should be very cautious about prescribing them, especially for patient with a documented CV disease or in the presence of CV risk factors.</td>
<td>2a-3</td>
<td>C</td>
</tr>
<tr>
<td>9. Glucocosteroids: use the lowest dose possible.</td>
<td>3</td>
<td>C</td>
</tr>
<tr>
<td>10. Recommend smoking cessation.</td>
<td>3</td>
<td>C</td>
</tr>
</tbody>
</table>
ACE, angiotensin-converting enzyme

Anti-CCP, anti-cyclic citrullinated peptide

AT-II, angiotensin II

TC, Total cholesterol

SCORE, Systematic Coronary Risk Evaluation. This is a chart which demonstrates 10-year risk of fatal CV disease in patients at high CV disease risk. The chart is based on TC/HDL, systolic blood pressure, age, smoking status and sex (110).

This body of work therefore contributes to the growing body of evidence linking inflammation, atherosclerosis and RA. It demonstrates that patients with RA have increased arterial stiffness, thereby reminding physicians of the need to screen regularly for traditional CV risk factors and to then treat any identified risk factors appropriately. It also highlights the ability to improve arterial stiffness with intensive RA treatment, most likely due to successful suppression of inflammation and active RA disease. This gives incentive to both patient and physician to aim for disease remission.

Understanding atherosclerosis and the relationship with inflammation will be enhanced with studies looking at other inflammatory conditions, both in the acute and chronic setting and in rheumatological and non-rheumatological conditions. The relative contributions of traditional and disease specific risk factors to CV disease and mortality in RA is still uncertain. Finding a biomarker or composite of biomarkers would assist in identifying which patients are at higher risk for CV disease. Monitoring for CV risks and treatment options for RA patients could therefore be better targeted.

The three musketeers; RA, CV disease and inflammation appear to have a strong yet complex relationship. The impact of drug therapies commonly used in RA on CV risk assists with trying to understand the complexities of this relationship. Future clinical trials of therapy in RA should include vascular endpoints as well as the usual markers of inflammation, function and joint damage.
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Title:
Rheumatoid arthritis, cardiovascular disease and inflammation.... and the effects of rheumatoid arthritis drug therapies on this musketeer trio

Date:
2010

Citation:
Wong, M. (2010). Rheumatoid arthritis, cardiovascular disease and inflammation.... and the effects of rheumatoid arthritis drug therapies on this musketeer trio. Doctorate, Faculty of Medicine, Dentistry and Health Sciences, Department of Medicine, The University of Melbourne.

Persistent Link:
http://hdl.handle.net/11343/35664

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