Cardiovascular Disease in Rheumatoid Arthritis

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Diploma (RcompN), PgDip (HSM)

A thesis submitted for the degree of Doctor of Philosophy at

The University of Queensland in 2015

The University of Queensland, Diamantina Institute
Abstract

This thesis focuses on cardiovascular (CV) disease (CVD) risk in rheumatoid arthritis (RA). Inflammation and CV risk factors are increased in RA patients. This population has a higher carotid intima-media thickness and more CV events than the general population and kynurenine (KYN) concentration levels are higher in RA patients compared to healthy controls. Therefore, in this thesis inflammation and CVD risk factors are investigated for increased risk of future RA development. RA and type2 diabetes (T2D) carotid intima-media thickness (CIMT) are compared and measurements of serum KYN for CVD, cancer and mortality are studied. First I tested the hypothesis that inflammation, CVD and CV risk factors are associated with increased risk of future RA development. Data from the Norwegian HUNT population health survey were obtained to compare groups with RA at baseline and follow-up (HUNT2 and HUNT3, n = 429) or follow up alone (HUNT3, n = 786), or without RA at both times (n = 33,567). Results showed female gender, age, smoking, body mass index (BMI), and history of previous CVD were associated with self-reported incident RA (previous CVD: odds ratio 1.52 (95% confidence interval 1.11-2.07). The findings regarding previous CVD were confirmed in sensitivity analyses excluding participants with psoriasis (odds ratio (OR) 1.70 (1.232.36)) or restricting the analysis to cases with hospital diagnosis of RA (OR 1.90 (1.10-3.27)) or carriers of the shared epitope (OR 1.76 (1.134-2.74)). History of previous CVD was not associated with increased risk of osteoarthritis (OR 1.04 (0.86-1.27)). I then compared the characteristics of CV risk and progression of CIMT in 78 RA patients from an outpatient Rheumatology Clinic and 212 patients with T2D who attended a community or hospital diabetes clinic in Brisbane. We found that the burden of risk varied between the 2 cohorts. RA patients were older, had a higher proportion of smokers, females and previous CVD. T2D patients had a higher body mass index (BMI), diastolic blood pressure, triglycerides, lower high density lipoprotein and a higher statin use. At baseline, CIMT measurements were similar in the RA and T2D cohorts. In an adjusted linear regression model, RA was significantly associated with lower CIMT at follow-up. Despite a shorter follow-up, 91 %
of the T2D cohort had increased CIMT at follow-up compared to 54 % of the RA cohort. In the RA cohort, disease modifying anti-rheumatic drug (DMARD) use at baseline was associated with significantly lower CIMT values at follow-up. Finally we hypothesized that increased KYN in RA patients may predispose to CVD, infections and cancer. We measured KYN in 129 RA patients followed for 10 years for development of CVD, cancer and death. Median KYN concentrations were significantly higher in RA patients than in controls, but there was large overlap between groups. There were no variables in our data set that could explain the differences in KYN concentrations among the RA patients and KYN concentrations did not predict development of CVD, new malignancies or death. The number of pack-years of smoking was the only variable associated with death in logistic or Cox regression analysis. In summary, CVD risk factors are associated with increased risk of future RA development. The burden of risk varies in RA and T2D, and CIMT progression is slower for RA than for T2D. Although serum KYN concentrations are significantly higher in RA than in controls, they were not associated with CVD, new malignancies or death.
Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Publications during candidature

Publication in peer-reviewed journal

Pahau H, Brown MA, Paul S, Thomas R, Videm V. Cardiovascular disease is increase prior to onset of rheumatoid arthritis but not osteoarthritis: the
population-based Nord-Trøndelag health study (HUNT). *Arthritis Res Ther.* 2014 Apr 2;16(2)


**Conference abstracts**

CIMT in individuals with Rheumatoid Arthritis compared to individuals with Type 2 Diabetes Mellitus [abstract].

**Helen Pahau**¹, Brian Haluska³, Leanne Short³, Vibeke Videm*², and Ranjeny Thomas*¹ (Oral presentation at New Zealand Rheumatology Conference, 2014)

CIMT in individuals with Rheumatoid Arthritis compared to individuals with Type 2 Diabetes Mellitus [abstract].

**Helen Pahau**, Brian Haluska, Leanne Short, Vibeke Videm, and Ranjeny Thomas

(Poster presentation at Princess Alexandra Hospital Symposium, 2014)

CIMT in individuals with Rheumatoid Arthritis compared to individuals with Type 2 Diabetes Mellitus [abstract].

**Helen Pahau**, Brian Haluska, Leanne Short, Vibeke Videm, and Ranjeny Thomas

(Poster presentation at Australia Rheumatology Conference, 2014)

Cardiovascular disease is increased prior to onset of rheumatoid arthritis but not osteoarthritis: the population-based Nord-Trøndelag health study (HUNT) [abstract]
Cardiovascular disease is increased prior to onset of rheumatoid arthritis but not osteoarthritis: the population-based Nord-Trøndelag health study (HUNT) [abstract].

Helen Pahau, Matthew A Brown, Sanjoy Paul, Ranjeny Thomas and Vibeke Videm
(Oral presentation at the Princess Alexandra Hospital Symposium, Brisbane 2012)

Outcome of an intensive treat-to-target disease modifying drug regimen in early rheumatoid arthritis within in the first month sets the response trajectory for one year [abstract]. Douglas White, Helen Pahau, Emily Duggan, Sanjoy Paul & Ranjeny Thomas. (Poster presentation at the Australian Rheumatology Conference, 2011).
Publications included in this thesis

Pahau H, Brown MA, Paul S, Thomas R, Videm V. Cardiovascular disease is increase prior to onset of rheumatoid arthritis but not osteoarthritis: the population-based Nord-Trondelag health study (HUNT). *Arthritis Res Ther.* 2014 Apr 2;16(2) – incorporated as Chapter 2 (complete article in Appendices).

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<tr>
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<th>Statement of contribution</th>
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<tr>
<td>Author-Pahau H (Candidate)</td>
<td>Wrote the paper (85%)</td>
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<td>Statistical analysis of data (80%)</td>
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<td>Author-Videm V</td>
<td>Wrote and edited paper (10%)</td>
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<td>Statistical analysis of data (15%)</td>
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<tr>
<td>Author-Paul S</td>
<td>Statistical analysis of data (5%)</td>
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<tr>
<td>Author-Thomas R</td>
<td>Wrote and edited paper (5%)</td>
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Contributions by others to the thesis

**Chapter 3:** Dr Sanjoy Paul Bio statistician Director School of Population Health, The University of Queensland, assisted with statistical analysis.

**Chapter 4:** Dr Brian Haluska, Leanne Short, Dr Carly Jenkins, CIRCA, The University of Queensland performed the ultrasound images

**Chapter 5:** HPLC on serum of the RA and healthy controls were completed by DR Rowsan Ara. Rowsan assisted with reviewing of patients medical records and writing the methods.

Significant contributions to the thesis as a whole have been made by Professor Vibeke Videm and Professor Ranjeny Thomas in the capacity of
my PHD supervisor. This includes conceptual and statistical analysis, writing and editing and advice on thesis content.

**Statement of parts of the thesis submitted to qualify for the award of another degree** None

**Acknowledgements**

E rere ana ngā mihi huri haere i te tihi o maunga Hikurangi maringi ana ki te awa ō Waiapu hei whārikihia ngā kōiwi ō āku mātua típuna o Ngati Porou.

Ko tōku oranga he tāonga tuku iho mai rā nō. Ko tēnei te tino hā o tōku tino rangatiratanga. Mai te timatanga o te pō, ki te ao mārama.

Kō au tēnei he kākano i ruia mai Rangiatea.

He tāonga tēnei hei kohia ki ōku Tuakana ā Patricia TeoArani Wilson rāua kō Ellamein Matariki Phillips me ōku tungane ā Moana Pahau rāua kō Tapara Pahau.

*My acknowledgements flow around the tip of my mountain Hikurangi, into the Waiapu river which nurture the bones of my beloved parents and tipuna.
My being is a gift formed long ago. This is the breath of my existence. From the beginning of time to today.
I am a seed sown from Rangiatea.
This treasure I gift to my sisters Trish and Ellamein and my brothers Moana and Tapara.*

There is no doubt that this thesis would not be possible without the support, encouragement and guidance of my supervisor Professor Ranjeny Thomas. Thank you for believing in me.

My sincere gratitude to Professor Vibeke Videm who guided me through the world of statistics and provided valuable advice and friendship. Dr Douglas White who never faulted in believing I would be able to finish this thesis. You are a great mentor and I will be forever grateful.

Helen B thank you for the last minute readings, the feedback, friendship and unrelated PHD talks and great coffee. So I am so fortunate to have met you, you provided me with laughter and joy and of course the final nudge at the
end. To my colleagues of the Thomas Lab, thank you for allowing me to share your journeys and for being a part of mine.

Thank you to Professor Don Mathieson, Georgina Paerata, Terry Ehau, Professor Jim Mann, Dr Kirsten Coppell, Dr Rawiri Tipene-Leach, Dr Sally Abel and all the dinosaurs (you know who you are), who were instrumental in my career pathway.
I would like to acknowledge the financial support I received from the University of Queensland – Research Scholarship and the Diamantina Institute – Research Scholarship.
To my loving parents, who gave us unconditional love and support and encouraged us to be whoever we wanted to be. I am forever grateful and fortunate. You are the best parents in the world.

To Shane and Kewene, Aunty Ma, and our beautiful neighbours at 50 Somerset St, I am forever grateful for your support and help with TeWhaikura.

Finally I acknowledge my sons Tapara and Hamuera, who have grown up to be special young men, we are very proud of you both. My girl TeWhaikura, who quietly reminded me that life was not only about my PHD, thank you my girl for keeping me real.
To my husband Sam, always supportive, always encouraging, I love you.
Keywords
rheumatoid arthritis, cardiovascular disease, carotid intima-media thickness, kynurenine

Australian and New Zealand Standard Research Classifications (ANZSRC)
ANZSRC code: 110322, Rheumatology and arthritis 50%
ANZSRC code: 110201, Cardiology (Cardiovascular Diseases), 50%

Fields of Research (FoR) Classification
FoR code: 1103, Medical and Health Science, 50%
FoR code: 1102, Cardiorespiratory medicine and haematology, 50%

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<th>Description</th>
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<tbody>
<tr>
<td>HCQ</td>
<td>Hydrochloroquine</td>
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<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
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<tr>
<td>HLA</td>
<td>Human leukocyte antigen</td>
</tr>
<tr>
<td>HUNT</td>
<td>North Trondelag Health Study</td>
</tr>
<tr>
<td>ICA</td>
<td>Internal carotid artery</td>
</tr>
<tr>
<td>IDO1</td>
<td>Indoleamine 2,3 dioxygenase 1</td>
</tr>
<tr>
<td>IDO2</td>
<td>Indoleamine 2,3 dioxygenase 2</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon Gamma</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin antibodies</td>
</tr>
<tr>
<td>IHD</td>
<td>Ischemic heart disease</td>
</tr>
<tr>
<td>IMT</td>
<td>Intima media thickness</td>
</tr>
<tr>
<td>KYN</td>
<td>Kynurenine</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>MCE</td>
<td>Major coronary events</td>
</tr>
<tr>
<td>MCP</td>
<td>Metacarpophalangeal</td>
</tr>
<tr>
<td>MHC</td>
<td>Major Histocompatibility complex</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial Infarction</td>
</tr>
<tr>
<td>MMPs</td>
<td>Metalloproteinase’s</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MTP</td>
<td>Metatarsalphalangeal</td>
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<td>MTX</td>
<td>Methotrexate</td>
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<tr>
<td>NSAID drugs</td>
<td>Non steroidal anti inflammatory</td>
</tr>
<tr>
<td>OA</td>
<td>Osteoarthritis</td>
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<tr>
<td>PIP</td>
<td>Proximal interphalangeal</td>
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<td>RA</td>
<td>Rheumatoid arthritis</td>
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<tr>
<td>SCORE</td>
<td>Systemic coronary risk evaluation</td>
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<tr>
<td>SE</td>
<td>Shared epitope</td>
</tr>
<tr>
<td>SLE</td>
<td>Systemic lupus erythematosus</td>
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<tr>
<td>SMR</td>
<td>Standard mortality ratio</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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</tr>
<tr>
<td>SSZ</td>
<td>Sulphasalazine</td>
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<td>T2D</td>
<td>Type 2 diabetes</td>
</tr>
<tr>
<td>TDO</td>
<td>Tryptophan 2,3 dioxygenase</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>TRP</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very low density lipoprotein</td>
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</table>
Chapter 1: Literature Review

Rheumatoid Arthritis

1.1 Introduction
Rheumatoid Arthritis (RA) is a common systemic inflammatory disorder primarily involving the joints. This arthritis is progressive and if inadequately treated, significant functional disability can occur within 10 years [1]. The pattern of disease activity varies in patients, and often includes periods of high disease activity interspersed with periods of low disease activity. The precise aetiology of RA is unknown, however a combination of environmental and genetic factors are implicated [2]. There is a reduced median life expectancy of 7-10 years for people with RA and premature cardiovascular disease (CVD) is primarily responsible [3]. Despite treatment advances, RA patients continue to have significantly higher mortality and morbidity rates than the general population [4]. At present there is no cure for RA. Current goals of treatment include (1) early and intensive management; (2) suppression of disease activity; (3) limitation of disease progression and (4) adequate treatment of comorbidities. Continued research and subsequent advances in therapeutics have recently enhanced effective treatment of this disease.

1.2 Epidemiology
The annual incidence of RA is reported to be approximately 0.03 percent [5]. Although RA can affect any age group the peak age of onset is between 30 and 55 years [6]. Women are affected two to three times more often than men [7]. There is currently an overall decline in the incidence of RA and this is more evident in the female population [8]. The disease prevalence is approximately 1 percent in Caucasians, but varies between ethnicities including 0.1 percent (in rural Africans) and 56 percent (in Pima and Chippewa Indians) [5].

1.3 Aetiology
The exact cause of RA is unknown however a number of factors are implicated in the aetiology. Current evidence suggests that both genetic and environmental factors are involved.
1.3.1 Genetic Factors
Increasing evidence suggests RA is highly heritable. There is greater disease concordance in monozygotic twins (12-15%) than dizygotic twins (3.5%) [9, 10]. Recent population health studies have also shown that prevalence of RA is higher in first degree relatives of people with RA [11, 12]. These twin and first-degree relative studies suggest genetic factors have a significant impact on susceptibility to RA. Estimated genetic contribution to RA is thought to be as high as 50% to 60% [13, 14].
The best-established genetic association is with the human leukocyte antigen (HLA) locus, in particular the HLA-DR B1 chains known as the shared epitope (SE) [15]. The SE are known to be associated with RA susceptibility [16], specifically susceptibility to Anti-citrullinated protein antibody (ACPA) positive RA [17].

The presence of anti-citrullinated protein antibody (ACPA) measured by anti-cyclic citrullinated antibodies (anti-CCP) is highly specific for RA [18, 19] and are reported to be a good predictor for the development of RA [20]. In addition to the major histocompatibility complex (MHC) alleles (HLA in humans), there are non MHC alleles that are associated with RA risk and also other autoimmune diseases [21]. For instance PAD 14 is associated only with RA, whilst PTPN22 is linked to RA, systemic lupus erythematosus, type 1 diabetes and other autoimmune diseases [22-24].

1.3.2 Environmental factors
Smoking is the most established and studied environmental risk factor for RA [25, 26]. There is a strong association with smoking and sera-positive RA, [27, 28] with established interactions between the HLA-DR shared epitope (SE) in ACPA positive RA patients [29]. This gene environment interaction does appear to differ between ethnic groups. A cross-sectional study involving African Americans with early RA, did not find an association between smoking and ACPA-positive RA patients [30]. Furthermore RA cases in an Asian cohort demonstrated that the combination of SE alleles and smoking was associated with risk of RA, however ACPA status was not [31].
The association between HLA SE and smoking appears to hold irrespective of the number of cigarettes smoked. A recent study showed that there is a 40% higher risk among ever smokers compared to never smokers [32], indicating that light or heavy exposure increases risk of developing RA, likely due to activation of the immune system against citrullinated protein antigens [33].

1.4 Pathogenesis

Alteration in immune function leads to joint inflammation, degradation and destruction in RA. CD4+ T cells are regarded as the protagonists in initiating the inflammatory response in RA [34]. Numerous cytokines are expressed in synovial tissue and an imbalance between pro and anti-inflammatory cytokines induces chronic inflammation [35]. The inflamed synovium contains various macrophage and fibroblast derived cytokines that further support the activation of CD4+ T cells. Tumor necrosis factor (TNF-α) and interleukin-1 (IL-1β), are considered key pro-inflammatory cytokines in the pathogenesis of RA [36, 37]. In vitro studies suggest that IL-1β cause cartilage destruction by stimulating the release of matrix metalloproteinases and other degradative products increases bone reabsorption by stimulating osteoclast differentiation and activation [38]. Damage to articular cartilage in RA begins at the cartilage pannus interface, with progressive erosions occurring into subchondral bone [38].
1.5 Clinical Features

The symptoms of RA predominantly consist of pain, swelling and stiffness of the joints. RA typically begins insidiously, with the signs and symptoms developing slowly over weeks to months. However in a third of the patients, onset is rapid and can occur over days or weeks [39].

The metacarpophalangeal (MCP), proximal interphalangeal (PIP), metatarsalphalangeal joints (MTP) and the wrist are the most common joints affected. In some patients the shoulders, elbows, knees and ankles may also be affected. If this disease is left untreated, deformity of the joint and disability can occur within 10 years of onset of disease [40]. However achieving remission is within reach, and the earlier that RA treatment is started the better the odds are of reaching remission.
RA patients with extra articular manifestations have increased morbidity and mortality [41] and extra articular manifestations appear to be most common in patients with severe disease activity [42]. Severe weight loss, osteoporosis and CVD are examples of extra articular manifestations [43].

Table 1-1: Criteria for inclusion as extra-articular manifestations of RA

<table>
<thead>
<tr>
<th>Criteria for inclusion as extra-articular manifestations of RA</th>
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<tbody>
<tr>
<td><strong>Pericarditis</strong></td>
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<tr>
<td>(A) Clinical judgment and exudation verified by echocardiography</td>
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<tr>
<td>If ultrasound not available: criteria according to Hara <em>et al</em>5</td>
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<tr>
<td>(B) Clinical criteria: (1 required)</td>
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<tr>
<td>Typical pericardial pain, peripheral oedema,</td>
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<tr>
<td>dyspnoea/orthopnoea, ascites, dysrhythmia (heart rate &gt;140/min,</td>
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<td>atrial flutter/fibrillation, 2–3 degree atroventricular block,</td>
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<td>ventricular tachycardia)</td>
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<td>Objective criteria compatible with pericarditis: (1 required)</td>
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<td>Cardiac catheterisation findings</td>
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<tr>
<td><strong>Pleuritis</strong></td>
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<tr>
<td>Clinical judgment and exudation verified by x-ray examination</td>
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</tr>
<tr>
<td><strong>Felty’s syndrome</strong></td>
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<tr>
<td>Splenomegaly (clinically evident or measured by ultrasound) and</td>
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<td>neutropenia (&lt;1.8×10⁹/l) on two occasions</td>
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<tr>
<td>Other causes improbable, such as drug side effect or infection</td>
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<tr>
<td><strong>Major cutaneous vasculitis</strong></td>
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<td>Diagnostic biopsy or clinical judgment by dermatologist</td>
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<tr>
<td><strong>Neuropathy</strong></td>
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<td>Clinical judgment by doctor and signs of polyneuropathy/mononeuropathy at</td>
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<tr>
<td>electromyography/electroneurography</td>
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<tr>
<td>Other causes, such as diabetes or alcohol abuse, improbable</td>
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<tr>
<td><strong>Scleritis, episcleritis or retinal vasculitis</strong></td>
</tr>
<tr>
<td>Clinical judgment by ophthalmologist</td>
</tr>
<tr>
<td><strong>Glomerulonephritis</strong></td>
</tr>
<tr>
<td>Clinical judgment by nephrologist and positive biopsy</td>
</tr>
<tr>
<td><strong>Vasculitis affecting other organs</strong></td>
</tr>
<tr>
<td>Clinical judgment by organ specialist and biopsy compatible with</td>
</tr>
<tr>
<td>vasculitis</td>
</tr>
<tr>
<td><strong>Amyloidosis</strong></td>
</tr>
<tr>
<td>Clinical judgment and positive biopsy from affected organ</td>
</tr>
<tr>
<td><strong>Keratoconjunctivitis sicca</strong></td>
</tr>
<tr>
<td>Clinical judgment</td>
</tr>
<tr>
<td>Positive rose-bengal staining or result of Schirmer’s test &lt;5</td>
</tr>
<tr>
<td>mm/min</td>
</tr>
<tr>
<td><strong>Xerostomia</strong></td>
</tr>
<tr>
<td>Clinical judgment</td>
</tr>
<tr>
<td>Abnormal sialometry, sialography, salivary scintigraphy or salivary gland biopsy with lymphocytic infiltrate</td>
</tr>
<tr>
<td><strong>Secondary Sjögren’s syndrome</strong></td>
</tr>
<tr>
<td>Two of three criteria:</td>
</tr>
<tr>
<td>Keratoconjunctivitis sicca (see above)</td>
</tr>
<tr>
<td>Xerostomia (see above)</td>
</tr>
<tr>
<td>Condition</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Serological evidence: rheumatoid factor, ANA, anti-Ro (SSA), anti-La (SSB) positive, or hypergammaglobulinaemia</td>
</tr>
<tr>
<td>Pulmonary fibrosis</td>
</tr>
<tr>
<td>Bronchiolitis obliterans organising pneumonia</td>
</tr>
<tr>
<td>Cervical myelopathy</td>
</tr>
<tr>
<td>Subcutaneous rheumatoid nodules</td>
</tr>
<tr>
<td>Rheumatoid nodules in other locations</td>
</tr>
</tbody>
</table>
1.5.1 Classification Criteria

The 1987 American College of Rheumatology (ACR) criteria were used to classify patients with RA until 2010 (Table 2). For the diagnosis of RA, patients must fulfil 4 out of 7 criteria including morning stiffness 1 hour, arthritis of 3 joint areas, arthritis of hand/wrist joints, symmetrical arthritis, rheumatic nodules, serum rheumatoid factor (RF) and radiographic changes [44]. The criteria distinguished patients with established RA from other arthritides and identified patients with moderate to severe disease.

Table 1-2: The 1987 revised criteria for the classification of RA by the ACR

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Morning Stiffness</td>
<td>Morning stiffness in and around the joints, lasting at least one hour before maximal improvement</td>
</tr>
<tr>
<td>2. Arthritis of 3 or more joint</td>
<td>At least three joint areas (out of 14 possible; right or left PIP, MCP, wrist, elbow, ankle, MTP joints)</td>
</tr>
<tr>
<td>areas</td>
<td>simultaneously have had soft tissue swelling or fluid (not bony overgrowth) as observed by a physician</td>
</tr>
<tr>
<td>3. Arthritis of hand joints</td>
<td>At least one area swollen (as defined in criterion two) in a wrist, MCP, or PIP joint</td>
</tr>
<tr>
<td>4. Symmetric arthritis</td>
<td>Simultaneous involvement (as in criterion two) of the same joint areas on both sides of the body (bilateral involvement of PIP, MCP, or MTP joints without absolute symmetry is acceptable)</td>
</tr>
<tr>
<td>5. Rheumatoid nodules</td>
<td>Subcutaneous nodules over bony prominences or extensor surfaces, or in juxta-articular regions as observed by a physician</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>6. Serum rheumatoid factor</td>
<td>Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in ≤5% of normal control subjects</td>
</tr>
<tr>
<td>7. Radiographic changes</td>
<td>Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal body decalcification localized in, or most marked adjacent to, the involved joints (osteoarthritis changes alone do not qualify)</td>
</tr>
</tbody>
</table>

In 2010 the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) revised the classification criteria to allow capture of RA patients with early and milder disease, while still allowing application to patients with established and more severe disease (Table 3) [45].
Table 1-3: The 2010 American College of Rheumatology / European League Against Rheumatism classification criteria for rheumatoid arthritis

<table>
<thead>
<tr>
<th>Target population (Who should be tested?): Patients who</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) have at least 1 joint with definite clinical synovitis (swelling)</td>
<td></td>
</tr>
<tr>
<td>2) with the synovitis not better explained by another disease</td>
<td></td>
</tr>
<tr>
<td>Classification criteria for RA (score-based algorithm: add score of categories A–D); a score of ≥6/10 is needed for classification of a patient as having definite RA</td>
<td></td>
</tr>
<tr>
<td>A. Joint involvement</td>
<td></td>
</tr>
<tr>
<td>1 large joint</td>
<td>0</td>
</tr>
<tr>
<td>2-10 large joints</td>
<td>1</td>
</tr>
<tr>
<td>1-3 small joints (with or without involvement of large joints)</td>
<td>2</td>
</tr>
<tr>
<td>4-10 small joints (with or without involvement of large joints)</td>
<td>3</td>
</tr>
<tr>
<td>&gt;10 joints (at least 1 small joint)</td>
<td>5</td>
</tr>
<tr>
<td>B. Serology (at least 1 test result is needed for classification)</td>
<td></td>
</tr>
<tr>
<td>Negative RF and negative ACPA</td>
<td>0</td>
</tr>
<tr>
<td>Low-positive RF or low-positive ACPA</td>
<td>2</td>
</tr>
<tr>
<td>High-positive RF or high-positive ACPA</td>
<td>3</td>
</tr>
<tr>
<td>C. Acute-phase reactants (at least 1 test result is needed for classification)</td>
<td></td>
</tr>
<tr>
<td>Normal CRP and normal ESR</td>
<td>0</td>
</tr>
<tr>
<td>Abnormal CRP or normal ESR</td>
<td>1</td>
</tr>
<tr>
<td>D. Duration of symptoms</td>
<td></td>
</tr>
<tr>
<td>&lt;6 weeks</td>
<td>0</td>
</tr>
<tr>
<td>≥6 weeks</td>
<td>1</td>
</tr>
</tbody>
</table>
1.5.2 Diagnosis and disease activity
The diagnosis of RA is made via a thorough assessment of patient symptoms, physical examination and additional investigations in accordance with the classification criteria.

1.6 Rheumatoid Factor
Rheumatoid factor (RF) is a complex of antibodies directed against the conserved end of immunoglobulin (IgG) antibodies. It occurs in 60% of patients at diagnosis and rises to approximately 80% with time. It is associated with more severe disease and a greater risk of atherosclerosis. The specificity for RF is however relatively low (60-70%) and is also detected in patients with other diseases and also in the healthy population.

1.6.1 Anti- citrullinated protein antibodies
ACPA are auto-antibodies directed against peptides and proteins that are citrullinated. ACPA are used as a clinical biomarker for the diagnosis of RA, and have a 55-80% sensitivity and specificity of 90-98% for RA [46, 47]. The presence of ACPA is also a prognostic marker and is associated with more aggressive disease [48], preceding the clinical manifestations of RA by up to 10 years along with RF [19, 49]. Furthermore, there is evidence of significant interactions between the HLA-DRB1 shared epitope and cigarette smoking in the development of ACPA-positive RA [50, 51]. Sero-positive RA is more common (70%) than sero-negative disease (30%) and is associated with more aggressive disease and extra- articular manifestations [52].

1.6.2 CRP and ESR
C - reactive protein (CRP) is a member of the pentraxin family, a pattern-recognition protein and a host-defence-related component of the innate immune system [53-55]. CRP was discovered in 1929 and is the most characteristic acute phase protein in the human body [56]. CRP is a sensitive marker of systemic inflammation, and the plasma concentration levels are elevated in RA patients and in other conditions with both acute and chronic inflammation [56-58]. Although CRP is a non-specific test, doctors may
utilise the test to assist the effectiveness of a specific arthritis treatment and to monitor periods of disease flare-up.

Erythrocyte sedimentation rate (ESR) measures how fast red blood cells fall through a column of blood. It is an indirect index of acute-phase protein concentrations (particularly fibrinogen) and is a sensitive but non-specific test used to assist in the detection of inflammation associated with autoimmune diseases, infections and cancers [59]. Similar to CRP, doctors may utilise ESR to test the effectiveness of specific arthritis treatment and to follow inflammation levels.

1.7 Disease Activity Scores

In order to evaluate the need for, and the effect of treatment in patients with RA, it is valuable to measure the disease activity at diagnosis and follow-up in a standardized manner. For this purpose several scores have been developed. The Disease Activity Score (DAS) was developed in Europe as a continuous activity score [60]. This score takes into account the patient’s general health, the number of swollen and tender joints as well as the ESR: a non-specific measure of inflammation. The DAS28 takes into account 28 different joints. A value >5.1 is regarded as high disease activity, <3.2 is regarded as low activity and <2.6 is regarded as remission. Figure 2 by comparing a patient’s DAS28 score over multiple time points, one can substantiate his/her improvement or response. The EULAR response criteria are shown in Figure 3.
Figure 1-2: Disease activity score 28
From: DAS-Score.nl. Available at http://www.das-score.nl/www.das-score.nl/index.html

Table 1-4: DAS 28

<table>
<thead>
<tr>
<th>PRESENT DAS28</th>
<th>DAS28 IMPROVEMENT OVER TIME POINTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;1.2</td>
</tr>
<tr>
<td>&lt;3.2</td>
<td>Good response</td>
</tr>
<tr>
<td>3.2-5.1</td>
<td>Moderate response</td>
</tr>
<tr>
<td>&gt;5.1</td>
<td>Moderate response</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>DAS Score</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>DAS28 &gt;5.1     = high disease activity</td>
</tr>
<tr>
<td>DAS28 &lt;3.2     = low disease activity</td>
</tr>
<tr>
<td>DAS28 &lt;2.6  = remission</td>
</tr>
</tbody>
</table>

Treatment response over time is also a crucial component of patient assessment, although largely a tool for clinical trial ACR score measures % treatment response, i.e., ACR20 requires at least a 20 percent improvement in both the number of swollen and tender joints, as well as a 20 percent
improvement in three of the following five parameters: ESR, health assessment questionnaire (HAQ), physician’s global assessment of disease activity, patient’s global assessment and patient’s pain [61]. Correspondingly, the ACR50 requires a 50% improvement and the ACR70 a 70% improvement. Furthermore, the ACR has defined remission criteria based on the same variables [62].

1.8 Imaging
Radiography remains the mainstay of imaging in the diagnosis and follow-up of RA. RA patients should have a hand and wrist x-ray, and baseline x-rays of affected joints to document any future erosive changes. Erosions often develop in the first year but may occur at any time. A previous study showed radiographically demonstrable erosions were present in 30% of their patients at diagnosis, and in 70% 3 years later [63]. The use of newer imaging modalities such as ultrasonography, magnetic resonance imaging (MRI) and computerized tomography (CT) are increasingly being used to assist in the diagnosis and continuing management of RA [64]. Many of the imaging methods offer greater sensitivity in detecting synovitis, effusions, joint subluxations, joint space narrowing and bone edema then standard x-ray [65]. Furthermore, when compared with conventional methods, the increasing use of ultrasound has allowed for a greater success rate when aspirating joints, and greater accuracy when injecting steroids into affected joints [65].

1.9 Treatment
RA rarely spontaneously remits. As the exact pathophysiology is unknown and there is currently no cure, treatment aims to suppress and modulate the inflammatory disease. Supportive ancillary treatments such as physiotherapy, occupational therapy, podiatry and surgery are used as adjuncts. Medications used in RA aim to suppress the ‘overactive’ immune system. Different drugs with various mechanisms of action are utilised and often a combination of therapies is needed.

Non-steroidal anti-inflammatory agents (NSAIDs) have analgesic and anti-inflammatory effects. They are used effectively to assist with RA
symptoms. They also have significant gastric and cardiac toxicity [66]. The gastrointestinal toxicity appears to be related to cyclooxygenase 1 (COX) inhibition. However COX-2 inhibitors with an improved gastrototoxicity profile have been found to increase the risk of atherothrombosis even with short-term use.

Current medications used to treat RA span the history of medicine, from intramuscular gold to targeted cytokine blocking agents. The exact mechanism of action of the many of the disease modifying agents (DMARDs) is incompletely understood. Disease modifying (or slow acting) antirheumatic drugs include Methotrexate (MTX), Sulphasalazine (SSZ), Leflunomide, Hydroxchloroquine (HCQ), Gold, Cyclosporine, Azathioprine and Corticosteroids. Biologic (B)DMARDs are newer therapeutics and they interfere with the immune system in a more selective way, targeting cytokines or signalling pathways. However, both DMARDs and B-DMARDs suppress immune and inflammatory pathways and hence RA activity.

Methotrexate is the most frequently used DMARD in RA and the foundation therapeutic for DMARD and biologic combinations [67]. Results of multiple double-blind, randomised studies have indicated that MTX improves the health of patients (e.g. improving health assessment questionnaire scores and those of other quality of life measures), and decreases systemic inflammation (e.g. erythrocyte sedimentation rate, concentrations of CRP and signs of clinical inflammation [68, 69]. Patients react differently to anti-rheumatic medications and often require varying doses or combinations to manage their disease. Adverse effects are not uncommon. The medications used to treat RA mostly take several months or longer to achieve remission. However over the last decade, the development of new and effective treatments and treatment regimens has substantially improved the prognosis for patients with RA. Many patients now experience lower disease activity and remission than in the 1970s and 1980s [70].
1.10 Early arthritis clinics and ‘treat to target’ regimens

1.10.1 Early arthritis clinics

There is substantial evidence that early treatment with DMARDs can lead to better outcomes, including the suppression of synovitis, prevention of bone destruction, and the reduction of functional disability and mortality rates. The late 80s saw the introduction of the concept of ‘early intervention’ [71]. Soon after, specialised early arthritis clinics (EAC) [72] were created in multiple centres around the world, ensuring early diagnosis and treatment. The EAC most often represents a structured environment where patients can be assessed, reviewed and treated in a protocol-driven manner, utilising best-practice therapeutics and technology.

1.10.2 Treat to target regimens

In 2008, an international expert panel primarily of Rheumatologists reviewed available goal directed therapy in RA, resulting in the International Treat to Target initiative [73]. The panel introduced overarching principles (Figure 3) and 10 recommendations for “Treat Arthritis to Target” (Figure 4). There are extended guidelines from both European and American Rheumatology expert groups which, although generally similar, vary in the type of medications used. The aim of the treat-to-target therapeutic approach is to bring RA under control quickly and to prevent joint damage and disease progression.

**Overarching principles:**

a. The treatment of rheumatoid arthritis must be based on a shared decision between patient and rheumatologist.

b. The primary goal of treating the patient with rheumatoid arthritis is to maximize long-term, health-related quality of life through control of symptoms, prevention of structural damage, normalization of function and social participation.

c. Abrogation of inflammation is the most important way to achieve these goals.

d. Treatment to target by measuring disease activity and adjusting therapy accordingly optimizes outcomes in rheumatoid arthritis.

*Figure 1-3: Recommendations*
1. The primary target for treatment of rheumatoid arthritis should be a state of clinical remission.
2. Clinical remission is defined as the absence of signs and symptoms of significant inflammatory disease activity.
3. While remission should be a clear target, based on available evidence low disease activity may be an acceptable alternative therapeutic goal, particularly in established long-standing disease.
4. Until the desired treatment target is reached, drug therapy should be adjusted at least every 3 months.
5. Measures of disease activity must be obtained and documented regularly, as frequently as monthly for patients with high/moderate disease activity or less frequently (such as every 3–6 months) for patients in sustained low disease activity or remission.
6. The use of validated composite measures of disease activity, which include joint assessments, is needed in routine clinical practice to guide treatment decisions.
7. Structural changes and functional impairment should be considered when making clinical decisions, in addition to assessing composite measures of disease activity.
8. The desired treatment target should be maintained throughout the remaining course of the disease.
9. The choice of the (composite) measure of disease activity and the level of the target value may be influenced by consideration of co-morbidities, patient factors, and drug-related risks.
10. The patient has to be appropriately informed about the treatment target and the strategy planned to reach this target under the supervision of the rheumatologist.

Figure 1-4: Ten recommendations on treating rheumatoid arthritis to target based on both evidence and expert opinion: From: Ann Dis Rheum. 2010, 69:631-637 [73]

An algorithm was proposed for treating RA to target (Fig 4) and is based on the recommendation in Fig 3.

Figure 1-5: Algorithm for treating RA to target. Indicated as separate threads are the main target (remission and sustained remission) and the alternative target (low disease activity in patients with long-term disease), but the approaches to attain the targets and sustain them are essentially identical. Adaptation of therapy should usually be done by performing control examinations with appropriate frequency and using composite disease activity measures which comprise joint counts.


1.11 Mortality and Morbidity in RA

Patients with RA have increased mortality and morbidity when compared to healthy controls [74, 75]. The increased mortality predominantly relates to increased cardiovascular disease [76-79]. It was found in a retrospective study of an inception cohort of RA patients that they were at increased risk of cardiovascular death, ischemic heart disease and heart failure compared
to age and sex matched community controls. The incidence of cardiovascular events was significantly higher among those with RA compared with controls (3.43 versus 0.59 per 100 patient-years) [80]. Another study of 3501 patients with RA with follow-up for 35 years, found that mortality was increased twofold, resulting in a decreased lifespan of 7 to 10 years [81]. Mortality directly due to RA itself was low (9.8 percent of the deaths). Other studies suggest that increased inflammation associated with RA appears to contribute substantially to increased cardiovascular mortality [82].

1.12 Cardiovascular Disease
CVD is the result of damage and dysfunction within the vascular tree. This damage occurs as a result of loss of vessel compliance, damage to components of vessel walls leading to plaques, evolution of aneurysms and exposure of thrombotic wall components with resultant clot formation. This damage causes the vascular tree to fail in different ways, leading to particular clinical states.

1.12.1 Epidemiology
CVD is common in the general population, affecting the majority of adults over the age of 60 years. The prevalence of coronary heart disease (CHD) is approximately 1/3 to 1/2 that of total CVD. The lifetime risk of CHD was illustrated in a study of 7733 participants, age 40 to 94, in the Framingham Heart Study who were initially free of CHD [83]. The lifetime risk for individuals at age 40 was 49% in men and 32% in women. Even those who were free from disease at age 70 had a lifetime risk of 35% in men and 24% in women [83]. While young patients in a large autopsy study had fatty streaks [84], not all adults develop clinically apparent CVD as fatty streaks progress at different rates in different individuals [85].

1.13 Atherosclerosis
Generalised thickening of the arteries with subsequent loss of wall compliance is known as arteriosclerosis. Atherosclerotic heart disease, otherwise known as coronary heart disease or coronary artery disease, is most often caused by atherosclerosis that occurs due to the deposition of
cholesterol in the arterial wall causing plaque accumulation. Resulting chronic inflammation in the plaques can lead to the thinning and rupture of the endothelial cap and exposure of thrombogenic core. This then leads to the formation of a platelet thrombosis often leading to vessel occlusion and tissue infarction [86]. The presence of atherosclerosis is associated with arteriosclerosis.

The vascular endothelium was previously considered to be an inert barrier between the blood and tissues, however increasing evidence suggests that the endothelium is an active biological interface. The single layer of continuous endothelium lining arteries and veins forms a unique thromboresistant layer between blood and potentially thrombogenic subendothelial tissues [87, 88].

The exact pathogenesis of endothelial damage is not known, however a range of risk factors has been shown to be associated with accelerated vascular damage. These include environmental and genetic factors such as smoking, hypertension, obesity, ageing, lack of exercise, inflammation, diabetes and family history. Intima media thickness (IMT) increase begins with endothelial dysfunction and is generally considered the earliest sign of atherosclerosis.

The activity of endothelial cells includes regulation of vascular tone and structure. This is accomplished by releasing molecules such as nitric oxide and prostacyclin, which control vasodilatation and vasoconstriction [89]. Endothelial cells also provide both a non-thrombotic and non-adherent arterial surface for leukocytes [88]. Endothelial dysfunction therefore is defined as impaired vasodilatation or vasoconstriction to stimuli, pro-inflammatory surface state, and prothrombotic surface state [90].

The changes usually occur slowly over time and vary greatly from individual to individual. The first observable change of atherosclerosis is the formation of a fatty streak. In one autopsy study in the US, for example, all 2876 men and women, aged 15 to 34 years, had aortic fatty streaks [91]. The fatty streak is caused by an excess deposition of lipid in macrophages in the vessel wall. With time these cells swell, there is deposition of
extracellular lipid and the plaque grows. Fibrous tissue is deposited, areas calcify and the plaque increases in size. The increasing plaque size can encroach on the vessel lumen, reducing blood flow and causing ischaemia of downstream tissue, and causing symptoms such as angina. With time, the fibrous cap thins, and with exposure to the shear stress of the turbulent blood flow, is at risk of rupture. Upon rupture, the thrombogenic contents rapidly cause clotting leading to vessel occlusion. This occlusion can lead to tissue death, such as a myocardial infarction (MI) [92].

Concurrent with the plaque formation there is generalised vascular damage where the lining of the vessel thickens. There is good correlation between generalised arteriosclerosis, focal atherosclerosis and risk of CVD [93]. This arteriosclerosis causes a loss of vessel wall compliance, which increases the load on the vessel and the heart. In healthy vascular trees this is a reflection of the normal systolic outflow. The wave of ejected blood from the heart is partially reflected back from the peripheral arteries. Normally this is reflected back in diastole and does not load the heart. However as the vessels lose compliance and stiffen this wave is reflected back earlier and eventually arrives in systole. This reflected wave adds additional load to the heart and the extra workload accelerates the progression of heart disease [94]. Decreased aortic compliance has been shown to be predictive of primary coronary events in hypertensive patients with normal renal function [95].

1.13.1 Risk Factors for atherosclerosis
There are many risk factors for atherosclerosis. These factors show individual variation and no single factor acts in isolation. The worldwide INTERHEART study of patients from 52 countries, identified nine potentially modifiable factors accounting for over 90 percent of the population with an attributable risk of a first MI [96]. These included smoking, dyslipidaemia, hypertension, diabetes, abdominal obesity, psychosocial factors, regular alcohol consumption, not consuming a daily intake of fruits and vegetables, and not regularly participating in physical activity. Non modifiable risk factors include male sex, age, and a family history of CVD.
1.13.2 CRP and atherosclerosis

The association of elevated CRP concentrations and increased risk of CVD is well documented [97]. CRP levels are elevated in response to inflammatory stimuli; therefore it is thought that CRP may play a direct role in the development of atherosclerosis in inflammatory disease such as RA [98, 99].

1.14 Effects of Dyslipidaemia in CVD

Lipid abnormalities play a critical role in the development of atherosclerosis. Lipids such as cholesterol and triglycerides are insoluble in plasma. Circulating lipid is carried in lipoproteins that transport the lipid to various tissues for energy utilisation, lipid deposition, steroid hormone production, and bile acid formation [100]. Lipoprotein consists of esterified and unesterified cholesterol, triglycerides, and phospholipids and protein [100]. The protein components of the lipoprotein are known as apolipoproteins. The different apolipoproteins serve as cofactors for enzymes and ligands for receptors. There are five major lipoproteins, each of which has a different function. In clinical practice measurement of the low density lipoprotein (LDL), high density lipoprotein (HDL) and total cholesterol level are the most relevant [101].

Animal studies have demonstrated accelerated atherosclerosis with a high cholesterol diet [102]. In humans, worldwide epidemiologic studies show an increasing incidence of atherosclerosis when serum cholesterol concentrations are > 3.9 mmol/L. The different lipoproteins have particular effects on vessel health. In the clinical context high levels of LDL and low levels of HDL are particularly important risk factors for CVD [103]. LDL cholesterol can promote inflammatory and immune changes via cytokine release from macrophages and antibody production. Circulating LDL rapidly accumulates in the cholesterol enriched macrophages (foam cells), but not directly in the lipid core of atherosclerotic plaque [104]. Oxidative modification of LDL is necessary for macrophage uptake via unregulated scavenger receptors and accelerated accumulation of cholesterol [105]. Macrophage uptake of LDL cholesterol may initially be an adaptive
response, which prevents LDL-induced endothelial injury [106].
Cholesterol accumulation in foam cells leads to mitochondrial dysfunction, apoptosis, and necrosis with resultant release of cellular proteases, inflammatory cytokines, and prothrombotic molecules [106]. Oxidized LDL can cause disruption of the endothelial cell surface [107], promote inflammatory and immune changes via cytokine release from macrophages and antibody production, and increase platelet aggregation. It may also play a role in plaque instability. Levels of oxidized LDL are increased in patients with an acute coronary syndrome and are positively correlated with the severity of the syndrome [108]. Low HDL levels are more common in patients with a first myocardial infarction than in age-matched controls without CHD (19 versus 4 percent) [109]. HDL, in contrast to LDL, has antiatherogenic properties that include reverse cholesterol transport, maintenance of endothelial function, protection against thrombosis, and maintenance of low blood viscosity through a permissive action on red cell deformability [110]. The net effect is that there is an inverse relationship between plasma HDL-cholesterol levels and cardiovascular risk. Values above 1.9 mmol/L are associated with a longevity syndrome [111].

Much of the anti-atherogenic effect of HDL is thought to be mediated by reverse cholesterol transport, a process whereby excess cholesterol in cells and in atherosclerotic plaques is removed [112]. Initially hepatic and intestinal cells synthesise small nascent HDL particles composed of phospholipids and apolipoproteins [113]. These HDL particles gather surface components (phospholipids, cholesterol, and apolipoproteins) from triglyceride-depleted chylomicron and very low density lipoprotein (VLDL) remnants [113]. They also acquire free (unesterified) cholesterol from tissue sites and other lipoproteins, as the initial HDL particles contain relatively little cholesterol. Apo A-I on the surface of HDL plays a central role in this process. It serves as a signal transduction protein to mobilize cholesterol esters from intracellular pools [113].
1.14.1 Lipid modification and CVD risk

There is extensive evidence that modification of dyslipidaemia can lower the risk of CVD. This evidence is both for primary (patients without evidence of CVD) and secondary (patients with CVD) prevention. There are multiple studies which assess the relationship between dyslipidaemia and CVD. A meta-analysis of 38 primary and secondary prevention trials found that for every 10 percent reduction in serum cholesterol, coronary heart disease mortality was reduced by 15 percent and total mortality risk by 11 percent. No increase in non-coronary heart disease mortality was seen [114, 115].

Lipid modification is achieved by medications, predominantly by HMG-CoA reductase inhibitors also known as statins. They lower cholesterol by inhibiting the enzyme HMG-CoA reductase, which is the rate-limiting enzyme of the mevalonate pathway of cholesterol synthesis [116]. Inhibition of this enzyme in the liver stimulates LDL receptors, resulting in an increased clearance of LDL from the bloodstream and a decrease in blood cholesterol levels. The first results can be seen after one week of use and the effect is maximal after four to six weeks [116]. However the mechanisms by which lipid-lowering therapy (particularly with statins) is beneficial, are incompletely explained by the serum LDL concentration at baseline or after treatment [117]. Although statins probably cause regression of atherosclerosis, an improvement in outcome can be demonstrated as early as six months: a time considered too early for significant plaque regression [118]. Among the non-lipid mechanisms that may be involved in CVD reduction are plaque stabilization, reduced inflammation, reversal of endothelial dysfunction, and decreased thrombogenicity [119].

One important effect of statin therapy may be maintenance of integrity of the fibrous cap of the plaque, thereby protecting against plaque rupture. This effect appears to be mediated by inhibition of macrophage proliferation, reduced expression of matrix metalloproteinases (MMPs) and tissue factor (which promotes thrombus formation) by macrophages, and an increase in tissue inhibitor of metalloproteinase-1 [120]. Statin therapy, given as primary or secondary prevention, reduces the serum CRP concentration, an
effect that is mostly unrelated to lipid levels at baseline or during therapy [121]. The fall in serum CRP begins within 14 days of treatment [122]. Additional evidence that statins may have an anti-inflammatory effect is provided by a randomized trial that found that patients with rheumatoid arthritis experienced modest clinical improvement in their RA with atorvastatin. Atorvastatin also reduced CRP levels and the ESR compared with placebo [123]. In clinical trials, statins appear to have greater effects in patients with evidence of inflammation at baseline. The potential importance of statin-induced reduction in serum markers of inflammation was illustrated by an analysis from the secondary prevention cholesterol and recurrent event (CARE) trial [124]. Patients with baseline serum concentrations of CRP and serum amyloid A in the highest quintile had a relative risk for a recurrent event 75 percent higher than those with levels in the lowest quintile.

How statins might interfere with the inflammatory response is not well understood. One possible mechanism is impairment of inflammatory cell adhesion by inhibition of the main beta-2 integrin, LFA-1 [125, 126]. Other contributing factors may include reduced lipidation of intracellular proteins and reduced expression of major histocompatibility complex class II molecules on antigen presenting cells in response to interferon, decreasing subsequent T-lymphocyte activation [127].

1.15 Assessment of CVD in RA

It is hard to power interventional studies in RA to look at definitive CVD endpoints. This is because it is difficult to obtain a large enough cohort of patients to show a treatment difference in mortality or cardiovascular events. Therefore a surrogate measure of a person’s risk for future CVD is ideal. In recent years, the evolution of non-invasive ultrasound techniques has allowed assessment of cardiovascular health. These techniques can detect vascular dysfunction, and appear to correlate well with future risk of CVD. An ideal situation to assess a person’s risk for CVD would be to know the exact state of their vascular tree. Currently there is no such technique. All current tests are necessarily approximate measures of vascular risk. Individual tests assess various aspects of vascular structure and function to
add weight to the knowledge of a persons’ CVD risk. No single test gives a complete assessment of CVD.

Cardiac catheter studies can demonstrate the vessel lumen, but these tests are expensive, invasive, have inherent risks and thus make them less useful for large scale progress studies. Other tests, such as CT assessment of coronary calcium scores, have good correlation with CVD but are also expensive and expose the patient to significant levels of radiation. Functional tests identify disease in the setting of significant coronary stenosis, which implies the presence of advanced disease.

Ultrasound provides a range of non-invasive techniques that assess peripheral vascular structure and function. It is commonly used to assess arterial structure and function by measuring arterial wall thickness, arterial wall reactivity and arterial stiffness. Observational studies have shown that these tests correlate well with future risk of CVD and current CVD burden. Atherosclerosis is a patchy process and it is difficult to gain an overall impression of the burden of disease. It has been recognised that there is good correlation between the risk of CVD and the thickness of the arterial wall. This thickness can be measured using ultrasound.

Arteries are divided into three layers: the intima, media and adventitia. Ultrasound detects changes in tissue boundaries and as such can clearly delineate the discrete areas of the arterial wall. This allows the thickness of the intima media to be measured. Measurement of the carotid artery is preferred because it allows clear visualisation of a large calibre artery that is relatively superficial, parallel and has a distinct anatomical area, the carotid bulb, to provide a reference point, thus giving the most reliable data [128].

Carotid ultrasonography has been used to obtain measurements of the thickness of the intima and media of the carotid arteries. Previous studies have shown cross-sectional associations between common-carotid-artery intima-media thickness and cardiovascular risk factors [129-131], the prevalence of cardiovascular disease, [130-132] and the involvement of other arterial beds with atherosclerosis [133] Changes in common-carotid-
artery intima-media thickness (CIMT) have also been adopted as a surrogate end point to determine the success of interventions that lower the levels of low-density lipoprotein cholesterol [134, 135]. CIMT has been shown to be associated with diabetes, fibrinogen level, body mass index, and clinically overt atherosclerosis [136]. There is evidence that CIMT can predict an increased risk of future myocardial infarction and stroke.

Increasing levels of CIMT are associated with an incremental risk of myocardial infarction and stroke. In one study, 5858 patients >65 years of age with no pre-existing cardiovascular disease were separated into quintiles on the basis of maximal CIMT, and followed for a mean period of 6.2 years. Over this period, 47 of 897 patients in the lowest quintile of IMT (<0.87 mm) had a myocardial infarction or stroke. In comparison, those in the highest quintile of IMT (≥1.18 mm) had 167 events. This equated to an age- and sex-adjusted relative risk of 2.85 [93].

In newly diagnosed patients with RA and subjects in a control group, a recent study found no signs of atherosclerosis; however after 18 months there was a significant increase in CIMT in RA patients compared to controls [137]. Hannawi et al [138] found that CIMT was significantly increased in their early RA cohort compared to controls (0.64 ± 0.13 mm versus 0.58 ± 0.09 mm, \( P = 0.03 \)). They repeated their analysis after removing patients with previous CV events and their controls and the results had not changed (0.63 ± 0.02 mm versus 0.57 ±0.01 mm, \( P = 0.03 \)). There are few studies investigating CIMT in early RA, indicating a need for further studies of CIMT in early and longstanding RA.

### 1.16 Kynurenine pathway

The kynurenine (KYN) pathway is a metabolic pathway leading to the production of nicotinamide adenine dinucleotide (NAD) from the degradation of the essential amino acid enzyme tryptophan (TRP) (Figure 3). The oxidation of TRP, initiating the KYN pathway, may be catalysed by one of three enzymes – tryptophan 2,3 dioxygenase (TDO) residing predominantly in the liver, indoleamine 2,3 dioxygenase 1 (IDO1) or a newly-discovered, related enzyme, indoleamine 2,3 dioxygenase 2 (IDO2).
IDO1 is the predominant extra-hepatic enzyme, found in numerous cells, including macrophages, microglia, neurons and astrocytes. IDO1 is stimulated by the pro-inflammatory cytokine interferon γ (IFNγ), which is activated by T lymphocytes during an immune response.

Figure 1-6: Schematic diagram of the kynurenine pathway

1.17 Kynurenine in RA
Through several pathways, KYN and its metabolites induce apoptosis of CD4+ Th1 T-cells and generation of Tregs, thereby providing negative feedback on T-cell responses. These effects may be helpful to maintain self-tolerance and to limit inflammatory disorders, but little is known about the relationship between disease activity and KYN levels in RA patients. Furthermore, increased concentrations of KYN have been linked to tumour cell immune escape and to poor prognosis in cancer patients [148]. One may speculate that immune down-regulation by KYN could increase the risk of infections, especially viral infections, where Th1 T-cell responses contribute an important part of host defence.
Increased IDO activity may be measured as the KYN/TRP ratio. In patients undergoing coronary angiography for suspected coronary artery disease, an increased KYN/TRP ratio in urine was strongly associated with major coronary events, acute myocardial infarctions, ischemic stroke, and all-cause mortality [149]. Similarly, an increased ratio was linked with more advanced atherosclerotic changes in patients on chronic haemodialysis due to kidney failure [150]. In these settings, KYN may be acting as a biomarker of inflammation. Taken together, KYN may play various roles in the interactions among inflammation, immune dysregulation, CVD risk and possibly the risks of cancer and infections in RA patients.

1.18 Rationale for the study
There are several studies that have reported and demonstrated CVD in established and early onset RA patients, CIMT progression in patients with RA compared to the general population, and the relationship between IDO and or KYN/TRP ratio in RA patients. However there are very few studies reporting the association of CVD and risk factors such as inflammation as an increased risk for future development of RA, or comparing CIMT progression between RA and another chronic disease with similar CV risk, or demonstrating the relationship in KYN and CVD, new CV events, malignancy and death in RA patients. This thesis will address some of these issues by examining CVD and risk factors in patients who later developed RA, comparing CIMT progression in RA and T2D and investigating KYN concentration levels in RA patients.

1.18.1 Aims
Aim 1. To investigate the effects of CV risk factors, and CV events on the development of RA, using a population-based cohort study design. As a control to assess whether any findings were specific to RA or related to arthritis in general, a parallel investigation was undertaken in participants with and without osteoarthritis.

Aim 2. To compare the characteristics of cardiovascular risk and progression of carotid intima-media thickness (CIMT) in individuals with RA and type 2 diabetes.
Aim 3. To measure serum kynurenine in baseline samples from an RA cohort with 10 year follow-up for cardiovascular events, and to investigate the relationship between kynurenine concentrations, malignancy, infection, cardiovascular disease and cause of death. These aims will be discussed in 3 separate chapters and will form the body of this thesis.
Chapter 2: Cardiovascular disease is increased prior to onset of rheumatoid arthritis but not osteoarthritis: HUNT population-based Norwegian health surveys.

Published in Arthritis Research & Therapy April 2014
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Full article (PDF) in appendices

2.1 Abstract

Objective: Patients with rheumatoid arthritis (RA) have increased risk of cardiovascular (CV) events. We sought to test the hypothesis that due to increased inflammation, CV disease and risk factors are associated with increased risk of future RA development.

Methods: The HUNT population-based health surveys were conducted among the entire adult population of Nord-Trøndelag, Norway to establish the health history of 75000 people. All inhabitants 20 years or older were invited, and information was collected through comprehensive questionnaires, a clinical examination, and blood samples. In a cohort design, data from HUNT2 (1995-1997, baseline) and HUNT3 (2006-2008, follow-up) were obtained to study participants with RA (n=786) or osteoarthritis (n=3586) at HUNT3 alone, in comparison with individuals without RA or osteoarthritis at both times (n = 33,567).

Results: Female gender, age, smoking, body mass index, and history of previous CV disease were associated with self-reported incident RA (previous CV disease: odds ratio 1.52 (95% confidence interval 1.11-2.07).
The findings regarding previous CV disease were confirmed in sensitivity analyses excluding participants with psoriasis (odds ratio 1.70 (1.23-2.36)) or restricting the analysis to cases with a hospital diagnosis of RA (odds ratio 1.90 (1.10-3.27)) or carriers of the shared epitope (odds ratio 1.76 (1.13-2.74)). History of previous CV disease was not associated with increased risk of osteoarthritis (odds ratio 1.04 (0.86-1.27)).

**Conclusion:** A history of previous CV disease was associated with increased risk of incident RA but not osteoarthritis.

### 2.2 Introduction

Rheumatoid Arthritis (RA) is a systemic, inflammatory autoimmune disorder that primarily involves the joints. Despite treatment advances, RA patients continue to have higher mortality and morbidity rates than the general population, predominantly related to increased atherosclerotic cardiovascular (CV) disease [77, 151]. The traditional CV risk factors include age, gender, family history, smoking, diabetes, hypertension, dyslipidemia, obesity and sedentary lifestyle. RA patients carry a higher burden of some of these factors. The disease occurs most commonly after 40 years of age and is associated with accelerated cellular ageing [152].

Smoking history is associated both with RA development and severity [43, 153, 154]. Physical inactivity is common in patients with RA and may increase co-morbidity due to obesity or diabetes, leading to an increased risk of CV mortality [155]. Some studies have also suggested that myocardial disease without atherosclerosis is more prominent in RA patients. For example, an echocardiography study demonstrated that RA patients asymptomatic for CV disease had ischemia more frequently than controls, but they also had fewer significant angiographic coronary artery stenoses [156]. Likewise, an autopsy study showed that RA patients had diffuse myocardial fibrosis or non-specific myocardial degeneration more frequently than controls after excluding individuals with other known causes [157].

Inflammation plays a central role in the pathogenesis of atherosclerosis, and the increase in CV disease and mortality in RA may partly be explained by
inflammatory factors associated with RA, even after adjustment for traditional CV risk factors [158, 159]. We and others showed that atherosclerosis is increased both in patients with established RA and at presentation in patients with recent-onset RA, as determined by increased carotid intima media thickness and plaque, and is associated with their inflammatory burden [160, 161]. The risk for MI and possible CV death is already increased within approximately 5 years after diagnosis of RA depending upon age and presence of CV risk factors, resulting in a 10-year absolute risk comparable to non-RA individuals who were 5-10 years older [14].

It has been demonstrated that inflammation pre-dates the onset of RA [162]. These data suggest that an increased risk of CV disease might also precede the onset of RA. The Rochester Epidemiology Project study demonstrated that RA patients were more likely to have been hospitalized because of MI prior to RA diagnosis [163]. However, a previous longitudinal cohort study found no difference in rate of MI, congestive heart failure or angina between pre-RA and control individuals [164].

We hypothesized that CV risk factors and events are more prominent in persons with incident RA, and that this augments the risk of future RA development by increasing inflammation. The aim of the study was therefore to investigate the effects of CV risk factors and CV events on the development of RA, using a population-based cohort study design. As a control to study whether any findings were specific to RA or related to arthritis in general, a parallel investigation was performed in participants with and without osteoarthritis.

2.3 Patients and methods
The study participants were from the HUNT population-based health surveys conducted in the county of Nord-Trøndelag in Norway. The county is fairly representative for Norway as a whole, with a stable and ethnically homogenous population (3% non-Caucasians). All inhabitants 20 years or older were invited, and information was collected through comprehensive
questionnaires and a clinical examination. The HUNT2 survey has previously been described in detail [165]. The HUNT3 survey had a similar design. In total, about 75,000 (70% of those invited) participated in HUNT2 (1995-1997), 51,000 (54% of those invited) participated in HUNT3 (2006-2008), and 37,071 participated in both HUNT2 and HUNT3. By design the participants were not seen during the years between inclusion in HUNT2 and HUNT3.

The study cohort consisted of all participants in both HUNT2 and HUNT3 who answered whether they had a diagnosis of RA or not (n=36,493, i.e. 98.4% of 37,071) and this number determined the study size. Incident cases of RA were identified, i.e. participants who reported a diagnosis of RA in HUNT3 but not in HUNT2. We also identified the incident cases of osteoarthritis for comparison, based on the question; “Has a doctor ever said that you have/have had any of these diseases: degenerative joint disease (osteoarthritis)?” The question also included the Norwegian colloquial term for osteoarthritis. Each patient group was compared to the remaining participants of the study cohort.

Participants of HUNT gave informed consent. Approval for the study was obtained from the Regional Committee on Medical Research Ethics, Central Norway, the Norwegian Data Safety Authorities, and the Norwegian Department of Health. Ethics approval was also obtained by the Metro South Ethics Committee Brisbane. Permission was granted from the two primary hospitals in Nord-Trøndelag, Levanger and Namsos hospitals, and the nearest secondary referral hospital, Trondheim University Hospital, to link the identified RA cases in our study to the hospital diagnosis registries for verification of diagnosis. The registered diagnoses are used for billing and reimbursement from the national insurance scheme. The ICD-9 code 714 and ICD-10 codes M05 and M06 with sub-codes were searched for. We did not have permission to access to the patients’ case notes in order to check that the current criteria for RA were correctly employed.

On enrolment in HUNT2 and HUNT3 the participants completed a questionnaire incorporating information on medical history, smoking habits,
and family history of CV disease, defined as a parent, sibling or child with previous MI or stroke. Information on use of lipid-lowering medications or non-steroid anti-inflammatory drugs was not available. Anthropometric and clinical measures included height, weight, waist and hip circumference, and blood pressure. Non-fasting blood samples were drawn and total cholesterol, low-density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides and serum glucose were measured using an autoanalyzer (Hitachi). Using DNA isolated at HUNT Biobank, participants with self-reported RA were genotyped for the Shared Epitope [166]. Samples were genotyped using the Illumina Immunochip microarray chip, and HLA-DRB1 genotypes then determined by imputation using the program HLA-IMP [167, 168]. Autoantibodies (anti-citrullinated peptide antibodies or ACPA, rheumatoid factor) and C-reactive protein were not measured in this survey, and classification into seropositive or seronegative RA was not possible. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or use of medication. Hypercholesterolemia was defined as total serum cholesterol > 6.2 mmol/L. Body mass index (BMI) was calculated as weight / height^2 (kg/m^2). Patients who reported using over-the-counter analgesics daily for 1 month or more during the last year before inclusion in HUNT2 were recorded as users of analgesics. Previous CV disease was defined as a composite of angina, MI or stroke. The relevant questions were; “Have you had or do you have angina pectoris?” “Have you had a stroke/brain hemorrhage?” and “Have you had a myocardial infarction?” The question about angina also included a Norwegian colloquial term for this diagnosis. The composite variable was used for previous CV disease as numbers of cases were too low for analysis of individual disease events. The level of missingness at baseline for most key variables was < 1%, with the exception of smoking (12.8% missing data). Missingness for smoking was evenly distributed among the subgroups of the study cohort. Non-complete cases were omitted from analysis.

2.4 Statistical methods
Data are presented as number (percentage), mean (standard deviation), or median (interquartile range), as appropriate. The chi-square test and the
Mann-Whitney U-test were used for between group comparisons of categorical and continuous study parameters, respectively. Risk factors associated with the development of RA and osteoarthritis between HUNT2 and HUNT3 were identified using multivariate logistic regression models. Linearity of logits for continuous variables was checked by plotting. P-values <0.05 were considered significant. Pearson’s correlation coefficient R was calculated to evaluate linear correlation.

Since this was a population-based survey and RA was self-reported, we performed several sensitivity analyses to support our main analysis. Because previous studies have indicated that few women with RA surveyed in the community report their diagnosis accurately [169], we repeated the analysis in several subgroups of the incident RA cases using additional criteria to remove false positive diagnoses, i.e. 1) excluding all who also reported having psoriasis (n=178), 2) including only patients where the diagnostic registries of the nearest hospitals showed a diagnosis of RA (n=216), 3) restricting the hospital-diagnosed cases to those where the diagnosis first occurred after 1999 or 4) after 2001, to avoid including patients who forgot to report RA in HUNT2, or 5) including only incident RA cases who carried the shared epitope (with and without additional exclusion due to a report of psoriasis). To represent the CV risk factors in another way, we developed alternative models including the Framingham risk score, which is based on age, serum cholesterol, hypertension, smoking and diabetes [170], as well as gender and history of CV disease. The Framingham risk score was preferred because it assigns higher risk with diabetes and there are no age limits, and because it may easily be calculated in large cohorts. The European HeartScore [171] is based on risk for patients between 40 and 65 years and requires separate input for each individual using charts or an online calculator, which was not practical in our large cohort. To verify that the Framingham risk score was applicable in our cohort, we calculated the PC-based HeartScore for 50 randomly selected incident RA cases and 50 controls [172]. Parallel Framingham risk scores and HeartScores were used to calculate an equation permitting estimation of the HeartScore in the entire cohort from their Framingham risk scores. An alternative logistic model was developed substituting the Framingham risk score with these estimated
HeartScores. In addition, we compared factors associated with incident cases of self-reported RA with incident cases of self-reported osteoarthritis.

2.5 Results
The baseline characteristics of the participants are presented in Table 1. At baseline (1995-1997) 33,567 participants reported not having RA, and of this group 786 (2.34%) reported RA at follow-up (2006-2008). This corresponds to an average annual incidence of 0.21% in women and 0.16% in men, respectively. If restricted to participants with cases identified in the local hospital registries (n=216), the annual incidence was 0.06% in women and 0.04% in men. As expected, incident cases of RA were older and more of them were current or previous smokers and had hypertension at baseline. Incident cases of RA had significantly elevated metabolic risk factors including blood pressure, BMI, serum cholesterol and triglyceride. The estimated median (interquartile range) Framingham risk scores at baseline were 11 (7, 16) and 8 (4, 13) respectively in incident cases and those who did not develop RA. Notably, a higher proportion of incident RA cases had a history of CV disease at baseline.

Female gender, age, smoking, BMI, and previous CV disease were more prominent in those developing RA (odds ratio 1.52 (1.11-2.07), Table 2, I - Main model). There was minimal change in the odds ratio for previous CV disease with inclusion of use of analgesics in this logistic regression model, when systolic and diastolic blood pressure were included as continuous variables instead of the categorical variable for hypertension, with additional adjustment for total cholesterol and HDL cholesterol concentrations, or when a variable for physical activity (low, moderate, high) was also included (data not shown). In all the alternative multivariate models for incident RA where the number of patients was restricted to better characterized subgroups, previous CV disease was significant and the odds ratios were higher than for the main model including all self-reported cases (Table 2).
# Table 2-1: Characteristics of individuals without rheumatoid arthritis (RA) or osteoarthritis (OA) at baseline

<table>
<thead>
<tr>
<th></th>
<th>All participants</th>
<th>Later developed RA</th>
<th>Did not develop RA</th>
<th>P-value</th>
<th>Later developed OA</th>
<th>Did not develop OA</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>33567</td>
<td>786</td>
<td>32781</td>
<td></td>
<td>3586</td>
<td>29981</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>46 (13)</td>
<td>51 (13)</td>
<td>46 (13)</td>
<td>**</td>
<td>52 (10)</td>
<td>45 (13)</td>
<td>**</td>
</tr>
<tr>
<td><strong>Sex (female)</strong></td>
<td>18207 (54.2%)</td>
<td>488 (62.1%)</td>
<td>17719 (54.1%)</td>
<td>**</td>
<td>2406 (67.1%)</td>
<td>15801 (52.7%)</td>
<td>**</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>8901 (26.5%)</td>
<td>256 (32.6%)</td>
<td>8645 (26.4%)</td>
<td></td>
<td>1015 (28.3%)</td>
<td>7886 (26.3%)</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>7908 (23.6%)</td>
<td>212 (27.0%)</td>
<td>7696 (23.5%)</td>
<td></td>
<td>945 (26.4%)</td>
<td>6963 (23.2%)</td>
<td></td>
</tr>
<tr>
<td>Never smoking</td>
<td>15017 (44.7%)</td>
<td>281 (35.6%)</td>
<td>14736 (45.0%)</td>
<td></td>
<td>1425 (39.7%)</td>
<td>13592 (45.3%)</td>
<td></td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td>12186 (36.3%)</td>
<td>339 (43.1%)</td>
<td>11847 (36.1%)</td>
<td>**</td>
<td>1547 (43.1%)</td>
<td>10639 (35.5%)</td>
<td>**</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td>454 (1.4%)</td>
<td>19 (2.4%)</td>
<td>435 (1.3%)</td>
<td></td>
<td>61 (1.7%)</td>
<td>393 (1.3%)</td>
<td></td>
</tr>
<tr>
<td><strong>Previous CV disease</strong></td>
<td>1060 (3.2%)</td>
<td>52 (6.6%)</td>
<td>1088 (3.1%)</td>
<td>**</td>
<td>158 (4.4%)</td>
<td>902 (3.0%)</td>
<td>**</td>
</tr>
<tr>
<td><strong>Angina</strong></td>
<td>673 (2.0%)</td>
<td>35 (4.5%)</td>
<td>638 (1.9%)</td>
<td>**</td>
<td>107 (3.0%)</td>
<td>566 (1.9%)</td>
<td>**</td>
</tr>
<tr>
<td><strong>MI</strong></td>
<td>441 (1.3%)</td>
<td>16 (2.0%)</td>
<td>425 (1.3%)</td>
<td></td>
<td>49 (1.4%)</td>
<td>392 (1.3%)</td>
<td></td>
</tr>
<tr>
<td><strong>Stroke</strong></td>
<td>223 (0.7%)</td>
<td>9 (1.1%)</td>
<td>214 (0.7%)</td>
<td></td>
<td>32 (0.9%)</td>
<td>191 (0.6%)</td>
<td></td>
</tr>
<tr>
<td><strong>Family risk of CV disease</strong></td>
<td>15692 (46.7%)</td>
<td>414 (52.7%)</td>
<td>15278 (46.6%)</td>
<td>**</td>
<td>2017 (56.2%)</td>
<td>13675 (45.6%)</td>
<td>**</td>
</tr>
<tr>
<td><strong>Framingham risk score</strong></td>
<td>9 (4-13)</td>
<td>11 (7-16)</td>
<td>8 (4-13)</td>
<td></td>
<td>11 (7-15)</td>
<td>8 (3-13)</td>
<td></td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>76 (14)</td>
<td>77 (14)</td>
<td>76 (14)</td>
<td></td>
<td>77 (13)</td>
<td>76 (14)</td>
<td></td>
</tr>
<tr>
<td><strong>BMI² (kg/m²)</strong></td>
<td>25.6 (23.5-28.1)</td>
<td>26.3 (24.3-29.0)</td>
<td>25.6 (23.4-28.1)</td>
<td>**</td>
<td>26.4 (24.2-29.0)</td>
<td>25.4 (23.3-27.8)</td>
<td>**</td>
</tr>
<tr>
<td><strong>Waist circumference (cm)</strong></td>
<td>85 (11)</td>
<td>86 (11)</td>
<td>85 (11)</td>
<td>*</td>
<td>86 (11)</td>
<td>85 (11)</td>
<td>**</td>
</tr>
<tr>
<td><strong>Hip circumference (cm)</strong></td>
<td>102 (8)</td>
<td>103 (8)</td>
<td>102 (8)</td>
<td>**</td>
<td>103 (8)</td>
<td>101 (8)</td>
<td>**</td>
</tr>
</tbody>
</table>
II – Alternative models A to F). A diagnosis of RA was confirmed in the hospital registries for 216 participants (27%) who had self-reported a new RA diagnosis in HUNT3. The characteristics of these participants were very similar to those reported for the entire group of incident RA cases (data not shown). In the 216 cases with a hospital diagnosis of RA, the diagnosis was found 1-2 years following enrolment in HUNT2 for 14 cases (6.5%), after 3 years for 24 cases (11.1%), after 4 years for 15 cases (6.9 %), after 5 years for 28 cases (13.0%), and after 6 years for 22 cases (10.2 %). For the remaining 113 cases (52.3%), the times were distributed from 7 years onwards to the inclusion in HUNT3 with numbers varying unsystematically from 15 to 24 cases (6.9-11.1%) per year. Thus, there was no obvious pattern regarding the number of years from HUNT2.

In a separate alternative multivariate logistic regression model containing gender, previous CV disease, and the Framingham risk score as covariates, the odds ratio for developing RA in subjects with previous CV disease was 1.65 (1.21-2.24)(p<0.01). This model also suggested the likelihood of developing RA was 6% greater with each 1 unit increase in Framingham risk score (odds ratio: 1.06, 95% CI: 1.05-1.08), p<0.001. The model did

<table>
<thead>
<tr>
<th>Waist/hip ratio</th>
<th>0.84 (0.08)</th>
<th>0.84 (0.08)</th>
<th>0.84 (0.08)</th>
<th>0.83 (0.07)</th>
<th>0.84 (0.08) **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure* (mmHg)</td>
<td>131 (120-144)</td>
<td>133 (122-146)</td>
<td>131 (120-144) *</td>
<td>133 (122-147)</td>
<td>131 (120-143) **</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79 (11)</td>
<td>80 (11)</td>
<td>79 (11) *</td>
<td>81 (11)</td>
<td>79 (11) **</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/L)</td>
<td>5.8 (1.2)</td>
<td>6.0 (1.2)</td>
<td>5.8 (1.2) **</td>
<td>6.1 (1.2)</td>
<td>5.8 (1.2) **</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.40 (0.39)</td>
<td>1.37 (0.39)</td>
<td>1.40 (0.39)</td>
<td>1.44 (0.40)</td>
<td>1.39 (0.38) **</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/L)</td>
<td>1.40 (0.98-2.07)</td>
<td>1.51 (1.10-2.24)</td>
<td>1.40 (0.97-2.06) **</td>
<td>1.46 (1.02-2.12)</td>
<td>1.37 (0.95-2.04) **</td>
</tr>
</tbody>
</table>

*Values are mean (standard deviation) or numbers (%), or †median (interquartile range). Non-respondents were excluded. CVD=cardiovascular disease. MI=myocardial infarction. BMI=body mass index. HDL=high density lipoprotein. * p-value <0.05, ** p-value <0.01, comparing individuals who developed RA and did not develop RA, or comparing individuals who developed OA and did not develop OA. All blood samples were non-fasting.
not change with adjustment for BMI or daily use of over-the-counter analgesics. In the model where the Framingham risk score was substituted with the estimated HeartScore, previous cardiovascular disease remained significant (odds ratio: 1.70 (1.25-2.32), p<0.01). In the 100 participants where the HeartScore was calculated using the PC-based calculator, the HeartScore (logarithmically transformed) was highly correlated with the Framingham risk score (R=0.86, p<0.001). The correlation was very similar in patients (R=0.89) and controls (R=0.86).

Table 2-2: Effects of risk factors on incident RA and osteoarthritis, by multivariate logistic regression

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female gender</td>
<td>1.50</td>
<td>1.29-1.75</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age</td>
<td>1.03</td>
<td>1.02-1.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current smoker</td>
<td>1.64</td>
<td>1.38-1.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Former smoker</td>
<td>1.32</td>
<td>1.10-1.59</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.97</td>
<td>0.82-1.15</td>
<td>0.76</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.34</td>
<td>0.83-2.18</td>
<td>0.23</td>
</tr>
<tr>
<td>Body mass index</td>
<td>1.04</td>
<td>1.02-1.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Previous CV disease</td>
<td>1.52</td>
<td>1.11-2.07</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

CI = Confidence Interval
Alternative models (sensitivity analyses) - Rheumatoid arthritis

<table>
<thead>
<tr>
<th></th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A – After exclusion of patients with self-reported psoriasis</strong> (n=573 patients and 30,829 controls)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous CV disease</td>
<td>1.70</td>
<td>1.23-2.36</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>B – Including patients with hospital diagnosis of RA</strong> (n=201 patients and 30,829 controls)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous CV disease</td>
<td>1.90</td>
<td>1.10-3.27</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>C – Including patients with hospital diagnosis of RA after 1999</strong> (n=178 patients and 30,829 controls)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous CV disease</td>
<td>2.26</td>
<td>1.26-3.99</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>D – Including patients with hospital diagnosis of RA after 2001</strong> (n=138 patients and 30,829 controls)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous CV disease</td>
<td>2.51</td>
<td>1.33-4.73</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>E – Including patients carrying the Shared Epitope</strong> (n=313 patients and 30,829 controls)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous CV disease</td>
<td>1.76</td>
<td>1.13-2.74</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>F – Including patients carrying the Shared Epitope and excluding patients with self-reported Psoriasis</strong> (n=257 patients and 30,829 controls)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous CV disease</td>
<td>1.96</td>
<td>1.24-3.10</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**II – Model for osteoarthritis** (n = 3364 patients and 24,631 controls)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female gender</td>
<td>2.36</td>
<td>2.15-2.56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age²</td>
<td>1.05</td>
<td>1.05-1.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current smoker</td>
<td>1.42</td>
<td>1.30-1.56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Former smoker</td>
<td>1.23</td>
<td>1.12-1.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.90</td>
<td>0.66-1.22</td>
<td>0.49</td>
</tr>
<tr>
<td>Body mass index²</td>
<td>1.06</td>
<td>1.05-1.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Previous CV disease</td>
<td>1.04</td>
<td>0.86-1.27</td>
<td>0.66</td>
</tr>
</tbody>
</table>

¹ Cases with complete data. ²Continuous variable, Odds ratio per 1 unit change. ³Multivariate model including the same variables as the main model for RA
At follow-up (2006-2008), 3,586 participants had developed osteoarthritis, corresponding to an average annual incidence of 1.05% in women and 0.64% in men respectively. These patients were older and the proportion of smokers, former smokers and of those with hypertension was higher than in the group that did not develop osteoarthritis. Systolic and diastolic blood pressure, BMI, hip and waist circumference, total cholesterol and triglycerides were higher for incident cases of osteoarthritis, whereas HDL cholesterol was lower in incident cases of osteoarthritis. Female gender, age and smoking were associated with development of osteoarthritis, whereas previous CV disease was not (Table 2. III).

2.6 Discussion
In the present large population-based health study we found that participants who developed RA between HUNT2 and HUNT3 had more CV disease and CV risk factors prior to the onset of RA (at HUNT2) compared to those without incident RA. A history of CV disease was a significant risk factor for incident RA, as well as the previously reported risk factors: female gender, increasing age, increasing BMI and smoking [173]. The finding of a positive association with the Framingham risk score may be related to smoking and age being incorporated into the score. The supplementary analysis showed that the Framingham score was equivalent to the estimated HeartScore in our population even though the Framingham score was not developed from European subjects. On the other hand, previous CV disease events did not contribute to the risk of future osteoarthritis in our study, indicating that the finding may be specific to RA. Smoking was associated with incident osteoarthritis.

2.6.1 CV disease and incident RA
The design of our study has several strengths. Since the data on the CV risk factors and events were collected at HUNT2, recall bias at HUNT3 was greatly reduced compared to a design where patients report on these factors when receiving an RA diagnosis at a hospital-based clinic. Furthermore, the observation time between HUNT2 and HUNT3 was approximately 10 years. The importance of a long observation time is underscored by the
finding that the odds ratio for the association of incident RA with previous CV disease increased when patients receiving a hospital diagnosis of RA during the first years following HUNT2 were excluded. It also seems biologically plausible that longer exposure to increased inflammation due to CV disease further increases the risk of incident RA. Furthermore, the statistical power is greatly improved in a population-based cohort study due to the large number of controls, and problems defining a relevant control group as seen with case-control studies of RA are avoided.

The major limitation of our study is that data were self-reported. Previous studies suggest that self-reported CV diagnosis and risk factors such as hypertension and cigarette smoking are reliable [174, 175]. However, the high incidence rate of self-reported RA in our study compared for example to recent Swedish data based on inpatient and non-primary outpatient care (0.056% in women, 0.025% in men) [176], confirms that there probably were many false-positive RA diagnoses. This would tend to decrease the power to detect true positive results in our study, but not bias towards false positive findings. When including only cases identified in the hospital registries, the incidence was close to the Swedish data. Furthermore, the incidence of RA increases with observation time [177]. Therefore different data may not be directly comparable.

Epidemiological studies also carry a risk of reverse causation, which could ensue if persons with undiagnosed RA in HUNT2 were erroneously included as incident cases of RA, because RA in itself increases the risk of CV disease and events [178]. It seems unlikely that reverse causation could explain our findings, given that the association with previous CV disease became stronger when the cases with shortest duration between HUNT2 and a hospital diagnosis of RA were removed from the analysis. Furthermore, we did not observe a tendency for a higher number of cases with a hospital diagnosis to occur during the earliest years following HUNT2, which would have been expected with substantial reverse causation.

While fatal CV events prior to onset of RA also limited the participants in our study to survivors of those events, this again would lead to under-
estimation of the impact of CV events on development of RA, or to restrict the population at risk of RA to those ageing with CV risk that was insufficient to lead to premature mortality. Autoantibodies were not measured and seropositive and seronegative cases of RA could not be distinguished, which impacts on the generalizability of our results compared to others, such as population-based cohorts where it was concluded that ischemic heart disease was not increased prior to RA onset [164]. There may also have been a selection bias regarding participation in HUNT, where sicker people or those with a lower socioeconomic status are less likely to join. These people have a higher risk both for CV disease and for RA, which may have biased our findings.

The results from our various sensitivity analyses unanimously supported our main analysis. The overall conclusion that a history of CV disease is associated with the risk for incident RA therefore seems reliable. Although our study may not be suitable to give an exact estimate of the size of this increased risk, the main analysis giving an OR of approximately 1.5 is probably conservative.

2.6.2 Risk factors for osteoarthritis

Our results identifying smoking as a risk factor for future osteoarthritis contradict some previous studies, which report an inverse association between smoking and osteoarthritis [179]. However, the longitudinal prospective Clearwater study designed to identify risk factors for OA development, which included 2,505 participants, did not support this inverse association with smoking for any of four joint sites (knee, hand, foot or spine) [180]. In another prospective study of 1,003 participants from the general population there was no association between smoking and radiologically confirmed OA at different sites [181]. The current study may have shown an association with smoking because it is larger and included a greater number of incident osteoarthritis diagnoses. Nevertheless, since our study relies on self-report, replication in a cohort ascertained for radiographic osteoarthritis and symptoms is needed. However in support of the accuracy of our case classification, we noted that higher BMI increased the risk for osteoarthritis development, as previously described [182].
2.6.3 Inflammation as a link between CV disease and incident RA

From a biological perspective based on the current studies, we hypothesize that inflammation may contribute to the parallel development of atherosclerosis and RA during the pre-clinical period in individuals exposed to common RA and CV risk factors, just as this interaction accelerates complication of RA by CV events after onset.

Inflammation is the most plausible link between previous CV disease or BMI and increased risk for RA. It is well established that the pathogenesis of CV disease, i.e. atherogenesis, includes chronic inflammation of the wall of muscular arteries and that inflammation is increased during acute coronary events, associated with unstable plaque and thrombosis [163]. Furthermore, obesity induces chronic inflammation in adipose tissue, increasing pro-inflammatory cytokines including interleukin-6 and tumour-necrosis factor [183]. In addition, a pro-inflammatory state is present before the clinical onset of RA.

CRP levels were found to be higher in individuals with pre-clinical RA compared to a control group [184]. Serum cytokines and chemokines were
also elevated preceding the onset of RA [185]. In at least some patients, periodontal inflammation may precede the onset of RA symptoms, also associated with ACPA [186]. Respiratory inflammation has also been described in ACPA positive individuals without RA [187]. Autoantibodies may also increase the risk of immune complex mediated vascular inflammation [188]. Thus, CV disease and CV risk factors increase the future risk of RA development in the context of systemic inflammation and autoantibody development [160]. Patients with seropositive RA are at greater risk of CV disease [43, 78], and it is possible that the RA genetic background is more permissive to atherosclerosis when associated with the “lifestyle” factors described here. In contrast to RA, in osteoarthritis inflammation is local and low-grade rather than systemic, and is not associated with autoantibodies.

Figure 1 proposes a model for the relationships among RA, CV disease, inflammation and various predisposing factors, based on the results from the present study, as well as previous studies demonstrating an increased risk of CV disease after diagnosis of RA. Given that our findings were robust when adjusting for known risk factors for RA, this hypothesis merits further investigation in studies designed to clarify pathogenetic mechanisms, which is not possible in an epidemiological study.

2.7 Conclusions
A history of previous CV disease was associated with increased risk of incident RA but not osteoarthritis. A probable explanation is that increased systemic inflammation may contribute to the parallel development of atherosclerosis and RA during the pre-clinical period in individuals exposed to common RA and CV risk factors, in a similar way that such interaction accelerates CV disease in patients with established RA. The association of previous CV events with the development of RA at the population level suggests presentation with CV events, especially in middle-aged female smokers or first-degree relatives of RA patients, should raise clinical suspicion to capture cases of undiagnosed early RA. Because CV risk
factors are increased at onset of RA, active cardiovascular risk management is important from the time of diagnosis.

**Transition to chapter 3**

We and others have previously reported that CVD was present in patients as early as at diagnosis of RA and in established RA patients. There are few studies comparing CIMT in RA patients compared to other diseases of similar CV risk. Therefore with the information from this chapter we decided to compare CIMT in RA patients to patients with T2D.
Chapter 3: T2D patients have a higher risk of carotid-intima media thickness progression than RA patients

3.1 Background
It is well known that patients with Rheumatoid Arthritis (RA) or type 2 diabetes (T2D) have increased risk of atherosclerosis and cardiovascular disease (CVD). Carotid ultrasound measurement of the intima media thickness (CIMT) is the most commonly used method to validate progression of atherosclerosis.

Aim: To compare the characteristics of cardiovascular risk and progression of CIMT in individuals with RA and T2D.

Methods: We measured CIMT using B-Mode ultrasonography of the common carotid artery, in 290 individuals (78 RA patients and 212 T2D patients). RA patients were attending routine clinic appointments and T1D patients were assessed as part of a lifestyle intervention study. We carried out a physical assessment, routine laboratory investigations and took a history of CV risk profile in all participants. CIMT was measured twice in individuals with RA and individuals with T2D, at a mean interval of 4.8 and 2.4 years respectively.

Results: The study comprised 290 individuals: 78 with RA and 212 with T2D. The RA cohort was older and had a higher proportion of smokers and previous CVD. The T2D cohort had higher BMI, diastolic blood pressure and triglycerides, lower HDL cholesterol and more statin use.

At baseline, CIMT measurements were similar in the RA and T2D cohorts (0.88 (0.19) mm vs. 0.86 (0.21) mm p=0.80) respectively. In an adjusted linear regression model, RA was significantly associated with lower CIMT at follow-up (p<0.0005). Despite a shorter follow-up, 91 % of the T2D cohort had increased CIMT at follow-up compared to 54 % of the RA cohort (p<0.0005). In a supplementary adjusted logistic regression analysis
where the outcome was CIMT progression compared to unchanged or reduced CIMT, the OR for progression in T2D was 11.4 (5.2-25.0) compared to RA. In the RA cohort, DMARD use at baseline was associated with significantly lower CIMT values at follow-up (p=0.04).

**Conclusion:** T2D patients have a much higher risk of CIMT progression than RA patients when adjusting for relevant risk factors and baseline CIMT.

### 3.2 Introduction:

There is substantial evidence that cardiovascular (CV) risk in RA is increased [189, 190]. However, whilst individuals with rheumatoid arthritis (RA) have higher carotid intima media thickness (CIMT) [138, 191] and develop more CV events than the general population [192, 193], it is not clear how rapidly cardiovascular disease (CVD) progresses relative to other diseases at increased CV risk. Patients with type 2 diabetes (T2D) also have an increased risk of CVD and diabetes is an independent risk factor for CV complications [194]. Diabetic patients without a prior history of myocardial infarction (MI) have the same risk for future events as non-diabetic patients who have had a prior MI [195]. Traditional CV risk factors and inflammation contribute to this increased risk in patients with RA and diabetes [196-199]. Obesity promotes insulin resistance, and obesity-related inflammation contributes to metabolic impairment and the risk of development of T2D [200, 201]. Several studies have shown associations between CIMT and CVD [129-131]. CIMT values measured by ultrasound have been shown to correlate with direct measurement of local and systemic atherosclerotic burden in pathology studies and with clinical CV endpoints [202, 203]. Current CVD burden B-mode ultrasound is also commonly used to assess arterial structure and function by measuring arterial wall thickness, arterial wall reactivity and arterial stiffness [204-206].

We found recent-onset RA patients had increased CIMT compared to healthy controls [138]. Similar increases were reported in patients with long-standing RA [191] and in T2D [207]. A recent study comparing T2D patients from the Hoorn study and RA patients from the Care study reported
that RA and T2D had similar CVD outcomes [208], however the pattern of CVD manifestations differed with RA having more coronary artery disease and T2D with more peripheral arterial disease [208]. Although RA and T2D may share some CV risk factors, differences exist, including presence of high-grade inflammation amongst those with RA [209]. Few studies have compared CIMT progression in two chronic diseases with elevated CV risk. With this in mind, the objective of the current study was to compare the rate of progression of CIMT in RA and T2D patients drawn from the same geographic location and in whom CIMT was measured using the same instrumentation and analysis techniques, and to relate this to CVD risk profile and CV events.

3.3 Materials and Methods
3.3.1 Participants
Participants consecutively attending an outpatient Rheumatology clinic at a tertiary hospital in Brisbane Australia diagnosed with RA by a rheumatologist according to the American college of Rheumatology (ACR) 1987 criteria [44], were recruited. A health assessment and CV questionnaire was completed by each individual. Demographic information was ascertained by a questionnaire and disease duration was determined by the length of RA symptoms. Patients were treated according to a response-driven step-up algorithm [210]. Most participants were treated with a disease-modifying anti-rheumatic drug (DMARD) or a biologic agent at baseline.

Participants with T2D were recruited from hospital community diabetes clinics in the same geographic area as RA participants. The participants with T2D participated in a randomised trial of exercise intervention for 4 weeks. 248 patients underwent screening for occult coronary artery disease using exercise echocardiography, which excluded 25 patients. Of the 223 patients only 212 had readable CIMT measurements [211].

All patients provided formal written consent for the carotid ultrasound measurements. Ethical approval for the current study was obtained from the
Metro South Human Ethics Committee. The CIMT was reported previously in some of the RA patients [138].

3.3.2 Cardiovascular risk factor measurement:
For the RA patients cigarette smoking status was obtained by questionnaire, which included past and present smoking history (1). In T2D patients, present smoking status was determined by reviewing patient medical notes. Body mass index (BMI) was calculated as weight (kilograms) divided by the square in height (metres). Fasting lipid, erythrosedimentation rate (ESR) and C-reactive protein (CRP) measurements were collected by the hospital pathology department on both the RA patients and T2D patients at baseline. Fasting glucose, insulin and HbA1c measurements collected in T2D patients were measured prior to and after the 4-week intervention period. Blood analyses were conducted using standard procedures by the Queensland Health Pathology Service (Brisbane, Australia), and all measurements were taken after an overnight fast and at least 24 h after the last exercise session. Insulin sensitivity [HOMAIR (homoeostasis model assessment of insulin resistance) and QUICKI (quantitative insulin check index)] and β-cell function [HOMAβ-Cell (homoeostasis model assessment of β-cell function)] were determined using formulae derived previously [273, 274].

In the RA patients, diagnosis of diabetes was by a physician or use of insulin or oral hypoglycaemic medications. Hypercholesterolemia and hypertension were identified if the diagnosis was recorded in medical notes by a physician or patients were taking lipid-lowering or anti-hypertensive medications at the time of assessment.

3.3.3 Cardiovascular events
To ascertain CV events, a CV questionnaire was completed by the patients for history, dates and treatments of MI, angina and stroke, as previously described (2) and these events were verified by reviewing the medical records. Patients with multiple events had only one event counted per person. MI was identified if patients developed either of the following clinical signs: a typical rise and fall in the levels of biochemical markers
consistent with myocardial necrosis, in addition to ischemic symptoms, development of pathologic Q waves on the electrocardiogram (ECG) and/or ECG changes indicative of ischemia (ST-segment elevation or depression); or either new pathologic Q waves on serial ECGs or pathologic changes of healed or healing MI [212]. Stroke was identified if patients had been admitted to the hospital with evidence of ischemic occlusion on computed tomography (CT) scanning, carotid endarterectomy, or symptoms of stroke, evidence of a significant plaque on carotid ultrasound and neurologic sequelae, with the exclusion of subarachnoid haemorrhage and space-occupying lesions. For the exercise intervention study in patients with T2D, those with serious co-morbidity or known CVD had been excluded [211]. CV events occurring after the baseline CIMT measurement were determined in patients with RA and T2D by review of hospital charts.

3.4 Measurement of intima media thickness
This hospital uses protocols established by The American College of Echocardiography (ASE) for measuring CIMT [213]. CIMT was measured from ultrasound images as earlier described using carotid duplex scanning and automated software [138]. Ultrasound images were frozen by ECG triggering (top of R-wave) to minimize variability depending on changes in the CIMT and lumen diameter occurring during the cardiac cycle [138, 214, 215]. Offline analysis of the images was performed on a workstation using standard software for automated measurement of the intima-media thickness (IMT) (QLab 4.2.1 with IMT plugin, version 1.1; Philips Ultrasound, Bothell, WA, USA). The algorithm uses a maximum-slope edge-detection technique, beginning in the vessel lumen and detecting the first maximum slope of the change in signal for the near and far walls, and repeating the analysis to identify the media–adventitia interface. The software calculates the mean and standard deviation for the IMT thickness in a region-of-interest box, placed by the observer perpendicular to the vessel walls, with the measurements performed on the R-wave of the ECG [138].

Examination and analysis of the CIMT were performed by sonographers BH and LS, both from the same institute. The intra-observer variation and the co-efficient of variance was $0.01 \pm 0.05 \text{ mm (5\%)}$ [138]. Data are given as
the total average CIMT over all measured vascular segments from anterior, lateral and posterior views from both the right and left common carotid arteries (n=6). Areas with plaque were not included and not quantified.

3.5 Statistical analysis
Analyses were performed with IBM SPSS (v.20), STATA (v.13.1) and the rms package in the statistical environment R. Data are presented as mean (SD) or median (inter-quartile range), or numbers (percentages). The t-test or Mann-Whitney U-test was used to compare continuous variables and the chi²-test was used to compare frequencies between patients with RA and T2DM.

Correlations between quantitative variables were analysed by Pearson’s correlation coefficient. P-values <0.05 were considered significant. We undertook multivariable robust linear and logistic regression modelling. The model included pre-defined variables to analyse the CIMT measurements, as univariate screening carries a risk of overfitting. Transformation to normality was performed if necessary, and baseline CIMT was included as a covariate because we expected an association with follow-up CIMT. For linear regression, model fit was checked by residual plotting. For logistic regression, linearity of logits for continuous variables was checked by addition of spline terms.

For comparison between the RA and T2DM groups, the main outcome was the mean follow-up CIMT. Secondary analyses were performed using the average yearly rate of change in CIMT as outcome, and alternatively using progression (positive mean yearly rate of change) or no progression (zero or negative mean yearly rate of change) as outcome in logistic regression. For comparisons between the two patient groups, adjustments could only be made for variables available in both groups, i.e. age, sex, blood lipids, BMI, blood pressure, smoking, ever use of statins, and time to follow-up. Because the RA and T2DM groups were not matched, we also performed sensitivity analyses on a subgroup of the patients, matching each RA patient to a T2DM patient of the same sex and age (+2 years). For 3 RA patients (2
women aged 74 and 75 years and 1 male aged 78 years), matching by age was not possible and these cases were omitted from the sensitivity analyses. We used STATA to calculate Framingham Risk Score (FRS) as an alternative way to represent CV risk [170]. A sensitivity analysis was undertaken for CIMT at follow-up using CIMT at baseline, ever use of statins, RA/T2D and the FRS as covariates. Age and sex were not included because they are used in the calculation of the FRS.

For analysis of medication effects on CIMT at follow-up in the RA patients, a step-wise modelling approach was used in order to reduce the final number of adjustment variables. This avoided overfitting due to a small sample size. In the first step, only baseline CIMT and classical risk factors were included, i.e. age, sex, diabetes (no/yes), smoking, hypertension, BMI and total cholesterol/HDL cholesterol ratio. The significant variables were included in the next step together with factors related to RA severity: RA duration, baseline ESR and baseline CRP. Again, only significant factors were carried on to the third step where we also included the variables of main interest, i.e. ever use of statins (no/yes), ever use of prednisone (no/yes), and baseline use of all or a combination of disease modifying anti-rheumatic drugs (DMARDs) i.e. methotrexate, hydroxychloroquine and sulphosalazine (no/yes). The coding of statins and prednisone was chosen because changes in medication during follow-up may have influenced CIMT at follow-up and detailed information on dosage was not available. Baseline DMARD use was chosen because we hypothesized early treatment to be beneficial. In alternative models, baseline DMARD use was exchanged with ever use of DMARDs or DMARD use at follow-up.

3.6 Results
3.6.1 Sample characteristics
The studied population consisted of 290 individuals, 78 with RA and 212 with T2D who had all undergone carotid ultrasonography for measurement of CIMT at two time points: mean 4.8 years apart in RA patients and 2.4 years apart in T2DM patients (p=0.001). Baseline characteristics of the participants are presented in Table 1. The two analysis time points are designated baseline and follow-up. Recruitment of patients occurred at a
new-onset or a follow-up clinic appointment, thus did not necessarily occur at diagnosis. The median disease duration for the RA group at follow up was 19.1 yrs (9.9-28) yr and for the T2D group was 6.4 (3.4-12.4) yrs (p=0.0005) respectively.

The burden of CV risk factors varied between the two patient groups at baseline. The RA group was older, and had higher proportions of females, smokers, and previous CV events. The T2D group had higher mean BMI, diastolic blood pressure and triglycerides, lower HDL cholesterol and higher statin use (Table 1).

At follow-up, HDL cholesterol remained lower, and systolic and diastolic blood pressure, BMI and triglyceride levels were higher in T2D than RA patients (Table 1). Although LDL cholesterol had decreased at follow-up for both RA and T2D patients, LDL cholesterol levels were higher in RA than T2D patients (Table 1). These data indicate that CVD risk factors were present in each group but the distribution of risk factors differed between groups.

**3.7 Comparison of CIMT in RA and T2D patients**

The mean CIMT at baseline was 0.88 (0.19) mm in the RA group and 0.86 (0.21) mm in the T2DM group (p=0.80). The mean CIMT at follow-up was 0.92 (0.16) mm in the RA group and 1.12 (0.25) mm in the T2DM group (p=0.001). In multivariable linear regression adjusted as previously described, higher follow-up CIMT was significantly positively associated with higher baseline CIMT (p<0.0005), age (p<0.05), diabetes (p<0.0005) and ever use of statins (p<0.05) (Figure 1). Time to follow-up was not significantly associated with CIMT at follow-up (p=0.94). The median FRS was higher for T2DM 16 (16-17) than for RA 13 (12-14) (p<0.001). In the sensitivity analysis including the FRS instead of separate cardiovascular risk factors, the FRS was not significant (p=0.32) and there were only minimal changes in the coefficients for the significant variables mentioned above.

The mean yearly rate of CIMT change was 0.003 (-0.009-0.024) mm in the RA group and 0.088 (0.054-0.170) mm in the T2D group (p<0.0005). In the regression model for yearly rate of CIMT change, the only significant
variables were diabetes (p<0.0005) and ever use of statins (p=0.01). Notably, baseline CIMT was not significantly associated with the yearly rate of CIMT change (p=0.52). With correction for multiple comparisons, however, the differences between statin users and non-users within each diagnosis group was no longer significant (Figure 2).

193 (91%) of the T2D patients had CIMT progression over the follow-up period as opposed to 42 (54%) of the RA patients (p<0.0005). In logistic regression, progression of CIMT was associated with diabetes (p=0.001, odds ratio: 6.34 (2.17-18.50)) and ever use of statins (p<0.01, odds ratio: 2.96(1.41-6.22)). Time to follow-up was not significant (p=0.23). This model was adjusted for baseline CIMT, hypertension, smoking and BMI. Given the differences in age and gender among RA and T2D groups at baseline and the potential for these to skew CIMT, we carried out a sensitivity analysis, in which data from 75 pairs of RA and T2DM patients matched for age and gender were compared. The average CIMT at baseline was 0.89 (0.17) mm in the RA group and 0.90 (0.17) mm in the matched T2D group (p=0.43). The average CIMT at follow-up was 0.92 (0.16) mm in the RA group and 1.15 (0.23) mm in the matched T2D group (p<0.0005). The average yearly rate of CIMT change was 0.003 (-0.002-0.012) mm in the RA group and 0.088 (0.076-0.113) mm in the matched T2DM group (p<0.0005). In the matched pairs, 72 (96%) of the T2D patients had CIMT progression over the follow-up period as opposed to 40 (53.3%) of the RA patients (p<0.0005). The findings using linear and logistic regression modelling were very similar to those reported above for the complete study cohort (data not shown).

3.8 Influence of medication on CIMT in RA patients
Characteristics of the RA patients are given in Table 2. At baseline, 7 women (13.5%) and 7 men (26.9%) (p=0.14) were not using DMARDs. Using the described stepwise approach for the linear regression model for follow-up CIMT in the RA patients, age, sex, smoking and baseline CIMT were carried on from step 1 and no factors related to RA severity were significant in step 2. In the final model from step 3, baseline use of DMARDs (p=0.02), and the interaction between baseline CIMT and gender
(p=0.03) were significantly associated with follow-up CIMT (Figure 3). Baseline use of DMARDs had a protective effect but ever use of prednisone was not significant (p=0.44). If RA duration was added to the final model, this was not significant (p=0.84). In alternative models, ever use of DMARDs or use at follow-up were not significant contributors to follow-up CIMT (p=0.28 and 0.27, respectively).

3.9 CV events
CV events were self-reported by our RA patients, and confirmed by reviewing all medical records. At baseline, 16.7% of RA and no T2DM patients had experienced a CV event. At follow-up, 30 (24.0%) T2DM patients and 10 (13.0%) RA patients had experienced a new CV event since baseline (p=0.09) (Table 2).

3.10 Discussion
CIMT measurement by B-mode ultrasound is a valid biomarker for CV risk in RA and T2D patients. In the present study we demonstrated that the rate of CIMT progression was significantly lower in RA than T2D patients, despite an observation time twice as long in RA patients. At baseline, CIMT measurements were similar in each group. Despite a shorter follow-up period, a significantly greater percentage of T2D patients had CIMT progression than RA patients. By logistic regression, where the outcome was CIMT progression compared to unchanged or reduced CIMT, OR for progression in T2D relative to RA was 11.4. We found diabetes and ever use of statins but not baseline CIMT were significantly associated with yearly rate of CIMT change. In the RA cohort, DMARD use at baseline was associated with significantly lower CIMT values at follow-up.

3.10.1 Differences in CV risk factors in RA and T2D
In our study RA patients were compared to T2D patients obtained from an exercise prevention study [211]. Our RA and T2D cohorts were non-matched, and the CV risk factor distribution was significantly different. However, the sensitivity analysis for CIMT at follow-up showed that the FRS was not significant, which fits well with our models where no CVD
risk factors significantly impacted follow-up CIMT or CIMT progression. Thus, regardless of how CVD risk factors other than diabetes were modelled, they did not significantly influence CIMT progression. To account for the age and gender differences, a sensitivity analysis was undertaken where we compared data from 75 pairs of RA and T2DM matched for age and gender. This did not alter the finding that CIMT progression was higher in T2D than RA patients. It therefore seems unlikely that the mentioned differences in risk factors, age or gender have biased our findings.

Although no CVD risk factors significantly impacted follow-up CIMT or CIMT progression in multivariate models, we found that CIMT progression in T2D was associated with use of statins. Although this may seem counter-intuitive, statins are generally prescribed early in treatment of T2D according to local guidelines [216], and thus statin use is likely reflective of the hyperlipidaemia risk in T2D and perceived higher CV risk in RA patients [217]. Figure 2 illustrates that the relationship between CITM progression and statin use is similar in RA and T2D patients.

At follow-up, 13% of RA patients had had a CV event compared to 24% of T2D patients (p=0.09). Although this was not a statistically significant difference, the current study may have been underpowered to detect differences in CV events, given their low frequency.

3.10.2 Comparison of CIMT in RA and T2D patients
A higher follow-up CIMT was positively associated with higher baseline CIMT as expected. The impact of the higher baseline on follow-up CIMT was eliminated by alternatively modelling CIMT yearly rate of change. A previous study found that yearly rate of change was higher in the first 6 years after RA diagnosis [218]. Our failure to find a significant effect of disease duration may relate to a higher proportion of longstanding RA patients in the current than the previous study. By logistic regression of all patients in our study, progression of CIMT was associated with diabetes and ever use of a statin. While the same previous study found that in RA patients with disease duration of 6yrs or longer, the change in yearly CIMT of
patients on a statin was comparable to those of similar disease duration and not on a statin, the statin effect in the current study is likely to arise from the diabetes group and not from RA duration.

3.10.3 Effect of anti-rheumatic drugs on atherosclerosis progression

Almost all studies exploring CIMT in RA have reported progression in CIMT, however some studies have found RA patients using TNF inhibitors have a reduction or no progression in CIMT [218-221]. In the present study we did not have sufficient numbers of patients (n=5) on a TNF inhibitor to assess the impact of TNF inhibition on CIMT progression [218]. However we found CIMT progression was reduced with DMARD treatment at baseline. Previous studies have shown that the use of DMARDs can improve CV risk in RA patients by influencing traditional risk factors of atherosclerosis directly or through controlling inflammation [222-224]. Furthermore the DMARD hydroxchloroquine has shown cardio-protective properties by reducing LDL and increasing HDL cholesterol levels [225]. Prednisone use in our study had no significant effect on CIMT progression, although in other larger studies it is suggested that glucocorticoid use may promote atherosclerosis [226, 227]. A recent study found an association between glucocorticoid use and carotid plaque in patients with systemic lupus erythematosus (SLE) [228]. In RA, the glucocorticoid dose is not normally as high as in SLE and it is possible that lower doses of glucocorticoids either have an anti-atherogenic effect, given the inflammatory nature of atherosclerosis, or have no effect on CIMT [229]. CIMT and carotid plaques have been found to be predictors of future CV events in RA, with a 2.5 increased risk of CV complications among unilateral plaques and 4.3 among those with bilateral plaques [230].

3.10.4 Advantages of CIMT as a measure of CVD

In 2010, EULAR published 10 key recommendations regarding CV risk screening and management in RA. Early detection of CV risk factors and annual CV risk assessment using national guidelines was recommended [231]. CV risk screening and management are based on CV risk score calculators like FRS or the systemic coronary risk evaluation (SCORE)
However a recent study found that carotid ultrasonography was more sensitive than SCORE for the detection of subclinical atherosclerosis in RA.

The ASE defines CIMT as the combined thickness of the intima and medial layers of the far interior wall of the carotid artery. ASE has set guidelines for measuring CIMT and states that carotid ultrasound imaging should follow a scanning and reporting protocol, similar to that of the Atherosclerosis Risk in Communities Study. Of importance, both cohorts were examined at the same centre using the same techniques in this study, showing good reproducibility of CIMT measurements.

A recent study supports the use of CIMT in the assessment of CV risk in patients with RA. However, another study measuring internal carotid artery (ICA) IMT found an association of CVD risk with rate of change in ICA-IMT but not common carotid artery (CCA) IMT in RA, suggesting a need to evaluate both carotid arteries. The standard procedure of measuring CIMT at this hospital is at the CCA because of its size, superficial location, ease of accessibility and limited movement when identifying subclinical vascular disease and evaluating CVD risk. We did not examine plaque in this study.

3.11 Limitations
This study was exploratory and the sample size was small, and thus further studies are needed to determine the generalisability of our findings. Nevertheless, a large effect of diabetes was observed despite this limitation. Our study groups were not matched for CV risk factors. This is not possible when comparing RA to diabetes, because diabetes is an independent risk factor for CVD. However, RA patients are more likely to smoke, to be older and to be female. Thus, without a very large sample it would be very difficult to match all subjects for CV risk except RA. The advantages of our study are that the groups were recruited from the same hospital. They are from the same geographical area, similar financial background and have experienced being at a specialist outpatient clinic at this hospital. All CIMT measurement for both groups was completed by the same sonographers. We
were able to match 75 RA patients by age and gender to the T2DM and the findings using linear and logistic regression modelling were very similar to those reported above for the complete study cohort (data not shown).

3.12 Conclusion:
In conclusion, our data suggest that the risk of CIMT progression and CV events in RA patients is lower than in T2DM patients when comparing patients with similar baseline CIMT. A higher burden of CV risk factors in T2D (including diabetes itself) and early use of DMARDs in RA may contribute to risk reduction in RA. Future studies in larger cohorts, and comparing ICA-IMT and carotid plaque in RA and T2D would be of interest.

Table 3-1: Characteristics of individuals with Rheumatoid Arthritis (n=78) and individuals with T2DM (n=212)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Rheumatoid Arthritis Baseline (78)</th>
<th>T2DM (212) Follow-up (78)</th>
<th>P-value</th>
<th>Rheumatoid Arthritis Follow-up (78)</th>
<th>T2DM (212) Follow-up (78)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, (yrs)</td>
<td>61 (10)</td>
<td>66 (10)</td>
<td>0.001</td>
<td>52 (67%)</td>
<td>58 (10)</td>
<td>0.001</td>
</tr>
<tr>
<td>Sex, (female)</td>
<td>52 (67%)</td>
<td>98 (46%)</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interval between CIMT measurements</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease Duration</td>
<td>13.7 (4.5-23)</td>
<td>19.1 (9.9-28)</td>
<td>0.001</td>
<td>13.7 (4.5-23)</td>
<td>6.4 (3.4-12.4)</td>
<td>0.0005</td>
</tr>
<tr>
<td>Ever Smoker</td>
<td>53.0 (68%)</td>
<td>57.0 (73%)</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>27.0 (35%)</td>
<td>39.0 (51%)</td>
<td>0.056</td>
<td>27.0 (35%)</td>
<td>64.0 (30%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>35.0 (47%)</td>
<td>27.0 (35%)</td>
<td>0.094</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV events ∞</td>
<td>13 (16.7%)</td>
<td>10 (13.7%)</td>
<td>0.001</td>
<td>13 (16.7%)</td>
<td>30 (24.0%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Statins (mg)</td>
<td>15.0 (19%)</td>
<td>24.0 (31%)</td>
<td>0.001</td>
<td>15.0 (19%)</td>
<td>71.0 (34%)</td>
<td>0.66</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75 (17.0)</td>
<td>76 (17.0)</td>
<td>0.001</td>
<td>75 (17.0)</td>
<td>92 (19.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27 (6.0)</td>
<td>28 (8.0)</td>
<td>0.001</td>
<td>27 (6.0)</td>
<td>32 (6.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>132 (19.0)</td>
<td>126 (13.0)</td>
<td>0.44</td>
<td>132 (19.0)</td>
<td>139 (18.0)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Inter-quartile ranges, T2DM=type 2 diabetes mellitus, NA=not available, ∞ myocardial infarction, angina, stroke

*New CV events exclusive of baseline CV events
### Table 3-2: Characteristics of RA patients (n=78) at baseline and follow up and data on CV events in RA and T2DM

<table>
<thead>
<tr>
<th></th>
<th>RA baseline (78)</th>
<th>T2D at baseline (212)</th>
<th>P-Value</th>
<th>RA at Follow-up (78)</th>
<th>T2D at follow up (126)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Early RA</strong></td>
<td>19.0 (24%)</td>
<td>19.0 (12.0)</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RA duration (yrs)</strong></td>
<td>14.7 (12.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RF</strong></td>
<td>43 (58%)</td>
<td>47 (68%)</td>
<td>0.44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Positive ACCP</strong></td>
<td>47 (68%)</td>
<td>48 (71%)</td>
<td>0.82</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Positive SE alleles</strong></td>
<td>12 (43%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CRP</strong></td>
<td>7.5 (4.0-13.0)</td>
<td>6.0 (3.0-14.0)</td>
<td>0.37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ESR</strong></td>
<td>20.0 (10.0-37.0)</td>
<td>17.0 (12.0-26.0)</td>
<td>0.24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prednisone</td>
<td>34 (44%)</td>
<td>25 (33%)</td>
<td>0.088</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DMARDs</strong></td>
<td>64 (82%)</td>
<td>65 (83%)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Biologic</strong></td>
<td>3.0 (3.8%)</td>
<td>6 (7.7%)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DMARDs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CV Events</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>7 (9.0%)</td>
<td>8 (10.0%)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angina</td>
<td>9 (11.5%)</td>
<td>0 &lt;0.001</td>
<td></td>
<td>7 (9.0%)</td>
<td>10 (4.7%)</td>
<td>0.83</td>
</tr>
<tr>
<td>Myocardial</td>
<td>9 (11.5%)</td>
<td>0 &lt;0.001</td>
<td></td>
<td>3 (4.1%)</td>
<td>14 (6.6%)</td>
<td>0.49</td>
</tr>
<tr>
<td>Infarction</td>
<td>0</td>
<td>0</td>
<td></td>
<td>3 (3.8%)</td>
<td>13 (6.1%)</td>
<td>0.45</td>
</tr>
</tbody>
</table>

ACCP=anti-cyclic citrullinated protein, CRP=C-reactive protein, ESR=erythrocyte sedimentation rate, \(^2\) Inter-quartile ranges, \(^\dagger\) ever use of 1 or combination of disease modifying anti-rheumatic drug, \(\pi\) ever use of a biologic DMARD.

T2DM=Type2 diabetes mellitus, \(\pi\) analysis excluded 1 pt that had angina at baseline and follow-up, \(\sigma\) analysis excluded 5 patients that had MI at baseline and follow up. Some patients had more than one type of CV event.
Figure 3-1: Relationship between mean CIMT at follow up and baseline in T2DM and RA

- Solid black line – Relationship between mean CIMT at follow up and baseline in T2DM
- □ Broken Line – Relationship between mean CIMT at follow up and baseline in RA.
- The model was adjusted for sex, ever use of statin, smoking, and baseline triglycerides, total cholesterol/HDL cholesterol ratio, body mass index and hypertension.
- For the figure, age was set at the median, i.e. 57 years. Data were back-transformed to the original scale.
- Thin broken lines give 95% confidence intervals.
Figure 3-2: Yearly rate of change in carotid intima-media thickness in diabetes and rheumatoid arthritis patients

1: Diabetes patients, no statin use, 2: Diabetes patients, ever statin users, 3: Rheumatoid arthritis patients, no statin users, 4: Rheumatoid arthritis patients, ever statin users.

Differences between diabetes and RA patients: p<0.001. Differences within diabetes and RA diagnosis group: p=0.12 and p=0.82, respectively.
Relationship between mean CIMT at follow-up and baseline for patients without DMARD (disease-modifying anti-rheumatic drug) treatment at baseline (solid line) and patients with DMARD treatment at baseline (broken line). The model was adjusted for ever use of statin, ever use of prednisone and smoking. For the figure, age was set at the median, i.e. 62 years. Data were back-transformed to the original scale. Thin broken lines give 95 % confidence intervals. Overlap of confidence intervals cannot be used to assess significance in the multivariate model.

Transition to Chapter 4

Infectious diseases (e.g. HIV), neurological disorders (e.g. AD, HD and ALS), affective disorders (e.g. schizophrenia, depression and anxiety), autoimmune diseases (e.g. MS and rheumatoid arthritis), peripheral conditions (e.g. cardiovascular disease) and malignancy (e.g. haematological neoplasia and colorectal cancer) are reported to be associated with the up-regulation of the kynurenine pathway. Indoleamine 2,3 dioxygenase is the first enzyme along the kynurenine pathway, and is strongly stimulated by inflammatory molecules. The final chapter we analysed serum KYN from a cohort of RA patients in a 10 year follow-up study.
Chapter 4: Kynurenine and tryptophan concentrations and kynurenine/tryptophan ratio in rheumatoid arthritis patients: A 10 year follow-up study.

4.1 Background
Indoleamine 2,3 dioxygenase catalyzes the rate-limiting step of tryptophan degradation along the kynurenine pathway. The depletion of tryptophan and generation of kynurenines play a modulatory role in the immune response. An increase in indoleamine 2,3 dioxygenase activity determined by tryptophan /kynurenine ratio has been shown to be associated with infectious diseases (e.g. AIDS), neurological disorders (e.g. amyotrophic lateral sclerosis), affective disorders (e.g. depression, anxiety), autoimmune disease (e.g. multiple sclerosis, rheumatoid arthritis), peripheral conditions (e.g. cardiovascular disease) and malignancy (e.g. hematological neoplasias and colorectal cancer).

Aim
To measure serum kynurenine in baseline samples from a rheumatoid arthritis cohort with 10 year follow-up for cardiovascular events, and to investigate the relationship between kynurenine concentrations, malignancy, infection, cardiovascular disease, cause of death and disease activity in RA.

Methods
Baseline sera from 129 rheumatoid arthritis patients were analysed in 2013. These 129 patients derived from an original cohort of 231 rheumatoid arthritis patients recruited and followed since 2003 (baseline). Medical records of the 231 patients were reviewed in 2013 to determine cardiovascular risk factors, new cardiovascular events, duration of rheumatoid arthritis and rheumatological medications between 2003 and 2013. An additional 32 healthy control sera were collected in 2003.
Kynurenine and tryptophan concentration levels were measured in patient and control sera by high performance liquid chromatography (HPLC).

**Results**
Kynurenine concentrations at baseline were higher in the RA patients than the healthy controls. Serum kynurenine concentrations were not related to features of rheumatoid arthritis severity, or associated with baseline and previous cardiovascular disease, new cardiovascular disease during follow-up, or the two combined. Furthermore there was no association between kynurenine and malignancies or death. The only variable associated with death was pack-years smoking.

**Conclusion**
Although serum kynurenine concentrations are significantly higher in RA than in healthy controls, they were not associated with CVD, new malignancies or death.
4.2 Introduction

It is now well recognized that patients with rheumatoid arthritis (RA) have increased morbidity and mortality due to premature cardiovascular (CV) disease (CVD). There is a 1.5-fold increase in the standardized mortality ratio due to CV events in RA patients compared with the general population [235]. Up to 50% of this excess CV mortality results from ischemic heart disease (IHD), and this is closely followed by cerebrovascular disease [102, 236, 237]. Immune dysregulation and systemic inflammation are believed to be integral to the development of accelerated atherogenesis in RA patients [198, 238, 239].

Under normal conditions, regulatory T-cells (Treg) suppress immune activation including self-antigen-driven inflammation, thus inhibiting autoimmunity. Some but not all studies indicate that Treg function is impaired in RA, possibly due to the high concentrations of the pro-inflammatory cytokine tumour-necrosis factor (TNF) [240]. Patients with RA have increased numbers of pro-inflammatory Th17 cells [241, 242]. Some studies have shown reduced and others increased numbers of Treg cells in RA [243]. There is also evidence that Treg may develop into Th17 cells in autoimmune arthritis [244], also supporting the concept of immune dysregulation in RA.

Atherosclerosis is the result of chronic inflammation in the arterial walls. Treg have been shown to inhibit atherosclerosis by affecting lipoprotein metabolism. Depletion of Treg in mice led to a more atherogenic lipoprotein profile and increased atherogenesis [245]. Thus, Treg impairment in RA patients may be one mechanism underlying accelerated atherosclerosis. Kynurenine (KYN), a breakdown product of the amino acid tryptophan (TRP), has emerged as a molecule with immunomodulatory effects [246]. Three different enzymes catalyse the formation of KYN from TRP: Indoleamine 2,3-dioxygenases (IDO) 1 and 2 and TRP 2,3-dioxygenase (TDO). IDO1 can be found in macrophages, microglia, neurons and astrocytes [143, 144, 247]. IDO1 is upregulated by certain cytokines and
inflammatory molecules, particularly interferon gamma (IFN-γ) [247, 248].

Indoleamine 2,3 dioxygenase 2 (IDO2) is a recently-discovered enzyme and its encoding genes are located next to IDO1. IDO2 possess similar structure and enzymatic activities to IDO1, but differs in its expression pattern and signalling, and is preferentially inhibited by D-1methyl-TRP [139, 140].

TDO resides primarily in the liver and is induced by TRP or corticosteroids [141]. Several cytokines, including TNF, activate IDO to promote KYN formation. Through several pathways, KYN and its metabolites induce apoptosis of CD4+ Th1 T-cells and generation of Tregs, thereby regulating T-cell responses. A recent study demonstrated that development of collagen-induced arthritis was associated with increased IDO activity and enhanced tryptophan catabolism in mice. Blocking IDO with its inhibitor, 1-Methyl-Tryptophan, aggravated the severity of arthritis and enhanced immune responses. These findings suggest that IDO may play an important and novel role in the negative feedback of collagen-induced arthritis and possibly in the pathogenesis of rheumatoid arthritis [250].

In patients undergoing coronary angiography for suspected coronary artery disease, an increased KYN/TRP ratio in urine was strongly associated with major coronary events, acute myocardial infarctions, ischemic stroke, and all-cause mortality [149]. Similarly, an increased ratio was linked with more advanced atherosclerotic changes in patients on chronic haemodialysis due to kidney failure [150]. In these settings, KYN may be acting as a biomarker of inflammation. Taken together, these studies suggest that KYN may play various roles in the interactions between inflammation, immune dysregulation, CVD risk and possibly the risks of cancer and infections in RA patients. Here we hypothesized that increased serum KYN in RA patients is associated with risk of CVD, cancer, serious infections and increased severity of RA. Therefore our aim was to investigate the relationship between kynurenine concentrations, malignancy, infection, CVD, cause of death and disease activity in RA.
4.3 Materials and methods:

4.3.1 Study Population

We recruited two hundred and thirty one RA patients who met the American College of Rheumatology (ACR) 1987 revised criteria for the classification of RA and presented for a scheduled appointment over a 5-month period (July to November 2003) at a tertiary hospital rheumatology clinic. This cohort has been previously described [251]. This cohort was followed for 10 years within this hospital clinic setting. Thirty two adult healthy controls were recruited from another study and from laboratory staff from whom written consent was obtained.

4.3.2 Baseline data:

At baseline, CV risk factors and events were collected by completion of a self-administered questionnaire detailing CV history, risk factors, treatment, and details of RA, as well as laboratory data. Each patient was clinically evaluated, with chart review to confirm history. The data on high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), triglycerides, systolic and diastolic blood pressure, weight, body mass index (BMI), smoking and previous cardiovascular disease (CVD) were recorded and previously described [251]. The percentage risk of CV events over the next 5 years was estimated using the ‘CV disease risk calculator’ derived from the Framingham study [251]. Serum was available from 129 patients at baseline, of whom 16 had died. No clinical data were available from the healthy controls.

4.3.3 10-year follow-up data:

At follow-up, hospital records of all 231 participants were reviewed to determine new cardiovascular disease events, death, malignancy and infection information over last 10 years. Thirty one participants died during follow-up.

4.4 Measurement of Kynurenine

Blood samples were collected at baseline and aliquots of serum were stored at - 80⁰C until analysis. Serum concentrations of KYN and TRP were analyzed by high performance liquid chromatography as described [4].
Briefly 50 µl of serum were diluted with 50 µl of normal saline and then 10 µl of 150mM sodium acetate buffer pH 4.0 was added and left at room temperature for 2 minutes. 27 µl (1/4 volume) of 30% TCA was added and mixed immediately with vortex. Sample mixtures were incubated on ice for 5 minutes before spinning on the pre-cooled microfuge (4°C) with max speed (14,000 rpm). After spinning, supernatants were used for chromatography. Standards were prepared with both low (1 µM and 0.1 µM) and high (100 µM and 10 µM) concentration of TRP and KYN. KYN and TRP were detected with UV detector at 360 nm and 285 nm excitation respectively. The ratio of KYN/TRP concentrations was calculated. This study was approved by Metro South Human Research Ethics Committee and the Australian Health & Welfare, National Death Index registry Human Research Ethics Committee.

4.5 Statistical Analysis:
For analysis, we used SPSS and Stata (version 13.0), and the “party” program in R. Categorical data are presented as number and percentages and chi square tests were used to compare baseline and follow-up frequencies. Continuous data are presented as mean and standard deviation or median and inter-quartile range (IQR) or 95% confidence intervals as indicated. T-tests or Mann-Whitney U-tests were used for comparisons depending on data distribution. Multivariate robust linear regression was used to analyze RA concentrations. Transformation to normality was performed if necessary, and model fit was checked by residual plotting.

For analysis of dichotomous endpoints we used multivariate logistic regression modelling, and for analysis of death, multivariate Cox regression. P-values less than 0.05 were considered to indicate significant differences. The number of possible explanatory variables was high compared to the number of positive outcomes. Therefore, we first performed random forest analysis with bootstrapping (n=2000) for feature selection. The following variables were included in the random forest analysis: On the next step using logistic regression or survival analysis, only the variables with an
association to the outcome stronger than random variation in the random forest analysis were included [252].

**Results**

The original cohort consisted of 231 RA patients [251], of whom serum KYN concentration levels were measured in 129. The follow-up characteristics of the cohort are in Table 1. At the 10 year follow-up, this cohort had more smokers and a higher body mass index compared to baseline (described previously). These patients had a higher frequency of angina, myocardial infarction, stroke, diabetes, new and old CV events.

Table 4-1: Characteristics of RA patients

<table>
<thead>
<tr>
<th></th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Females</strong></td>
<td>80.0 (62.0%)</td>
</tr>
<tr>
<td><strong>Age (yrs)</strong></td>
<td>62.0 (13.0)</td>
</tr>
<tr>
<td><strong>Weight</strong></td>
<td>75.0 (20.0)</td>
</tr>
<tr>
<td><strong>Body Mass Index</strong></td>
<td>28.0 (7.0)</td>
</tr>
<tr>
<td><strong>Current smoker</strong></td>
<td>29.0 (23.0%)</td>
</tr>
<tr>
<td><strong>Duration of Rheumatoid Arthritis</strong></td>
<td>14.0 (13.0)</td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td>39.0 (30.0%)</td>
</tr>
<tr>
<td><strong>Hyperlipidemia</strong></td>
<td>33.0 (26.0%)</td>
</tr>
<tr>
<td><strong>Previous Cardiovascular Disease</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Angina</strong></td>
<td>9.0 (7.0%)</td>
</tr>
<tr>
<td><strong>Myocardial Infarction</strong></td>
<td>8.0 (6.0%)</td>
</tr>
<tr>
<td><strong>Stroke</strong></td>
<td>10.0 (8.0%)</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td>16.0 (12.0%)</td>
</tr>
<tr>
<td><strong>New Cardiovascular event</strong></td>
<td>21.0 (16.0%)</td>
</tr>
<tr>
<td><strong>Old Cardiovascular event</strong></td>
<td>9.0 (7.0%)</td>
</tr>
<tr>
<td><strong>Old and New Cardiovascular event</strong></td>
<td>6.0 (5.0%)</td>
</tr>
<tr>
<td><strong>Systolic Blood Pressure</strong></td>
<td>132.0 (19.0)</td>
</tr>
<tr>
<td>Test</td>
<td>Value</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td><strong>Diastolic Blood Pressure</strong></td>
<td>77.0 (11.0)</td>
</tr>
<tr>
<td><strong>Inflammatory markers</strong></td>
<td></td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate</td>
<td>26.0 (23.0)</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>20.0 (34.0)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>5.1 (1.1)</td>
</tr>
<tr>
<td>Triglycerides*</td>
<td>1.3 (1.0-1.9)</td>
</tr>
<tr>
<td>High Density Lipoprotein</td>
<td>1.5 (0.5)</td>
</tr>
<tr>
<td>Low Density Lipoprotein</td>
<td>3.0 (0.9)</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>57.0 (28.0)</td>
</tr>
<tr>
<td>Kynurenine</td>
<td>1.21 (0.58)</td>
</tr>
</tbody>
</table>

Numbers (%), Mean (SD), *Median (IQR).

KYN concentrations in RA patients (median 95% CI) 1.09 (0.99-1.14) µM were higher than in healthy controls 0.91 (0.76-1.00) µM, p=0.007 (fig 1). TRP concentrations in RA patients 48.5 (46.0-53.0) µM were also higher than in controls 42.0 (37.0-47.0) µM, p=0.024 (fig 2). This difference was due to a proportion of RA patients with higher KYN levels that the upper limit for controls (Figure 1). The ratio for KYN/TRP did not differ between RA patients and controls (p = 0.52, figure 3).

![Figure 4-1: KYN concentrations in RA patients and healthy controls](image-url)
Figure 4-2: TRP concentrations in RA patients and healthy controls

Figure 4-3: KYN/TRP ratio of RA patients and controls
4.7 KYN concentrations in RA patients with and without the CV and malignancy endpoints of the study

4.7.1 A) New CV event
Of the 129 patients with baseline KYN concentrations, 21 RA patients experienced a new CV event in the subsequent 10 years. Baseline KYN concentrations did not predict future CV events: in patients with an event, median KYN was 1.06 (0.83-1.32) µM and in RA patients with no new event 1.09 (0.97-1.17) µM, p=0.91. In RA patients with previous CVD, median KYN was 0.99 (0.85-1.26) µM and in those without previous CVD 1.1 (0.92-1.16) µM, p=0.72. Based on these findings, logistic regression analysis was not necessary – no adjustments could change the lack of association of KYN with previous CVD or new CV events.

![Figure 4-4: KYN concentrations in RA patients with and without a new CV event between 2003 and 2013.](image)
4.7.2 B) New Malignancy
In the 21 RA patients with a new malignancy at follow-up, the median baseline KYN concentration was 1.26 (0.84-1.46) µM and in RA patients without a new malignancy 1.06 (0.97-1.13) µM, p=0.54.

Figure 4.5: KYN concentrations in RA patients with and without a new malignancy between 2003 and 2013.

4.7.3 C) Death
In the 16 RA patients who died during follow-up, the median KYN concentration was 1.04 (0.881.15) µM and in the RA patients still alive 1.09 (0.97-1.23) µM, p=1.00. Two patients with a new malignancy died, and 3 patients with a new CV event died.

Figure 4.6: KYN concentrations in RA patients dying or alive between 2003 and 2013.
4.8 Causes of death for the RA patients with KYN data:
There were too few cases for a meaningful analysis of the relationship between KYN and cause of death.

Table 4-2: KYN concentrations at baseline by cause of death

<table>
<thead>
<tr>
<th>Death Cause</th>
<th>Frequency</th>
<th>Percent of deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVD</td>
<td>7</td>
<td>43.75</td>
</tr>
<tr>
<td>Cancer</td>
<td>3</td>
<td>18.75</td>
</tr>
<tr>
<td>Respiratory</td>
<td>1</td>
<td>6.25</td>
</tr>
<tr>
<td>Renal Disease</td>
<td>1</td>
<td>6.25</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1</td>
<td>6.25</td>
</tr>
<tr>
<td>Not classified yet</td>
<td>3</td>
<td>18.75</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>100</td>
</tr>
</tbody>
</table>

4.8.1 Other variables (than KYN) related to death
Random forest analysis was used to select possible baseline explanatory variables predicting death of the 129 patients. Only pack-years of smoking and average prednisone dose were selected for logistic regression; age and sex were included as biologically important variables (Table 3). Average prednisone dose was not significant in logistic regression (p=0.17), which left the best model:

Table 4-3: Other variables (than KYN) related to death

<table>
<thead>
<tr>
<th>Death</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.06</td>
<td>1.00-1.12</td>
<td>0.07</td>
</tr>
<tr>
<td>Sex</td>
<td>1.48</td>
<td>0.40-5.47</td>
<td>0.56</td>
</tr>
<tr>
<td>Pack-years smoking</td>
<td>1.03</td>
<td>1.00-1.05</td>
<td>0.02</td>
</tr>
</tbody>
</table>
When adjusting for age and sex, pack-years of smoking significantly predicted death within the next 10 years with an OR of 1.03 (1.00-1.05), p=0.02, or a 3% increase in likelihood of death per pack-year. KYN concentration was included in this model, and as expected, was not significant (p=0.97), confirming the findings described above.

A Cox regression model was run using deaths as an outcome. The best model was very similar to the logistic regression model, in that the only risk factor for death was pack-years of smoking.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.05</td>
<td>1.00-1.11</td>
<td>0.07</td>
</tr>
<tr>
<td>Sex</td>
<td>1.36</td>
<td>0.46-4.06</td>
<td>0.58</td>
</tr>
<tr>
<td>Pack-years smoking</td>
<td>1.02</td>
<td>1.00-1.04</td>
<td>0.00</td>
</tr>
<tr>
<td>KYN</td>
<td>0.99</td>
<td>0.37-2.64</td>
<td>0.98</td>
</tr>
</tbody>
</table>
4.9 Discussion

Although KYN has been linked to poor prognosis in cancer, renal disease and CVD, there is little known about the relationship between disease activity and KYN concentration levels in patients with RA. We found KYN to be a more sensitive marker to distinguish RA patients from the healthy controls than TRP or KYN/TRP ratio. The results of this study demonstrate higher KYN concentrations in RA patients compared to healthy controls, similar to results shown in previous studies [253-255]. Like Bett et al (1962), we found KYN levels to be unrelated to RA disease duration or disease activity [256]. In an earlier study plasma TRP concentration was lower in RA than controls, whilst the urinary excretion of the tryptophan metabolites, KYN, xanthurenic acid and 3-hydroxyanthralinic was increased [257]. More recently, concentrations of TRP were measured in sera of RA patients and found to be lower than for healthy controls, whilst KYN did not significantly differ from controls [255].

4.9.1 The relationship between KYN and CVD

We found no significant relationship in the RA cohort between KYN and either presence of CVD or new CV events. Previous studies have reported KYN imbalances (an increase in the rate of formation or a relative decrease in its rate of degradation) in CVD [258, 259]. Urine KYN was found to be a significant predictor of major cardiovascular events, all-cause mortality and CVD mortality, however there was no relationship between urine KYN and stroke or non-CVD mortality [149]. KYN/TRP ratio has been suggested to provide a more reliable measure of IDO activation than the absolute concentrations of KYN [260]. A previous study has demonstrated that elevated plasma KYN/TRP ratio levels are associated with increased risk of major coronary events (MCEs) and mortality in patients with stable CAD, participating in the Bergen Coronary Angiography Cohort (BECAC) [261]. These studies are substantially larger than our study, which may explain the difference in our results in that our study is underpowered.
4.9.2 Malignancy and KYN
There were higher levels of KYN observed in patients diagnosed with colon carcinoma, adenoma tubule villosum, or tubular adenoma than in those from the control group in a recent study. [262]. In another study, KYN/TRYP ratio but not KYN were increased in colorectal cancer patients (n=66) compared to non-cancer controls (n=37) [263].

4.9.3 Previous studies of predictors of mortality in RA.
Mortality is higher in RA patients when compared to the general population, this excess in mortality is predominantly due to coronary artery disease, cerebrovascular atherosclerosis and other cardiovascular complications including heart failure [264] [265, 266]. CVD associated mortality risk is increased in both men and women who are seropositive RA [43]. CVD associated death could be as much as 50% higher in RA patients compared to controls, with the risk of ischemic heart disease and cerebrovascular disease increased to a similar degree [235]. The enhanced vascular risk is not restricted to individuals with established RA, because increased mortality in patients who are positive for the RF and have early inflammatory polyarthritis has been reported [43, 76].

We found in a population health survey that CV risk is increased pre onset of RA. RF-positivity early in RA is a strong predictor of mortality. This was demonstrated in an inception cohort of 130 RA patients, where RF-positive RA was associated with a 6-fold increased risk of mortality compared to patients who were RF negative at baseline [267]. In another study, RF-positive patients excess mortality was found to be due to respiratory and cardiovascular diseases [268]. Chronic obstructive pulmonary disease was the most common cause of respiratory death in the RF-positive patients. Compared to the general population the RF-positive group had a 3-fold increased mortality rate [Standard Mortality Rate (SMR) 3.36 (95% CI 2.02, 5.24)]. SMR for ischemic heart disease (IHD) including myocardial infarction were elevated [SMR 1.41 (95% CI 1.04, 1.88)] for RF-positive patients, but not in those who were persistently RF-negative [268].
It is well documented that smoking is a risk factor for RF-positive RA [26, 269]. In Finland they found through a questionnaire that people who were RF-positive and had arthritis, had increased mortality from all causes [270]. After adjusting for age, gender and smoking status the increased mortality risk persisted. They also found that people who were RF-positive in the absence of arthritis had increased cardiovascular mortality, which remained significant after adjusting for age, gender and smoking status.

4.10 KYN concentration level and mortality
In the present study there were no differences in KYN at baseline between the 16 patients who died during follow-up or the patients that survived and there were too few cases for a meaningful analysis of KYN and cause of death. In logistic regression and Cox regression analysis, after adjusting for age and sex, pack-years of smoking was the only significant predictor of death in our cohort. A recent study “Epidemiology and Cost-effectiveness of Out-Of-Hospital Cardiac Arrest in Finland (FINNRESUSCI)” found KYN correlated with intensive care unit death, that KYN was significantly higher in patients who died compared to those that survived, and that KYN was higher in patients with poor outcomes compared with those with good outcomes [271]. Even though this cohort had twice the sample size (n=245) of our own RA cohort, it is unlikely that doubling the size of the RA cohort would have been able to show a relationship between KYN and mortality based on the small differences observed here.

4.11 Limitations
The major limitation of this study is the small sample size and very few endpoints. Based on our data, one would require a very large study to get enough statistical power to demonstrate an association between KYN and RA complications. In the healthy controls, no clinical characteristics were available. Thus we could not compare them further to the RA cohort. There was a high probability that the healthy controls were much younger than the RA patients. In the present study KYN in serum has a very narrow concentration range, therefore this test has low capacity for questions of prediction.
4.12 Conclusion
Median KYN concentrations were significantly higher in RA patients than in healthy controls, but there was a large overlap between groups. There were no variables in our data set that could explain the differences in KYN concentrations among the RA patients. KYN concentrations were not related to previous or new CVD, new malignancies or death. The only variable predicting death in our 10 year follow-up of 129 RA patients in logistic or Cox regression was pack-years of smoking.
Chapter 5: Final Discussion

Most of the work presented in this thesis has already been discussed in the individual chapters (2, 3 and 4). The main aim of this chapter is to cohesively bring all the findings together to highlight an overall conclusion of the thesis, the impact of the findings and how they may lead to future research.

In this thesis, I report that CVD and CVD risk factors are significant predictors of RA, that the increase in CIMT is similar in patients with RA and T2D, and that KYN concentration levels are elevated in a subset of RA patients compared to healthy controls but are not associated with risk of death or CVD.

The most important pathophysiologic mechanism of CV risk in RA is the relationship between systemic inflammation and atherosclerosis. There is substantial evidence that there are influences beyond traditional risk factors that link RA and CVD and this has led to the notion that RA patients carry additional risk factors for CVD. I propose that inflammation and disease activity are the fundamental links for the 3 studies.

In chapter 2 I reported an association of previous CV events with the development of RA in individuals exposed to common RA and CV risk factors. The increase in CVD and CV risk factors also occurs in patients with established RA and at those at disease onset. My results concur with previous studies showing that female gender, increasing age, increasing BMI and smoking were risk factors for incident RA. The obvious caveat of this study is we have relied mainly on self-reported information to detect the presence of RA. The positive predictive value of self-reported RA has been studied in various populations including the elderly and disabled, and was found to be low, ranging between 21% and 34%. (3).
It is well documented that patients with RA and T2D develop more CV events than the general population, as reflected in CIMT measurements. However it is not clear how rapidly CVD progresses in RA relative to T2D, which has a similar increased CV risk. We demonstrated (chapter 3) that RA patients had similar baseline CIMT to T2D patients. Previous studies have shown that DMARDs can improve CV risk in RA patients, by influencing traditional risk factors of atherosclerosis directly or by controlling inflammation. In our RA cohort we reported that DMARD use at baseline was associated with significantly lower CIMT values at follow-up than in the T2D group at follow-up. The limitations for this study are 1. The small sample size, and 2. The cohorts were not fully matched. In a sensitivity analysis however, we were able to match 75 pairs and the results were similar.

Previous studies have found that the enzyme KYN correlated with intensive care unit death, and that KYN was significantly higher in patients who died compared to those that survived. Furthermore, KYN was higher in patients with poor outcomes compared with those with good outcomes (4). We did not find similar results in our cohort of RA patients. We did not undertake a preliminary power calculation, as the study was exploratory. However, the effect size of elevated KYN was small and we determined that we would require a very large study to get enough statistical power to demonstrate an association between KYN and RA complications. In the healthy controls, no clinical characteristics were available, thus we could not compare them further to the RA cohort. There was a high probability that the healthy controls were much younger than the RA patients. In the present study KYN in serum has a very narrow concentration range, therefore this test has low capacity for questions of prediction.

**Future research**

Further studies addressing predictors for cardiovascular disease are needed. When evaluating the extent of CV risk in RA, identifying markers of inflammation and disease activity might be useful. Given that previous CV events were associated with the development of RA, future studies should focus on the development of effective screening methods to identify patients
with high cardiovascular risk and who would benefit from close monitoring for early signs of RA requiring intervention, as well as management of CV risk factors. There should be clear guidelines for the management of CV risk factors in RA, similar to guidelines developed for diabetes.

From the data presented, it is unlikely that CIMT is a useful outcome measurement for following CVD in longitudinal studies in RA. Further outcome studies comparing CV events in RA and T2D patients would be more useful. However these would need to be of long duration and large size.

Taken all together, these results emphasize the complexity of the associations between inflammation, anti-rheumatic therapies, atherosclerotic burden and CVD in RA.

The main contributions to the field of RA and CVD research within this thesis were

- Demonstrating that CVD precedes the onset of RA
- Comparing CIMT in T2D and RA; T2D has a higher CIMT progression compared to RA
- Measuring KYN concentrations in RA found levels were higher for RA than for healthy controls.

To effectively prevent cardiovascular events in patients with RA, we must identify RA disease characteristics which signal an increased risk of CVD. Identification of patients at high risk will support an effective individualized strategy of targeted therapy. Our studies indicate that patients with active RA disease qualify for intensified action in order to prevent future CV events.
References


63. Dixey, J., et al., Is it possible to predict radiological damage in early rheumatoid arthritis (RA)? A report on the occurrence, progression, and prognostic factors of radiological erosions over the first 3 years in 866 patients from the Early RA Study (ERAS). J Rheumatol Suppl, 2004. 69: p. 48-54.


Appendices
Cardiovascular disease is increased prior to onset of rheumatoid arthritis but not osteoarthritis: the population-based Nord-Trøndelag health study (HUNT)

Helen Pahau1, Matthew A Brown1, Sanjoy Paul2,5†, Ranjeny Thomas1†* and Vibeke Videm3,4†

Abstract

Introduction: Patients with rheumatoid arthritis (RA) have increased risk of cardiovascular (CV) events. We sought to test the hypothesis that due to increased inflammation, CV disease and risk factors are associated with increased risk of future RA development.

Methods: The population-based Nord-Trøndelag health surveys (HUNT) were conducted among the entire adult population of Nord-Trøndelag, Norway. All inhabitants 20 years or older were invited, and information was collected through comprehensive questionnaires, a clinical examination, and blood samples. In a cohort design, data from HUNT2 (1995–1997, baseline) and HUNT3 (2006–2008, follow-up) were obtained to study participants with RA (n = 786) or osteoarthritis (n = 3,586) at HUNT3 alone, in comparison with individuals without RA or osteoarthritis at both times (n = 33,567).

Results: Female gender, age, smoking, body mass index, and history of previous CV disease were associated with self-reported incident RA (previous CV disease: odds ratio 1.52 (95% confidence interval 1.11-2.07)). The findings regarding previous CV disease were confirmed in sensitivity analyses excluding participants with psoriasis (odds ratio (OR) 1.70 (1.23-2.36)) or restricting the analysis to cases with a hospital diagnosis of RA (OR 1.90 (1.10-3.27)) or carriers of the shared epitope (OR 1.76 (1.13-2.74)). History of previous CV disease was not associated with increased risk of osteoarthritis (OR 1.04 (0.86-1.27)).

Conclusion: A history of previous CV disease was associated with increased risk of incident RA but not osteoarthritis.
myocardial degeneration more frequently than controls after excluding individuals with other known causes [9].

Inflammation plays a central role in the pathogenesis of atherosclerosis, and the increase in CV disease and mortality in RA may partly be explained by inflammatory factors associated with RA, even after adjustment for traditional CV risk factors [10,11]. We and others showed that atherosclerosis is increased both in patients with established RA and at presentation in patients with recent-onset RA, as determined by increased carotid intima media thickness and plaque, and is associated with their inflammatory burden [12,13]. The risk for myocardial infarction (MI) and possible CV death is already increased within approximately 5 years after diagnosis of RA depending upon age and presence of CV risk factors, resulting in a 10-year absolute risk comparable to non-RA individuals who were 5 to 10 years older [14].

It has been demonstrated that inflammation pre-dates the onset of RA [15]. These data suggest that an increased risk of CV disease might also precede the onset of RA. The Rochester Epidemiology Project study demonstrated that RA patients were more likely to have been hospitalized because of MI prior to RA diagnosis [16]. However, a previous longitudinal cohort study found no difference in the rate of MI, congestive heart failure or angina between pre-RA and control individuals [17].

We hypothesized that CV risk factors and events are more prominent in persons with incident RA, and that this augments the risk of future RA development by increasing inflammation. The aim of the study was therefore to investigate the effects of CV risk factors and CV events on the development of RA, using a population-based cohort study design. As a control to study whether any findings were specific to RA or related to arthritis in general, a parallel investigation was performed in participants with and without osteoarthritis.

Methods

The study participants were from the Nord-Trøndelag Health Study (HUNT) population-based health surveys conducted in the county of Nord-Trøndelag in Norway. The county is fairly representative for Norway as a whole, with a stable and ethnically homogenous population (3% non-Caucasians). All inhabitants 20 years or older were invited, and information was collected through comprehensive questionnaires and a clinical examination. The HUNT2 survey has previously been described in detail [18]. The HUNT3 survey had a similar design. In total, about 75,000 (70% of those invited) participated in HUNT2 (1995 to 1997), 51,000 (54% of those invited) participated in HUNT3 (2006 to 2008), and 37,071 participated in both HUNT2 and HUNT3. By design, the participants were not seen during the years between inclusion in HUNT2 and HUNT3.

The study cohort consisted of all participants in both HUNT2 and HUNT3 who answered whether they had a diagnosis of RA or not (n = 36,493, that is, 98.4% of 37,071) and this number determined the study size. Incident cases of RA were identified, that is, participants who reported a diagnosis of RA in HUNT3 but not in HUNT2. We also identified the incident cases of osteoarthritis for comparison, based on the question: “Has a doctor ever said that you have/have had any of these diseases: degenerative joint disease (osteoarthritis)?” The question also included the Norwegian colloquial term for osteoarthritis. Each patient group was compared to the remaining participants of the study cohort.

Participants of HUNT gave informed consent. Approval for the study was obtained from the Regional Committee on Medical Research Ethics, Central Norway, the Norwegian Data Safety Authorities and the Norwegian Department of Health. Ethics approval was also obtained by the Metro South Ethics Committee Brisbane. Permission was granted from the two primary hospitals in Nord-Trondelag, Levanger and Namsos hospitals, and the nearest secondary referral hospital, Trondheim University Hospital, to link the identified RA cases in our study to the hospital diagnosis registries for verification of diagnosis. The registered diagnoses are used for billing and reimbursement from the national insurance scheme. We searched for the ICD-9 code 714 and ICD-10 codes M05 and M06 with sub-codes. We did not have permission to access to the patients’ case notes in order to check that the current criteria for RA were correctly employed.

On enrolment in HUNT2 and HUNT3 the participants completed a questionnaire incorporating information on medical history, smoking habits and family history of CV disease, defined as a parent, sibling or child with previous MI or stroke. Information on the use of lipid-lowering medications or non-steroid anti-inflammatory drugs was not available. Anthropometric and clinical measures included height, weight, waist and hip circumference, and blood pressure. Non-fasting blood samples were drawn and total cholesterol, low-density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides and serum glucose were measured using an autoanalyzer (Hitachi Biocore Systems, Thornhill, ON, Canada). Using DNA isolated at HUNT Biobank, participants with self-reported RA were genotyped for the Shared Epitope [19]. Samples were genotyped using the Illumina Immunochip microarray chip, and HLA-DRB1 genotypes then determined by imputation using the program HLA-IMP [20,21]. Autoantibodies (anti-citrullinated peptide antibodies or ACPA, rheumatoid factor) and C-reactive protein were not measured in this survey, and classification into seropositive or seronegative RA was not possible. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or use of medication. Hypercholesterolemia
was defined as total serum cholesterol >6.2 mmol/L. Body mass index (BMI) was calculated as weight/height² (kg/m²). Patients who reported using over-the-counter analgesics daily for one month or more during the last year before inclusion in HUNT2 were recorded as users of analgesics. Previous CV disease was defined as a composite of angina, MI or stroke. The relevant questions were: “Have you had or do you have angina pectoris?” “Have you had a stroke/brain hemorrhage?” and “Have you had a myocardial infarction?” The question about angina also included a Norwegian colloquial term for this diagnosis. The composite variable was used for previous CV disease as numbers of cases were too low for analysis of individual disease events. The level of missingness at baseline for most key variables was <1%, with the exception of smoking (12.8% missing data). Missingness for smoking was evenly distributed among the subgroups of the study cohort. Non-complete cases were omitted from analysis.

Statistical methods

Data are presented as number (percentage), mean (standard deviation) or median (interquartile range), as appropriate. The chi-square test and the Mann-Whitney U-test were used for between-group comparisons of categorical and continuous study parameters, respectively. Risk factors associated with the development of RA and osteoarthritis between HUNT2 and HUNT3 were identified using multivariate logistic regression models. Linearity of logits for continuous variables was checked by plotting. P-values <0.05 were considered significant. Pearson's correlation coefficient R was calculated to evaluate linear correlation.

Since this was a population-based survey and RA was self-reported, we performed several sensitivity analyses to support our main analysis. Because previous studies have indicated that few women with RA surveyed in the community report their diagnosis accurately [22], we repeated the analysis in several subgroups of the incident RA cases using additional criteria to remove false-positive diagnoses, that is, 1) excluding all who also reported having psoriasis (n = 178), 2) including only patients where the diagnostic registries of the nearest hospitals showed a diagnosis of RA (n = 216), 3) restricting the hospital-diagnosed cases to those where the diagnosis first occurred after 1999 or 4) after 2001, to avoid including patients who forgot to report RA in HUNT2, or 5) including only incident RA cases who carried the shared epitope (with and without additional exclusion due to a report of psoriasis). To represent the CV risk factors in another way, we developed alternative models including the Framingham risk score, which is based on age, serum cholesterol, hypertension, smoking and diabetes [23], as well as gender and history of CV disease. The Framingham risk score was preferred because it assigns higher risk with diabetes and there are no age limits, and because it may easily be calculated in large cohorts. The European HeartScore [24] is based on risk for patients between 40 and 65 years and requires separate input for each individual using charts or an online calculator, which was not practical in our large cohort. To verify that the Framingham risk score was applicable in our cohort, we calculated the PC-based HeartScore for 50 randomly selected incident RA cases and 50 controls. Parallel Framingham risk scores and HeartScores were used to calculate an equation permitting estimation of the HeartScore in the entire cohort from their Framingham risk scores. An alternative logistic model was developed substituting the Framingham risk score with these estimated HeartScores. In addition, we compared factors associated with incident cases of self-reported RA with incident cases of self-reported osteoarthritis.

Results

The baseline characteristics of the participants are presented in Table 1. At baseline (1995 to 1997) 33,567 participants reported not having RA, and of this group 786 (2.34%) reported RA at follow-up (2006 to 2008). This corresponds to an average annual incidence of 0.21% in women and 0.16% in men, respectively. If restricted to participants with cases identified in the local hospital registries (n = 216), the annual incidence was 0.06% in women and 0.04% in men. As expected, incident cases of RA were older and more of them were current or previous smokers and had hypertension at baseline. Incident cases of RA had significantly elevated metabolic risk factors including blood pressure, BMI, serum cholesterol and triglyceride. The estimated median (interquartile range) Framingham risk scores at baseline were 11 (7, 16) and 8 (4, 13), respectively, in incident cases and those who did not develop RA. Notably, a higher proportion of incident RA cases had a history of CV disease at baseline.

Female gender, age, smoking, BMI and previous CV disease were more prominent in those developing RA (odds ratio 1.52 (1.11 to 2.07), Table 2, I - Main model). There was minimal change in the odds ratio for previous CV disease with inclusion of use of analgesics in this logistic regression model, when systolic and diastolic blood pressure were included as continuous variables instead of the categorical variable for hypertension, with additional adjustment for total cholesterol and HDL-cholesterol concentrations, or when a variable for physical activity (low, moderate, high) was also included (data not shown). In all the alternative multivariate models for incident RA where the number of patients was restricted to better characterized subgroups, previous CV disease was significant and the odds ratios were higher than for the main model including all self-reported cases (Table 2,
A diagnosis of RA was confirmed in the hospital registries for 216 participants (27%) who had self-reported a new RA diagnosis in HUNT3. The characteristics of these participants were very similar to those reported for the entire group of incident RA cases (data not shown). In the 216 cases with a hospital diagnosis of RA, the diagnosis was found one to two years following enrolment in HUNT2 for 14 cases (6.5%), after three years for 24 cases (11.1%), after four years for 15 cases (6.9%), after five years for 28 cases (13.0%) and after six years for 22 cases (10.2%). For the remaining 113 cases (52.3%), the times were distributed from seven years onwards to the inclusion in HUNT3 with numbers varying unsystematically from 15 to 24 cases (6.9 to 11.1%) per year. Thus, there was no obvious pattern regarding the number of years from HUNT2.

In a separate alternative multivariate logistic regression model containing gender, previous CV disease and the Framingham risk score as covariates, the odds ratio for developing RA in subjects with previous CV disease was 1.65 (1.21 to 2.24) \( (P < 0.01) \). This model also suggested the likelihood of developing RA was 6% greater with each one unit increase in Framingham risk score (odds ratio: 1.06, 95% CI: 1.05 to 1.08), \( P < 0.001 \). The model did not change with adjustment for BMI or daily use of over-the-counter analgesics. In the model where the Framingham risk score was substituted with the estimated HeartScore, previous cardiovascular disease remained significant (odds ratio: 1.70 (1.25 to 2.32), \( P < 0.001 \)). In the 100 participants where the HeartScore was calculated using the PC-based calculator, the HeartScore (logarithmically transformed) was highly correlated with the Framingham risk score.

Table 1 Characteristics of individuals without rheumatoid arthritis (RA) or osteoarthritis (OA) at baseline

<table>
<thead>
<tr>
<th>All participants without RA/OA</th>
<th>Later developed RA</th>
<th>Did not develop RA</th>
<th>P-value</th>
<th>Later developed OA</th>
<th>Did not develop OA</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>33,567</td>
<td>786</td>
<td>32,781</td>
<td>3,586</td>
<td>29,981</td>
<td></td>
</tr>
<tr>
<td>Age(^{a}) (years)</td>
<td>46 (13)</td>
<td>51 (13)</td>
<td>46 (13)</td>
<td>**</td>
<td>52 (10)</td>
<td>45 (13)</td>
</tr>
<tr>
<td>Sex (female)</td>
<td>18,207 (54.2%)</td>
<td>488 (62.1%)</td>
<td>17719 (54.1%)</td>
<td>**</td>
<td>2,406 (67.1%)</td>
<td>15,801 (52.7%)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>8,901 (26.5%)</td>
<td>256 (32.6%)</td>
<td>8,645 (26.4%)</td>
<td>1,015 (28.3%)</td>
<td>7,886 (26.3%)</td>
<td></td>
</tr>
<tr>
<td>Former smoker</td>
<td>7,098 (23.6%)</td>
<td>212 (27.0%)</td>
<td>7,696 (23.5%)</td>
<td>945 (26.4%)</td>
<td>6,963 (23.2%)</td>
<td></td>
</tr>
<tr>
<td>Never smoker</td>
<td>15,017 (44.7%)</td>
<td>281 (35.6%)</td>
<td>14,736 (45.0%)</td>
<td>1,425 (39.7%)</td>
<td>13,592 (45.3%)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>12,186 (36.3%)</td>
<td>339 (43.1%)</td>
<td>11,847 (36.1%)</td>
<td>**</td>
<td>1,547 (43.1%)</td>
<td>10,639 (35.5%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>454 (1.4%)</td>
<td>19 (2.4%)</td>
<td>435 (1.3%)</td>
<td>*</td>
<td>61 (1.7%)</td>
<td>393 (1.3%)</td>
</tr>
<tr>
<td>Previous CV disease</td>
<td>1,060 (3.2%)</td>
<td>52 (6.6%)</td>
<td>1,008 (3.1%)</td>
<td>**</td>
<td>158 (4.4%)</td>
<td>902 (3.0%)</td>
</tr>
<tr>
<td>Angina</td>
<td>673 (2.0%)</td>
<td>35 (4.5%)</td>
<td>638 (1.9%)</td>
<td>**</td>
<td>107 (3.0%)</td>
<td>566 (1.9%)</td>
</tr>
<tr>
<td>MI</td>
<td>441 (1.3%)</td>
<td>16 (2.0%)</td>
<td>425 (1.3%)</td>
<td>49 (1.4%)</td>
<td>392 (1.3%)</td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td>223 (0.7%)</td>
<td>9 (1.1%)</td>
<td>214 (0.7%)</td>
<td>**</td>
<td>32 (0.9%)</td>
<td>191 (0.6%)</td>
</tr>
<tr>
<td>Family risk of CV disease</td>
<td>15,692 (46.7%)</td>
<td>414 (52.7%)</td>
<td>15,278 (46.6%)</td>
<td>**</td>
<td>2,017 (56.2%)</td>
<td>13,675 (45.6%)</td>
</tr>
<tr>
<td>Framingham risk score(^{a})</td>
<td>9 (4 to 13)</td>
<td>11 (7 to 16)</td>
<td>8 (4 to 13)</td>
<td>*</td>
<td>11 (7 to 15)</td>
<td>8 (3 to 13)</td>
</tr>
<tr>
<td>Weight(^{1}) (kg)</td>
<td>76 (14)</td>
<td>77 (14)</td>
<td>76 (14)</td>
<td>**</td>
<td>77 (13)</td>
<td>76 (14)</td>
</tr>
<tr>
<td>BMI(^{2}) (kg/m(^{2}))</td>
<td>25.6 (23.5 to 28.1)</td>
<td>26.3 (24.3 to 29.0)</td>
<td>25.6 (23.4 to 28.1)</td>
<td>**</td>
<td>26.4 (24.2 to 29.0)</td>
<td>25.4 (23.3 to 27.8)</td>
</tr>
<tr>
<td>Waist circumference(^{1}) (cm)</td>
<td>85 (11)</td>
<td>86 (11)</td>
<td>85 (11)</td>
<td>*</td>
<td>86 (11)</td>
<td>85 (11)</td>
</tr>
<tr>
<td>Hip circumference(^{1}) (cm)</td>
<td>102 (8)</td>
<td>103 (8)</td>
<td>102 (8)</td>
<td>**</td>
<td>103 (8)</td>
<td>101 (6)</td>
</tr>
<tr>
<td>Waist/hip ratio(^{1})</td>
<td>0.84 (0.08)</td>
<td>0.84 (0.08)</td>
<td>0.84 (0.08)</td>
<td>0.83 (0.07)</td>
<td>0.84 (0.08)</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure(^{2}) (mmHg)</td>
<td>131 (120 to 144)</td>
<td>133 (122 to 146)</td>
<td>131 (120 to 144)</td>
<td>*</td>
<td>133 (122 to 147)</td>
<td>131 (120 to 143)</td>
</tr>
<tr>
<td>Diastolic blood pressure(^{1}) (mmHg)</td>
<td>79 (11)</td>
<td>80 (11)</td>
<td>79 (11)</td>
<td>*</td>
<td>81 (11)</td>
<td>79 (11)</td>
</tr>
<tr>
<td>Serum cholesterol(^{1}) (mmol/L)</td>
<td>5.8 (1.2)</td>
<td>6.0 (1.2)</td>
<td>5.8 (1.2)</td>
<td>**</td>
<td>6.1 (1.2)</td>
<td>5.8 (1.2)</td>
</tr>
<tr>
<td>HDL cholesterol(^{1}) (mmol/L)</td>
<td>1.40 (0.39)</td>
<td>1.37 (0.39)</td>
<td>1.40 (0.39)</td>
<td>1.44 (0.40)</td>
<td>1.39 (0.38)</td>
<td></td>
</tr>
<tr>
<td>Serum triglycerides(^{2}) (mmol/L)</td>
<td>1.40 (0.98 to 2.07)</td>
<td>1.51 (1.10 to 2.24)</td>
<td>1.40 (0.97 to 2.06)</td>
<td>**</td>
<td>1.46 (1.02 to 2.12)</td>
<td>1.37 (0.95 to 2.04)</td>
</tr>
</tbody>
</table>

\(^{a}\)Values are mean (standard deviation) or numbers (%), or \(^{2}\)median (interquartile range). Non-respondents were excluded. BMI, body mass index; CVD, cardiovascular disease; HDL, high density lipoprotein; MI, myocardial infarction; OA, osteoarthritis; RA, rheumatoid arthritis. \(^{1}\)P-value <0.05, \(^{2}\)P-value <0.001, comparing individuals who developed RA and did not develop RA, or comparing individuals who developed OA and did not develop OA. All blood samples were non-fasting.

II – Alternative models A to F). A diagnosis of RA was confirmed in the hospital registries for 216 participants (27%) who had self-reported a new RA diagnosis in HUNT3. The characteristics of these participants were very similar to those reported for the entire group of incident RA cases (data not shown). In the 216 cases with a hospital diagnosis of RA, the diagnosis was found one to two years following enrolment in HUNT2 for 14 cases (6.5%), after three years for 24 cases (11.1%), after four years for 15 cases (6.9%), after five years for 28 cases (13.0%) and after six years for 22 cases (10.2%). For the remaining 113 cases (52.3%), the times were distributed from seven years onwards to the inclusion in HUNT3 with numbers varying unsystematically from 15 to 24 cases (6.9 to 11.1%) per year. Thus, there was no obvious pattern regarding the number of years from HUNT2.
risk score (R = 0.86, P <0.001). The correlation was very similar in patients (R = 0.89) and controls (R = 0.86).

At follow-up (2006 to 2008), 3,586 participants had developed osteoarthritis, corresponding to an average annual incidence of 1.05% in women and 0.64% in men, respectively. These patients were older and the proportion of smokers, former smokers and of those with hypertension was higher than in the group that did not develop osteoarthritis. Systolic and diastolic blood pressure, BMI, hip and waist circumference, total cholesterol and triglycerides were higher for incident cases of osteoarthritis, whereas HDL cholesterol was lower in incident cases of osteoarthritis. Female gender, age and smoking were associated with development of osteoarthritis, whereas previous CV disease was not (Table 2 III).

**Discussion**

In the present large population-based health study we found that participants who developed RA between HUNT2 and HUNT3 had more CV disease and CV risk factors prior to the onset of RA (at HUNT2) compared to those without incident RA. A history of CV disease was a significant risk factor for incident RA, as well as the previously reported risk factors: female gender, increasing age, increasing BMI and smoking [25]. The finding of a positive association with the Framingham

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**Table 2 Effects of risk factors on incident RA and osteoarthritis, by multivariate logistic regression**

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I - Main model – Rheumatoid arthritis (n = 739 patients and 30,829 controls)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female gender</td>
<td>1.50</td>
<td>1.29 to 1.75</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age</td>
<td>1.03</td>
<td>1.02 to 1.04</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Current smoker</td>
<td>1.64</td>
<td>1.38 to 1.96</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Former smoker</td>
<td>1.32</td>
<td>1.10 to 1.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.97</td>
<td>0.82 to 1.15</td>
<td>0.76</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.34</td>
<td>0.83 to 2.18</td>
<td>0.23</td>
</tr>
<tr>
<td>Body mass index</td>
<td>1.04</td>
<td>1.02 to 1.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Previous CV disease</td>
<td>1.52</td>
<td>1.11 to 2.07</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>II - Alternative models (sensitivity analyses) - Rheumatoid arthritis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A – After exclusion of patients with self-reported psoriasis (n = 573 patients and 30,829 controls)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous CV disease</td>
<td>1.70</td>
<td>1.23 to 2.36</td>
<td>0.001</td>
</tr>
<tr>
<td>B – Including patients with hospital diagnosis of RA (n = 201 patients and 30,829 controls)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous CV disease</td>
<td>1.90</td>
<td>1.10 to 3.27</td>
<td>0.02</td>
</tr>
<tr>
<td>C – Including patients with hospital diagnosis of RA after 1999 (n = 178 patients and 30,829 controls)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous CV disease</td>
<td>2.26</td>
<td></td>
<td>1.26 to 3.99</td>
</tr>
<tr>
<td>D – Including patients with hospital diagnosis of RA after 2001 (n = 138 patients and 30,829 controls)</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Previous CV disease</td>
<td>2.51</td>
<td>1.33 to 4.73</td>
<td></td>
</tr>
<tr>
<td>E – Including patients carrying the Shared Epitope (n = 313 patients and 30,829 controls)</td>
<td></td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>Previous CV disease</td>
<td>1.76</td>
<td>1.13 to 2.74</td>
<td></td>
</tr>
<tr>
<td>F – Including patients carrying the Shared Epitope and excluding patients with self-reported psoriasis (n = 257 patients and 30,829 controls)</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Previous CV disease</td>
<td>1.96</td>
<td>1.24 to 3.10</td>
<td></td>
</tr>
<tr>
<td><strong>III - Model for osteoarthritis (n = 3,364 patients and 24,631 controls)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female gender</td>
<td>2.36</td>
<td>2.15 to 2.56</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age</td>
<td>1.05</td>
<td>1.05 to 1.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Current smoker</td>
<td>1.42</td>
<td>1.30 to 1.56</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Former smoker</td>
<td>1.23</td>
<td>1.12 to 1.35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.90</td>
<td>0.66 to 1.22</td>
<td>0.49</td>
</tr>
<tr>
<td>Body mass index</td>
<td>1.06</td>
<td>1.05 to 1.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Previous CV disease</td>
<td>1.04</td>
<td>0.86 to 1.27</td>
<td>0.66</td>
</tr>
</tbody>
</table>

1Cases with complete data.
2Continuous variable, Odds ratio per one unit change.
3Multivariate model including the same variables as the main model for RA. CV, cardiovascular; RA, rheumatoid arthritis.
risk score may be related to smoking and age being incorporated into the score. The supplementary analysis showed that the Framingham score was equivalent to the estimated HeartScore in our population even though the Framingham score was not developed from European subjects. On the other hand, previous CV disease events did not contribute to the risk of future osteoarthritis in our study, indicating that the finding may be specific to RA. Smoking was associated with incident osteoarthritis.

**CV disease and incident RA**

The design of our study has several strengths. Since the data on the CV risk factors and events were collected at HUNT2, recall bias at HUNT3 was greatly reduced compared to a design where patients report on these factors when receiving an RA diagnosis at a hospital-based clinic. Furthermore, the observation time between HUNT2 and HUNT3 was approximately 10 years. The importance of a long observation time is underscored by the finding that the odds ratio for the association of incident RA with previous CV disease increased when patients receiving a hospital diagnosis of RA during the first years following HUNT2 were excluded. It also seems biologically plausible that longer exposure to increased inflammation due to CV disease further increases the risk of incident RA. Furthermore, the statistical power is greatly improved in a population-based cohort study due to the large number of controls, and problems defining a relevant control group as seen with case-control studies of RA are avoided.

The major limitation of our study is that data were self-reported. Previous studies suggest that self-reported CV diagnosis and risk factors, such as hypertension and cigarette smoking, are reliable [26,27]. However, the high incidence rate of self-reported RA in our study compared, for example, to recent Swedish data based on inpatient and non-primary outpatient care (0.056% in women, 0.025% in men) [28], confirms that there probably were many false-positive RA diagnoses. This would tend to decrease the power to detect true positive results in our study, but not bias towards false-positive findings. When including only cases identified in the hospital registries, the incidence was close to the Swedish data. Furthermore, the incidence of RA increases with observation time [29]. Therefore, different data may not be directly comparable.

Epidemiological studies also carry a risk of reverse causation, which could ensue if persons with undiagnosed RA in HUNT2 were erroneously included as incident cases of RA, because RA in itself increases the risk of CV disease and events [10]. It seems unlikely that reverse causation could explain our findings, given that the association with previous CV disease became stronger when the cases with shortest duration between HUNT2 and a hospital diagnosis of RA were removed from the analysis. Furthermore, we did not observe a tendency for a higher number of cases with a hospital diagnosis to occur during the earliest years following HUNT2, which would have been expected with substantial reverse causation.

While fatal CV events prior to the onset of RA also limited the participants in our study to survivors of those events, this again would lead to under-estimation of the impact of CV events on development of RA, or to restrict the population at risk of RA to those ageing with CV risk that was insufficient to lead to premature mortality. Autoantibodies were not measured and seropositive and seronegative cases of RA could not be distinguished, which impacts on the generalizability of our results compared to others, such as population-based cohorts where it was concluded that ischemic heart disease was not increased prior to RA onset [17]. There may also have been a selection bias regarding participation in HUNT, where sicker people or those with a lower socioeconomic status are less likely to join. These people have a higher risk both for CV disease and for RA, which may have biased our findings.

The results from our various sensitivity analyses unanimously supported our main analysis. The overall conclusion that a history of CV disease is associated with the risk for incident RA therefore seems reliable. Although our study may not be suitable to give an exact estimate of the size of this increased risk, the main analysis giving an OR of approximately 1.5 is probably conservative.

**Risk factors for osteoarthritis**

Our results identifying smoking as a risk factor for future osteoarthritis contradict some previous studies, which report an inverse association between smoking and osteoarthritis [30]. However, the longitudinal prospective Clearwater study designed to identify risk factors for OA development, which included 2,505 participants, did not support this inverse association with smoking for any of four joint sites (knee, hand, foot or spine) [31]. In another prospective study of 1,003 participants from the general population there was no association between smoking and radiologically-confirmed OA at different sites [32]. The current study may have shown an association with smoking because it is larger and included a greater number of incident osteoarthritis diagnoses. Nevertheless, since our study relies on self-report, replication in a cohort ascertained for radiographic osteoarthritis and symptoms is needed. However, in support of the accuracy of our case classification, we noted that higher BMI increased the risk for osteoarthritis development, as previously described [33].

**Inflammation as a link between CV disease and incident RA**

From a biological perspective based on the current studies, we hypothesize that inflammation may contribute to
the parallel development of atherosclerosis and RA during the pre-clinical period in individuals exposed to common RA and CV risk factors, just as this interaction accelerates complication of RA by CV events after onset.

Inflammation is the most plausible link between previous CV disease or BMI and increased risk for RA. It is well established that the pathogenesis of CV disease, that is, atherosclerosis, includes chronic inflammation of the wall of muscular arteries and that inflammation is increased during acute coronary events, associated with unstable plaque and thrombosis [16]. Furthermore, obesity induces chronic inflammation in adipose tissue, increasing pro-inflammatory cytokines including interleukin-6 and tumour-necrosis factor [34]. In addition, a pro-inflammatory state is present before the clinical onset of RA. C reactive protein (CRP) levels were found to be higher in individuals with preclinical RA compared to a control group [35]. Serum cytokines and chemokines were also elevated preceding the onset of RA [36]. In at least some patients, periodontal inflammation may precede the onset of RA symptoms, also associated with ACPA [37]. Respiratory inflammation has also been described in ACPA positive individuals without RA [38]. Autoantibodies may also increase the risk of immune complex-mediated vascular inflammation [39]. Thus, CV disease and CV risk factors increase the future risk of RA development in the context of systemic inflammation and autoantibody development [12]. Patients with seropositive RA are at greater risk of CV disease [6,40], and it is possible that the RA genetic background is more permissive to atherosclerosis when associated with the "lifestyle" factors described here. In contrast to RA, in osteoarthritis inflammation is local and low-grade rather than systemic, and is not associated with autoantibodies.

Figure 1 proposes a model for the relationships among RA, CV disease, inflammation, atherosclerosis and predisposing factors. CV, cardiovascular; RA, rheumatoid arthritis.

Conclusions
A history of previous CV disease was associated with increased risk of incident RA but not osteoarthritis. A probable explanation is that increased systemic inflammation may contribute to the parallel development of atherosclerosis and RA during the pre-clinical period in individuals exposed to common RA and CV risk factors, in a similar way that such interaction accelerates CV disease in patients with established RA. The association of previous CV events with the development of RA at the population level suggests presentation with CV events, especially in middle-aged female smokers or first-degree relatives of RA patients, should raise clinical suspicion to capture cases of undiagnosed early RA. Because CV risk factors are increased at the onset of RA, active cardiovascular risk management is important from the time of diagnosis.

Abbreviations
ACPA: Anti-citrullinated peptide antibodies; BMI: Body mass index; CV: Cardiovascular; HDL: High-density lipoprotein; HUNT: Nord-Trøndelag Health Study; LDL: Low-density lipoprotein; MI: Myocardial infarction; OA: Osteoarthritis; RA: Rheumatoid arthritis.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
HP was responsible for conception and design, data collection and analysis, and manuscript writing. MAB contributed to conception and design, data collection, and critical revision of the manuscript. SP contributed to conception and design, and critical revision of the manuscript. VV was responsible for conception and design, data collection and analysis, and critical revision of the manuscript. All authors read and approved the final manuscript.

Acknowledgements
The HUNT study is a collaboration among the HUNT Research Centre (Faculty of Medicine, Norwegian University of Science and Technology NTNU), Nord-Trøndelag County Council, Central Norway Health Authority, and the Norwegian Institute of Public Health. We are grateful to Leivanger, Namos and Trondheim University hospitals for giving access to their diagnostic registries. This research was supported by NHMRC grant 569938, an Australian Postgraduate Award (HP) and an ARC Future Fellowship (RT). MAB is funded by an NHMRC Senior Principal Research Fellowship and Queensland Premier’s Fellowship.

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References


Dear Prof Thomas

HREC Reference number: HREC/13/QPAH/604
Protocol Title: Cardiovascular events and death in rheumatoid arthritis patients – a ten-year follow up study

Thank you for submitting the above research protocol to the Metro South Health Human Research Ethics Committee for ethical and scientific review. This protocol was considered by the Low Risk Review Panel and will be ratified at the next Metro South HREC meeting.

You are reminded that this letter constitutes ethical approval only. You must not commence this research protocol at a site until separate authorisation from the Hospital Health Service Chief Executive (CE) or Delegate of that site has been obtained.

A copy of this approval must be submitted to the Research Governance Office(r)/Delegate of the relevant institution with a completed Site Specific Assessment (SSA) Form for authorisation from the CE or Delegate to conduct this research at the Princess Alexandra Hospital.

I am pleased to advise that the Low Risk Review Panel of the HREC has granted approval of this research protocol and a waiver of consent. The documents reviewed and approved include:

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Please note the following conditions of approval:

1. The Principal Investigator will immediately report anything which might warrant review of ethical approval of the protocol in the specified format, including unforeseen events that might affect continued ethical acceptability of the protocol. Serious Adverse Events must be notified to the HREC as soon as possible. In addition the Investigator must provide a summary of the adverse events, in the specified format, including a comment as to suspected causality and whether changes are required to the Patient Information and Consent Form. In the case of Serious Adverse Events occurring at the local site, a full report is required from the Principal Investigator, including duration of treatment and outcome of the event.

2. Amendments to the research protocol which may affect the ongoing ethical acceptability of a protocol must be submitted to the HREC for review. Major amendments should be reflected in a revised online NEAF (accompanied by all relevant updated documentation and a cover letter from the principal investigator).
investigator, providing a brief description of the changes, the rationale for the changes, and their implications for the ongoing conduct of the study). Hard copies of the revised NEAF, the cover letter and all relevant updated documents, with tracked changes, must also be submitted to the HREC office as per standard HREC SOP. (Further advice on submitting amendments is available at

3. Amendments to the research protocol which only affect the ongoing site acceptability of the protocol are not required to be submitted to the HREC for review. These amendment requests should be submitted directly to the Research Governance Office/r.

4. Proposed amendments to the research protocol which may affect both the ethical acceptability and site suitability of the protocol must be submitted firstly to the HREC for review and, once HREC approval has been granted, then submitted to the Research Governance Office/r.

5. Amendments which do not affect either the ethical acceptability or site acceptability of the protocol (e.g. typographical errors) should be submitted electronically (track changes) and in hard copy (final clean copy) to the HREC Coordinator. These should include a cover letter from the Principal Investigator or Study Co-ordinator providing a brief description of the changes and the rationale for the changes, and accompanied by all relevant updated documents with tracked changes.

6. The HREC will be notified, giving reasons, if the protocol is discontinued at a site before the expected date of completion.

7. The Coordinating Principal Investigator will provide an annual report to the HREC and at completion of the study in the specified format.

8. If you require an extension for your study, please submit a request for an extension in writing outlining the reasons. Note: One of the criteria for granting an extension is the compliance with the approval’s conditions including submission of progress reports.

9. Any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes (WHO / ICMJE 2008 definition) should be registered, including early phase and late phase clinical trials (phases I-III) in patients or healthy volunteers (WHO Recommendation / ICMJE policy). If in doubt, registration is recommended. All studies must be registered prior to the study’s inception, i.e. prospectively.
http://www.anzctr.org.au/

This HREC approval is valid for three years from the date of this letter.

Should you have any queries about the HREC’s consideration of your protocol please contact Ethics Secretariat on 07 3443 8049.

Please note that the Metro South HREC is constituted and operates in accordance with the National Health and Medical Research Council’s (NHMRC) National Statement on Ethical Conduct in Human Research (2007), NHMRC and Universities Australia Australian Code for the Responsible Conduct of Research (2007) and the CPMP/ICH Note for Guidance on Good Clinical Practice. Attached is the HREC Composition with specialty and affiliation with the Hospital (Attachment I).

The HREC Terms of Reference, Standard Operating Procedures, membership and standard forms are available from the following websites:

Once authorisation to conduct the research has been granted, please complete the Commencement Form (Attached) and return to the Metro South Human Research Ethics Committee.
The Metro South HREC wishes you every success in your research.

Yours sincerely,

A/Professor Richard Roylance
Deputy Chair
Metro South Hospital and Health Service
Human Research Ethics Committee (EC00167)
Centres for Health Research
Princess Alexandra Hospital

[Signature]
Professor Ranjeny Thomas  
UQ Diamantina Institute  
Level 6  
Translational Research Institute  
37 Kent Street  
WOOLLOONGABBA QLD 4102

Dear Professor Thomas

Research Title: Cardiovascular events and death in rheumatoid arthritis patients - a 10-year follow up study.  
HREC Number: HREC/13/QPAH/604

I am writing to inform you that your request for access to confidential health information for the above project has been approved under the delegation of the Director-General. In accordance with Section 284 of the Public Health Act 2005 the researchers listed in your application, dated 11 August 2013 can access and use the specified confidential information, providing they act within the limits detailed in your submission.

This approval (RD004860) commences on the date of this letter and is valid to 2016.

This approval relates to data for the period from 01 January 2003 to 31 March 2014 from Princess Alexandra Hospital.

This approval means that you must undertake the responsibilities and obligations of confidentiality of the information under the provisions of the Public Health Act 2005. You must take all reasonable steps necessary to ensure that the confidential information is kept confidential, including storing or disposing of all data, information, documents and associated correspondence in a secure manner. Unauthorised use or disclosure of confidential information may incur a penalty under the laws of the Queensland Government. These obligations include providing notification of any change in the names of persons who will be given the information for the research.

When conducting research within the Queensland public health system, a copy of this Approval Letter must be provided to the relevant Research Governance Officer as part of your research governance application.

Please display this letter and a copy of your application when requesting the confidential information from the relevant data custodian.
You are required to provide an annual progress report and a final report at the completion of your project, to Health and Medical Research, Preventive Health Unit. Templates can be found on the web page http://www.health.qld.gov.au/ohmr/html/regu/aces_conf_hth_info.asp

Should you wish to extend your research project beyond this time or amend the study protocol, you will need to seek approval of these amendments from the approving HREC and re-apply for approval of the release of confidential data. This includes disclosing this information to and recruiting additional people to this project. Please provide a copy of your HREC approval of the amendments when re-applying.

Please feel free to contact Health and Medical Research, Preventive Health Unit on email HMR@health.qld.gov.au or phone 07 3328 9866 if you have any queries on this matter.

Yours sincerely,

Kaye Pulsford
Executive Director, Preventive Health Unit
Chief Health Officer Branch
Health Services and Clinical Innovation Division

14/11/2013