Studies exploring the relationship between methotrexate use and blood pressure and arterial function in the rheumatoid arthritis population

by

Leena Rashad Baghdadi

Thesis
Submitted to Flinders University
for the degree of

Doctor of Philosophy

College of Medicine and Public Health

August 2017

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

THESIS ABSTRACT

Rheumatoid arthritis (RA) is a chronic disabling inflammatory disease affecting up to 2% of the population. Methotrexate (MTX) use has been associated with reduced cardiovascular morbidity and mortality in patients with RA. Although MTX has anti-inflammatory effects, there is little evidence to support additional salutary effects of MTX on markers of cardiovascular risk such as blood pressure (BP), and markers of arterial function including arterial wave reflection (augmentation index, AIx), and pulse wave velocity (PWV). Observational studies have suggested a lower systolic BP (SBP) in MTX users; however, it is unknown whether these differences remain constant over time and whether they are independent of systemic markers of inflammation.

The studies presented in this thesis sought to determine whether there is an association between MTX and BP/arterial function in the RA patients and whether it is independent of inflammation. In addition, it explores the role of asymmetric dimethylarginine (ADMA), intracellular MTX polyglutamates (MTXPGs), and genetic polymorphism of transporters and target enzymes in modulating the effects of MTX on BP and arterial function. An initial cross-sectional study was conducted followed by a repeat cross-sectional study, where the same RA patients were followed for 8 months to examine changes in BP and arterial function. Mobil-O-Graph monitors were used to record 24-hour BP, AIx and PWV. The SphygmoCor was used to measure AIx and central BP.

The findings of these studies revealed that clinical BP was significantly lower in MTX patients compared to non-MTX patients at both baseline and 8 months. Similar differences were found for central BP and PWV. Patients treated with MTX had significant lower average 24-h and daytime, but not night-time, peripheral and central BP and PWV when compared to RA patients on other DMARDs. The changes in outcomes between visits were mostly no different between groups, although the 24-h central SBP was lowerd more in MTX than in non-MTX users. There were no significant associations between the two systemic inflammatory markers ESR and CRP and clinic SBP, or between DAS28 and clinic SBP. Heterozygous genotype of MTX *ABCG2* (i.e. mutant gene) showed higher concentration of MTXPGs and was associated with lower BP and arterial function. The associations between MTX and low BP and PWV have pharmacological, genetic and preventive attractions.

PUBLICATIONS AND AWARDS

Research publications:

1. **Baghdadi, LR, Woodman, RJ, Shanahan, EM & Mangoni, AA 2015, 'The impact of traditional cardiovascular risk factors on cardiovascular outcomes in patients with rheumatoid arthritis: a systematic review and meta-analysis', *PLoS One*, vol. 10, no. 2, p. e0117952. doi: 10.1371/journal.pone. eCollection 2015.**
2. **Mangoni, AA, Baghdadi, L, Wiese, MD, Shanahan, EM, Tommasi, S, Elliot, D & Woodman, RJ 2017, "Methotrexate, blood pressure and markers of arterial function in patients with rheumatoid arthritis: a repeated cross-sectional study", *under review.***

Conference abstract:

1. **Baghdadi LR, Woodman RJ, Shanahan EM, Mangoni AA 2015, 'Methotrexate, blood pressure and arterial wave reflection in rheumatoid arthritis', [abstract]. *Arthritist & Rheumatology*, vol. 67, no. 10. http://acrabstracts.org/abstract/methotrexate-blood-pressure-and-arterial-wave-reflection-in-rheumatoid-arthritis.**
2. **Baghdadi LR, Woodman RJ, Shanahan EM, Mangoni AA 2016 Methotrexate use, blood pressure, arterial function and inflammation in rheumatoid arthritis: repeated cross-sectional study', paper presented to the annual conference of Australian Society for Medical Research, Adelaide, 8 June.**

Awards:

2016 Best Research Higher Degree Student Publication for the paper: *The impact of traditional cardiovascular risk factors on cardiovascular outcomes in patients with rheumatoid arthritis: a systematic review and meta-analysis.*

DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Signature: Leena Baghdadi

Date: 15/05/2017

ACKNOWLEDGMENTS

It is with great pleasure that I acknowledge the many outstanding people who made this thesis possible; they have supported me throughout my study at Flinders University from 2013 to 2017.

I owe my deepest gratitude to my distinguished supervisor, Professor Arduino Mangoni, who has provided his time, wisdom, expertise, knowledge and continued encouragement in mentoring me throughout my study. He always offers his extensive guidance and insightful comments. I have been inspired and motivated by him, and learned so much from his experience. Without his advice and support, this thesis would not have come into being. A hearty thanks to Professor Arduino for his continued support, patience and encouragement.

I would like to extend a very special thanks to my great co-supervisors Professor Richard Woodman and Associate Professor Michael Shanahan for their consistent support, expert supervision and encouragement throughout my PhD study. I express my infinite thanks to Professor Woodman who supported me during the process of the data analysis and provided me with worthy comments. Richard, you deserve my everlasting gratitude. Sincere and heartfelt thanks to Associate Professor Shanahan for believing in my capabilities, his generous hospitality at the Repatriation General Hospital and his understanding and positive attitude. Thank you Michael, I could not wish for a friendlier supervisor.

My sincere gratitude goes to all the rheumatologists, staff, and colleagues at the Repatriation Hospital for their kind help in recruiting patients. Special thanks to Professor Malcolm Smith, Dr Mihir Wechalekar, Dr Jenny Walker and Dr Eliza Pontifex. I am also truly grateful to Mrs Helen Weedon who has been not only a lab mentor, but also a true friend who supported me emotionally. Many thanks for Ms Thornton at the Academic Editorial Services for her wonderful assistance in editing my thesis.

To my father and mother, I cannot thank you enough for your unconditional love and limitless support, even while you are at the other end of the world. All the credit for the success and what I have achieved goes to you as you always been the secret of my happiness and your positive attitude gave me wings to fly.

This thesis would have never been possible without my friendly, loving and loyal husband, Emad. Warm thanks to your support, patience and guidance every single day. You were always there cheering me on, repeating over and over again that I am capable of accomplishing my PhD, I love you and I cannot imagine my life without you.

Special thanks to my brothers, sisters, son and daughter, who are my real eyes that has let me see a brighter life. Looking at your faces washes the pain and stress away. You are the greatest companion gift for my PhD. I love you all.

Finally, many lovely, charming and delightful thanks to my wonderful friends in Australia and overseas for showing me their love and support throughout my PhD.

LIST OF ABBREVIATIONS

7-OH-MTX: 7-hydroxymethotrexate

ABC: ATP-binding cassette transporters

ACR: American College of Rheumatology

ADMA: Asymmetric dimethylarginine

ADORA2a: Adenosine receptor subtype A2a

AIx: Augmentation index

AMP: Adenosine monophosphate

AMPD1: Adenosine monophosphate deaminase 1

Anti-CCP: Anti-citrullinated protein antibody

AP: Augmentation pressure

APCs: Antigen-presenting cells

ARA: American Rheumatism Association

ARG: L-Arginine

ATIC: 5-aminoimidazole-4-carboxamide ribonucleotide formyltrans-ferase

ATP: Adenosine triphosphate

BH4: Tetrahydrobiopterin

BHS: British Hypertension Society

BMI: Body mass index

BP: Blood pressure

Bpm: Beats per minute

Ca2+: Calcium

cAIx: central augmentation index

CBS: Cystathionine-β synthase

cDBP: central diastolic blood pressure

CE: Conformité Européenne

cGMP: Cyclic guanosine monophosphate

CHF: Congestive heart failure

CI: Confidence interval

CIT: L-citrulline

CLAs: Conjugated linoleic acids

CMV: Cytomegalovirus

COX: Cyclooxygenase

cPP: Central pulse pressure

CRP: C-reactive protein

cSBP: central systolic blood pressure

CV: Cardiovascular

CVA: Cerebrovascular accident

CVD: Cardiovascular disease

DALY: Disability-adjusted life year

DAS28: Disease activity score using 28 joint count

DBP: Diastolic blood pressure

DDAH: Dimethylarginine dimethylaminohydrolases enzyme

DHE: Docosahexaenoic acid

DHF: Dihydrofolate

DHFR: Dihydrofolate reductase

DI: Disability index

DMARDs: Disease-modifying anti-rheumatic drugs

DNA: Deoxyribonucleic acid

DPA: Docosapentaenoic acid

DQES v2: Dietary Questionnaire for Epidemiological Studies Version 2

dTMP: Deoxythymidine monophosphate

dTTP: Deoxythymidine triphosphate

dUMP: Deoxyuridine monophosphate

DVT: Deep vein thrombosis

EBV: Epstein barr virus

eGFR: Estimated Glomerular Filtration Rate

ELISA: Enzyme-linked immunosorbent assay

eNOS: Endothelial NO synthase

EPA: Eicosapentaenoic acid

ESC: European Society of Cardiology

ESH: European Society of Hypertension

ESI: Electrospray ionisation

ESR: Erythrocyte sedimentation rate

EULAR: European League Against Rheumatism

FDA: Food and Drug Administration

FMC: Flinders Medical Centre

FMD: Flow-mediated dilatation

FPGS: Folypolyglutamyl synthetase enzyme

FRS: Framingham Risk Score

GGH: Gamma-glutamyl hydrolase enzyme

h: Hour

HAQ: Stanford health assessment questionnaire

HDLc: High-density lipoprotein cholesterol

HR: Hazard ratio

HARG: L-homoarginine

HLA: Human leucocyte antigen gene

HMA: N(G)-hydroxymethyl-arginine

ICD-9: International Classification of Diseases 9th Revision

ID: Identification number

IL: Interleukins

IMT: Intimal medial thickness

IR: Incident rate

LDLc: Low-density lipoprotein cholesterol

L-NMMA: NG-methyl-L-arginine

M: Million

MCP: Metacarpophalangeal joint

MHC: Major histocompatibility complex class II molecule

MI: Myocardial infarction

mmHg: Millimetre of mercury

MMP: Metalloproteinase

m/s: Metre per Second

MRI: Magnetic resonance imaging

MRP: Multidrug resistance associated protein

MTHFD1: Methylenetetrahydrofolate dehydrogenase 1

MTHFR: Methylenetetrahydrofolate reductase enzyme

MTP: Metatarsophalangeal joint

MTX: Methotrexate

MTXPGs: MTX polyglutamates

NADPH: Nicotinamide-adeninedinucleotide phosphate

NATSIHS: National Aboriginal and Torres Strait Islanders Health Survey

NO: Nitric oxide

NSAIDs: Nonsteroidal anti-inflammatory drugs

OAT: Organic anion transporter

OR: Odds ratio

ORN: Ornithine arginine

PGA: Patient global assessment analogue scale

PGs: Prostaglandins

PIP: Proximal interphalangeal joint patient

PP: Pulse pressure

PRMT: Protein arginine methyl transferase enzymes

PWA: Pulse wave analysis

PWV: Pulse wave velocity (PWV)

QRISK2: Risk prediction algorithm

RA: Rheumatoid arthritis

RBCs: Red blood cells

RF: Rheumatoid factor

RFC1: Reduced folate carrier 1

RGH: Repatriation General Hospital

RR: Relative risk

RRS: Reynolds Risk Score

ROS: Reactive oxygen species

SBP: Systolic blood pressure

SDMA: Symmetric dimethyl arginine

sGC: Guanylate cyclase

SJC: Swollen joint count

SNPs: Single-nucleotide polymorphisms

SPE: Solid phase extraction

T2D: Type 2 diabetes mellitus

TC: Total cholesterol

TH1: T-helper cells

TIMP: Tissue inhibitors of metalloproteinases

TJC: Tender joint count

TNF-α: Tumour necrosis α

TS: Thymidylate synthase

TYMS: Thymidylate synthase enzyme

TXA-2: Thromboxane A-2

UPLC-MS: Ultra-performance liquid chromatography mass spectrometry

USA: United States of America

WBCs: White blood cells

WHO: World Health Organization

CHAPTER 1: REVIEW OF THE LITERATURE

1.1 Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown origin. It usually affects small joints of the hands and feet, but it can also affect larger joints such as the hips and knees (Aletaha et al. 2010). The onset of RA is believed to be triggered by an autoimmune response (Firestein 2003) leading to inflammation and progressive joint destruction. Autoantibodies, such as the rheumatoid factor (RF), have been found in the blood of RA patients several years before the diagnosis (Aho et al. 1991; Aho et al. 1993; Nielen et al. 2004; Rantapaa-Dahlqvist et al. 2003); thus, RA is considered an autoimmune disease (Firestein 2003; Smolen et al. 2007). About 63% of affected patients are women (Australian Institute of Health and Welfare 2010); the incidence rate of RA peaks between age 30 and 50 years. If not adequately treated, extra-articular complications are most likely to develop. This section provides an overview of RA highlighting its epidemiology, pathogenesis, clinical diagnosis and complications.

1.1.1 Epidemiology

RA is a common type of chronic arthritis and globally, the prevalence of RA is recorded to be 1% (Rindfleisch & Muller 2005). RA was rated, in 2010, as the 42nd highest cause of global disability; disability-adjusted life year (DALY) was 4.8 million (M) (95% confidence interval [CI] 3.7 M, 6.1 M) (Cross et al. 2014).

In Australia, the prevalence of RA is higher than that recorded globally. Based on the 2014-15 National Health Survey, the prevalence of all types of arthritis in Australia was 15.3% affecting about 3.5 M Australian adults; and around 12% of those patients with arthritis were diagnosed with RA (Australian Bureau of Statistics 2015; Australian Institute of Health and Welfare 2010). In 2005, about 4% of the government expenditure was spent on RA (Australian Institute of Health and Welfare 2009) with a cost of $355 million spent on health care (Australian Bureau of Statistics 2002). Based on the 2004–05 National Aboriginal and Torres Strait Islanders Health Survey (NATSIHS), higher prevalence of RA was reported in indigenous Australians compared to non-indigenous population (Minaur et al. 2004; Roberts-Thomson & Roberts-Thomson 1999; Australian Institute of Health and Welfare 2009).

There is a variation, however, in the epidemiology of RA in different areas of the world. Compared to Australia, the prevalence of RA is higher in the United States of America (USA)

and Finland (Ong et al. 2013). The USA statistics for arthritis during the period 1999–2008 showed that the prevalence of RA was 17.9 % among Americans with arthritis aged 40 years or more (Ong et al. 2013); this study has forecasted this prevalence to increase as the USA population ages. This variation might be due to different genetic makeup, or ethnic and geographic variations, or environmental and dietary differences (Alamanos, Voulgari & Drosos 2006). The incidence of RA, however, is decreasing over time even though some countries such as Finland and the USA are known to have the highest prevalence of RA. Another explanation for the reported epidemiological variation is the lack of robust epidemiological data in developing countries. This might be explained by several factors, such as lower incidence and prevalence of RA cases, no national databases, absence of best practice in diagnosing RA, or scarce research on RA in these countries.

1.1.2 Aetiology and pathogenesis

Although the aetiology of RA is still unknown, there are some proposed theories that include inflammatory, genetic, environmental, and lifestyle influences.

1.1.2.1 Inflammation

RA is an autoimmune disease associated with inflammation. These two features are responsible for RA pathogenesis and joint destruction. Both RF and anti-citrullinated protein antibody (anti-CCP) are sensitive in detecting RA. Indeed, anti-CCP can detect RA before the onset of symptoms (Brink et al. 2015). In addition to activation of the autoimmune system, inflammation plays a major role in triggering the pathogenesis of RA. The T-helper cells (TH1), also known as $CD4^+$, are the prime initiators of the inflammatory response (Choy & Panayi 2001). In RA, the presence of antigens triggers the autoimmune response. These antigens can be detected by TH1 cells by the assistance of antigen-presenting cells (APCs), such as macrophages. After detecting the antigen, TH1 cells become activated and stimulate the release of pro-inflammatory cytokines, namely, tumour necrosis α (TNF-α) and interleukins (IL-1, IL-6 and IL-17) (Choy & Panayi 2001). Subsequently, activated TNFα and IL-6 stimulate the expression of adhesion molecules enhancing the formation of new blood vessels (Nassonov et al. 2000; Ospelt & Gay 2008). The latter increase the delivery of inflammatory cells to the synovium, causing oedema and joint swelling (Figure 1-1).

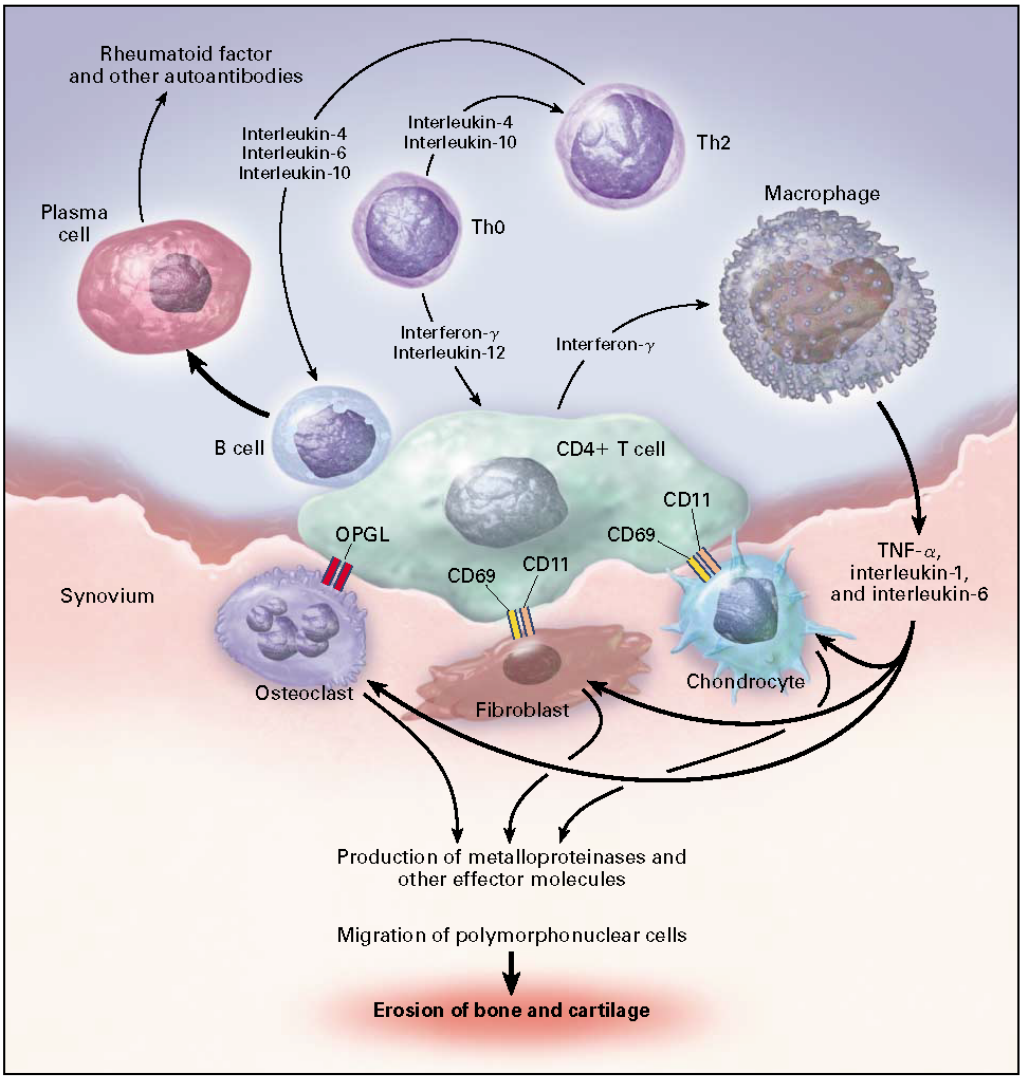


Figure 1-1 Pathogenesis of rheumatoid arthritis and inflammation

From Choy & Panayi 2001, p.908. Th2 = type 2 helper T cell, Th0 = precursor of type 1 and type 2 helper T cells, OPGL = osteoprotegerin ligand

1.1.2.2 Genetic factors

Evidence also points to genetic factors in the aetiology of RA. Several studies suggest a strong familial tendency in developing RA, especially in the first-degree relatives of a patient with RA (Lawrence 1970; Worthington et al. 1994). This factor increases the susceptibility of developing RA by 60% (Kurko et al. 2013; MacGregor et al. 2000). Moreover, identical twins have 15% higher risk of RA compared to non-identical twins (Silman et al. 1993). Although some researchers have reported that the heritability did not predict the development of RA (de Vries

2011; Frisell et al. 2016; Kurko et al. 2013; van der Woude et al. 2009), recent studies confirm the role of genetic factors in RA pathogenesis (Jiang et al. 2015).

Gene alleles play a role in RA susceptibility with eight genes currently linked to RA predisposition (Bowes & Barton 2008; O'Rielly & Rahman 2010). The human leucocyte antigen (HLA) gene (more specifically HLA-DRB1 alleles) is the prime gene responsible for 37% of RA susceptibility (Kurko et al. 2013). This gene is a major histocompatibility complex class II molecule (MHC), which is encoded by HLA on chromosome 6. In the presence of a pathogen, antigen-presenting cells (APCs) engulf that antigen; subsequently, the HLA-DRB1 gene encodes viral peptides. These peptides help in identifying the engulfed antigen by T cells released by the immune system. Once the T cells recognise the engulfed pathogen, they destroy the infected cells (Shiina et al. 2009). Thus, the susceptibility of developing RA depends on the risk of having alleles on the HLA-DRB1 gene (Gregersen, Silver & Winchester 1987). This risk has been estimated to range between 30% (Gregersen, Silver & Winchester 1987) and 37% (Deighton et al. 1989; Kurko et al. 2013). More specifically, the tendency to RA development is associated with HLA-DRB1*0401 subtype (Bowes & Barton 2008).

1.1.2.3 Hormonal factors

Hormonal changes have been proposed to influence RA development; for example, hormonal changes during pregnancy have been thought to protect women from developing RA (Nicholas & Panayi 1988). In fact, remission has been observed among RA pregnant women thus supporting the favourable effects of female sex hormones (Ostensen & Villiger 2007). Compared with females who had conceived during their life, those who were nulliparous have shown higher risk of RA (Hazes 1991). The protective effects of female sex hormones is however, still a controversial issue (Qi et al. 2014).

1.1.2.4 Environmental and lifestyle factors

Many environmental and lifestyle influences have been examined to understand the pathogenesis of RA. These factors include smoking, past history of infection and body weight.

Smoking is the most important environmental factor triggering RA disease (Silman, Newman & MacGregor 1996; Stolt et al. 2003; Sugiyama et al. 2010; Vessey, Villard-Mackintosh & Yeates 1987). Although the exact biological mechanism of smoking and RA development is still unclear, it has been postulated that the interaction between genetic factors and smoking might explain the observed high risk of RA among smokers (Padyukov et al. 2004). One of the

claimed processes is the interaction between HLA-DRB1 gene, as a major contributor in RA pathogenesis, and smoking. Even though there is no clear evidence of such interaction, protein citrullination is one of the proposed factors linking such interaction (Klareskog et al. 2006a; Klareskog et al. 2006b); in the process of citrullination, protein modification occurs. Arginine, which is a basic amino acid constituent in protein, is deaminated into citrulline. This results in alteration of the protein structure and function. In most inflammatory diseases, antibodies are usually formed against citrullinated proteins. Anti-CCP antibody is an example of such antibody formation. As this biomarker of RA has been detected in the blood before the onset of RA, the interaction between smoking and citrullination might contribute to RA development (Aho et al. 2000; Berglin et al. 2004; Bridges 2004; Rantapaa-Dahlqvist et al. 2003).

Infectious agents, such as viruses, have also been investigated in the pathogenesis of RA (Benedek 2006); however, the available evidence is inconclusive. Few studies have examined the relationship between RA pathogenesis and infection with cytomegalovirus (CMV) (Jorgensen et al. 2008), rubella and parvovirus (Zentilin et al. 2002), and Epstein Barr virus (EBV) (Barzilai et al. 2007). Among all these viruses, EBV has been given great attention to support the cause–effect hypothesis. Molecular mimicry has been proposed as a mechanism involved in RA pathogenesis (Barzilai et al. 2007; Wucherpfennig 2001). In this type of mechanism, EBV is thought to contain the HLA-DRB1 epitope, which is structurally similar to that seen in inflamed joints. Thus, the immune system is confused by the EBV epitope, which activates the immune system.

In addition to smoking and infection, body weight has been claimed to be one of the causal factors in RA development but the relationship between these factors is still debatable. Recently, it has been shown that being overweight (body mass index [BMI] 25.00–29.99), or obese (BMI >30) increases the risk of developing RA by 15% and 31%, respectively (Qin et al. 2015). More specifically, for every 5 kg/m^2 increase in the BMI, the risk of RA increases by 13% (relative risk [RR] 1.13, 95% CI 1.01, 1.26) (Feng et al. 2016). This increased risk has been plausibly explained by the shared pathway between obesity and inflammation. Thus, obesity is considered an inflammatory disease associated with high levels of TNF-α, IL-6 (Das 2001) and adipokines (Versini et al. 2014), specifically leptin (Trayhurn & Wood 2005).

1.1.3 Clinical features

Joint stiffness, pain and swelling are the hallmark symptoms of RA. The onset of the disease, on average, occurs over weeks to months. Nevertheless, it could progress more rapidly, over days,

among one-third of the RA population (Plant et al. 1998). Although joint symptoms are the most common presentation, patients might also present with extra-articular manifestations (Aletaha et al. 2010).

Proximal joints are most commonly affected, including metacarpophalangeal, proximal interphalangeal and metatarsophalangeal joints; shoulders, knees and elbows are also possible joints affected. Progression of RA leads to joint destruction, deformities and disabilities (Scott et al. 2000). Disease duration of 10 years or longer was found to be associated with severe disability in 16% of RA patients (Sherrer et al. 1986).

Conversely, patients with RA might present with extra-articular symptoms, with these manifestations ranging between general fatigue and loss of weight to several systemic organ involvements (Table 1-1) (Turesson et al. 2002).

Table 1-1 Extra-articular manifestations of rheumatoid arthritis from (Turesson et al. 2002)

System	Manifestation
Cardiovascular	Pericarditis, vasculitis, coronary artery disease and myocardial infarction (MI)
Respiratory	Pleuritis, pulmonary fibrosis, pulmonary nodules, bronchiolitis obliterans, organising pneumonia
Blood	Felty's syndrome, thrombocytosis, large granulocytic leukaemia, splenomegaly, anaemia and non-Hodgkin's lymphoma
Central nervous	Neuropathy, and cervical myelopathy
Eye	Keratonconjunctivitis sicca, scleritis, episcleritis, and retinal vasculitis
Muscle	Rheumatoid cachexia
Bone	Osteopaenia and osteoporosis
Renal	Glomerulonephritis
Skin	Subcutaneous rheumatoid nodules, cutaneous vasculitis
Salivary gland	Secondary Sjögren's syndrome

1.1.4 Diagnosis

Patients' presenting symptoms and signs, imaging and laboratory tests can help in diagnosing RA.

1.1.4.1 Rheumatoid arthritis diagnostic criteria

It is mandatory in clinical practice to have criteria for diagnosing and defining cases of RA (Aletaha et al. 2010). Standardised criteria to diagnose RA have been established since the early 1950s and, at that time, RA was diagnosed based on a clinical grading system (Kellgren, Lawrence & Aitken-Swan 1953). The American College of Rheumatology (ACR), formerly the American Rheumatism Association (ARA), was developed to establish uniform criteria for RA diagnosis. ACR launched the first diagnostic criteria in 1956, based on the clinical experience of committee members, and these were subsequently revised in 1958 (Table 1-2) (Ropes et al. 1957, 1958). These criteria were classified into three groups namely, definite, probable and possible. In 1983, these criteria were revised again by ARA when a single category "Rheumatoid Arthritis" replaced the previous three groups (Table 1-3) (Arnett et al. 1988). As early changes in early stage of the disease were not considered in the 1987 ACR diagnostic criteria, critics argued about the sensitivity of these criteria in diagnosing RA. Therefore, modifications were made by ACR in the "2010 American College of Rheumatology/European League against rheumatism classification criteria for rheumatoid arthritis" (Table 1-4) (Aletaha et al. 2010).

Table 1-2 1958 American Rheumatism Association diagnostic criteria for rheumatoid arthritis from Ropes et al. (1956) and Ropes et al. (1958)

1. Morning stiffness
2. Pain on motion or tenderness in at least one joint (observed by a physician)
3. Swelling (soft tissue thickening or fluid, not bony overgrowth alone) in at least one joint (observed by a physician)
4. Swelling (observed by a physician) of at least one other joint (the interval between two joint involvements must be no more than 3 months)
5. Symmetrical joint swelling (observed by a physician) with simultaneous involvement of the same joint on both sides of the body (bilateral involvement of the mid-phalangeal, MCP or MTP joints is acceptable without absolute symmetry). Terminal IP joint involvement will not satisfy the criterion.
6. Subcutaneous nodules (observed by a physician) over bony prominences, on extensor surfaces or in juxta-articular regions

7. X-ray changes typical of rheumatoid arthritis (which must include at least bony decalcification localized to or greatest around the involved joints)

8. Positive agglutination test—demonstration of RF by any method which in two laboratories has been positive in not over 5% of normal controls—or positive streptococcal agglutination test (1956 criteria: positive sheep cell agglutination or positive streptococcal agglutination test)

9. Poor mucin precipitate from synovial fluid

10. Characteristic histologic changes in synovial membrane

11. Characteristic histologic changes in nodules

Classical RA requires 7/11 criteria with 1–5 present continuously for 6 weeks *(not included in 1956 criteria)*

Definite RA requires 5/11 criteria with 1–5 present continuously for 6 weeks

Probable RA requires 3/11 criteria with at least one of 1–5 present continuously for 6 weeks *(1956 criteria: 4 weeks)*

Possible RA requires two of the following criteria and a total duration of joint symptoms of at least three weeks: (1) Morning stiffness (2) Tenderness or pain on motion (observed by a physician) with a history of recurrence or persistence for three weeks (3) History or observation of joint swelling (4) Subcutaneous nodules (observed by a physician) (5) Elevated sedimentation rate or C-reactive protein (6) Iritis.

Exclusions: (1) Typical rash of SLE, (2) High concentration of LE cells, (3) Histologic evidence of periarteritis nodosa, (4) Weakness of the neck, trunk and pharyngeal muscles or persistent muscle swelling of dermatomyositis (5) Definite scleroderma (6) A clinical picture of rheumatic fever (7) A clinical picture of gouty arthritis (8) Tophi (9) Acute infectious arthritis (10) Evidence of joint tuberculosis (11) A clinical picture characteristic of Reiter's syndrome (12) A clinical picture characteristic of shoulder-hand syndrome (13) A clinical picture of hypertrophic pulmonary osteoarthropathy (14) Neuroarthropathy (15) Homogentisic acid in the urine (16) Histologic evidence of sarcoid or a positive Kveim test (17) Multiple myeloma (18) Erytbema nodosum (19) Leukaemia or lymphoma (20) Agammaglobulinaemia *(not included in 1956 criteria).*

Table 1-3 The revised 1987 American Rheumatism Association criteria (from Arnett et al. 1988)

1. Morning stiffness	Morning stiffness in and around the joints, lasting at least one hour before maximal improvement
2. Arthritis in three or more joint areas*	Soft tissue swelling or fluid (not bony overgrowth) observed by a physician, present simultaneously for at least 6 weeks
3. Arthritis of hand joints	Swelling of wrist MCP or PIP for at least 6 weeks
4. Symmetrical arthritis	Simultaneous involvement of the same joint areas (defined in 2) on both sides of the body (bilateral involvement of PIPs, MCPs or MTPs is acceptable without absolute symmetry) for at least 6 weeks
5. Rheumatoid nodules	Subcutaneous nodules over bony prominences, or extensor surfaces, or in juxta-articular regions, observed by a physician
6. Rheumatoid factor	Detected by a method positive in less than 5.0% normal control
7. Radiographs changes	Typical of RA on posteroanterior hand and wrist radiographs which must include erosions or unequivocal bony decalcification localized to or most marked adjacent to the involved joints (OA changes alone do not qualify)

* Proposed areas: right or left PIP, MCP, wrist, elbow, knee, ankle, MTP.

At least four criteria must be fulfilled for classification as RA.

Patients with two clinical diagnoses are not excluded.

Table 1-4 The 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for rheumatoid arthritis (from Aletaha et al. 2010)

Classification criteria Score
Target population (Who should be tested?): Patients who **1. have at least 1 joint with definite clinical synovitis (swelling)*** **2. with the synovitis not better explained by another disease†** **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)‡** **A. Joint involvement§** **1 large joint¶ 0** **2-10 large joints 1** **1-3 small joints (with or without involvement of large joints)# 2** **4-10 small joints (with or without involvement of large joints) 3** **>10 joints (at least 1 small joint)** 5** **B. Serology (at least 1 test result is needed for classification)††** **Negative RF and negative ACPA 0** **Low-positive RF or low-positive ACPA 2** **High-positive RF or high-positive ACPA 3** **C. Acute phase reactants (at least 1 test result is needed for classification)‡‡** **Normal CRP and normal ESR 0** **Abnormal CRP or abnormal ESR 1** **D. Duration of symptoms§§** **<6 weeks 0** **≥6 weeks 1**

* The criteria are aimed at classification of newly presenting patients. In addition, patients with erosive disease typical of RA with a history compatible with prior fulfilment of the 2010 criteria should be classified as having RA. Patients with longstanding disease, including those whose disease is inactive (with or without treatment) who, based on retrospectively available data, have previously fulfilled the 2010 criteria should be classified as having RA.

† Differential diagnoses vary among patients with different presentations, but may include conditions such as systemic lupus erythematosus, psoriatic arthritis, and gout. If it is unclear about the relevant differential diagnoses to consider, an expert rheumatologist should be consulted.

‡ Although patients with a score of ☐ 6/10 are not classifiable as having RA, their status can be reassessed and the criteria might be fulfilled cumulatively over time.

§ Joint involvement refers to any swollen or tender joint on examination, which may be confirmed by imaging evidence of synovitis. Distal interphalangeal joints, first carpometacarpal joints, and first metatarsophalangeal joints are excluded from assessment. Categories of joint distribution are classified according to the location and number of involved joints, with placement into the highest category possible based on the pattern of joint involvement.

¶"Large joints" refers to shoulders, elbows, hips, knees, and ankles.

"Small joints" refers to the metacarpophalangeal joints, proximal interphalangeal joints, second through fifth metatarsophalangeal joints, thumb interphalangeal joints, and wrists.

** In this category, at least 1 of the involved joints must be a small joint; the other joints can include any combination of large and additional small joints, as well as other joints not specifically listed elsewhere (e.g., temporomandibular, acromioclavicular, sternoclavicular, etc.).

†† Negative refers to IU values that are less than or equal to the upper limit of normal (ULN) for the laboratory and assay; low-positive refers to IU values that are higher than the ULN but ≤3 times the ULN for the laboratory and assay; high-positive refers to IU values that are >3 times the ULN for the laboratory and assay. Where rheumatoid factor (RF) information is only available as positive or negative, a positive result should be scored as low-positive for RF. ACPA≡ anti citrullinated protein antibody.

‡‡ Normal/abnormal is determined by local laboratory standards. CRP = C-reactive protein; ESR = erythrocyte sedimentation rate.

§ § Duration of symptoms refers to patient self-report of the duration of signs or symptoms of synovitis

(e.g., pain, swelling, tenderness) of joints that are clinically involved at the time of assessment, regardless of treatment status.

1.1.4.2 Imaging

New radiological investigations facilitate RA diagnosis and monitoring. Unlike conventional X-ray, ultrasonography and magnetic resonance imaging (MRI) are highly sensitive in detecting soft tissue inflammation, effusion, and erosive joint destruction (Østergaard, Ejbjerg & Szkudlarek 2005; Østergaard, Pedersen & Døhn 2008).

1.1.4.3 Assessment of disease activity

Until recently, no cure was available for RA patients, with one way of mitigating patients' symptoms was to control the disease activity. Regular evaluation of the erythrocyte sedimentation rate (ESR) and/or C-reactive protein (CRP) is commonly performed to monitor the progression of RA. Other tools such as disease activity score and disability index score have, however, been widely and simultaneously used with the level of acute phase reactants.

1.1.4.3.1 Acute phase reactants

Increased ESR (Caruso et al. 1990; Dixey et al. 2004; Mottonen 1988; Scott et al. 1984) and/or CRP (Aman et al. 2000; Emery et al. 2007; Jansen et al. 2001; Jansen et al. 2000; Plant et al. 2000; van Leeuwen et al. 1993) correlate with high RA activity and disease outcomes. High ESR has been linked to destruction of joints and its progression with time as well as RA activity

(Caruso et al. 1990; Dixey et al. 2004; Mottonen 1988; Scott et al. 1984). While elevated ESR is associated with high RA activity and disability, elevated CRP is correlated to radiographic progression besides functional disability (Wolfe & Hawley 1998). Recent evidence suggests that there is no relationship between RA activity and the levels of acute phase reactants (Kay et al. 2014). In this observational study, almost half of the RA patients assessed showed no increase in the ESR or CRP despite the disease activity.

1.1.4.3.2 Rheumatoid factor and anti-CCP antibodies

For almost half a century, RF has been used as a marker for diagnosing RA. The sensitivity and specificity of RF analysed by first-generation enzyme-linked immunosorbent assay (ELISA) CCP1 is about 50–75% and 90–95% in diagnosing RA, respectively (Schellekens et al. 1998). Other studies, however, recorded higher sensitivity of RF for RA using CCP2 assay but similar specificity (Jansen et al. 2002). Although anti-CCP antibodies have shown a comparable sensitivity and specificity to RF, they are disease-specific antibodies found positive even before the onset of RA (Brink et al. 2015; Rantapaa-Dahlqvist et al. 2003). Anti-CCP antibodies are surrogate markers for not only diagnosing RA, but also for assessing disease progression (Krootde et al. 2000; Nishimura et al. 2007). RF is of more diagnostic value if it is combined with anti-CCP level. While RF might be detected in various diseases, anti-CCP is more specific for RA (Nishimura et al. 2007). Using anti-CCP as a marker for aggressive treatment approaches in early RA is still unclear. Moreover, the relationship between these antibodies and different therapies used to treat RA is still under investigation.

1.1.4.3.3 Disease activity score

Measuring RA activity is complex as this disease is multifactorial. There is no ‘gold standard’ quantitative tool to measure the RA activity (Pincus & Sokka 2004; van Riel & Schumacher 2001). In the 21st century, several disease activity measures were used ranging from joint count and hand grip to laboratory acute phase reactants (Blackburn 1994); however, having different endpoint measures is difficult for interpretation. Thus, a disease activity index, which combines all disease measures, has been developed for ease of interpretation (Boers & Tugwell 1993), and for detecting changes of disease activity over time (Cheah et al. 1996; Goldsmith, Smythe & Helewa 1993; Roberts 1993). This composite index is a measure of the level of RA activity presented as a score; with higher scores pointing to worse RA activity. Disease activity score using 28-joint count (DAS28) is an example of the composite index, which has been extensively validated and widely used to determine a patient’s disease activity (Aletaha & Smolen 2006;

Harrington 2009; Prevoo et al. 1995; Smolen et al. 1995; Soubrier & Dougados 2005; van Riel & Schumacher 2001). In Australia, DAS28 is the main tool used to assess RA activity based on acute phase reactant (i.e. ESR or CRP) (Littlejohn et al. 2015). This validated assessment tool is not only efficient but also easy to use. It considers the number of tender (TJC) as well as swollen joints (SJC); the 28 joints include: shoulders, elbows, wrists, fingers, and knees. Additionally, both visual patient global assessment analogue scale (PGA) assessing the global health of the patient and ESR are included in the calculation of the disease activity score (van der Heijde et al. 1990). The formula used to calculate the DAS28 is DAS28 = 0.56 * √(28TJC) + 0.28 * √(28SJC) + 0.70 * log [ESR] + 0.014 * PGA. The obtained score ranges from zero to ten. The disease activity is graded to remission, low, moderate and high disease activity if the DAS28 score is ≤ 2.6, 2.6 < DAS28 ≤ 3.2, 3.2 < DAS28 ≤ 5.1, and DAS28 >5.1, respectively. Although CRP can be used to calculate the DAS28, ESR is preferable and has been fully validated and used constantly in the research field (Hensor et al. 2010). ESR is better than CRP as incorporating CRP in DAS28 score calculation has been found to underestimate the actual disease activity and overestimate RA improvement (Matsui et al. 2007). A recent study, however, showed that using ESR and CRP is comparable (Nielung et al. 2015).

1.1.4.3.4 Stanford Health Assessment Questionnaire (HAQ)

RA activity and severity can be assessed using a validated index score such as the Stanford Health Assessment Questionnaire (HAQ) (Kirwan & Reeback 1986). The HAQ has two versions: short HAQ-DI (Disability Index) and full HAQ. It examines the patient's ability in performing 20 tasks of eight types of daily activities; these domains include dressing/grooming, rising, eating, walking, hygiene, reach, grip and errands/tasks. The ability of doing these tasks is classified into four grades ranging from 'unable to do it' to 'no difficulty in doing it'. Additionally, receiving assistance from people or by the aid of appliances is considered in calculating the final score. The assessment of these tasks is based on the patient's ability in that last week prior to the appointment. Patients' answers are rated from zero to three; zero indicates that there is no difficulty in doing the task; one represents mild difficulty in performing the task; two means there is much difficulty in completing the task; and three indicates that the patient is unable to do the task at all. Aids and devices such as cane and walker used to help in completing the tasks are included in the calculated score of HAQ. Moreover, aid from another person are also included. Visual analogue scales for pain and for general health are incorporated into the HAQ.

The scoring system for the HAQ is based on obtaining the highest score given to the tasks in each of the eight domains; then the total of the highest score is summed up. If the patient marked any one of the aids, the number of these aids is added into the highest score of the domain. The obtained score from each domain is then divided by eight, which is the total number of the main domains.

1.1.5 Complications of rheumatoid arthritis

Patients with RA are expected to suffer from numerous complications, including disability (Scott et al. 1987) with the latter frequently associated with work absence (Pincus et al. 1984; Rindfleisch & Muller 2005). The estimated percentage of Australians with RA who were absent from their work due to disability was 18% in 2004–2005 (Australian Institute of Health and Welfare 2009). In addition to functional disability, multisystem organ morbidities and mortalities have been associated with RA (Gabriel, Crowson & O'Fallon 1999a). One of the most common and serious complications affects the cardiovascular system, accounting for the higher mortality rate among RA population.

1.1.5.1 Morbidity

Rheumatoid arthritis is known to associate with extra-articular complications impacting cardiovascular, immune, respiratory, and renal systems. As RA has an immunosuppressive nature, aggravated by using medications such as glucocorticoids, RA patients are at risk of developing infections. For example, pneumonia and other interstitial lung diseases are common among those with RA (Horton 2004; Van Doornum et al. 2006) as are renal diseases (Van Doornum et al. 2006). Amyloidosis is one of the complications affecting the renal system as an influence of chronic inflammation associated with RA or as a complication of drug used (Owlia 2006). Most importantly, RA is associated with several cardiovascular (CV) morbidities (Avina-Zubieta et al. 2008) and this CV risk associated with RA will be discussed in detail later.

1.1.5.2 Mortality

The life expectancy of patients diagnosed with RA is shorter compared to the general population. The median life expectancy among Australian females and males diagnosed with RA is reduced by 6 and 7 years, respectively (Lassere et al. 2013). It has been estimated that mortality rates among RA patients start to increase after approximately 7 years since the diagnosis (Gabriel et al. 2003; Krootvan et al. 2000). Unfortunately, the mortality gap between the general population and patients with RA is widening (Gonzalez et al. 2007). The causes of death have been investigated since 1950s and, at that time, Cobb et al. (1953) was the first to

report the higher mortality rate in RA (Cobb, Anderson & Bauer 1953), mainly due to infections and renal diseases. Since then, the excessive mortality rate seen in patients with RA has been explained by several studies (Allebeck 1982; Allebeck, Ahlbom & Allander 1981; Callahan et al. 1996; Duthie et al. 1964; Gabriel, Crowson & O'Fallon 1999b; Isomaki, Mutru & Koota 1975; Jacobsson et al. 1993; Kvalvik, Jones & Symmons 2000; Lewis et al. 1980; Mitchell et al. 1986; Monson & Hall 1976; Mutru et al. 1985; Myllykangas-Luosujarvi, Aho & Isomaki 1995; Pincus et al. 1984; Prior et al. 1984; Reilly et al. 1990; Sokka, Mottonen & Hannonen 1999; Symmons et al. 1998; Uddin, Kraus & Kelly 1970; Wallberg-Jonsson, Ohman & Dahlqvist 1997; Wolfe et al. 1994).

Some of these studies supported the evidence that infection (Duthie et al. 1964; Hakoda et al. 2005; Myllykangas-Luosujarvi, Aho & Isomaki 1995; Uddin, Kraus & Kelly 1970), and renal diseases (Allebeck 1982; Monson & Hall 1976; Mutru et al. 1985; Prior et al. 1984; Vandenbroucke, Hazevoet & Cats 1984) contribute to a higher mortality rate. Other studies have reported that the higher mortality rate among RA is linked to respiratory diseases (Monson & Hall 1976; Prior et al. 1984), gastrointestinal illness (Allebeck 1982; Monson & Hall 1976), and neoplasms (Goodson et al. 2002). The latter study showed that women and men with RA were more likely to die from lung cancer and oesophageal cancer, respectively; but cardiovascular disease (CVD) was the most common cause of death. About 50% of mortality among RA patients has been attributed to CVD, mainly MI and stroke (Maradit-Kremers et al. 2005). Despite the advances in pharmacological management of RA, CVD is still the leading cause of death (Gabriel et al. 2003) and no improvement in the survival has been seen (Gonzalez et al. 2007).

1.1.6 Rheumatoid arthritis and dietary intake

RA is an inflammatory disease and although there is some evidence showing that specific type of food and dietary nutrients are “pro-inflammatory”, other nutrients improve inflammation in RA patients. The effects of antioxidants, unsaturated fatty acids, probiotics, and omega-3 fatty acids on inflammation and CV risk have been extensively studied. In this section the effect of these nutrients on inflammation is summarised, but its impact on CV risk will be discussed later.

Antioxidants including selenium, zinc, vitamin A, vitamin C, and vitamin E have shown to have anti-inflammatory effects in RA patients (Jalili et al. 2014). These effects are not limited to inflammatory markers, but also involve the disease activity measured by DAS28. Additionally, unsaturated fatty acid such as conjugated linoleic acids (CLAs) (Aryaeian et al. 2009), and

probiotics such as lactobacillus casei have shown beneficial effects similar to antioxidants (Alipour et al. 2014; Vaghef-Mehrabany et al. 2014).

The main food nutrient examined among RA patients is omega-3 fatty acids including docosahexaenoic acid (DHE), eicosapentaenoic acid (EPA), and docosapentaenoic acid (DPA), but the effect of these fatty acids on clinical symptoms of RA and inflammation is uncertain (Cleland et al. 1988; Kjeldsen-Kragh et al. 1992; Kremer et al. 1987; Kremer et al. 1990; Kromann & Green 1980; Nielsen et al. 1992; Skoldstam et al. 1992; van der Tempel et al. 1990). While the first meta-analysis published in 1995 showed a modest effect of omega-3 supplementation on inflammation in RA (Fortin et al. 1995), it was followed by a study claiming that there was no such an effect (MacLean et al. 2004). The study by Fortin et al. (1995) found that the use of omega-3 for at least 3 months significantly reduced the number of tender joints and the duration of morning stiffness. There was, however, no significant effect documented on other markers of RA activity, such as ESR, or number of swollen joints. In line with the finding by Fortin et al. (1995), a recent meta-analysis of control trails showed that omega-3 fatty acids can be considered as an adjunctive treatment for managing pain and morning stiffness in patients with RA (Goldberg & Katz 2007). Furthermore, it reduces not only the number of tender joints, but also the need to take analgesics if it was given in a dose of > 2.7 g/day (Goldberg & Katz 2007; Lee, Bae & Song 2012).

The observed reduction in inflammation and joint pain might be explained by the effect of omega-3 fatty acids on pro-inflammation. In RA, inflammatory symptoms (pain and tenderness of the synovial joints) are due to the activation of pro-inflammatory eicosanoids (i.e. prostaglandin E_2 and leukotriene B_4) (Smith et al. 1998). The availability of these eicosanoids stimulates the release of inflammatory cytokines such as TNF-α. The production of these pro-inflammatory markers can be competitively inhibited by omega-3 fatty acids (Calder 2006). As a consequence, the activation of inflammatory cytokines (TNFα and IL-1) is inhibited reducing the pain and inflammation in the joint.

1.2 Management approaches in rheumatoid arthritis

Early diagnosis of RA and prompt treatment are recommended and this management approach is vital to prevent further joint destruction and to avert systemic complications (Emery et al. 2002). The goal of treatment is not only symptomatic but also therapeutic in aiming to reduce inflammation and prevent relapses. This means that treating RA at an early stage (Huizinga &

Landewe 2005) via mitigating disease activity or achieving remission (McInnes & O'Dell 2010; Saag et al. 2008; Singh et al. 2012; Smolen et al. 2010) is vital. The current treatment guidelines include non-pharmacological and pharmacological approaches.

1.2.1 Non-pharmacological approaches

RA patients experience several symptoms ranging from mild arthralgia to severe disability. Therefore, non-pharmacological treatment is recommended to improve quality of life. As muscle weakness is one of the pathological features associated with RA, physical exercise including aerobic and strengthening exercises has been found beneficial (Stenstrom & Minor 2003). The evidence-base recommendations for aerobic and strengthening exercises suggest increasing the intensity of exercise gradually up to 60–85% of maximum heart rate and 50–80% of a maximal voluntary contraction, respectively.

1.2.2 Pharmacological approaches

1.2.2.1 Disease-modifying anti-rheumatic drugs

The guidelines for managing RA are evolving. Initially, the recommendation was to treat patients with monotherapy and mostly with traditional disease-modifying anti-rheumatic drugs (DMARDs); however, this has been recently changed due to the introduction of biologic DMARDs (Table 1-5). Currently, the concept of treating RA is based on reaching the treatment target goal, namely remission or low disease activity. New recommendations therefore advise initiating treatment aggressively by combining and even switching between different traditional DMARDs and biologic agents (McInnes & O'Dell 2010; Saag et al. 2008; Singh et al. 2012).

Table 1-5 Different types of traditional disease-modifying anti-rheumatic drugs (DMARDs) and biologic agents adopted from 2012 American College of Rheumatology recommendations

DMARDs	Biologics	
Hydroxychloroquine (Plaquenil) Leflunomide (Arabloc, Arava) Methotrexate (Hospira, Methoblastin) Minocycline (Minocin) Sulfasalazine (Pyralin EN, Salazopyrin, Salazopyrin EN Cyclosporine (Gengraf, Neoral, Sandimmune)	**Non-TNF**	**Anti-TNF**
	Abatacept(Orencia) Rituximab (Mabthera) Tocilizumab (Actemra)	Adalimumab (Humira) Etanercept (Enbrel)) Infliximab (Remicade) Certolizumab pegol (Cimzia) Golimumab (Simponi)

1.2.2.2 Nonsteroidal anti-inflammatory drugs

Traditional nonsteroidal anti-inflammatory drugs (NSAIDs) are most commonly prescribed for symptomatic relief of inflammation in RA, but not as a therapy preventing disease progression. Traditional NSAIDs act by inhibiting cyclooxygenase (COX) with COX1 being responsible for platelet aggregation and COX2 for inflammation (Grosser, Fries & FitzGerald 2006). As traditional NSAIDs such as ibuprofen cause many side effects, practice has shifted towards the use of selective COX2 inhibitors (e.g. Celebrex) associated with fewer side effects, especially on the gastrointestinal tract. Selective COX2 inhibitors have, however, been linked to higher CV risks (Kearney et al. 2006). Inhibiting the COX2 enzyme, which has an anti-thrombotic effect and can promote vasodilation, can be detrimental in the contex of maintained COX1 enzyme activity, which promotes vasoconstriction and thrombosis (Bunting, Moncada & Vane 1983). Nevertheless, traditional NSAIDs (Hernandez-Diaz, Varas-Lorenzo & Garcia Rodriguez 2006), and selective COX2 inhibitors have both been associated with CV adverse effects. As RA is associated with inflammation and higher risk of thrombosis and arterial stiffness, using selective COX inhibitors might increase the risk of CV events. Longer usage of such medications is therefore not recommended (Antman et al. 2007).

1.2.2.3 Glucocorticoids

Glucocorticoids are another class of medications that control inflammation and prevent disease progression. Despite providing symptomatic control, glucocorticoids can cause many side

effects, including weight gain, osteoporosis, muscle weakness, cataract (Saag, Kenneth G. et al. 1994) and, most importantly, hypertension (Panoulas et al. 2008). Additionally, glucocorticoids have been found to be associated with arterial stiffness and carotid plaque formation independent of the presence of traditional CV risk factors (del Rincon et al. 2004).

1.2.3 Recommendations for rheumatoid arthritis management

1.2.3.1 European League Against Rheumatism (EULAR) recommendations

Although RA management has improved over the last decades, some inconsistencies in the management approaches have been recently documented in the annual European Congress of Rheumatology (Schoels et al. 2010). This urged the European League Against Rheumatism (EULAR) to formulate algorithms for managing RA as early as possible (Fig. 1-2). The recommendations were divided into three phases in treating RA. In the first phase, patients should be immediately commenced on one of the synthetic DMARDs after RA diagnosis; the first drug of choice was MTX alone or combined with a low dose of glucocorticoids. The aim was to achieve low disease activity or remission within three to six months of initiating the treatment regime. If the patient failed to reach a remission status, phase two of the treatment plan should be considered. Phase two was based on the level of circulating autoantibodies (anti-CCP and RF). If the level of these autoantibodies was normal, one of the traditional DMARDs was added to the initial treatment plan. If there was an evidence of anti-CCP or RF elevation, however, an alternative approach was recommended where one of the biological DMARDs was combined with the treatment regime, TNF inhibitors being the most common drug of choice. If no response was achieved within six months, progressing to the third stage was advised. In this final stage, combination of both traditional and biological DMARDs was initiated to achieve the maximum efficacy and response rate.

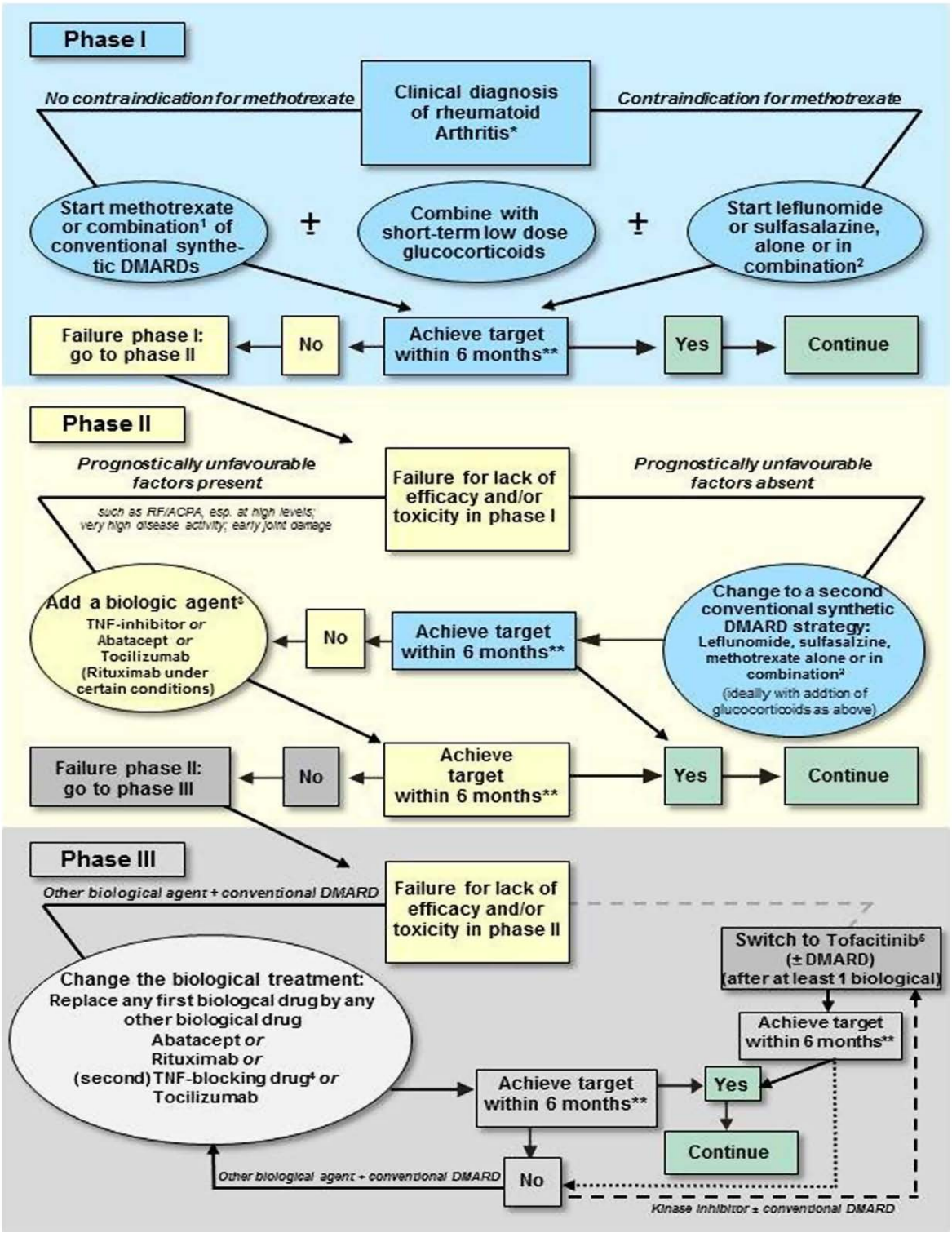


Figure 1-2 2013 European League Against Rheumatism recommendations on rheumatoid arthritis management

From Smolen et al. 2010, p.970

1.2.3.2 American College of Rheumatology recommendations

The American College of Rheumatology published extensive guidelines in 2008 on initiating conventional DMARDs or changing to biologicals in RA patients (Saag et al. 2008). In 2012, these recommendations for treating new and well-established RA were updated (Singh et al. 2012). The recommendations are based on the level of disease activity as well as prognostic features of the disease.

In early RA (less than 6 months), there were three main recommendations (Figure 1-3). First, if the patient presented with low, moderate or high disease activity, but had no evidence of poor prognostic features, DMARDs monotherapy was initiated. Second, if the patient showed moderate or high disease activity with poor prognostic features, DMARDs combined therapy was recommended. Third, in case of high disease activity and poor prognostic features, anti-TNF biologic was started with or without MTX.

Conversely, established RA (more than 6 months) should be managed differently (Figure 1-4). First, one of the following DMARDs: MTX, leflunomide or hydroxychloroquine, might be added to patients showing deterioration to moderate or high disease activity after three months of initiating DMARDs monotherapy, but still have no prognostic features. If patients showed no improvement after three months of such combination, adding another non-MTX DMARDs was recommended. Last, patient might be switched into one of the biologic DMARDs if disease activity deteriorated. The anti-TNF biologics, abatacept or rituximab were then recommended.

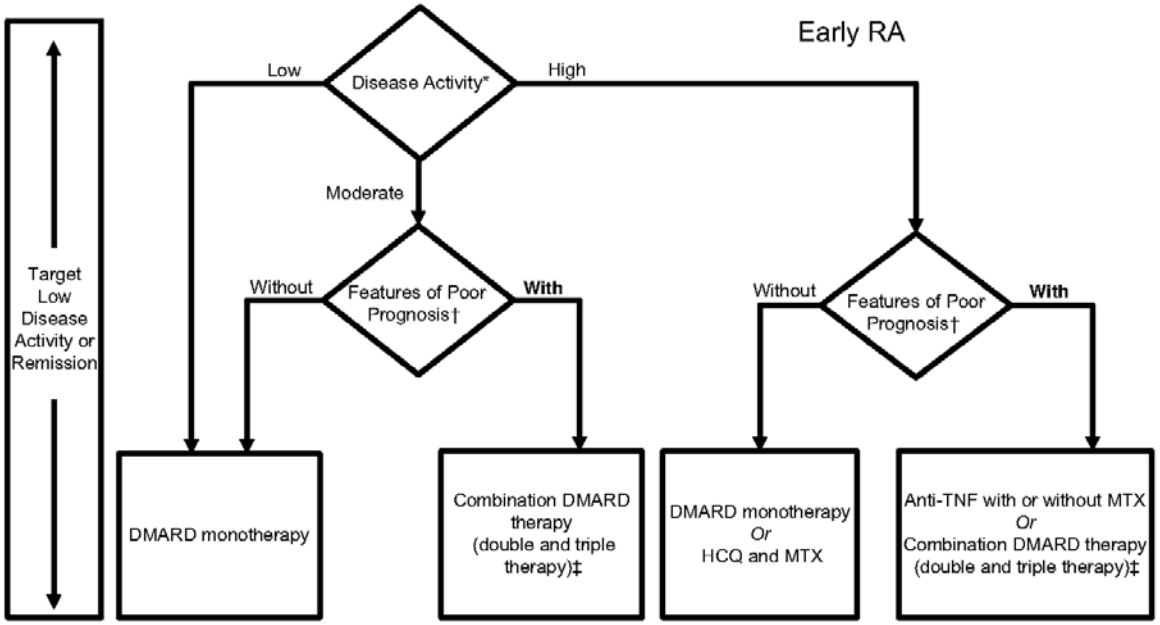


Figure 1-3 2012 American College of Rheumatology recommendations for treating early rheumatoid arthritis

From Singh et al. 2012, p. 632

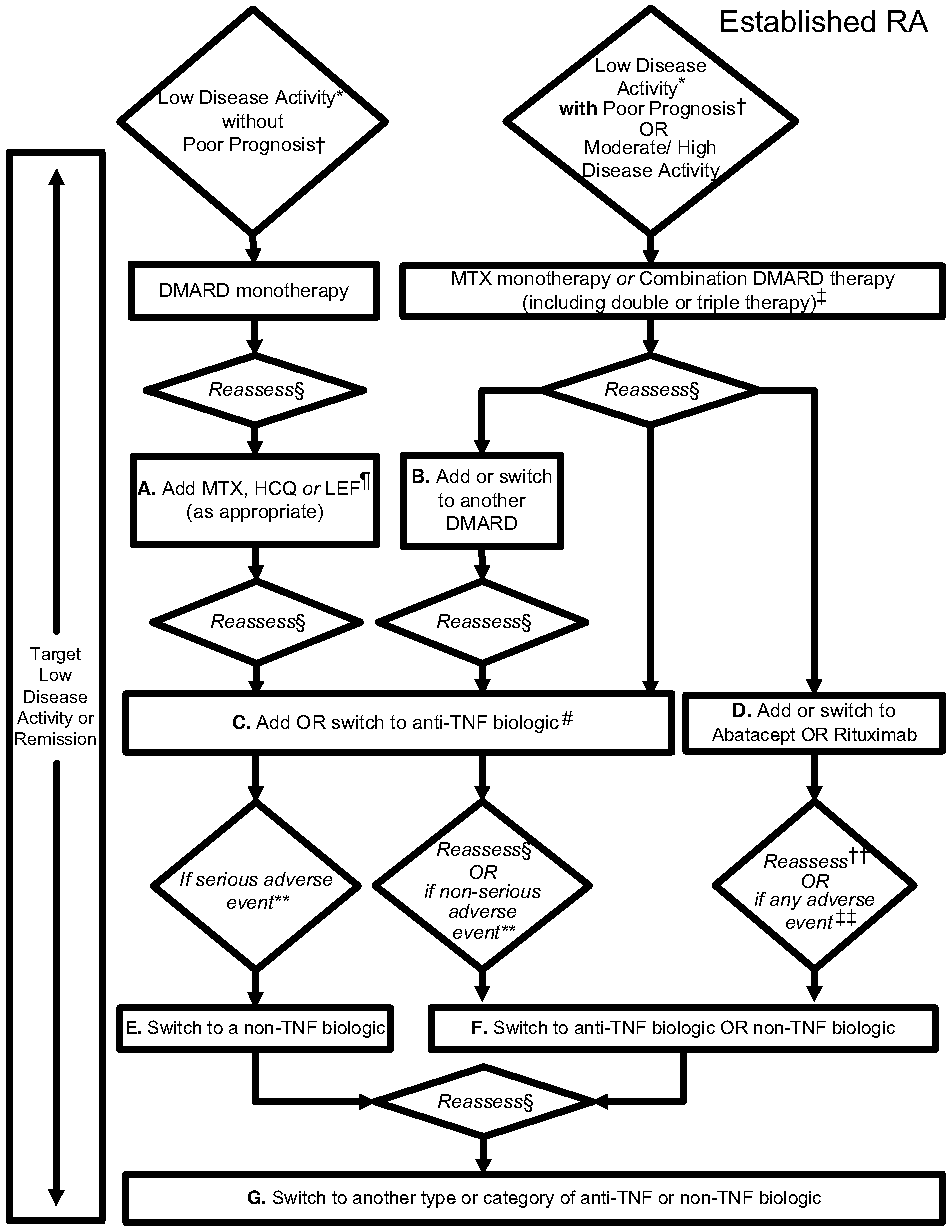


Figure 1-4 2012 American College of Rheumatology recommendations for treating established rheumatoid arthritis

From Singh et al. 2012, p. 633

In January 2016, the ACR published new recommendations for managing early and established RA (Singh et al. 2016) that are graded into strong and conditional recommendations (Figure 1-5). The recommendation is classified as strong if the panel was confident that benefits of following such recommendation outweigh the side effects. Conditional recommendations, in

contrast, indicate that following them might probably be beneficial and its effects most likely outweigh undesirable effects; therefore, sharing decision- making is mandatory in this situation.

	Strong recommendation	*Conditional recommendation*
Patients	**Most people in your situation would want the recommended course of action and only a small proportion would not**	*The majority of people in your situation would want the recommended course of action, but many would not**
Clinicians	**Most patients should receive the recommended course of action**	*Be prepared to help patients to make a decision that is consistent with their own values*
Policy makers	**The recommendation can be adapted as a policy in most situations**	*There is a need for substantial debate and involvement of stakeholders*

Figure 1-5 Grading of 2016 American College of Rheumatology rheumatoid arthritis management recommendations

From Singh et al. 2016, p. 6

The management approach for early and established RA overlaps to some extent. First, the ARC recommends using a “treat-to-target approach” regardless of the level of RA activity. Second, DMARDs monotherapy is strongly recommended for RA patients who are DMARDs naïve and have low disease activity. Conversely, combination therapy of DMARDs and biologics or anti-TNF (with or without MTX) is recommended for RA patients who are on DMARDs but who present with moderate or high disease activity. This recommendation includes RA patients who are taking glucocorticoids with DMARDs.

Despite similarities in the recommendations for managing both early and established RA, there are some differences depending on the disease activity. First, in early RA with moderate or high disease activity, the ACR conditionally recommends adding a low dose of glucocorticoids (i.e. ≤10mg/day) despite DMARDs or biologic drugs use. Aternatively, if RA patients are experiencing a disease flare, the ACR conditionally recommends commencing patients on the lowest dose of glucocorticoids for the shortest duration (i.e. less than three months).

In addition to this difference, in patients with established RA on TNF therapy and who have moderate or high disease activity, adding one or two DMARDs is strongly recommended. If those patients with established RA and on TNF monotherapy who are still suffering from moderate or high disease activity, adding one and then multiple non- TNF biologics is

conditionally recommended by the ACR. If the later management plan of adding multiple non-TNF biologics fails, the ACR conditionally recommends commencing a TNF drug. The next step of the management is to add another TNF drug if the patient is still showing moderate to high disease activity, but if this approach is unsuccessful then adding a non-TNF biologic is conditionally recommended. Finally, if RA patients are still suffering from moderate or high disease activity while they are on DMARDs or biologics therapy, commencing a low-dose glucocorticoid is conditionally recommended. It should be highlighted that all these recommendations for established RA include treatment with or without MTX. Additionally, the ACR strongly recommends that patients with established RA and in remission should continue taking their medications.

1.2.3.3 Rheumatoid arthritis response criteria

The ACR and EULAR response criteria are commonly used by rheumatologists to evaluate response to treatment or to monitor disease activity (Anderson et al. 2012; Felson et al. 1995; van Gestel et al. 1996). The ACR criteria include improvement of tender and swollen joints by 20% or more. Additionally, this response criteria consider an additional 20% improvement in the following measures: 1) acute phase reactants (CRP or ESR), 2) patient self-assessed function, 3) patient global assessment, and 4) patient assessment of pain (Felson et al. 1995). Improvement is classified into: ACR20, ACR50 and ACR70 indicating improvement in at least five of the above parameters by 20%, 50% or 70%, respectively. Conversely, baseline DAS28 has been incorporated in EULAR response criteria, where patients are categorised into non-responders, moderate or good responders (van Gestel et al. 1996).

1.2.4 Methotrexate in rheumatoid arthritis

Methotrexate (MTX) is one of the conventional DMARDs and, in the early 1950s, MTX was the treatment of choice for RA (Gubner, August & Ginsberg 1951). Its use was, however, minimal and not well-established for about 30 years after that report. In the 1980s, low dose MTX was prescribed as an anti-inflammatory drug for rheumatic diseases (Weinblatt et al. 1985; Williams et al. 1985), and it was considered the anchor drug for RA in the 1990s (Pincus, Cronstein & Braun 2010). Since then, low dose MTX has been the first-line treatment in managing patients with RA (Braun 2011). MTX is an anti-folate drug with low permeability and solubility. The chemical structure of MTX is composed of p-aminobenzoic acid, pteridine ring, and glutamic acid (2,4-diamino-N10-methyl propyl glutamic acid). MTX has a similar structure to folic acid except at the C4 carbon in the pteridine ring; in this location, the hydroxyl group, which is part of

folic acid structure, is substituted by the amine (Figure 1-6). To understand the pharmacology of MTX, both the kinetics and the transportation of MTX should be considered.

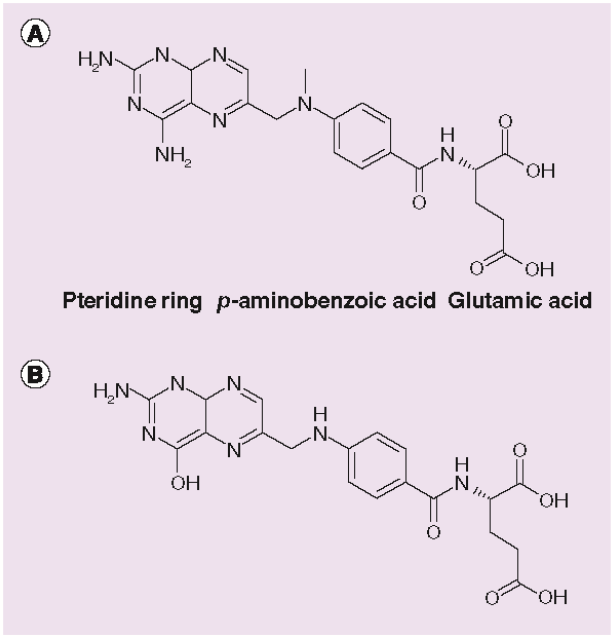


Figure 1-6 Methotrexate (A) and folic acid (B) chemical structure

From Lima et al. 2014, p. 1612

1.2.4.1 Pharmacokinetics

In RA patients, MTX is usually administered orally or intramuscularly, but it can also be given subcutaneously and is given as a single weekly dose ranging between 5 to 25 mg. The absorption of MTX is faster via the parenteral rout (Goodman, Cronstein & Bykerk 2015) but MTX absorption varies greatly between RA patients. After oral ingestion of MTX, absorption occurs in the gastrointestinal tract, specifically in the proximal jejunum. MTX absorption is limited as it is usually affected by food intake, drug ingestion (e.g. antibiotics) and conditions affecting gastrointestinal motility, such as constipation and diarrhoea (Tian & Cronstein 2007). Due to the variability in MTX absorption, the bioavailability of this anti-inflammatory drug ranges between 30% and 90% (Braun 2010; Hoekstra et al. 2004; van Roon & van de Laar 2010). The study by Hoekstra et al. (2004) noted that the bioavailability of MTX is reduced with increases in the oral dose. This decline in the bioavailability is due to the involvement of drug transporters (i.e. reduced folate carrier 1 [RFC1]) as this carrier has an action in regulating MTX transportation from the gastrointestinal tract (Matherly & Goldman 2003). In the liver, the small dose of MTX is converted into 7-hydroxymethotrexate (7-OH-MTX) (Figure 1-7); 90–95% of this

form of MTX is bound to plasma albumin, as compared with parental MTX (about 50% albumin bound) (Tian & Cronstein 2007). MTX is mainly eliminated in urine but a small amount of this drug is excreted in the bile (Swierkot & Szechinski 2006; Tian & Cronstein 2007). Tubular urinary excretion of MTX is facilitated by an organic anion transport system (Sekine et al. 1997; Williams & Huang 1982). Many transporters are identified, including organic anion transporter-1 and 2 (OAT1 and OAT2) (Sekine et al. 1998; Sekine et al. 1997), multidrug resistance associated protein 1 and 2 (MRP1 and MDRP2) (Hooijberg et al. 1999; Schaub et al. 1997), and kidney-specific organic anion transporters (OAT-K1 and OAT-K2) (Masuda et al. 1999; Saito, Masuda & Inui 1996). When MTX reaches the proximal tubule in the kidneys, the secretion process occurs where 90% of this drug is excreted in urine in an unchanged form (Mikkelsen et al. 2011). Usually, the serum half-life of MTX drug ranges between 6 and 8 hours after ingestion; however, this time might increase in a few conditions. As MTX is mainly cleared by the kidneys, renal impairment, where glomerular filtration rate is reduced, and use of NSAIDs might increase the half-life; this slow elimination process then leads to a higher possibility of drug toxicities. Nevertheless, recent evidence has shown that concurrent use of NSAIDS and MTX is safe in RA patients if drug toxicity is closely monitored (Colebatch et al. 2012). Being polyglutamated inside the cell, MTX polyglutamates (MTXPGs) have the ability to inhibit aminoimidazole carboxamide ribonucleotide transformylase (ATIC).

MTX can be transported in and out of the cells via influx and efflux transporters, respectively. The RFC1—also known as SLC19A1—is used by MTX as a vehicle to enter the cell (Cannella & O'Dell 2012; Dalrymple et al. 2008). As soon as MTX enters the cell, it is converted from inactive monoglutamate form to the biologically active polyglutamate form (MTXPGs) by the effect of the folypolyglutamyl synthetase (FPGS) enzyme (Cannella & O'Dell 2012). The efflux of MTX from the cell is mediated by ATP-binding cassette transporters (ABC). ABCC2, ABCB1 and ABCG2 are responsible for transporting MTX from the cell to the intestinal tract, but ABCC1 and ABCC3 transport MTX to the bloodstream (Inoue & Yuasa 2014).

1.2.4.2 Pharmacodynamics

1.2.4.2.1 Methotrexate and anti-inflammatory mechanism of action

Inside the cells, MTX plays an important role in controlling inflammation in RA. First, MTX interacts with enzymes using folate as a cofactor (Cutolo et al. 2001). These cellular enzymes include dihydrofolate reductase (DHFR), 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (AICAR), and thymidylate synthase (TS). Inhibiting DHFR enzyme inhibits purine

and pyrimidine synthesis, which subsequently inhibits deoxyribonucleic acid (DNA) synthesis, especially in inflammatory cells associated with RA. Additionally, a high concentration of AICAR enzyme plays a role in increasing not only the metabolism of purine, but also the extracellular release of adenosine (Cutolo et al. 2001). Being polyglutamated inside the cell, MTXPGs have the ability to inhibit 5-aminoimidazole-4-carboxamide ribonucleotide formyltrans-ferase (ATIC). This inhibition leads to an increase in adenosine levels, not only intracellularly but also extracellularly (Figure 1-7). Although adenosine can interact with most of the receptors located on inflammatory cells, the main site of interaction occurs with the A2 receptor; this receptor has been found to be associated with a different type of genetic polymorphisms that influence the release of adenosine and subsequently its anti-inflammatory effects (Cutolo et al. 2001). Therefore, adenosine has been considered a powerful inhibitor of inflammation in RA (Varani et al. 2010).

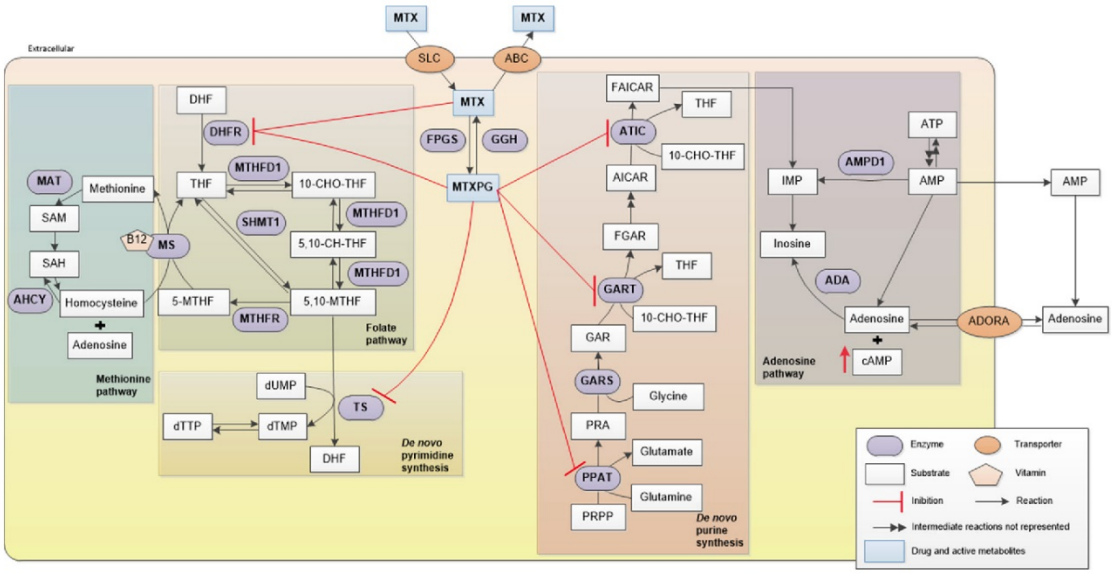


Figure 1-7 Methotrexate mechanism of action in rheumatoid arthritis

From Romao et al. 2014, p. 292

There are several mechanisms of MTX in controlling inflammation (Figure 1-8) (Cutolo et al. 2001). These include the following: a) inhibiting the growth of monocyte by increasing its apoptosis; b) reducing the availability of IL-1,2 and 6, TNF-α and interferon gamma; b) inhibiting the synthesis of COX2 (indirect action); and d) modulating cytokines, which indirectly inhibits the production of metalloproteinase (MMP) by stimulating their tissue inhibitors (TIMP). In active RA, the level of homocysteine is increased by MTX, which subsequently inhibits the growth and

the proliferation of most inflammatory cells (van Ede et al. 2002). In addition to reducing inflammatory cytokines, MTX inhibits several adhesion molecules (Tian & Cronstein 2007). Reducing inflammatory cytokines has, however, been associated with CV benefits in animal models; inhibiting cytokines, mainly IL-6, was associated with a lower risk of cardiac fibrosis (Zhang et al. 2009). It also enhances the blood flow and improves vascular function (Noguchi et al. 2011); while blood flow was increased by releasing adenosine, better vascular function resulted from a higher production of nitric oxide (NO). Furthermore, MTX exerts an atheroprotective effect in RA modulated via adenosine A2 receptor activation promoting the efflux of cholesterol (Reiss et al. 2008). Therefore, the mechanisms of anti-inflammatory effects of MTX used in RA are complex, showing also potential CV benefits.

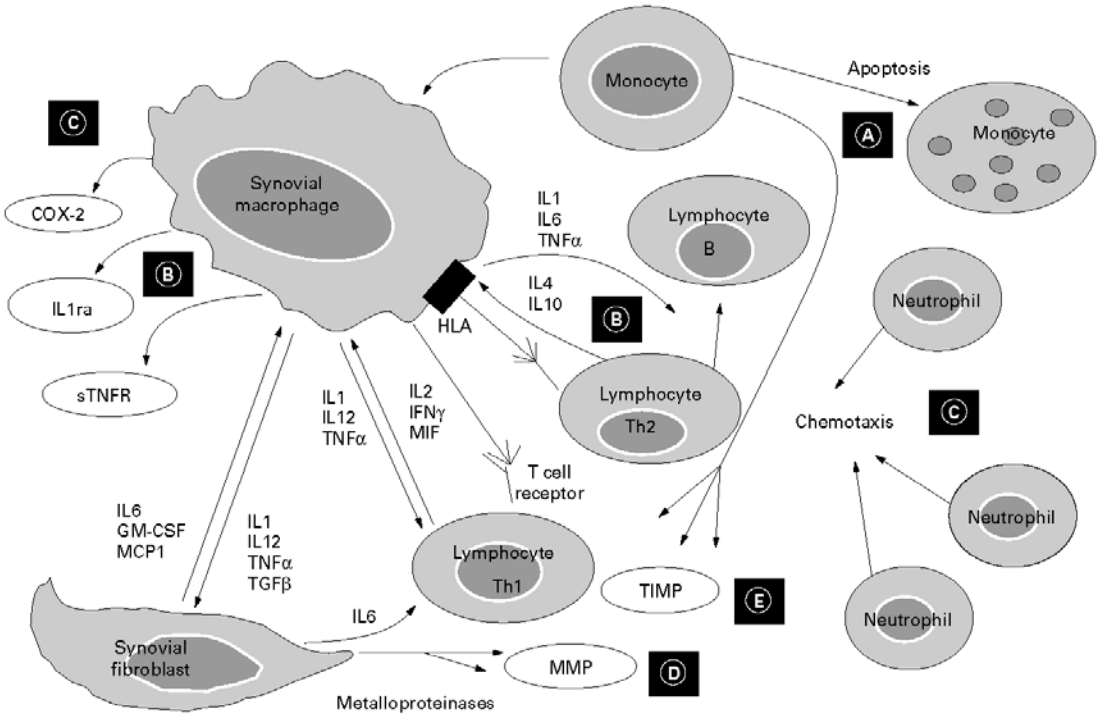


Figure 1-8 Anti-inflammatory effects of methotrexate in rheumatoid arthritis

From Cutolo et al. 2001, p. 731

1.2.4.2.2 Intracellular methotrexate polyglutamates concentration

The elimination half-life of MTX is important in determining MTXPGs concentration. When the concentration of MTXPGs inside the red blood cells (RBCs) reaches 90% (known as steady state) it is the ideal time for measuring the accurate level of MTX in the blood (Dalrymple et al. 2008). The median half-life of this accumulation in patients with RA has been found to be

between 1.9 to 45.2 weeks, however, MTX becomes undetectable in the RBCs after 15 weeks. The relationship between MTXPGs and RA activity is arguable. Some studies have claimed that there is no correlation between the disease activity and MTXPGs, subsequently, defining the optimal treatment would be difficult (Lafforgue et al. 1995). Yet other studies have shown that concentration of MTXPGs is correlated to the level of RA activity (Angelis-Stoforidis, Vajda & Christophidis 1999; Dervieux et al. 2005; Dervieux et al. 2004). Lower levels of MTXPG concentration measured in the RBCs (40 nmol/l) were found to be associated with a higher number of painful and tender joints, and a higher score of disease activity obtained by health assessment questionnaire (Dervieux et al. 2005).

In RA, there are several factors potentially affecting the intracellular concentration of MTXPGs. These include age and gender of the patients, disease activity, dose and duration of taking MTX, smoking history, the level of estimated glomerular filtration rate (eGFR) and anti-CCP or RA factors, BMI, and taking DMARDs, or prednisolone, or NSAIDs (Stamp et al. 2009). Increased age, higher dose and longer duration of using MTX and using prednisolone were found to be associated with higher concentration of MTXPGs. Conversely, lower concentration of MTX was associated with smoking and using NSAIDs, but understanding the influence of these factors on MTX concentration is complex and still to be explored.

1.2.4.2.3 Therapeutic effects of methotrexate in rheumatoid arthritis

RA is a progressive disease and therefore treat-to-target strategy is mandatory to prevent joint destruction and extra-articular complications (Steunebrink et al. 2016). There is a window in early RA to prevent such complications if adequate treatment is initiated. Low dose MTX is well-known as a gold standard treatment for RA, indeed, most combination therapy has included MTX (Furst 1997). There is evidence that commencing patients on MTX at the early stage of RA resulted in remission and less frequent flare-ups (Li et al. 2016). Moreover, MTX was associated with better tenderness and swelling scores, and pain analogue scale as compared to placebo (Furst 1997). Compared to RA patients on leflunomide, those on MTX showed greater improvement in their symptoms in the long term (Donahue et al. 2008).

1.2.4.3 Side effects

MTX can cause side effects among RA users (Schnabel, Armin & Gross 1994) and these contribute to up to 30% of MTX discontinuation (Wolfe, Hawley & Cathey 1990). Most common possible side effects involve the gastrointestinal tract (GIT) namely, nausea, vomiting, diarrhoea, and mouth ulcers. Side effects involving the GIT occur during the first two years at a

rate ranging between 20% and 70% (Schnabel, Armin & Gross 1994) and are dose-dependent (Goodman, Cronstein & Bykerk 2015). It was found that higher doses of MTX (≥25 mg/week) were associated with a significant higher rate of GIT side effects compared to the initial dose of 15 mg/week (28% vs 17%, $P < 0.05$) (Schnabel et al. 1994); taking folic acid supplement usually reduces the rate of developing these side effects. Other common side effects include dry skin or rash (20%), tiredness and headache (30%). Rare, but possible side effects, might affect the following: blood cells causing leukocytopenia or thrombocytopenia (22%), the liver (70%) and the lung (25%) (Schnabel, Armin & Gross 1994).

The most common liver toxicity is elevated liver enzymes. Typically, the transaminases are increased but do not exceed three times the normal level. In most of the cases, this elevation is transient and regresses spontaneously (Schnabel, Armin & Gross 1994), however, persistent elevation of any of the liver enzymes which exceeds three times the normal values mandate MTX discontinuation. Nevertheless, the rate of MTX withdrawal due to liver enzyme elevation is low in many studies (<5%) (Fehlauer et al. 1989; Singal, Chaturvedi & Brar 2005). Liver cirrhosis is another hepatic toxicity from using MTX (Zachariae 2000; Zachariae, Sogaard & Heickendorff 1996). Cirrhosis induced by MTX is, however, relatively benign and induces significantly less damage to the liver histology in the first 2 to 3 years of using MTX (Thomas & Aithal 2005; Zachariae 2000). Usually, the histopathological findings are stable on repeated hepatic biopsies (Zachariae, Sogaard & Heickendorff 1996). Alcohol abuse (≥ 2 standard alcohol drinks per day) is more important than an accumulative dose of MTX in determining the liver damage and its progression in RA (Whiting-O'Keefe, Fye & Sack 1991); this meta-analysis reported that heavy drinkers had advanced liver histopathology (17.8% vs 4.5%, $p = 0.0003$), which was progressive (73.3% vs 25.9%, $p = 0.0002$).

Pulmonary side effects of MTX range between non-progressive symptoms and progressive lung disease. Most RA patients who develop cough and dyspnoea and wheezing recover quickly after MTX discontinuation (Schnabel, Armin & Gross 1994). MTX might, however, induce fatal hypersensitivity reactions such as pneumonitis, which requires immediate assessment and treatment; compared to liver toxicity, pneumonitis is less predictable (Saravanan & Kelly 2004). The best approach to minimise progression to pulmonary fibrosis is to stop MTX once pneumonitis is suspected (Saravanan & Kelly 2004). A prevention technique was proposed to minimise the occurrence of pneumonitis (Saravanan & Kelly 2004) that included screening for lung illnesses by conducting lung function tests before commencing RA patients on MTX. Another lung disease associated with MTX use in RA is interstitial lung disease. This disease

ranges between subclinical lung inflammation to fatal lung fibrosis (Ascherman 2010; Carson et al. 1987). Additionally, lower respiratory infections such as infectious pneumonia and bronchitis are MTX side effects (BarreraLaan, et al. 1994; BarreraVan Ede, et al. 1994; Roux et al. 1996; Wolfe, Caplan & Michaud 2006). The risk of acquiring pneumonia is increased among RA patients on prednisone (Wolfe, Caplan & Michaud 2006).

There are many risk factors that aggravate the development of most of these side effects (Table 1-6). For example, renal insufficiency increases the rate of developing all side effects as the main route of MTX excretion is via renal tubules. RA patients are prone to immune suppression and this makes them more susceptible to infection; additionally, this might be aggravated by using MTX, which is associated with low count of white blood cells (WBCs) (McLean-Tooke et al. 2009); this might explain the recorded high risk of infection among RA patients on MTX (Greenberg et al. 2010). The relative risk of infection requiring hospitalisation is 1.10 (95% CI 0.98, 1.23) (Bernatsky, Hudson & Suissa 2007). The pulmonary system might also be affected with the risk of respiratory infection found to be higher among MTX users recording an incident rate of 38.9/1,000 person years (Cannon et al. 2004). Patients on MTX not only do show a higher risk of hepatitis, but also of liver cirrhosis (Diouf et al. 2001; Whiting-O'Keefe, Fye & Sack 1991). Furthermore, the risk of lymphoma increases with time among MTX users (Wolfe & Michaud 2004b). One of the MTX toxicities is related to a high concentration of homocysteine in the blood, with a consequent increase in the CV risk among RA patients (Boers 2000; Eikelboom et al. 1999; Welch & Loscalzo 1998).

Table 1-6 Risk factors aggravating side effects of methotrexate in rheumatoid arthritis

From Schnabel, Armin & Gross 1994

Risk factor	Effect
Renal insufficiency	Increased risk for all side effects
Regular alcohol consumption	Increased hepatotoxicity
Pre-existing leukocytopenia	increased myelotoxicity
Viral infection	increased myelotoxicity
Pre-existing interstitial lung disease	Increased pulmonary toxicity
Major trauma or surgery	Impaired wound healing
Peptic ulcer	Impaired healing

1.2.4.4 Contraindications

Most of the contraindications to MTX use were adopted from observational studies and randomised controlled trials, according to the American College of Rheumatology 2008 recommendations (Saag et al. 2008). MTX contraindications can be grouped into five categories: infection, liver injury, haematological or oncological disorders, renal injury, and pregnancy. First, as infection is one of MTX side effects, it is contraindicated to initiate or continue on this anti-folate drug in cases of acute serious bacterial infection or active life-threatening fungal infection. Other serious infections such as herpes zoster and latent tuberculosis are also contraindications. The presence of clinical interstitial pneumonitis, as well as pulmonary fibrosis, are contraindications to the use of MTX in RA patients. Second, patients with liver disease should not use MTX; for example, MTX is contraindicated in acute hepatitis B or C and in patients with higher transaminase levels. Moreover, MTX use is contraindicated in haematological or oncological diseases such as leukopenia, thrombocytopenia, myelodysplasia and lymphoproliferative. As the primary clearance of MTX is via the kidneys, renal impairment with a creatinine clearance of 30 ml/minute or less has been considered as a contraindication

for MTX use. Categorised as level C, the MTX drug is contraindicated in women planning for pregnancy or currently pregnant or breastfeeding.

1.2.4.5 Methotrexate cellular pathway

The MTX cellular pathway helps us to understand the MTX mechanism of action (Figure 1-9). As MTX is a folate analogue, it competes with folate for RFC1 transporter (i.e. SLC19A1) on the cell membrane to be transported into the cells. The MTX glutamation process is reversible. The ϒ-glutamyl hydrolase enzyme (GGH) is able to catalyse the removal of polyglutamates link and subsequently the efflux of MTX. The polyglutamated form of MTX has several functions (Ranganathan et al. 2008). First, it plays an important role in retaining MTX inside the cells leading to better MTX efficacy. Second, it has the ability of inhibiting folate dependent enzymes directly. For instance, inhibiting thymidylate synthase (TYMS), ATIC, and dihydrofolate reductase resulted in releasing adenosine, having a powerful anti-inflammatory effect, and interrupting the synthesis of purine and pyrimidine, respectively (Ranganathan et al. 2008).

To exit the cell, MTX uses ABC family transporters. These adenosine triphosphate (ATP) binding transporters are composed of 48 proteins divided into ABC A to G subclasses (Borst & Elferink 2002). ABCB1, ABCC1-4 and ABCG2 are the main ABC subclasses, which help in efflux MTX from the cell (Lima et al. 2014; Takano, Yumoto & Murakami 2006).

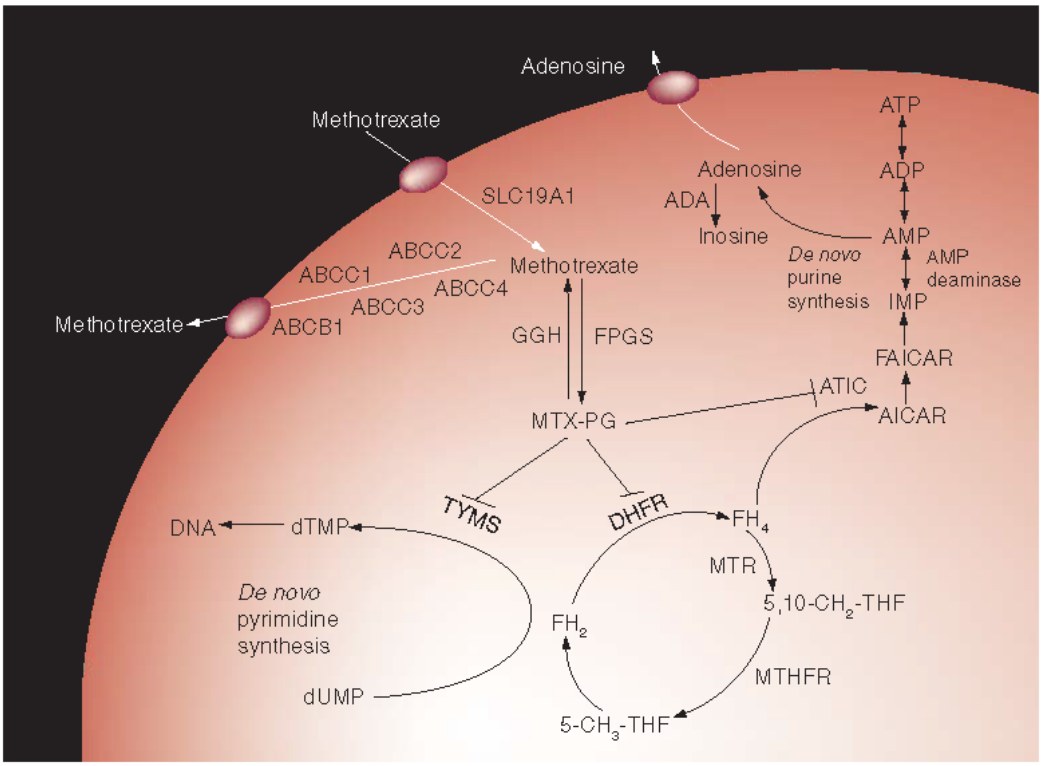


Figure 1-9 Cellular pathway of methotrexate

From Ranganathan 2008, p 441.

SLC19A1 = solute carrier family 19, member 1 (also known as reduced folate carrier); ABCB1, ABCC1-4 = ATP-binding cassette transporters; GGH = γ-glutamyl hydrolase; FPGS = folylpolyglutamate synthase; MTX-PG = MTX polyglutmate; TYMS = thymidylate synhase; dUMP = deoxyuridine monophosphate; dTMP = deoxythymidine monophosphate; DHFR = dihydrofolate reductase; FH2 = dihy-drofolate; 5-CH3-THF = 5-methyltetrahydrofolate; MTHFR = methylenetetrahydrofolate reductase; 5, 10-CH2-THF = 5, 10-methylenete-trahydrofolate; MTR = methyltetrahydrofolate reductase; AICAR = aminoimidazole carboxamide ribonucleotide; FAICAR = 10-formyl AICAR; ATIC = AICAR transformylase; IMP =inosine monophosphate; AMP = adenosine monophosphate; ADP = adenosine diphosphate; ATP = adenosine triphosphate; ADA = adenosine deaminase.

1.2.4.6 Methotrexate pharmacogenetics

Although MTX is an anchor drug for RA, its exact mechanism as a disease-modifying drug is still not fully understood; the anti-proliferative and immunosuppressive effects of MTX are known to be linked to several enzymes in the folate pathway (Brinker & Ranganathan 2010). Despite the anti-inflammatory effects of MTX, some RA patients do not achieve adequate disease control despite being on treatment for a prolonged period. This indicates that there is variability in MTX efficacy among RA patients. Response to MTX varies between 46% and 65% (Bathon et al. 2000; Strand et al. 1999) based on ACR response criteria (Felson et al. 1995). Differences in responses to the same drug among individuals could be partially inherited. Such inherited variability among MTX users might be related to gene polymorphisms known as

"pharmacogenetics" (Brinker & Ranganathan 2010). Therefore, polymorphisms in the genes encoding enzymes responsible for MTX metabolism or transmembrane transportation might influence the efficacy of this drug.

1.2.4.7 Genetic polymorphisms of transporters involved in methotrexate metabolism

1.2.4.7.1 SLC19A1 (RFC)

SLC19A1 is a solute carrier family 19, member 1, which is known as RFC. It plays a major part in transporting MTX inside the cell. It is encoded by the *SLC19A1* gene located on chromosome 21q22.3. Polymorphisms in this gene lead to inactivation or malfunction of the transporter. The most common polymorphism of SLC19A1 transporter is rs1051266 leading to a change of G to A at position 80 (80G>A) (Chango et al. 2000). Until recently, the effect of such polymorphism has remained controversial. A vitro study, however, showed that MTXPGs level were lower in RA patients with *SLC19A1* 80A/A genotype compared with RA patients with other genotyping (i.e. 80G/G and G/A) (Dervieux et al. 2004; Dervieux et al. 2004). As this polymorphism leads to reduced RFC activity, MTX transportation into the cell is reduced.

In another study by the same research group, RA patients with *RFC* 80A/A genotype showed well-controlled disease activity compared with other genotypes; they reported not only lower numbers of swollen joints, but also lower pain scores on a visual analogue scale (Dervieux et al. 2005). While some studies claim that A homozygotes had no effect on MTX toxicity (Chatzikyriakidou et al. 2007; Dervieux, Greenstein & Kremer 2006; Drozdzik et al. 2007; Owen et al. 2013; Plaza-Plaza et al. 2012; Stamp et al. 2010; Takatori et al. 2006; Wessels et al. 2006), others have found that RA patients with such single-nucleotide polymorphisms (SNPs) are at lower risk of overall MTX toxicity (Bohanec Grabar et al. 2012; Bohanec Grabar et al. 2008). Conversely, Wessels et al. (2006) claim that there is no association between MTX efficacy and *RFC* genotype. G homozygotes (80G/G) are found to be associated with low influx of MTX in lymphocytes (Baslund, Gregers & Nielsen 2008) and this type of SNPs shows a negative functional impact on the level of intracellular MTXPGs. It has been found that MTXPGs' concentration in RBCs is low among those with G homozygotes (Dervieux et al. 2004)—other SNPs of *RFC* included rs1131596. In this form of SNPs, T is replaced by C nucleobase (Chatzikyriakidou et al. 2007). The expression of RFC transporter is found to be reduced in RA patients, especially in lymphoblastic cells, with C homozygotes (Bohanec Grabar et al. 2012); therefore, the influx capability of this membrane transporter is decreased (Chatzikyriakidou et al. 2007). While this leads to less overall MTX toxicity (Bohanec Grabar et

al. 2012), Chatzikyriakidou et al. (2007) found no such low level of MTX toxicity among C homozygotes carriers.

1.2.4.7.2 ABCB1

ABCB1 is also known as P-gp or MDR1. The gene encoded for this unidirectional transporter is located on chromosome 7q21. This transmembrane protein transporter is expressed in the apical membrane of endothelial cells and peripheral blood lymphocytes. Its function is mainly related to drugs absorption and distribution (Stamp et al. 2010). ABCB1 has been found to play a role in MTX transportation (Kim 2002). Patients with refractory RA have shown higher expression of ABCB1 compared with those patients with well-controlled RA and taking DMARDs (Diaz-Borjon et al. 2000). The most common and studied SNP of *ABCB1* is rs1045642. At position 3435 of the chromosome, C nucleobase of amino acid is substituted by T leading to SNPs in the transporter. This change of the structure of the ABCB1 leads to impairment in the efflux function of this transporter. As a result, MTX accumulates inside the cells leading to a higher concentration of this drug (Wang et al. 2005). Such high MTXPGs' concentration is associated with good response in RA patients with T homozygotes. While Takatori et al. (2006) claimed that T homozygotes SNPs of *ABCB1* are associated with nonresponse to MTX in RA patients, other studies show improvement in MTX response in RA patients, who are on MTX monotherapy (de Rotte et al. 2012; Kato et al. 2012; Moya et al. 2016), or on MTX combined regimen (de Rotte et al. 2012), or on sulfasalazine (Pawlik et al. 2004).

1.2.4.7.3 ABCC1-4

1.2.4.7.3.1 ABCC1

The ABCC1 transporter is known as MRP1. This polytopic membrane protein is encoded by the *ABCC1* gene located on chromosome 16p13. The basolateral plasma membrane of the intestinal tract is the most common location of this membrane transporter (Takano, Yumoto & Murakami 2006). Additionally, there is some evidence that this membrane transporter is found in synovial fluid macrophages in RA patients and is expressed on $CD3^{+}$ T cells lymphocytes (van der Heijden et al. 2009). Its main function is to transport different drugs, including MTX (Breuninger et al. 1995). The impact of SNPs of this transporter is still controversial, however, the most common polymorphism of *ABCC1* is rs35592, which includes substituting T for C. Even though few studies showed no association between this SNPs and therapeutic outcomes of MTX (de Rotte et al. 2012; Stamp et al. 2010), others have found that RA patients, who were C carriers, showed no response to MTX therapeutic dose (Warren et al. 2008). Similarly, the

impact of other types of SNPs of *ABCC1*—including rs246240, rs2074087, and rs3784864—is still unknown. There is, however, some evidence that rs2230671 SNP might alter the function of ABCC1 transporter. In this type of SNPs, the G is substituted by A nucleobase; where G homozygous was found to associate with a higher rate of MTX efflux (Lee et al. 2010; Lima et al. 2014).

1.2.4.7.3.2 ABCC2

ABCC2 is an efflux pump known as MRP2 or cMOAT1 (Takano, Yumoto & Murakami 2006). It is encoded by the *ABCC2* gene located at chromosome 10q24. The apical membrane of epithelial cells of kidneys and small intestine, as well as hepatocytes, are the main locations of this transporter. Similar to other transporters in the ABCC family, the impact of SNPs of the gene encoding for the *ABBC2* is still unclear. There is emerging evidence that the ABCC2 influences MTX pharmacokinetics as it determines the elimination of MTX and its metabolites (i.e. 7-OH-MTX) (Vlaming et al. 2009). Patients with *ABBC2* mutations (heterozygous) showed 7OH-MTX accumulation in the kidneys and liver. In rs717620 SNPs, the G nucleobase is substituted by A nucleobase at 5-UTR. Although there is some evidence that A homozygotes carriers show a higher clearance of drugs (Fromm et al. 2000), others found that such SNPs have no impact on neither MTX clinical response (de Rotte et al. 2012; Kato et al. 2012) nor its toxicity among RA patients (Ranganathan et al. 2008).

1.2.4.7.3.3 ABCC3

This transporter is known as MRP3 or cMOAT2. The *ABCC3* gene located on 17q22 chromosome encodes it. The ABCC3 transporter is presented in the liver and the small intestine basolateral plasma membrane. In the absence of the ABCC2 transporter, the ABCC3 takes over the function of transporting MTX and its metabolites (i.e. 7-OH-MTX). It transports MTX across the sinusoidal membrane of the hepatocyte and releases it to the circulation. Thus, it provides an alternative route of excreting MTX through urine (Vlaming et al. 2008). The well-known SNP is rs4793665, where C nucleobase is altered to T at 5-UTR location. A pharmacogenetics study reported that T homozygotes are associated with better MTX response in rheumatic diseases (de Rotte et al. 2012).

1.2.4.7.3.4 ABCC4

ABCC4 plays a major role in transporting MTX and folate outside the cell. It is also known as MRP4, which is encoded by the *ABCC4* gene on chromosome 13q32.1. There is high expression of this transporter in the liver as well as the kidneys (van Aubel et al. 2002). This

renal transporter has an affinity for MTX, which facilitates renal clearance of MTX (Mikkelsen et al. 2011). Therefore, SNPs in *ABCC4* have been found to be associated with delayed clearance of MTX (Hulot et al. 2005; Rau et al. 2006; Vlaming et al. 2009). Until recently, there is no study examining the effect of *ABCC4* SNPs on therapeutic outcomes of MTX in RA.

1.2.4.7.4 ABCG2

ABCG2 is known as BCRP or multidrug resistance efflux transporter ATP-binding. It is encoded by the *ABCG2* gene located on 4q22 chromosome (Takano, Yumoto & Murakami 2006). It is present in the apical membrane of various tissues namely, hepatocytes, renal cells, and enterocytes (Stamp et al. 2010; Takano, Yumoto & Murakami 2006; van Aubel et al. 2002). The prime function of this membrane transporter is to reduce excessive exposure to medications and toxins. Thus, the availability of ABCG2 transporter is associated with reduced response to DMARDs (van der Heijden et al. 2009). Additionally, ABCG2 plays a role in eliminating MTX and its metabolites in the absence of ABCC2 (Vlaming et al. 2009). Most importantly, ABCG2 transports not only MTX, but also its plolyglutamates, specifically polyglutamate 2 and 3 (Volk & Schneider 2003). The most common SNP is rs2231142. This gene polymorphism is characterised by substituting C nucleobase to A at position number 421 (C421A) (Lima et al. 2014). Although few studies have claimed that this form of SNPs of *ABCG2* had no impact on clinical response to MTX (Kato et al. 2012; Stamp et al. 2010), the work by Zhang et al. (2013) has proven otherwise. In the latter study, A homozygotes have reduced function of the ABCG2 transporter (i.e. reducing the efflux capability of MTX) (Zhang et al. 2013). This results in greater MTX accumulation. Another SNP of *ABCG2* is rs13120400. At the intronic region, T is substituted to C nucleobase. Until recently, there was no evidence examining the relationship between this SNP and MTX response rate among the RA population, however, there is some evidence indicating that psoriatic patients show better response to MTX (Warren et al. 2008). The third type of SNPs is rs17731538 (G is replaced by A nucleobase at the intronic region). Again, there is no strong evidence supporting the impact of this type of SNP on clinical efficacy of MTX. Warren et al. (2008) reported that A carriers of this SNP poorly respond to MTX treatment. Similarly, no association between this SNP and toxicity or MTX clinical response has been found (Stamp et al. 2010).

1.2.4.8 Genetic polymorphisms in the polyglutamation pathway

1.2.4.8.1 GGH

Gamma-glutamyl hydrolase enzyme (GGH) plays an important role in transporting MTX outside the cell (Tian & Cronstein 2007). The efflux of MTX occurrs due to the removal of glutamic acid residue from MTX polyglutamates. Until recently, the impact of genetic polymorphism of *GGH* on intracellular polyglutamation of MTX and subsequent accumulation was controversial. First of all, an SNP of rs719235, characterised by altering G to T, has been associated with increased expression of the *GGH* gene (Chave et al. 2003). This change means that the rate of MTX removal from the cells is high, leading to a reduction in level of intracellular MTXPGs. Therefore, RA patients, who are T carriers, show not only lower levels of intracellular MTXPGs (odds ratio (OR) 4.8, 95% CI 1.8, 13.0, p-value 0.002) (Dervieux et al. 2005), but also a lower risk of MTX toxicity (Jekic et al. 2013). Conversely, GGH activity is reduced in RA patients with a T allele of rs11545078 SNPs (Dervieux et al. 2004; van der Straaten et al. 2007). This reduction in GGH activity promotes the accumulation of MTXPGs intracellularly, especially the long chain ones. Despite the MTX accumulation, there is no evidence reporting an increase in MTX toxicity (Jekic et al. 2013; Stamp et al. 2010; van der Straaten et al. 2007; Wang & Cooper 2007).

1.2.4.8.2 FPGS

FPGS is responsible for the polyglutamation process of MTX inside the cells by adding glutamic acid (Hoekstra et al. 2003; Tian & Cronstein 2007). It is encoded by a gene located on chromosome 9q34. Available evidence about SNPs and MTX toxicity and efficacy in RA is inconsistent. While some studies found no association between SNPs in rs10106 (substitution of C by T) and MTX toxicity (Davis et al. 2014), others have found that the toxicity of the drug is increased in RA patients with rs1054774 and rs4451422 polymorphisms (Owen et al. 2013).

1.2.4.9 Genetic polymorphisms in the folate and methionine pathways

1.2.4.9.1 DHFR

The *DHFR* gene is located on chromosome 5q11 (Swierkot & Szechinski 2006). The most common SNPs of the gene encoding for DHFR are rs1650697 and rs1232027, which are characterised by a substitution of G to A and A to G, respectively. Although these SNPs have been extensively studied, their impact on the clinical response and toxicity of MTX has been found to be insignificant (Wessels et al. 2006).

1.2.4.9.2 MTHFD1

The prime function of methylenetetrahydrofolate dehydrogenase 1 (MTHFD1) is to provide folate cofactor in MTX metabolisation during thymidylate and de novo purine synthesis (Krajinovic et al. 2004). Its gene is located at 14q24 chromosome (Stamp & Roberts 2011; Swierkot & Szechinski 2006). The most common SNP is rs2236225, where G is altered to A at 1958 location. The impact of this SNP on MTX clinical response and toxicity is uncertain. While Bohanec Grabar et al. (2008) found that there is no association between A allele and MTX toxicity, Stamp et al. (2010) documented a higher risk of MTX toxicity among RA patients with this polymorphism.

1.2.4.9.3 MTHFR

The methylenetetrahydrofolate reductase enzyme (MTHFR) converts 5, 10-MTHF into 5-MTHF in the folate pathway; it catalyses the remethylation of homocysteine to methionine by donating a carbon atom (van Ede et al. 1998). The gene responsible for coding MTHFR is located on chromosome 1p36. There are two common SNPs, including rs1801133 (A1a is substituted to Val) and rs1801131 (A is substituted to C). They have been linked to the level of MTHFR activity and MTX clinical effects (Stamp & Roberts 2011; Swierkot & Szechinski 2006), however, the available evidence is inconsistent. T homozygotes and T carriers of rs1801133 SNPs show lower levels of MTHFR activity (Frosst et al. 1995) leading to higher risk of MTX toxicity (Plaza-Plaza et al. 2012; Ranganathan et al. 2008). Likewise, C allele of rs1801131 SNPs is found to reduce the activity of MTHFR (van der Put et al. 1998). While Bohanec Grabar et al. (2008) found that SNPs of *MTHFR* modified MTX toxicity in RA patients, other studies claim that it increases the toxicity (Davis et al. 2014; Mena et al. 2011; Wessels et al. 2006).

Mutation in the *MTHFR* gene is associated with reduced activity of MTHFR enzyme. This reduction in enzyme activity reduces methyl group donation, which hinders homocysteine remethylation. Therefore, hyperhomocysteinemia is highly prevalent in patients with homozygosity of this gene (Frosst et al. 1995). Being one of the MTX toxicities, hyperhomocysteinemia has been linked to higher CV risk (Boers 2000; Eikelboom et al. 1999; Welch & Loscalzo 1998). It promotes coagulation, oxygen peroxide production and LDL oxidation impairing endothelial function.

1.2.4.9.4 SHMT1

Serine hydroxymethyltransferase 1 (SHMT1) is enzyme encoded by *SHMT1* gene located at chromosome 17p11. It has a major role in synthesising purine and thymidylate by encoding

vitamin B6 affording a carbon atom (van Ede et al. 2001). The most common SNP is rs1979277, which is characterised by replacing C by T at position 1420. T carriers are found to have high level of folic acid in RBCs and plasma (Heil et al. 2001), and subsequent lower risk of MTX toxicity (Weisman et al. 2006).

1.2.4.10 Genetic polymorphisms in the de novo pyrimidine synthesis pathway

1.2.4.10.1 TYMS

Thymidylate synthase enzyme (TYMS) plays a role in cell proliferation and DNA synthesis. Its main function is to synthesise deoxythymidine monophosphate (dTMP) and dihydrofolate (DHF) (Krajinovic et al. 2005; Lima et al. 2014; Touroutoglou & Pazdur 1996). Converting deoxyuridine monophosphate (dUMP) and 5, 10-MTHF is the key process in synthesising dTMP and DHF. After that conversion, phosphorylation of dTMP is followed leading to the formation of deoxythymidine triphosphate (dTTP); this dTTP is used to form DNA. Thus, reduction in dTMP synthesis leads to chromosomal damage and subsequent cell death (Ranganathan & McLeod 2006). TYMS is encoded by *TYMS* gene located on 18p11 chromosome. There are few SNPs studied including rs34743033 (Marsh et al. 2001), rs2853542 (Mandola et al. 2003), and rs34489327 (Pullmann et al. 2006; Zhang et al. 2004). Based on the pain VAS score, better MTX response is observed in RA patients who were homozygous of two repeat copies of the *TYMS* gene (Dervieux et al. 2004). Nevertheless, the same research group could not confirm this result after conducting a longitudinal prospective cohort study (Dervieux, Greenstein & Kremer 2006). In fact, higher dose of MTX was needed for patients who were homozygous for three copies of the repeat vs those with two copies (Kumagai et al. 2003); however, current evidence about the impact of these SNPs on MTX clinical response and toxicity is still controversial.

1.2.4.11 Genetic polymorphisms in the adenosine pathway

1.2.4.11.1 ADORA2a

The most common transporter of adenosine is adenosine receptor subtype A2a (ADORA2a) encoded by the gene located on 22q11.23 chromosome (Chan & Cronstein 2002; Kremer 2004). Several SNPs of *ADORA2a* gene have been found to affect the function of this transporter. These SNPs include: rs5760410 G>A, rs3761422 C>T, rs2298383 C>T, rs2236624 C>T, and rs2267076 T>C (Hider, Bruce & Thomson 2007; Hider et al. 2008). Hider et al. (2008) reported increased MTX toxicity among RA patients who have one of these SNPs.

1.2.4.11.2 AMPD1

Adenosine monophosphate deaminase 1 (AMPD1) is the enzyme involved in catalysing adenosine monophosphate (AMP) conversion into inosine monophosphate (IMP) (Wessels et al. 2006). It is encoded by a gene located on 1p13 chromosome. The most common SNP is rs17602729, where T is altered to C at position 34; this transition in nucleotides created a less active AMPD1 enzyme (Morisaki et al. 1992). Therefore, adenosine is released once the AMPD1 is deficient (Wettergren et al. 2010). Like other polymorphisms, the effect of SNPs of *AMPD1* on MTX clinical response and toxicity is still unclear. While T carriers are found to experience more incidents of MTX toxicity (Stamp et al. 2010), studies have not confirmed such findings (Bohanec Grabar et al. 2008; Owen et al. 2013; Wessels et al. 2006).

1.2.4.12 Genetic polymorphisms in the de novo purine synthesis pathway

1.2.4.12.1 ATIC

The most common enzyme involved in the purine synthesis pathway is ATIC encoded by the gene localised on chromosome 2q35 (Dervieux et al. 2004). Among all SNPs, rs2372536 C>G is the most frequent one examined, yet the exact effect of this polymorphism is uncertain. Although a few studies found that MTX toxicity was expected in RA patients, who were G homozygotes or G carriers (Bohanec Grabar et al. 2008; Dervieux, Greenstein & Kremer 2006; Weisman et al. 2006; Wessels et al. 2006), others found no such effect (Dervieux et al. 2009; Owen et al. 2013; Stamp et al. 2010; Takatori et al. 2006).

In summary, there is no conclusive evidence about polymorphisms in the genes encoding enzymes responsible for MTX metabolism and transmembrane transportation among the RA population. While most published studies examined the effect of several polymorphisms on MTX clinical response and toxicity, few studies assessed the genetic variations on the level of intracellular MTXPGs. While genetic polymorphisms in *SLC19A1*, *ABCC2* and *GGH* were associated with lower level of MTXPGs, a higher concentration of MTXPGs was reported for RA patients with *ABCB1* and *ABCG2* genetic polymorphism.

1.3 Rheumatoid arthritis and cardiovascular risk

RA is associated with an increased CV risk and has, in fact, been considered as an independent risk factor for CVD (del Rincon et al. 2001) although the exact mechanism of this excess risk is yet unclear. The process of developing CV events seems complex; beside the presence of chronic inflammation (Del Rincon et al. 2003), and uncontrolled traditional CV risk

factors (Innala et al. 2011), CV events are related to morphological (Park et al. 2002), and functional (Wong et al. 2003) changes in the vascular tree. First, RA is a disease associated with high inflammation and oxidative stress. These stimuli inhibit the function of the endothelial cells by reducing the availability of NO leading to vasoconstriction and increased vascular resistance. Second, RA sufferers are prone to atherosclerosis and increased arterial stiffness and these alterations increase the oxygen demand required by the heart. When there is a mismatch between the cardiac demands of oxygenated blood and the amount of blood supply to the heart, ischaemia wil more probably occur; this ischaemia is the prime cause of cardiac tissue necrosis leading to the development of ischaemic heart diseases. This leads to the development of angina, in the short-term, or major CV events in the long-term (Figure 1-10). Thus, in this section, the relationship between RA and clinical CVD, traditional CV risk factors and arterial stiffness will be discussed.

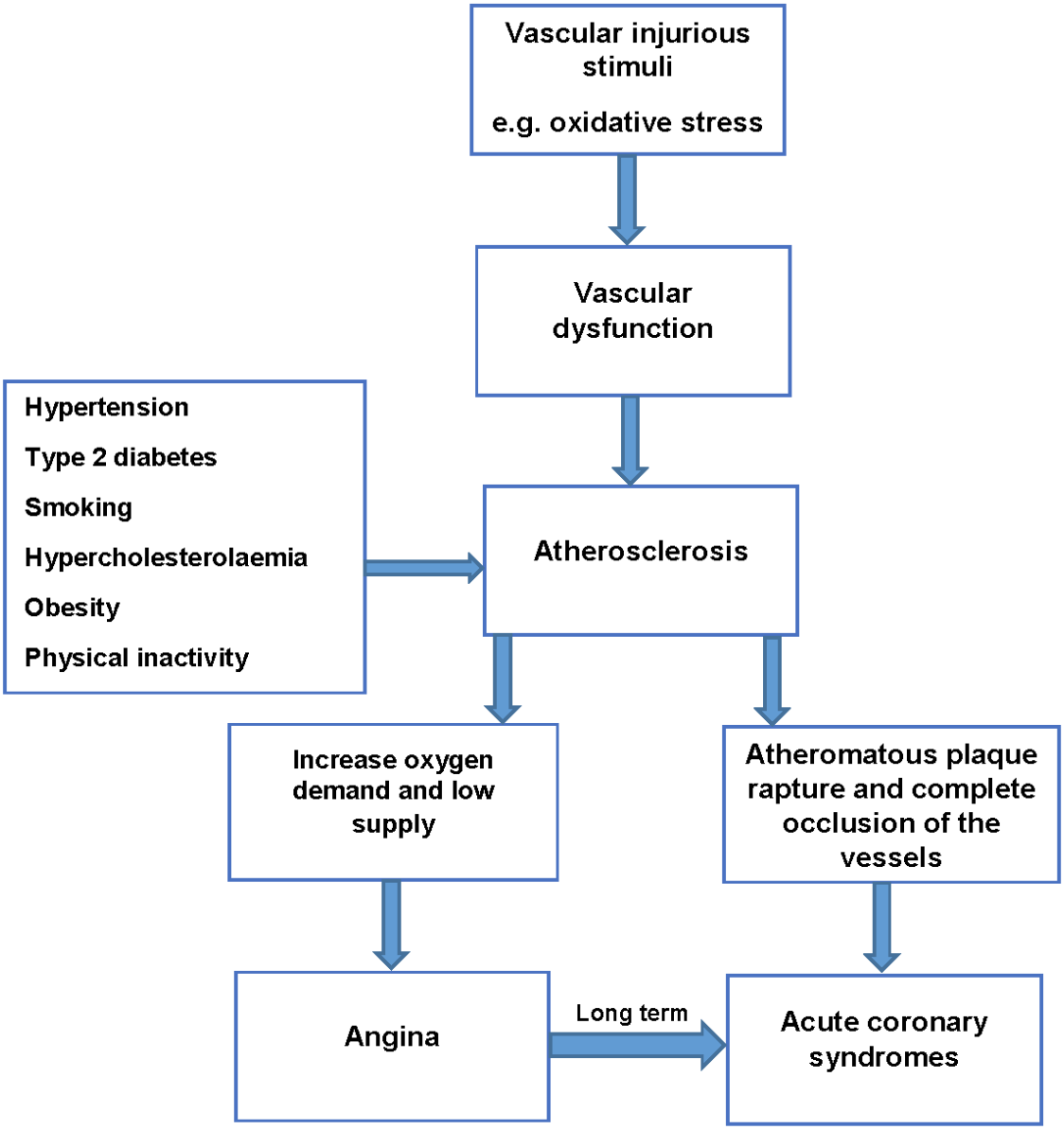


Figure 1-10 Pathophysiology of cardiovascular disease

1.3.1 Clinical cardiovascular morbidities

The risk of developing CVD is high in RA patients. Based on the World Health Organization (WHO) classification, these CV events include: coronary artery disease, heart failure, cerebrovascular diseases, and peripheral vascular diseases (WHO 2003). RA patients are at high risk of CV morbidity and mortality, with the majority of patients with RA suffering from subclinical CVD. Almost 50% of them are asymptomatic at the time of the diagnosis (Owlia

2006). Compared with the general population, RA patients show a 48% excess risk of developing incident CVD (Avina-Zubieta et al. 2012; Sakai et al. 2015). This excess risk of CV morbidity contributes to a higher mortality rate among the RA population (Boers et al., 2004). Mortality rate in RA patients is twice that of controls (RR = 2.0, 95% CI 1.6, 2.5) (Riise et al. 2001).

1.3.1.1 Coronary artery diseases

The first study examining the RA-associated morbidities showed that there was an increase in likelihood of developing coronary artery disease in RA patients (Cathcart & Spodick 1962). Among all CVDs, MI shows as the highest prevalence among RA patients (Maradit-Kremers et al. 2005b; Solomon et al. 2003; Watson, Rhodes & Guess 2003). The study by Wolfe et al. (2003) has shown that RA patients are 14 times more likely to develop MI compared with those without this inflammatory disease, recording an OR of 2.14 (95% CI 1.48, 3.09) (Wolfe, Freundlich & Straus 2003). This risk doubled during their life, registering an OR of 1.28 (95% CI 1.24, 1.33). Additionally, the incident cases of MI are increased by 68% in RA patients (RR 1.68, 95% CI 1.40, 2.03) (Avina-Zubieta et al. 2012) but unfortunately, most RA patients presented with undiagnosed MI (Maradit-Kremers et al. 2005b). This is because RA patients are less likely to report their chest pain (i.e. angina) (OR 0.58, 95% CI 0.34, 0.99) leading to high risk of unrecognised MI (hazard ratio (HR) = 2.13, 95% CI 1.13, 4.03) and subsequent sudden death (HR = 1.94, 95% CI 1.06, 3.55). The same research group conducted an inception cohort including a large number of RA patients (n = 603) who were followed for 15 years. The main finding was that the risk of CV death was significantly higher in RA patients reporting a hazard ratio of 2.03 (95% CI 1.45, 2.83).

1.3.1.2 Heart failure

There is abundant of evidence of the excess risk of congestive heart failure (CHF) in the RA population (Gabriel, Crowson & O'Fallon 1999a; Mutru et al. 1989; Myasoedova et al. 2011; Nicola et al. 2005; Wolfe, Freundlich & Straus 2003; Wolfe & Michaud 2004a). Some of these studies were population-based (Gabriel, Crowson & O'Fallon 1999a; Nicola et al. 2005), and others were hospital-based cohorts (Mutru et al. 1989; Wolfe., Freundlich & Straus 2003; Wolfe & Michaud 2004a). It should be noted that two of these studies used the same population (Gabriel, Crowson & O'Fallon 1999a; Nicola et al. 2005). In the study conducted by Gabiel et al. (1999), RA patients were 60% more likely to develop incident CHF compared with those without RA (RR 1.60, 95% CI 1.12, 2.27) (Gabriel, Crowson & O'Fallon 1999a). The same trend was confirmed by Nicola et al. (2005), where incident cases of CHF were more likely to be

diagnosed in both RF positive and negative patients recording a HR of 2.29 and 1.34, respectively. Similarly, the lifetime prevalence of CHF was found to be higher among the RA population (Wolfe, Freundlich & Straus 2003). The CHF in RA patients has been found to be associated with the same CV risk factors documented in the general population namely, hypertension, type 2 diabetes mellitus (T2D), smoking, age, and sex (Giles et al. 2005).

1.3.1.3 Cerebrovascular disease

There is an association between RA and stroke (cerebrovascular accident, CVA), however, there are some inconsistencies in the evidence reporting such an association. RA patients are found to be at higher risk of developing stroke compared with those without RA (Nadareishvili et al. 2008; Solomon et al. 2006; Solomon et al. 2003; Wolfe, Freundlich & Straus 2003), but few observational studies examined the occurrence of CVD in RA and claimed that there is an increase in the incident rates of MI but not CVA (Solomon et al. 2003; Turesson, Jarenros & Jacobsson 2004). This contradiction has been re-examined by Avina-Zubieta et al. (2012) (Avina-Zubieta et al. 2012), who reported that both MI and CVA incident risks are higher in RA compared with the general population. While the risk of MI in RA patients was increased by 68% (RR = 1.68, 95% CI 1.40, 2.03), the risk of CVA increased by 41% (RR = 1.41, 95% CI 1.14, 1.74).

1.3.1.4 Peripheral vascular disease

There are two main types of peripheral vascular diseases: obstruction (thrombosis), and narrowing (atherosclerosis) of the peripheral blood vessels. Although it is well-known that RA increases the risk of CVD, evidence about the effect of RA on peripheral vascular disease is still emerging. Few observational studies have reported that the prevalence of peripheral vascular disease is high (del Rincon et al. 2005; Henke et al. 2003; Shen et al. 2014). The frequency of developing peripheral obstructed and incompressible arteries (especially in cases with limb deformities) has been found to be 6% ($p = 0.005$), and 15% ($p < 0.001$), respectively (del Rincon et al. 2005), however, peripheral disease seems to be subclinical until the advanced stage (Stamatelopoulos et al. 2010). This higher risk of peripheral arterial diseases could be explained by systemic atherosclerosis associated with RA, even though there is no clear evidence yet.

1.3.2 Traditional cardiovascular risk factors and rheumatoid arthritis

Data about the effects of traditional CV risk factors in RA have been published in the PLoS ONE: The impact of traditional cardiovascular risk factors on cardiovascular outcomes in

patients with rheumatoid arthritis: a systematic review and meta-analysis (published previously in PLoS ONE.2015;10(2): e0117952 (Appendix-1).

Evidence shows that the high CV risk in the RA population might be explained by the presence of traditional CV risk factors. These factors include: hypertension, T2D, obesity, smoking, dyslipidaemia, and physical inactivity—therefore, screening for these risk factors to identify high risk patients is mandatory. The current recommended guidelines do not, however, provide sufficient information to diagnose and manage CV risk factors in patients with RA (Barber et al. 2015). For example, the Framingham Risk Score (FRS) is not useful in identifying high-risk RA patients (Chung et al. 2006; Kawai et al. 2015). This scoring system was modified to include RA as an independent factor known as risk prediction algorithm (QRISK2) (Hippisley-Cox et al. 2008), however, it was not accurate to detect the CV risk in the RA population (Monk et al. 2013). In fact, the CV risk estimated by the FRS among patients with RA was found to be underestimated (Crowson et al. 2012). Therefore, unrecognised CV risk factors among RA patients might exert additional effects on CVD development. Modifying the FRS by including the important factors which are RA-specific was suggested; for instance, adding the level of CRP to the model might improve the CV prediction in RA. Actually, the limitation of the FRS in estimating CV risk was addressed and modified for patients with Reynolds disease. The Reynolds Risk Score (RRS) accounts for the level of CRP (Ridker et al. 2007; Ridker et al. 2008). Although the CV risk in RA patients has been explained by the presence of traditional CV risk factors, the relative impact of individual CV risk factors on CV risk, beyond that caused by RA alone, is still unclear. Additionally, evidence regarding the effects of traditional CV risk factors on CV morbidity and mortality in RA patients is contradictory.

1.3.2.1 Hypertension

1.3.2.1.1 Epidemiology

Worldwide, the burden of hypertension is high with about one billion adults affected globally (Panoulas et al. 2008). It has been estimated that the prevalence of hypertension will increase by 60% in 2025 (Kearney et al. 2005). In the Australian population, high BP is the most frequent health problem encountered by general practitioners and accounts for about 8% of prescriptions (Britt et al. 2010). The Australian Diabetes, Obesity and Lifestyle Study (AusDiab) found that the prevalence of hypertension among adult Australians was 28.6 per 100 population (95% CI 25.0, 32.3) (Briganti et al. 2003). About half of Australians with high BP were untreated (Briganti et al. 2003). Hypertension among patients with RA is also prevalent with the rate ranging between

52% and 73% (Chung et al. 2012; Gonzalez et al. 2008; Panoulas et al. 2007). The available evidence about whether the prevalence of hypertension among RA is higher than that observed in the general population is still controversial (del Rincon et al. 2001; Han et al. 2006; McEntegart et al. 2001; Wolfe, Freundlich & Straus 2003). This variability might be due to recruiting older individuals with RA than controls (Chung et al. 2012; del Rincon et al. 2001), or to selection bias where patients and controls were selected from secondary care and community, respectively (McEntegart et al. 2001). The higher prevalence of hypertension among RA (34%) compared to general population (23.4%) found by Han et al. (2006), however, involved a large sample size and hypertension was defined based on standardised International Classification of Diseases 9th Revision, Clinical Modification (ICD-9) code.

1.3.2.1.2 Cardiovascular risk

Few studies have found an increased CV risk in hypertensive RA patients in contrast to the general population (Innala et al. 2011; Kremers et al. 2008; Serelis et al. 2011). Similar to the general population, there was a significant association between ischaemic stroke and the history of hypertension (OR of 1.98, 95% CI 1.16, 3.37) (Nadareishvili et al. 2008). Our meta-analyses showed an increased risk of MI in hypertensive RA patients (Baghdadi et al. 2015). The RR is 1.84 (95% CI 1.38, 2.46) thereby implying an 84% higher risk of MI among RA patients with hypertension compared with non-hypertensive RA patients. We also found that hypertensive RA patients are at higher risk of combined CV morbidity, namely through MI, angina pectoris, heart failure, stroke, and peripheral arterial disease. RA patients with hypertension show an overall RR of 2.24 (95% CI 1.42, 3.06), implying that hypertensive RA patients are twice as likely to experience combined CV morbidity compared with non-hypertensive patients.

1.3.2.1.3 Clinical measurements of blood pressure

Diagnosing and controlling high blood pressure (BP) early is thus an important target in reducing CV risk among the RA population. The diagnosis of hypertension is based on measurements using three main methods: clinic auscultatory BP, home self-report BP monitoring, and 24-h ambulatory BP monitoring. First, it is recommended that BP readings measured at the clinic are ideally taken while the patient is rested for at least 5 minutes. Measuring the BP three times and averaging the last two readings is another recommendation in The European Society of Hypertension/European Society of Cardiology (ESH/ESC) guidelines (Mancia et al. 2013). Even though this way of measuring the BP is a common

practice, it has several disadvantages (Sharman et al. 2015). Almost all physicians lack confidence in diagnosing hypertension based on clinic BP (Howes et al. 2010). Additionally, its reproducibility and prognostic value is low. Despite its availability and its low cost, it has no ability in identifying masked hypertension, white coat syndrome, and nocturnal BP. Although measuring BP in clinic only is considered an unreliable method in diagnosing high BP and assessing CV risk, many studies published data about CV risk in RA using this method (Briganti et al. 2003; Chae et al. 2001; Panoulas et al. 2007). Another way of measuring BP is home self-report BP monitoring. According to the High Blood Pressure Research Council of Australia, this method is adequate in assessing the risk of hypertension, including masked hypertension and white coat syndrome (Sharman et al. 2015). BP readings are optimal when a validated BP monitor is used, and the BP is recorded morning and night at around the same time for a week or so.

Additionally, monitoring BP at home is claimed to be an alternative to 24-h BP monitoring in assessing and managing patients with high BP (Hackam et al. 2013; Mancia et al. 2013; Parati et al. 2010). The medical decision in treating and monitoring high BP measured by this method is, however, based on awake (daytime) BP assessment. In many diseases, patients usually suffer from end organ damage; for example, RA patients more frequently have subclinical CVD (Stamatelopoulos et al. 2010). The presence of CVD might be associated with abnormal sleep-time BP where the BP does not decrease versus daytime (Boggia et al. 2007; Ohkubo et al. 2002; Verdecchia et al. 1994). Non-dippers are defined as those with a flattened 24-h BP profile that exposes them for a longer duration of elevated levels of BP over the 24 hours (Verdecchia, Schillaci & Porcellati 1991). In the non-dippers, the vascular resistance was higher during the night compared to the day, which explains the non-dippers phenomenon (Cavelaars et al. 2004). In normotensive individuals, however, both systolic BP (SBP) and diastolic BP (DBP) are usually the lowest during sleep (about ≥10% reduction) and increase upon awakening, reaching the highest during the daytime (Hermida et al. 2014). This rise in the BP in early morning is influenced by sympathetic activity (a surge in the level of norepinephrine and epinephrine) (Lakatua et al. 1986). Nocturnal BP, however, cannot be detected by home BP monitoring and therefore ambulatory 24-h BP monitoring is considered a better prognostic tool in detecting CV risk (Boggia et al. 2007). In addition to early detection of CV risk, changing clinical practice by using ambulatory BP monitors instead of single readings has been encouraged in recent years due to several aspects (Head et al. 2010; O'Brien et al. 2003); monitoring the BP during awake and sleep times has the ability to detect white coat syndrome, variability in BP during daily

activities, disease progression and therapies adjustment (Head et al. 2012; Head et al. 2010; Sharman et al. 2015).

Until recently, it has been claimed that conventional brachial BP measurement is reflective of central BP (Lewington et al. 2002). This assumption is supported by observational findings showing that peripheral pressure determinants including cardiac output governed by peripheral vascular resistance, heart rate and stroke volume can predict CV morbidity (White 2001) and mortality (Wright et al. 2004). Pressure in the central arteries, however, depends on other parameters in addition to peripheral vascular resistance and cardiac output. These additional factors include arterial stiffness and reflective waves' time and magnitude (Izzo 2004; Mitchell et al. 2003; O'Rourke 1995; O'Rourke & Mancia 1999; Williams et al. 2006). There is evidence that central BP is superior to peripheral BP in predicting CV risk (Agabiti-Rosei et al. 2007; Roman et al. 2007; Wang et al. 2010) and therefore, measuring both central and peripheral BP parameters are more accurate in assessing CV risk. Traditionally, the central BP was measured invasively by inserting a catheter in the ascending aorta (Philippe et al. 2002). For more than a decade, this invasive technique has been replaced by an accurate non-invasive method using the "SphygmoCor" device (AtCor Medical, Sydney, Australia) (O'Rourke, Pauca & Jiang 2001). This tool uses tonometry in recording the radial pulse waveform directly; this is then transformed to calculate central aortic pressure waveform (O'Rourke et al. 2002). The central BP obtained by this non-invasive technique is correlated to BP obtained by the invasive intra-aortic measurement (correlation coefficient = 0.91, $p < 0.001$) (Ding et al. 2011).

Recently, a new oscillometric ambulatory BP monitor, the "Mobil-O-Graph (Stolberg, Germany)", has been introduced. It monitors not only peripheral BP, but also central BP and PWA. It is approved by Food and Drug Administration (FDA) and Conformité Européenne (CE), and the ambulatory BP was validated based on British Hypertension Society (BHS) protocol and European Society of Hypertension (ESH) international protocol (Franssen & Imholz 2010; Jones et al. 2000; Wassertheurer et al. 2010; Wassertheurer, Mayer & Breitenecker 2008; Wei et al. 2010; Westhoff et al. 2005). It uses a general transfer function to calculate the central BP (this will be discussed in detail later), however, there is no study using this monitor in the RA population.

1.3.2.1.4 Pathway of hypertension in rheumatoid arthritis

Although hypertension is highly prevalent, control of hypertension is far from optimal—not only in the RA population (Panoulas et al. 2007), but also in the general population (Chobanian et al.

2003). The exact mechanism of high BP in the RA population is yet to be explored. There are several factors, however, which might explain the high prevalence of uncontrolled BP in this patient group that have been proposed. These factors include inflammation, physical inactivity and drug treatment (Panoulas et al. 2008).

First, inflammation is associated with high BP. CRP, a global inflammatory marker, is found to be inversely related to BP (Bautista et al. 2001; Chae et al. 2001), and to predict future risk of developing hypertension (Sesso et al. 2003). There are several pathways which might explain how inflammation associated with RA can increase BP. First and foremost, inflammation associated with RA (especially high level of CRP) increases the risk of vasoconstriction and subsequently increases the BP. It inhibits the production of NO from the endothelium (Verma et al. 2002). NO has several biological roles including as an anti-inflammatory, anti-atherosclerotic, anti-thrombotic, and vasorelaxation (Ignarro 2002; Ignarro & Napoli 2004; Wilkinson, Franklin & Cockcroft 2004). The reduced level of NO therefore leads to vasoconstriction increasing peripheral resistance; subsequently, this process increases the BP (Figure 1-11). CRP can also affect the renin–angiotensin system by upregulating angiotensin type 1 receptors increasing the BP (Wang et al. 2003), however, the reverse mechanism has been suggested (Hansson 2005). Increased BP, especially high oscillatory shear stress, stimulates pro-inflammatory cytokines production initiating the inflammatory cascade in the blood vessels. Eventually, the liver is stimulated to produce acute phase reactants such as CRP. This, in turn, reduces the level of NO by inhibiting its production from the endothelial cells creating a vicious circle (Figure 1-11).

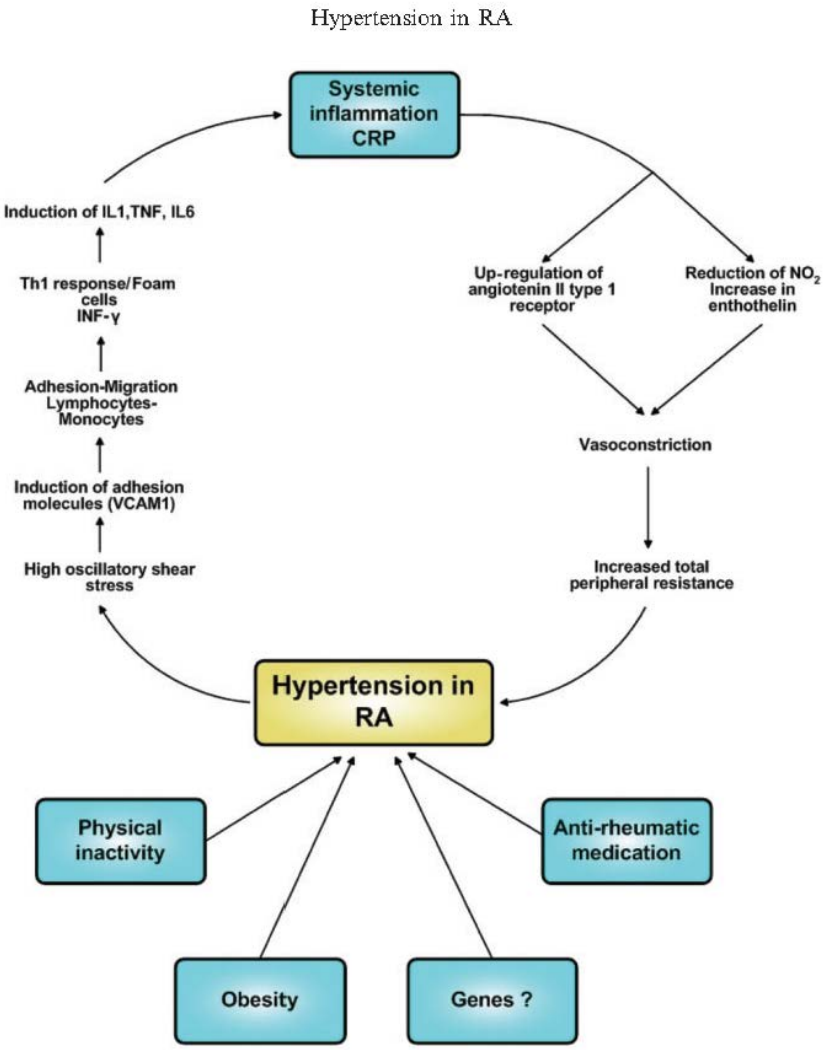


Figure 1-11 Pathways of hypertension in rheumatoid arthritis

From Panoulas et al. 2008, p. 1289. NO = nitric oxide; VCAM = vascular cell adhesion molecule; Th = T-helper lymphocytes.

Although chronic inflammation (high CRP) associated with RA has been proposed to increase BP directly, it also might increase the BP indirectly as a result of arterial elasticity impairment. Inflammation promotes the development of arterial stiffness manifested as a significantly higher percentage of central augmentation index (Klocke et al. 2003) predisposing RA patients to high BP (Franklin 2005). RA is a chronic inflammatory disease associated with increased response to acute phase reactants protein such as CRP and fibrinogen. This inflammatory status inhibits the production of NO from the endothelium (Verma et al. 2002) leading to vasoconstriction and high BP. Increased BP among the RA population could be at least partially explained by arterial

stiffness associated with this inflammatory disease. Thus, the relationship between inflammation, RA and high BP needs further investigation.

The second factor which might explain the high prevalence of hypertension in RA population is physical inactivity. As RA is associated with joint pain and stiffness, most patients with this disease avoid physical activity as it aggravates their pain. A sedentary lifestyle and inactivity have been linked to obesity (Fox & Hillsdon 2007), which is an independent risk factor for developing high BP (Panoulas et al. 2007); obesity was found to significantly increase the BP among RA patients ($p = 0.02$) and it was shown that it is an important mediator of hypertension. Obesity has been found to not only induce inflammation (Niskanen et al. 2004), but also to be preceded by inflammation (Weisberg et al. 2003). Obesity alters the function of adipose tissues, which increases the release of pro-inflammatory molecules. The latter increase the expression of TNF-α and IL-6. As the number of adipocytes are increased in obese subjects, the level of pro-inflammatory cells is also increased. This inflammatory response activated by adipocytes with the chronic inflammation in RA inhibit the function of NO, leading to vasoconstriction and subsequent high BP. Medications used among RA patients might also be considered as contributors to high BP; for example, while NSAIDs and glucocorticoids stimulate the production of angiotensinogen (Pandey et al. 2015), they inhibit the prostaglandin production (Rhen & Cidlowski 2005). These mechanisms lead to sodium and water retention by the kidney, and subsequently increase the volume of blood and causing hypertension (Figure 1-12).

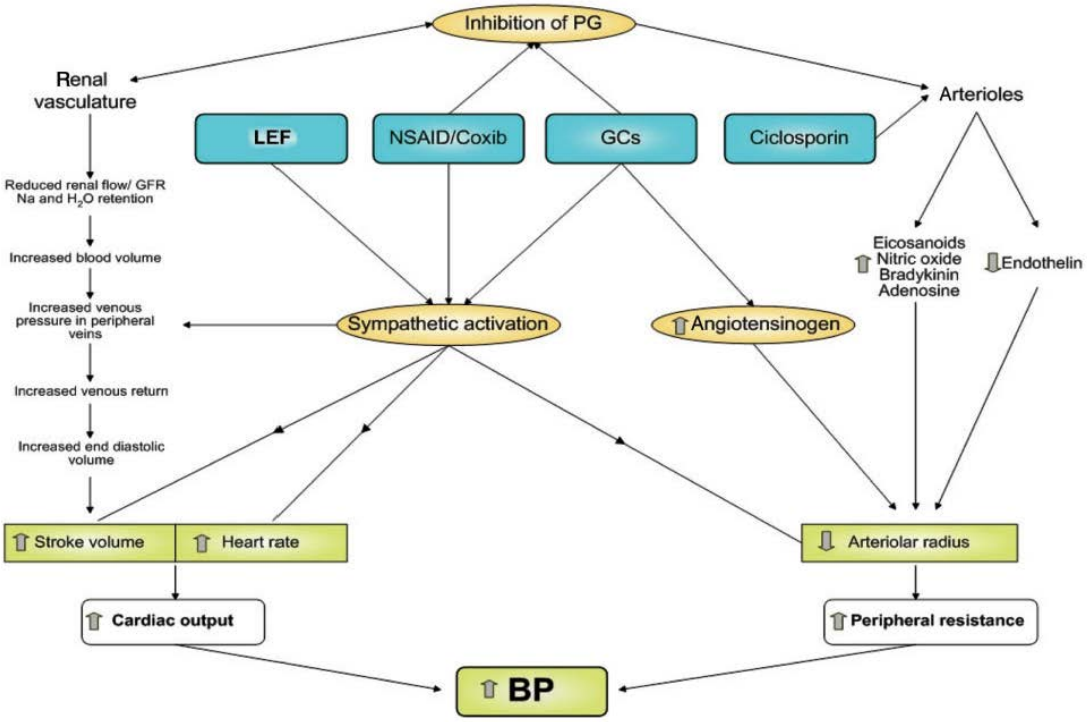


Figure 1-12 Mechanisms of anti-rheumatic drugs in increasing blood pressure

From Panoulas et al. 2008, p.1290. PG = prostaglandin

Another drug that has potentially increased BP in RA is cyclosporine, one of the DMARDs (Taler et al. 1999; Textor et al. 1994). This drug induces hypertension via several mechanisms: high vascular resistance as a consequence of vasoconstriction; vasodilation impairment as a result of a suppression in either NO or prostaglandins levels; high level of sodium retention; and vasoconstriction in renal vasculature, leading to glomerular filtration rate reduction (Figure 1-12). Leflunomide is another DMARD explaining high BP in RA (Fox et al. 1999). It increases the BP due to renal damage (Rozman et al. 2002); although renal toxicity is one of the rare side effects of leflunomide (Schiff & Whelton 2000), there is evidence indicating that its overdose might cause interstitial nephritis (Haydar et al. 2004). This type of renal injury is associated with activation of angiotensin-converting enzyme-2 and subsequent elevation in the BP (Mezzano et al. 2003). Therefore, careful monitoring of RA patients on these medications is vital in controlling the BP and preventing CV events.

RA is therefore a complex inflammatory disease associated with higher risk of developing hypertension. The mechanism of BP increase in this vulnerable population is still unclear despite the several proposed pathways.

1.3.2.2 Type 2 diabetes mellitus

T2D is a well-known risk for CVD, however, a limited number of studies have investigated the relationship between T2D and RA (Gonzalez et al. 2008; Han et al. 2006; Solomon et al. 2010). While a longitudinal cohort based on medical records claimed that there was no risk of T2D among the RA population (Gonzalez et al. 2008), other studies indicated that RA patients are at higher risk of T2D (Han et al. 2006; Solomon et al. 2010). The study by Han et al. (2006) was a large cohort study based on health insurance claims and included 28,208 RA patients and 112,832 controls. It found that RA patients were 1.4 times more likely to develop T2D compared to controls (OR = 1.4, 95% CI 1.4, 1.3). The study by Solomon et al. (2010) included a large cohort of RA (n = 48,718) in which the incident rate (IR) and HR for T2D were 8.6 per 1000 (95% CI 8.5 to 8.7), and 1.5 (95% CI 1.4 to 1.5), respectively.

Despite the observed high risk of T2D in RA, the exact mechanism is still controversial. The interaction between inflammation, insulin resistance and metabolic syndrome was argued to be the initiator of T2D in the RA population. First, the increased level of CRP might explain the increased risk of T2D in RA (Schmidt et al. 1999). A large prospective cohort study studied almost 6,000 patients and found that this inflammatory marker was increased in diabetics (Barzilay et al. 2001); after following these patients for three years it was found that they were twice as likely to develop T2D if the level of CRP was elevated. In fact, this observed inflammatory status plays an important role in insulin resistance associated with T2D (Natali et al. 2006). Findings by the Insulin Resistance Atherosclerosis Study (IRAS) showed that CRP is an independent predictor for insulin resistance (Festa et al. 2000), which is one of the components of metabolic syndrome (Grundy et al. 2004). Based on the Adult Treatment Panel III report (ATP III) definition, this syndrome comprises six conditions: abdominal obesity, atherogenic dyslipidaemia, raised BP, insulin resistance, pro-inflammatory state, and pro-thrombotic state. The same association was found between metabolic syndrome and inflammation (Festa et al. 2000). These data suggest that RA might increase the risk of T2D.

The relationship between T2D and CV risk in RA is contradictory. While hypertension has been found to be a strong predictor for CVD, T2D has shown no CV effects (Nadareishvili et al. 2008). There is no conclusive evidence on the presence, and magnitude, of an association between T2D and CV morbidity in RA. While some authors argue that the increased CV risk observed in RA patients is unrelated to the presence of T2D (Brady et al. 2009; del Rincon et al. 2001), others have documented that its presence increases CV risk (Innala et al. 2011; Koivuniemi et al. 2013; Kremers et al. 2008; Mohammad et al. 2010; Nadareishvili et al. 2008).

Our meta-analysis, however, shows an increased risk of MI in RA patients with T2D, leading to a combined RR of 1.89 (95% CI 1.36, 2.63). The same trend is documented for combined CV risk. RA patients with T2D are almost twice as likely to experience CV events compared with non-diabetic patients (RR1.94, 95% CI 1.58, 2.30) (Baghdadi et al. 2015).

1.3.2.3 Obesity

Obesity is a global epidemic and its prevalence is still increasing (Caballero 2007). In 2014–2015 almost two out of three Australians (63.4%) were obese or overweight, according to the Australian Bureau of Statistics' Australian Health Survey (Australian Bureau of Statistics 2015). The prevalence of obesity is increased since 1995 (56.3%) becoming the 2nd contributor to the burden of diseases. Although obesity is a well-known risk factor for several diseases including CVD, the magnitude of effect of this metabolic disease on developing RA is contradictory. While few studies argue that there is no effect of increasing body weight on RA development (Cerhan et al. 2002; Rodriguez et al. 2009; Wesley et al. 2013), others find that obesity aggravates the onset of RA thereby highlighting the importance of modifying this risk factor among the RA population (Bhole et al. 2012; Uhlig, Hagen & Kvien 1999; Qin et al. 2015).

Same contradiction was reported in the literature regarding the CV impact of obesity among RA regardless of its contribution to CVD development in the general population (Ajeganova, Andersson & Hafström 2013; Finckh & Turesson 2014; Lu et al. 2014; Metsios et al. 2009; Serelis et al. 2011). Despite these inconsistencies in the literature reporting the impact of obesity on CV risk, we have found a significant negative impact of obesity in the RA population, with the magnitude of effects similar to that for the general population (Baghdadi et al. 2015). We reported that obese RA patients are 16% more likely to develop CV morbidity compared to non-obese patients (RR 1.16, 95% CI 1.03, 1.29).

The exact mechanism explaining the positive association between RA, obesity and CV risk is still unknown, however, adiposity associated with obesity might explain that relationship (Wells 2012). Adipose tissue is known as 'toxic' tissue associated with numerous chronic diseases. It is an endocrine organ regulating growth, immune function, and inflammation. It produces and releases a wide range of inflammatory cytokines and chemokines, including TNF-α and IL-6 (Fantuzzi 2005). The adipocytes also produce hormones and proteins including adipokines; the latter include adiponectin and leptin, which have been considered as pro-inflammatory markers (Sonnenberg, Krakower & Kissebah 2004). Indeed, the secretion of these factors is increased with expansion of the adipose tissue leading to high inflammatory status in the obese person. In

fact, obese RA patients show worse disease activity, functional disability and physical inactivity (Ajeganova, Andersson & Hafström 2013), thus inflammation is a shared pathway between obesity and RA, which contributes to higher CV risk. The adipokines, mainly leptin and adiponectin, might explain the high CV risk in obese RA patients. Leptin deficiency has been associated with reduced inflammation among RA patients (Busso et al. 2002). Leptin concentrations have been positively correlated with BMI in RA patients (Mirfeizi et al. 2014; Wislowska et al. 2007). In addition to the pro-inflammatory effects, adiponectin also favours insulin resistance (Ouchi et al. 2003; Pittas, Joseph & Greenberg 2004).

1.3.2.4 Dyslipidaemia

For the last few decades, there has been some interest in understanding the link between RA and altered lipid metabolism. Similar to other chronic inflammatory diseases, metabolic syndrome, a cluster of CV risk factors that include abdominal obesity, increased BP, increased triglycerides and low-density lipoprotein cholesterol (LDLc), decreased high-density lipoprotein cholesterol (HDLc), and increased fasting glucose, is common among patients with RA (Chimenti et al. 2015; Karvounaris et al. 2007) with a prevalence of 40% (Gremese & Ferraccioli 2011; Metsios et al. 2008). Noticeably, changes in blood lipids are similar to that seen in sepsis and cancer (Amezaga Urruela & Suarez-Almazor 2012; Choy & Sattar 2009). The level of lipid components—including total cholesterol (TC), LDLc, and HDLc—is reduced in RA patients; one study found that these lipid markers were significantly reduced ($p \leq 0.015$) despite high concentrations of CRP (Toms et al. 2011). In fact, this change in lipid profile has been observed several years before the onset of RA (Myasoedova et al. 2010). Despite the reduction in lipid concentrations, the CV risk is still high in the RA population (Myasoedova et al. 2011; Toms, Symmons & Kitas 2010). This phenomenon is so-called the ‘lipid paradox’ (Amezaga Urruela & Suarez-Almazor 2012).

The mechanism behind this lipid paradox and CV risk in RA is still far from clear, but pro-inflammation and immune responses are two of the proposed suggestions (Amezaga Urruela & Suarez-Almazor 2012). LDLc moves into and out of the blood vessels; when moved outside the arterial wall, LDLc is oxidised and becomes pro-inflammatory. Additionally, RA is an inflammatory condition associated with high inflammatory products, mainly cytokines (TNF-α) and IL-1 and IL-6. These cytokines are involved in engulfing the oxidised LDLc leading to the formation of foam cells. As a consequence, these foam cells accelerate the formation of arterial plaques predisposing RA patients to atherosclerosis. In fact, the negative impact of

dyslipidaemia on CV risk might be underestimated in RA as the level of TC, LDLc, and HDLc are reduced.

Compared to other traditional CV risk factors, contradictive results were reported in the literature regarding the impact of hypercholesterolemia on CV risk in RA (Innala et al. 2011; Kremers et al. 2008; Mohammad et al. 2010). We showed a significant negative impact of hypercholesterolaemia in the RA population, however, with the magnitude of effects similar to that for the general population. The risk of CV morbidity is 73% in RA patients with hypercholesterolaemia compared to those with normal cholesterol levels (RR 1.73, 95% CI 1.03, 2.44) (Baghdadi et al. 2015).

1.3.2.5 Smoking

Although the prevalence of smoking among Australians is decreasing, it is still 14.5% (Australian Bureau of Statistics 2015). Smoking as a traditional CV risk factor has been considered as a strong predictor for RA pathogenesis (Ruiz-Esquide & Sanmarti 2012). Actually, the risk of developing seropositive RA is high among smokers compared to non-smokers. A case-control study found that female and male smokers, which included passive and ex-smokers, were more likely to develop RA (OR = 1.7, 95% CI1.2, 2.3), and (OR = 1.9, 95% CI 1.0, 3.5), respectively (Stolt et al. 2003). Another study found that smoking was associated with high RA activity ($p = 0.002$), and with highest pain scores ($p < 0.0001$) (Manfredsdottir et al. 2006).

The impact of smoking on CV risk in the RA population is still unclear, despite its well-established role as CV risk factor. Reported evidence in RA showed that smoking had a weak (Gonzalez et al. 2008), or non-significant association with CV risk (Boyer et al. 2011; Innala et al. 2011). Conversely, we found a significant negative impact of smoking in the RA population, with the magnitude of effect similar to that for the general population. CV morbidity is increased with smoking. RA smokers are 50% more likely to develop CVD compared to non-smokers (RR 1.50, 95% CI 1.15, 1.84) (Baghdadi et al. 2015).

Inflammation provoked by smoking has been considered one of the explanatory mechanisms in developing RA and CVD. Smoking increases the inflammatory response leading to production of serum fibrinogen, acute phase reactants and pro-inflammatory cytokines (TNF-α and IL6) (Sopori & Kozak 1998). Smoking also exposes RA patients to extra oxidative stress (Costenbader & Karlson 2006). This type of stress leads to LDL oxidation (Baka, Buzas & Nagy

2009), and subsequent atherosclerosis (Johnson et al. 2016). Therefore, reducing exposure to smoking might prevent the development of CVD in the RA population.

1.3.2.6 Physical inactivity

Physical activity in RA patients is limited due to joint pain and functional disabilities, however, physical activity might also improve the joint stiffness and overall physical function. Although few researchers have argued that high intensity exercise aggravated joint damage in RA (Nordemar et al. 1981), other investigators have proven otherwise (de Jong et al. 2004; Lemmey et al. 2009). They recommend aerobic exercise in combination with muscle strength training for RA patients. This recommendation might help RA patients in improving joint symptoms as well as reducig CV risk. Even though there is no evidence showing excess CV risk among physically inactive RA patients (Baghdadi et al. 2015; Benatti & Pedersen 2015; Brandt & Pedersen 2010; Naranjo et al. 2008), Metsios et al. (2009) found that physical activity significantly improves CV risk in RA ($p < 0.05$). Although the link between physical inactivity and the excess CV risk is still not clear, loss of muscle mass (Roubenoff et al. 1994), and adiposity (Wells 2012) associated with RA might contribute to CV risk. In rheumatic cachexia, the muscle mass is reduced as a result of chronic inflammation (Roubenoff et al. 1994), which subsequently predisposes RA patients to atherosclerosis. In addition, adiposity (as discussed earlier) increases CV risk. As high-intensity exercise strengthens muscle mass and prevents rheumatic cachexia and adiposity, CV risk might be reduced in RA patients following aerobic and muscle strength training.

In summary, patients with RA are at higher risk of CVD. Although there are inconsistencies in the literature reporting the impact of traditional CV risk factors in RA patients on MI and CV morbidity, there is a significant negative impact of hypertension, T2D, smoking, hypercholesterolaemia and obesity in this population, with the magnitude of effects being similar to that for the general population. The presence of high CV risk suggests that a careful diagnosis and management of CV risk factors should be considered as important as the management of the symptoms of RA in mitigating the risk of CV morbidity and mortality among these patients.

1.3.3 Vascular regulation, endothelial dysfunction, and inflammation

The blood vessel wall is composed of three layers: inner layer (intima), middle layer (tunica media), and outer layer (tunica externa). The health of blood vessels can be evaluated at two levels: morphological and functional. Morphological evaluation of the vascular tree reflects the

degree of atherosclerosis, and is assessed by measuring the intimal medial thickness (IMT). Conversely, augmentation index (AIx) and pulse wave velocity (PWV) obtained by pulse wave analysis (PWA) predict the degree of arterial function and vascular health (Figure 1-13). These markers of arterial function will be discussed in the next section.

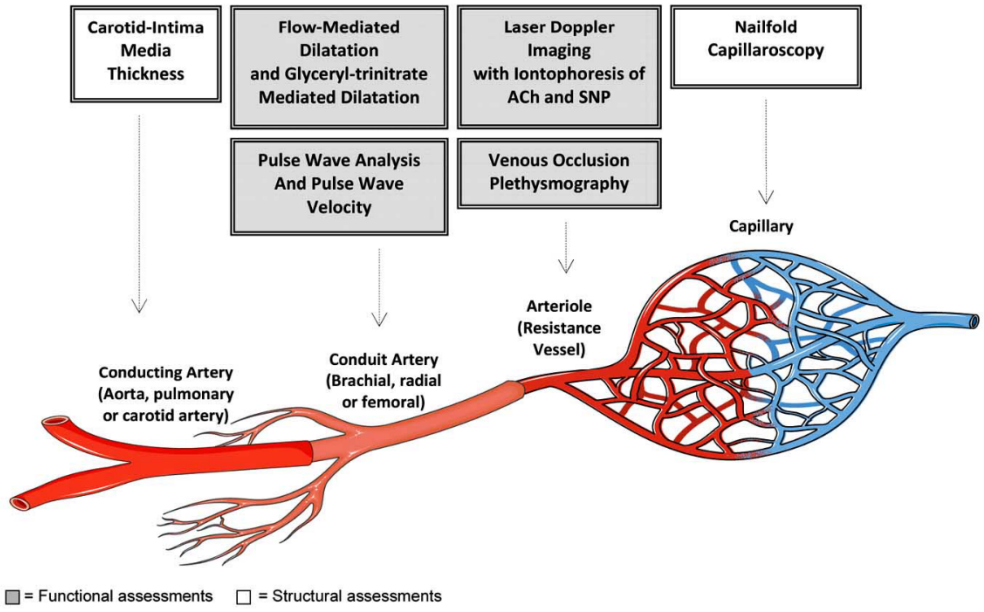


Figure 1-13 Assessment of vascular function in different vascular beds

From Sandoo et al. 2010, p. 2127. ACh = Acetylcholine, SNP = Sodium nitroprusside

1.3.3.1 Vascular tone regulation

The intima consists of endothelial cells. Being a large paracrine organ, the endothelium plays a fundamental role in controlling physiological vascular tone, homeostasis, vasodilatation, cell growth, platelet function and inflammation (Corretti et al. 2002; Ignarro 2002; Vane, Änggård & Botting 1990). One of the vital endogenous relaxing factors synthesised by the endothelium is NO via the enzyme endothelial NO synthase (eNOS). NO has several functions including as its anti-inflammatory, anti-atherosclerotic, anti-thrombotic, and vasorelaxation effects (Ignarro 2002; Ignarro & Napoli 2004; Wilkinson, Franklin & Cockcroft 2004). Its vasorelaxation effects depend on the availability of intracellular calcium (Ca^{2+}); usually, the inactive form of eNOS is attached to a protein called ‘caveolin’ (Bucci et al. 2000). The eNOS detaches from the bounded protein and becomes activated when the level of intracellular Ca^{2+} increases. When the level of intracellular Ca^{2+} is depleted, membrane receptors will receive a signal to open Ca^{2+} channels to permit the diffusion of extracellular Ca^{2+} into the cells (Lambert, Kent & Whorton 1986; Schilling,

Cabello & Rajan 1992; Schilling & Elliott 1992). Then, eNOS converts $_{L}$-arginine into NO (Palmer, Ashton & Moncada 1988). This pathway of producing NO via Ca2+ regulation is known as ‘capacitative Ca2+ entry’ (Figure 1-14) (Putney 1986; Sandoo et al. 2010).

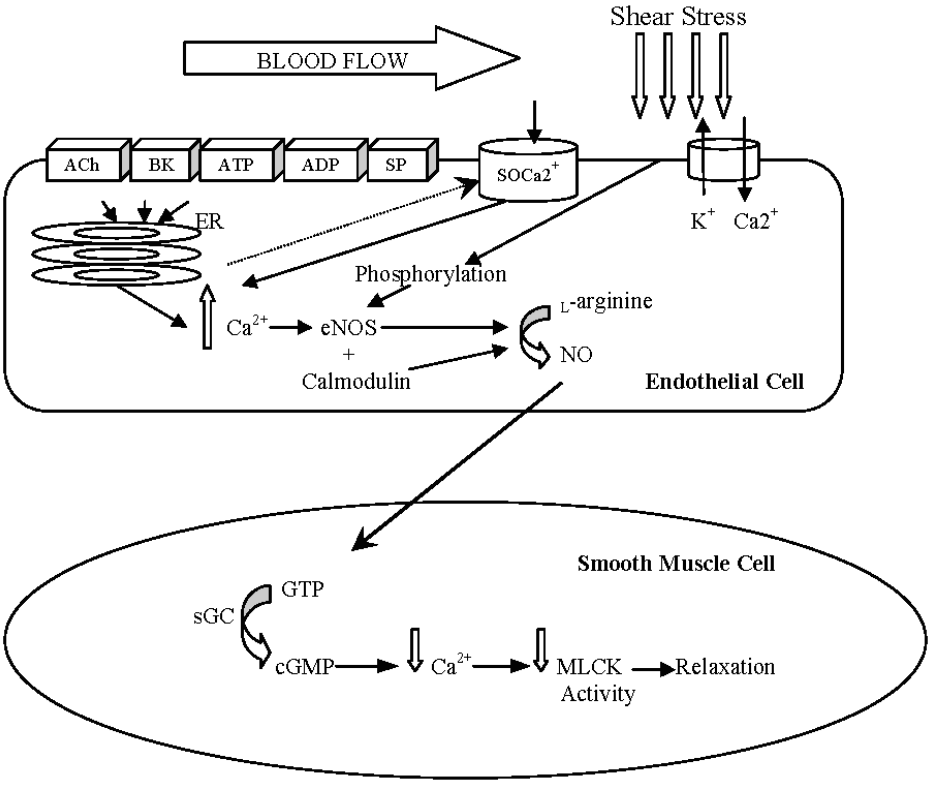


Figure 1-14 Pathway of nitric oxide synthesis via Ca^{2+} regulation

Adapted from Sandoo et al. 2010, p. 304. Endothelial nitric oxide production and its actions in the vascular smooth muscle cell. ACh = acetylcholine; BK = bradykinin; ATP = adenosine triphosphate; ADP = adenosine diphosphate; SP = substance P; SOCa2+ = store-operated Ca2+ channel; ER = endoplasmic reticulum; NO = nitric oxide; sGC = soluble guanylyl cyclase; cGMP = cyclic guanosine-3’, 5-monophosphate; MLCK = myosin light chain kinase. *When Ca2+ stores of the endoplasmic reticulum are depleted a signal is sent to SOCa2+ channel which allows extracellular Ca2+ into the endothelial cell.

Once NO is produced, it binds to guanylate cyclase (sGC), which is a soluble enzyme located in the adjacent smooth muscle cells (Ignarro et al. 1986). This activated enzyme increases the synthesis of cyclic guanosine monophosphate (cGMP) (Arnold et al. 1977); this process reduces the production of intracellular Ca^{2+} with subsequent vascular smooth muscle relaxation (Behrendt & Ganz 2002). Therefore, NO production and activation are crucial in regulating vessel wall tone and reducing arterial stiffness.

1.3.3.2 Endothelial dysfunction and inflammation

Exposure to any injurious stimuli, such as an infiltration by pro-inflammatory mediators, disturbs the function of the endothelial cells leading to endothelial dysfunction (Lerman & Zeiher 2005). Inflammation has been associated not only with endothelial dysfunction in the early stage of atherosclerosis, but also with plaque rupture and subsequent thrombosis (eventual stage of atherosclerosis) (Libby, Ridker & Hansson 2009; Libby, Ridker & Maseri 2002; Wilson et al. 2007). Therefore, both RA and atherosclerosis share the same pathophysiological pathway in terms of inflammation. In the 20th century, Pasceri et al. (1999) summarised the similarities between these inflammatory diseases (Pasceri & Yeh 1999) and are shown below (Table 1-7).

Table 1-7 Similarities between atherosclerosis and rheumatoid arthritis

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

NFα = indicates tumour necrosis factor-alpha; HSP = heat shock protein; 1 = increased; and 11 = marked increased. UA = indicates systemic markers found increased in patients with unstable angina. Other factors are expressed in atherosclerotic plaques. Adapted from Pasceri & Yeh 1999, p. 2125.

Not only can both disease states stimulate inflammatory cells and cytokines, but they also increase adhesion molecules' expression (Pasceri & Yeh 1999). These inflammatory factors include: activated T cells, TNFα, IL-6, and IL-18. Additionally, both RA and atherosclerosis activate adhesion molecules: intercellular adhesion molecules (ICAM-1), soluble vascular cell adhesion molecules (sVCAM-1), oxidised LDL, lipoprotein-a, lipoprotein associated phopholipase A2 (Lp-PLA2), and leptin. The presence of IL6, CRP and TNFα activate T cells and macrophages.

Additionally, CRP has been linked to higher risk of atherosclerosis and CV events (Ridker et al. 1998; Ridker et al. 2000) and was found to be one of the key markers for inflammatory process in atherosclerosis (Ernst & Resch 1993). Although CRP has been widely used as an acute phase reactant to assess progression and inflammatory status in RA, Ridker and colleagues suggested that it is also a marker for CV risk (Ridker et al. 2000; Ridker, Stampfer & Rifai 2001). In fact, CRP was considered an independent marker for predicting CV morbidity and mortality (Ridker et al. 2000; Ridker, Stampfer & Rifai 2001; Wilson, Ryan & Boyle 2006; Wilson et al. 2007). CRP interacts with several adhesion molecules and neutrophils, and stimulates the expression of tissue factor and complement activation (Wilson et al. 2007). These interactions lead to plaque formation in the vessels wall (i.e. atherosclerosis), susceptible to rupture at any stage of RA disease and thus initiating CV events.

Additionally, inflammation might provoke endothelial dysfunction. The activation of the pro-inflammatory cytokines, high level of white blood cells, and CRP inhibit the function of NO (Haruna et al. 2006), which increases the risk of oxidant stress, vasoconstriction and consequent endothelial dysfunction. Therefore, inflammation associated with RA eventually leads to endothelial dysfunction.

1.3.3.3 Endothelial dysfunction and homocysteine

Homocysteine is an amino acid formed as a result of methyl transfer reaction catalysed by MTHFR. This reaction liberates one methyl group used for proteins and deoxyribonucleic acid synthesis (Dimitroulas et al. 2016). It is metabolically converted into methionine, which is involved in the protein synthesis; besides, it is catalysed into cysteine which is used to synthesise protein. Elevated levels of homocysteine lead to hyperhomocysteinemia. This elevation has four possible causes: ingestion of protein high in methionine; deficiency of vitamin B_{12} or folate; cystathionine-β synthase (CBS) genetic variation; and impairment in renal excretion of cysteine (Figure 1-15) (Sen et al. 2010).

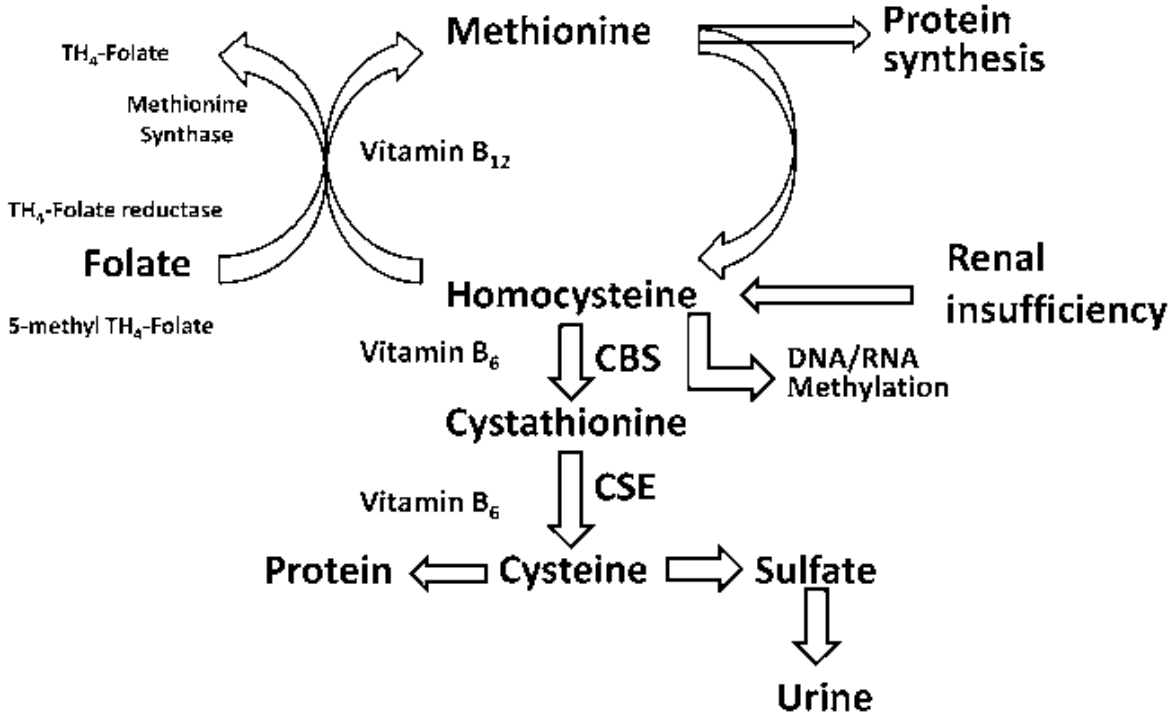


Figure 1-15 Hyperhomocysteinemia and methionine metabolism

From Sen et al. 2010, p. 50

Hyperhomocysteinemia promotes coagulation, oxygen peroxide production and LDL oxidation impairing the endothelial function. The literature shows that there are two pathways of endothelial dysfunction associated with hyperhomocysteinemia: via increasing the BP, and via impairing vasodilatation of endothelial NO (Sen et al. 2010). Almost half of methionine obtained by diet is metabolised into homocysteine, increasing the BP (Rolland et al. 1995); conversely, following a diet low in methionine such as fruits and vegetables has been found to improve not only the BP (Appel et al. 1997), but also vascular function (Woo et al. 1999). In fact, elevated levels of homocysteine independently increase the SBP (Sutton-Tyrrell et al. 1997), and impair endothelial function (Chambers et al. 1998); it is considered an independent risk factor for atherosclerosis. Recent studies showed that homocysteine concentration is strongly and positively correlated with BP, arterial stiffness measured by PWV ($\beta = 0.713$, $p = 0.004$) (Xiao et al. 2014), and AIx ($\beta = 0.236$, $p < 0.001$) (Vyssoulis et al. 2010). Similarly, homocysteine concentrations have been found significantly elevated in RA (Avalos et al. 2007), especially in those with hypertension (OR 2.9, 95% CI 1.5, 5.5, $p = 0.001$) (Manavathongchai et al. 2013). Yet, studies examining the impact of elevated homocysteine concentrations on arterial stiffness

in RA are scarce. A case-control study reported that elevated homocysteine level is common among RA patients diagnosed with atherosclerosis assessed by the degree of coronary artery calcification (Chung et al. 2005) or carotid IMT (Park et al. 2002). Although there is little evidence that the level of homocysteine is positively correlated with PWV among patients with systemic lupus erythematosus ($p = 0.034$) (Sabio et al. 2009), there is no clear evidence showing such association in RA.

Although the exact mechanism of elevated homocysteine in RA is still unclear, metabolic disturbance of vitamin B12 and folate, and genetic mutation in *MTHFR* might partially explain such elevation. B12 deficiency is common in patients with longstanding RA (Bournia et al. 2012). As B12 is involved in the metabolism of homocysteine, its depletion leads to homocysteine accumulation and subsequent increase in BP. Moreover, folate is another element involved in homocysteine metabolism. In RA patients taking an anti-folate drug (i.e. MTX), the level of folate decreases leading to hyperhomocysteinemia. Additionally, *MTHFR* 677C>T homozygous or heterozygous genotype have been linked to hyperhomocysteinemia (Essouma & Noubiap 2015). In this polymorphism, the activity of MTHFR is reduced which impairs homocysteine methylation and subsequent inhibition of methionine formation (Fujimaki et al. 2009). This results in homocysteine accumulation. Therefore, impairments in homocysteine metabolism might explain the increase CV risk in the RA population.

1.3.3.4 Endothelial dysfunction and traditional cardiovascular risk factors

In addition to inflammation, inactivation of eNOS has been linked to presence of the traditional CV risk factors namely, hypertension, T2D, dyslipidaemia, obesity and smoking (Goligorsky 2005; Higashi et al. 2009; Pepine 2009; Tomiyama & Yamashina 2010). This inhibition of eNOS is evident due to the oxidant stress associated with these CV risk factors. Additionally, these factors stimulate the activity of nicotinamide-adeninedinucleotide phosphate (NADPH) oxidase, which increases the production of reactive oxygen species leading to NO inactivation (Forstermann & Munzel 2006). Therefore, the observed increased CV risk in patients with RA can be attributed to endothelium dysfunction stimulated by the presence of traditional CV risk factors (Totoson et al. 2016).

1.3.4 Rheumatoid arthritis treatments and cardiovascular risk

There is a growing body of evidence examining the relationship between common treatments prescribed for RA and the risk of CVD. These drugs include traditional DMARDs, biologic DMARDs, glucocorticoids, traditional and new NSAIDs, and statins.

1.3.4.1 Disease-modifying anti-rheumatic drugs

It is well-known that the risk of CV morbidity and mortality is reduced with DMARDs treatment (Roubille et al. 2015; van Halm et al. 2006). Although many DMARDs such as sulfasalazine have been found to reduce CV risk (Choi et al. 2002; van Halm et al. 2006), most studies examining such effect of DMARDs have focused on MTX therapy. MTX was found to have impacts on arterial stiffness and on the development of clinical CVD.

1.3.4.1.1 Methotrexate and atherosclerosis

To date, there is no evidence about the effect of MTX monotherapy on arterial stiffness. Data about the effect of MTX on atherosclerosis and endothelial function in RA patients are conflicting. While Kumeda et al. (2002) argued that MTX used by RA patients showed no significant effect on reducing the risk of atherosclerosis, their claim was made as there was no difference in IMT between RA patients using MTX and those taking other medications. However, MTX was recently found to reduce the risk of atherosclerosis assessed by measuring IMT ($p = 0.0002$) (Kisiel et al. 2015). The observed effect of MTX was independent of the presence of any traditional CV risk factors. The magnitude of atherosclerosis risk was dependent on MTX dose, however, and it was found that the IMT was significantly reduced with higher doses of MTX (i.e.≥ 20mg/week). A possible explanation of the discrepancies between these studies might relate to the assessment technique. While both studies (Kisiel et al. 2015; Kumeda et al. 2002) examined the common carotid and femoral arteries, measurements protocol and definitions of the IMT of both arteries were different in these studies. Additionally, the populations in both studies were different: European (Kisiel et al. 2015) and Asian population (Kumeda et al. 2002). In fact, the risk of atherosclerosis has been found to be significantly higher in Asians compared to Europeans (OR = 4·51, 95% CI 1·46, 13·89, p = 0·02) (Anand et al. 2000). For example, several studies have shown that Japanese RA patients are at higher risk of developing atherosclerosis (Gonzalez-Juanatey et al. 2003; Kumeda et al. 2002; Nakajima et al. 2010). Ethnicity, genetic and lifestyle heterogeneity between the two populations might contribute to this variation in the CV risk (Anand et al. 2000). Moreover, about 30% of the East Asian population lives in low- to middle-income countries where poor lifestyle was reported (Iso 2011), and the access to health care is therefore limited (Ezzati et al. 2006); in fact, the incident rate of CVD has increased in Japan, especially in the suburbs of Osaka, with this being related to lifestyle factors. Interestingly, about 60% of increased CV morbidity in Japan was related to high BP (Nguyen et al. 2013).

1.3.4.1.2 Methotrexate and cardiovascular diseases

Besides the potent anti-inflammatory effects of MTX, it has shown positive impacts on CV morbidity and mortality among RA populations. A recent systematic review and meta-analysis has shown a possible protective effect of MTX by reducing the risk of CV morbidity in patients with RA (Roubille et al. 2015). This finding was supported by abundant evidence (Kisiel et al. 2015; Mason & Libby 2015; Ogdie et al. 2015; Roubille et al. 2015), however, a recent cross-sectional study conducted in Sub-Saharan Africa claimed that RA patients had no excess CV risk compared to the general population (Singwe-Ngandeu et al. 2016). Noticeably, MTX draws the researchers' attention as it reduces the CV morbidity and mortality in the RA population.

In addition to reducing the risk of developing CV events, there is emerging evidence that MTX might be involved in the mechanism of reducing one of the important CV risk factors (i.e. hypertension) in RA. RA patients who were not taking MTX or other DMARDs showed a 39% higher risk of CVD (HR 1.39, 95% CI 1.28,1.50) compared to patients on DMARDs (Ogdie et al. 2015). The exact mechanism of MTX in reducing CV risk is still not understood. Most published studies have examined the effect of MTX on CV risk in RA populations and reported the prevalence of hypertension, the use of anti-hypertensive medications, or have reported the mean BP as one of the baseline characteristics. For example, a randomised double-blind placebo-controlled trial found that combining MTX with glucocorticoid or cyclosporine showed better outcomes (rapid control to disease activity) compared to MTX monotherapy (Hetland et al. 2006). The baseline and one-year follow-up data showed that the prevalence of hypertension was lower among RA patients using MTX monotherapy. Another controlled trial showed a similar lower percentage of hypertension among those using MTX alone compared to combination therapies (van der Heijde et al. 2006). Similarly, few studies have reported the effect of MTX on hypertension as one of the diseases in the metabolic syndrome in RA (Bilecik et al. 2014; Chaer et al. 2012).

1.3.4.1.2.1 Methotrexate and blood pressure

Although MTX was found to reduce the risk of CV events in RA patients, evidence about specific effects on BP is yet to be explored. There are few observational studies suggesting a lower SBP in MTX users (Table 1-8) (Ajeganova et al. 2013; Choi et al. 2002; Cuchacovich & Espinoza 2009; Hansel et al. 2003; Panoulas et al. 2007; Rho et al. 2009; van Halm et al. 2006). MTX reduces the BP despite it being an anti-folate drug reducing the level of folate in plasma and red blood cells and increasing the level of homocysteine; hyperhomocysteinemia

has been linked to endothelial dysfunction as it increases the BP and impairs endothelial vasodilatation (as discussed earlier). The anti-inflammatory effect of MTX is well-known (Hasko & Cronstein 2004), however, it is not fully understood whether the cardio-protective effect of MTX is independent from the anti-inflammatory characteristics of MTX.

Table 1-8 Observational studies examined the effect of methotrexate on blood pressure in patients with rheumatoid arthritis

Author	Study design	MTX effect	Findings
(Rho et al. 2009b)	Cross-sectional	Lower BP	There is a lower SBP and DBP in those using MTX compared to non-users, even though the difference was not statistically significant for SBP (131.6±21 vs 137.5±18 mmHg, p = 0.55). and DBP (73.7±11.1 vs 77.9±9.5, p = 0.09)
(Panoulas et al. 2007)	Cross-sectional	Lower prevalence of hypertension	At the beginning of the study, the percentage of RA patients without hypertension was higher in those who were taking MTX (60.2%)
(van Halm et al. 2006)	Case-control	Lower prevalence of hypertension	RA Patients who were using MTX had lower percentage of hypertension compared to MTX naïve (12% vs 24%)
(Choi et al. 2002)	Prospective cohort	Lower prevalence of hypertension	RA Patients who were using MTX had lower percentage of hypertension compared with MTX naïve (13% vs 15%)
(Hansel et al. 2003)	Case-control	Lower BP	Lower SBP among MTX cases compared to controls (132±5 vs 130±5 mmHg, p = 0.58)
(Cuchacovich & Espinoza 2009)	Cross-sectional	Lower BP	Lower SBP among MTX RA users compared to MTX naïve (120.1±15.5 vs 123.6±12.2 mmHg)

(Mean± standard deviation)

One of the mechanisms of actions of MTX is via increasing adenosine concentrations (Bouma, van den Wildenberg & Buurman 1996; Feoktistov 2002). As soon as MTX enters the cell, it is

converted from inactive mono-glutamate form to the biologically active polyglutamate form (MTXPGs) by the effect of FPGS enzyme (Cannella & O'Dell 2012). Being polyglutamated inside the cell, MTXPGs has the ability to inhibit ATIC. This inhibition leads to an increase in adenosine concentrations both intracellularly and extracellularly. Adenosine is known to have not only anti-inflammatory effects (Hasko & Cronstein 2004), but also vasodilatory effects (Collis 1989; Drury & Szent-Gyorgyi 1929; Li et al. 1998; Newby 1988; Smits et al. 1995). As the main function of adenosine is tissue protection, its release is increased when there is a tissue injury caused by inflammation or ischaemia (Hasko & Cronstein 2004). An in vivo study postulated that the mechanism of actions of adenosine, prostaglandins (PGs) and NO are interdependent (Ray et al. 2002). This study showed that adenosine released after injurious stimuli acts on the vascular endothelium via its receptors A1 and d A2A. In the endothelium, adenosine stimulates the release of PG, which enhances the synthesis of NO. The produced NO has the ability to cause vasodilation (Figure 1-19). There is good evidence that adenosine plays a role in causing vasodilation via releasing NO (Bryan & Marshall 1999; Danialou et al. 1997; Merkel et al. 1992; Skinner & Marshall 1996; Vials & Burnstock 1993).

MTX prescribed for RA patients might have a vasodilatory effect, which helps in reducing BP. This physiological mechanism occurs as a result of increasing the level of adenosine. The latter acts on vascular endothelium and stimulates the production of PGs causing the eventual release of NO.

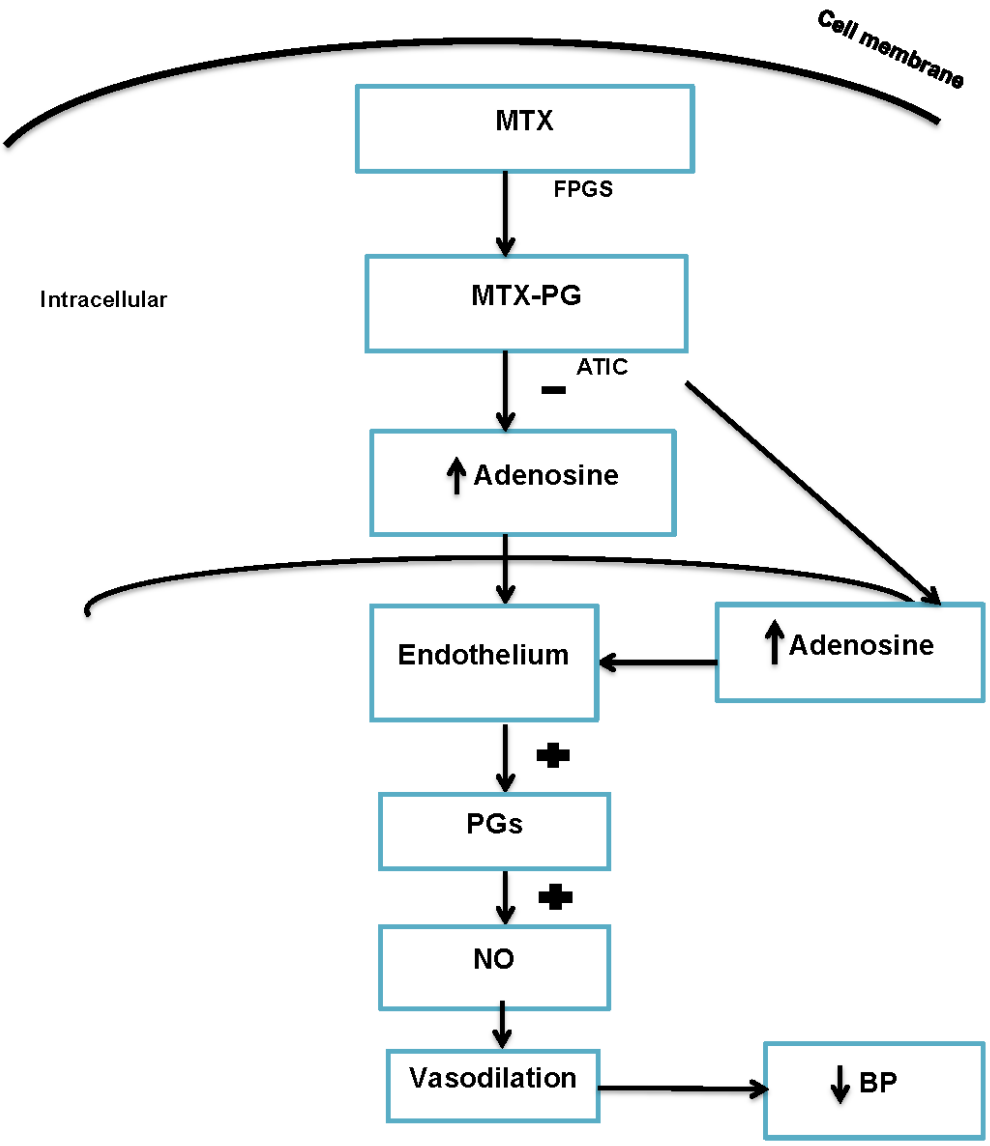


Figure 1-16 Vasodilation effect of methotrexate via increasing adenosine

FPGS = folylpolyglutamate synthase, ATIC = aminoimidazole carboxamide ribonucleotide transformylase. PG = prostaglandins

1.3.4.2 Biologics

1.3.4.2.1 Tumour necrosis factor (TNFα) inhibitors

There is good evidence supporting the beneficial effects of different types of biologics. These genetically engineered drugs were introduced recently in the treatment of RA patients inadequately controlled on traditional DMARDs. The main mechanism of action of biologics is

supressing inflammation in RA. As both RA and atherosclerosis are inflammatory conditions, these therapies might help in preventing CVD in RA.

The widely known and used biologic is TNF-α inhibitors (infliximab). Atherosclerosis is caused by immune response related to TNF-α. In the presence of inflammation, T-helper cells T_H1 are activated. These activated cells promote the production of TNF-α, which is capable of stimulating the release of pro-inflammatory mediators; the latter stimulate endothelial cells and increase the expression of adhesion molecules (Hansson & Libby 2006). Subsequently, thrombus is formed in the blood vessels. Therefore, inhibiting TNF-α reduces not only the degree of inflammation, but also the risk of developing atherosclerosis. TNF-α inhibitors have been found to reduce the risk of developing atherosclerotic CVD among RA patients. This class of drugs also reduce the risk of CV events (Dixon et al. 2007; El-Barbary et al. 2011; Listing et al. 2008; Roubille et al. 2015).

TNF-α inhibitors have also been linked to the improvement of the surrogate markers of CVD. Endothelial dysfunction, which is a surrogate marker for CVD and atherosclerosis, was improved among RA patients taking TNF-α inhibitors (Hurlimann et al. 2002; Komai et al. 2007; Maki-Petaja et al. 2006) especially if it is taken for at least 12 weeks (Tikiz et al. 2010). Arterial stiffness is another independent predictor for CVD. It seems that this inhibitor improves PWV among RA patients (Kerekes et al. 2011; Maki-Petaja et al. 2006; Protogerou et al. 2011). Studies have shown the CV-protective effect of TNF-α inhibitors despite the claim proposed by Van Doornum, McColl and Wicks (2005) and colleagues against these protective effects. In fact, it has the ability to reduce aortic stiffness among RA patients to values comparable to normal individuals (Angel et al. 2010; Galarraga et al. 2009; Wong et al. 2009). This improvement in aortic stiffness was evident by measuring PWV (Maki-Petaja et al. 2006)

1.3.4.2.2 Tocilizumab

Tocilizumab is one of the biologic DMARDs and its main mechanism of action in reducing inflammation is through inhibiting interleukin IL-6 receptor. Besides the anti-inflammatory effect of this biologic drug, a few studies have shown that it might reduce CV risk in RA patients (Kume et al. 2011; Provan et al. 2015). It was found that RA patients who were on tocilizumab showed reduction in PWV, but no Aix, compared to other RA patients who were on other biologics (Provan et al. 2015). A control trial, however, showed that the tocilizumab group recorded a reduction in AIx (Kume et al. 2011). In addition to improvement in arterial stiffness, as evidenced by better PWA among RA patients taking tocilizumab, these patients showed a

reduction in BP (Provan et al. 2015). In RA patients using tocilizumab, there was a reduction of about 11 and 7 mmHg in the systolic BP and diastolic BP (-11.5±18.6, p = 0.15 vs -6.7±9.3, p = 0.10, respectively).

1.3.4.3 Glucocorticoids

Glucocorticoids, such as prednisolone, are usually prescribed to RA patients during disease flares or as a long-term therapy in combination to other DMARDs. The main mechanism of action of these drugs is reducing inflammation. Glucocorticoid users were at high CV risk (Fardet, Petersen & Nazareth 2011). Several side effects associated with its use include hypertension, obesity, fat distribution, insulin resistance, lipid profile disturbance, and coagulation proteins impairment might explain the excess CV risk observed. CV risk was higher among those taking the drug either in higher doses (Wei, MacDonald & Walker 2004), or continuously (Souverein et al. 2004). Recent studies have shown that the risk of all CV events was 47% higher among RA using glucocorticoids (RR 1.47. 95% CI 1.34, 1.60, p < 0.001) (Roubille et al. 2015). While high dose of glucocorticoids was associated with increased CV risk, the effect of low-dose glucocorticoids on CV risk in RA patients is still controversial (Conn 2001; Girod & Brotman 2004; Pieringer & Pichler 2011; Raynauld 1997; Saag 2001).

Evidence about the effect of glucocorticoids on vascular function in RA patients is lacking. As glucocorticoids have an anti-inflammatory effect, atherosclerosis is expected to reduce. Prednisolone was found, however, to predispose RA patients to atherosclerosis (del Rincon et al. 2004); this effect was inversely related to time of exposure. Additionally, a recent study assessed the relationship between the use of acute and chronic low dose of prednisolone and arterial stiffness in RA (Radhakutty et al. 2016). It showed that low-dose prednisolone showed either no effect or reduced markers of CV risk (e.g. AIx). The authors of this study suggested that the reduction in these markers might be due to the reduction in sympathetic nervous system activity induced by prednisolone.

1.3.4.4 Nonsteroidal anti-inflammatory drugs and COX-2 inhibitors

Evidence about CV effect of traditional NSAIDs and COX-2 inhibitors is still controversial. While few studies have found that these anti-inflammatory drugs increased the risk of CV events (Hippisley-Cox & Coupland 2005; Silverstein et al. 2000), others claimed that it reduced atherosclerotic risk as evident by improved endothelial dysfunction (Belhassen et al. 2003; Chenevard et al. 2003).

The higher risk of CV events can be explained by the mechanism of action of NSAIDs, especially selective COX-2 inhibitors. The conversion of arachidonic acid to prostaglandins (i.e.PGI-2) is catalysed by COX-2. As the main action of this prostaglandin is vasodilation and anti-aggregation, this process leads to vasoconstriction. Inhibiting COX-2 selectively means that the function of COX-1 is preserved, which increases the production of thromboxane A-2 (TXA-2) mediating platelet aggregation. Theoretically, the decrease in PGI2 and increase in TXA-2 predispose the patients to thrombosis and subsequent CV events (Wong, Chowienczyk & Kirkham 2005). There is some evidence, however, showing that NSAIDs might reduce the risk of atherosclerosis by improving the endothelial function (Chenevard et al. 2003). The observed improvement in endothelial function could be related to anti-inflammatory effects of this type of analgesics as shown by reduced concentrations of CRP and oxidised LDL.

1.3.4.5 Statins

Statins were found to reduce CV risk (Ridker et al. 2008) with this effect thought to be mediated by the anti-inflammatory effect of statins. The TARA randomised control trial found that taking atorvastatin by RA patients can modify CV risk via improving inflammation (McCarey et al. 2004). Not only do statins reduce the risk of CV events, but also arterial stiffness. Evidence has shown that atorvastatin reduces arterial stiffness among RA patients (El-Barbary et al. 2011; Van Doornum, McColl & Wicks 2004). The work by Van Doornum et al. (2004) was the first to find the beneficial effect of statins on vascular function in RA, which was explained by cholesterol reduction. This finding was recently confirmed (Ikdahl et al. 2016). This study showed that long-term use of rosuvastatin among patients with inflammatory joint diseases improves not only the arterial stiffness, but also BP. The mechanism behind the observed improvement in arterial stiffness and wave-reflection (i.e. reduction in PWV and Aix) was believed to be due to the anti-hypertensive characteristic of statins (Ferrier et al. 2002; Shige, Dart & Nestel 2001).

1.3.5 Arterial stiffness

1.3.5.1 History and definition

Arterial stiffening is also known as arterial rigidity. Physiological changes such as ageing, and conditions such as hypertension are the most common predisposing factors for arterial stiffening (Lee & Oh 2010). Its pathogenesis involves endothelial dysfunction and inflammation (Jain et al. 2014). As RA is an inflammatory condition, it predisposes patients to develop arterial stiffening. In fact, arterial stiffening has been thought to increase CV risk independently (Provan et al.

2011). Additionally, it is associated with increased risk of developing CVD including angina, MI, left ventricular hypertrophy and heart failure (Nichols 2005). Thus, early detection of arterial stiffening might reduce CV morbidity and mortality.

Arterial stiffening is associated with reduced arterial elasticity, which negatively impacts on the pressure waves generated by the heart. In the normal cardiac cycle, each cardiac contraction generates pressure waves, which travel forward in the arterial tree; these waves are known as “forward waves”. These waves travel forward until they reach branch points, where they are reflected back toward the heart, therefore producing “backward waves”. In individuals with healthy vessels, these backward waves usually arrive during diastole, supporting blood supply to the coronary vessels. In the case of arterial stiffness, however, the backward waves travel faster and arrive back to the heart earlier during the systole while the heart is still contracting. This results in augmentation of the forward wave, with an increase in afterload (Figure 1-16).

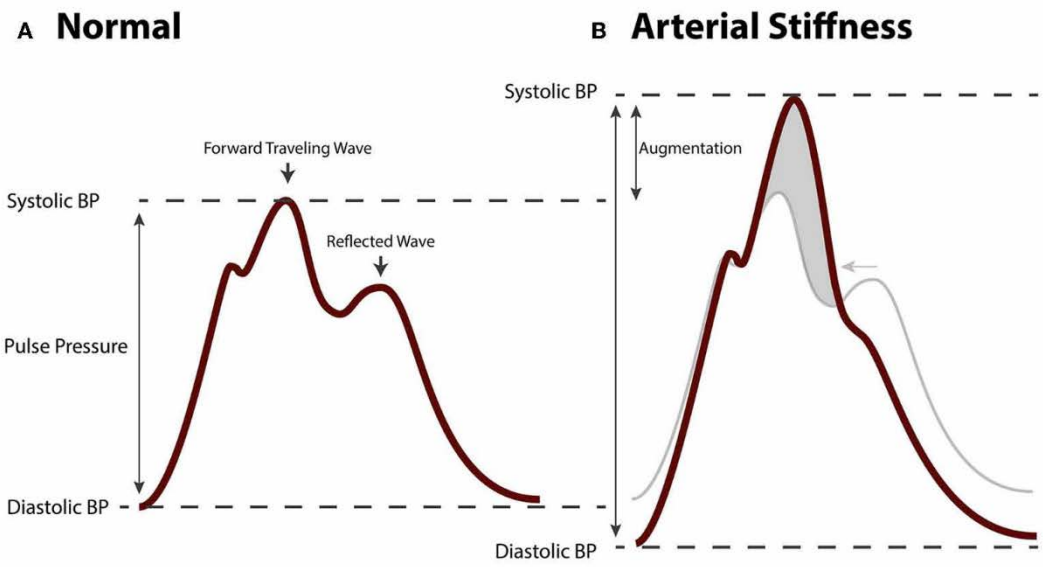


Figure 1-17 Arterial waveforms in arterial stiffening

From van Varik et al. 2012, p. 6. (A) Aortic blood pressure waveform of a healthy, normotensive person. The forward travelling wave precedes the (backward travelling) reflected wave. (B) Aortic pressure waveform of a person with arterial stiffness. Due to increased pulse wave velocity, the forward travelling wave and reflected wave are summated leading to augmented pulse pressure.

Understanding arterial stiffness and its relation to pressure waves was established in the 18th century (Mahomed 1872). Fredrick Akbar Mahomed was the pioneer in establishing the foundation of PWA. He was the first to describe the normal radial pressure waveform and showing the effect of hypertension on this waveform. He was the first to recognise the difference

between peripheral and central pressure waves. This phenomenon was later explained by McDonald (1960) based on the wave reflection theory. The elasticity and fluid viscosity of vessels was the foundation of understanding PWA. PWV has been considered a surrogate marker for arterial stiffness (Westenberg et al. 2012). First, Young's modulus of elasticity was one of the estimators of the arterial stiffness by calculating the PWV. While Young's modulus considers arterial wall thickness, it assumes wall homogeneity. This assumption is incorrect as arterial wall thickness is non-homogenous. Thus, arterial smooth muscle tone varies from one artery to another affected one because of a number of factors, such as autonomic nervous system activity, hormonal changes, and vasoactive substances such as NO (Bank & Kaiser 1998; Safar et al. 1986). This variation contributes to different degrees of arterial stiffness in the arterial tree. Second, the Bramwell-Hill model is another estimator of arterial stiffness linking aortic distensibility, pulse pressure (Malone & Reddan 2010) and PWV (Bramwell & Hill 1922). The PWV equation was modified to consider the variation in the arterial wall thickness (Bramwell & Hill 1922; Nichols & O'Rourke 1998); PWV = $\sqrt{E \times h/2rp}$, where p is the density of fluid in the blood (approximately = 1.05) and h/2r is the thickness of the arterial wall. While the clinical importance of PWA remained underestimated until the beginning of the new millennium, its application in clinical practice is progressing.

1.3.5.2 Assessing arterial stiffness

For decades, arterial stiffness was assessed using invasive techniques. The gold standard method is to assess the shape and velocity of pressure waves via inserting a catheter in the aorta during cardiac surgery (Tian & Chesler 2012). This invasive method is impractical and poses higher risk of complications and therefore, the clinical importance of assessing arterial stiffness non-invasively has received attention in the last century. Since then, many non-invasive techniques have been introduced and these include: ultrasound, MRI, and waveform analysis; the latter includes tonometric and oscillometric methods (Stoner, Young & Fryer 2012).

1.3.5.2.1 Waveform analysis by applanation tonometry

Waveform analysis is the most commonly used technique for assessing the impact of reflected waves on central blood pressure. It is widely used in clinical research due its reproducibility and accuracy (Wilkinson et al. 1998). First, PWA is recorded by using applanation tonometry, which was originally used in ophthalmology to measure intraocular pressure (O'Rourke, Pauca & Jiang 2001). It measures radial artery pressure waveforms by flattening the artery without occluding it (i.e. tonometric). Calibration of these waveforms is done against standard brachial BP. This

provides the systolic and diastolic pressure indicated by the maximum and minimum points of the pressure wave, respectively. Then, the radial pressure waveform is mathematically transformed into the central waveform (ascending aortic) using a generalised transfer function. This waveform encloses first and second systolic peaks (Figures 1-16, 1-17). These peaks result from the augmentation of generic pulse wave generated by the heart due to early arrival of the reflected wave. The difference between the second and the first systolic peak is known as AIx, which is expressed as percentage (%) of pulse pressure (PP). AIx is calculated by dividing the augmentation pressure (AP) by the PP; the equation is AIx = AP/PP. The AP results from the augmented wave and it is the difference between second and first systolic peak. The PP is the difference between diastolic and systolic blood pressure (Wilkinson et al. 2002). A higher percentage of AIx indicates a higher degree of arterial stiffness (Avalos et al. 2007; van Varik et al. 2012).

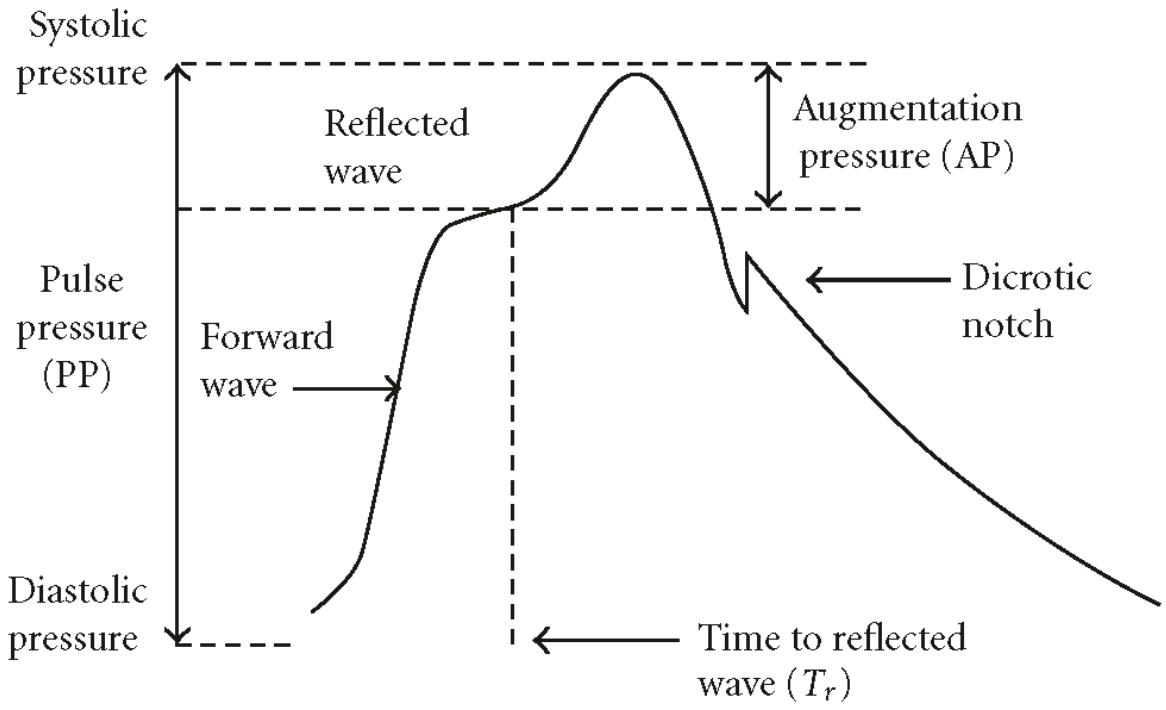


Figure 1-18 Aortic pulse pressure waveforms

From Stoner, Young & Fryer 2012, p.2

Again, PWV, a marker of arterial stiffness, can be measured by using the applanation tonometry technique. PWV is expressed as “the speed of travel of the pulse along an arterial segment” (PWV= distance+ t (m/s) (O'Rourke & Mancia 1999, p. 2). Using applanation tonometry,

waveforms are obtained from two arterial locations, with the carotid and femoral arteries the most common sites. Then, the distance between those arteries is usually recorded; then the measurement of the wave transit time between the two points is obtained to calculate the PWV. In fact, there is some evidence showing that atherosclerosis is significantly correlated with faster PWV (more than 13 m/s) (Vlachopoulos et al. 2013).

Currently, the gold standard applanation tonometric machine is the SphygmoCor (version 7·1, AtCor Medical, Sydney, Australia) (Actor Medical 2011). This non-invasive tool has been clinically validated against other invasive techniques such as aortic catheterisation (Shapiro et al. 2008). Additionally, this technique was used in the Conduit Artery Function Evaluation (CAFE) study, which was a large prospective, randomised, blinded, controlled trial that recruited more than 2,000 participants (Williams et al. 2006). It uses a pin-like tonometer to capture the radial pressure waveforms. It enables researchers to assess not only the arterial stiffness (i.e. PWV), but also the central aortic BP and the marker of arterial wave reflection (AIx). It is, however, an operator-dependent tool requiring a trained person to obtain high-quality waveforms; the tonometer needs to be placed on the maximum pulsating point on the radial artery to flatten the arterial wall without occluding the blood flow.

1.3.5.2.2 Waveform analysis by oscillometric method

Originally, the oscillometric technique was described by Marey (1876). It was defined as repeated fluctuation in the systolic and diastolic BP above and below the mean of intra-arterial pressure (Mauck et al. 1980); the maximum oscillation point corresponds to this mean. A novel oscillometric method has been recently introduced to determine AIx and PWV using a common BP cuff (Franssen & Imholz 2010; Wassertheurer et al. 2010; Weber et al. 2011; Wei et al. 2010; Weiss et al. 2012). Currently, there are two validated oscillometric devices: Mobil-O-Graph PWA (Stolberg, Germany) (Wei et al. 2010), and CardioMon (Vienna, Austria) (Wassertheurer et al. 2010).

This innovative oscillometric method is non-invasive and measures peripheral and central BP, and arterial stiffness at once. At first, researchers at the Austrian Institute of Technology in Vienna (ARCSolver) measured AIx and PWV using a three-level mathematical algorithm (Wassertheurer et al. 2010; Wassertheurer, Siegfried, Mayer & Breitenecker 2008); two separate cycles are followed to obtain data for the algorithms. In the first cycle, SBP and DBP are measured using a conventional oscillometric method. After the first BP reading, the second cycle begins, where the pulse wave is recoded using a pressure sensor. The sensor measures

the strength of the pulse signal during the continuous deflation (for about 10 seconds) of the brachial BP cuff from a highest SBP level to zero. The recorded signals are generated by a generalised transfer function. The aim of using this transfer function is to modify the frequency range in the obtained signal and to catch the aortic pressure wave. Then, both AIx and PWV are calculated using mathematical equations. The difference between the DBP and SBP is used to obtain the percentage of the AIx. The time difference between the first and second peak of the SBP is measured to calculate the PWV.

1.3.5.2.3 Pressure waveform and central blood pressure

Authors suggest that central BP might be used in practice in diagnosing hypertension and in adjusting anti-hypertensive treatment (Agabiti-Rosei et al. 2007), however, there is no convincing evidence yet that central BP can be used for these purposes. The peripheral SBP has been found to be higher (about 40 mmHg) than the aortic central SBP; conversley, the mean and diastolic BP are fairly constant in the arterial tree (Kroeker & Wood 1955; Ohte et al. 2007). This amplification in the brachial SBP has been explained by the changes occurring in the arterial tree due to arterial stiffness. The forward pressure wave generated by the heart during the systole travels from the highly elastic artery (i.e. aorta) to stiffer peripheral arteries (i.e. brachial and radial). Thus, the upper part of the wave becomes narrower and more prominent and the BP increases (Figure 1-18). This phenomenon is known as “systolic pressure amplification” (McEniery et al. 2014). This change, combined with early arrival of the reflected wave during the systolic phase of the cardiac cycle, augment the BP by increasing the SBP. As the degree of this augmented pressure in relation to the central pulse pressure is used to calculate the AIx, central BP can be obtained from AIx. Invasive aortic catheterisation is the gold standard method of measuring central BP, however, it can be calculated by generalised transfer function incorporated in 24-h BP monitors such as Mobil-O-Graph.

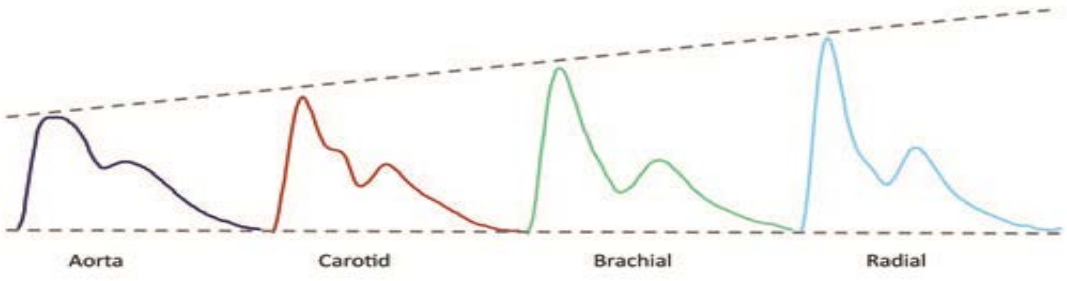


Figure 1-19 Amplification of the pressure waveform moving from the aorta to the radial artery

From McEniery et al. 2014, p. 1720

1.3.5.3 Arterial stiffness and cardiovascular risk in rheumatoid arthritis

The risk of CV morbidity and mortality is high in the RA population. Arterial stiffness was independently associated with higher CV morbidity in RA patients (Provan et al. 2011). As CVD takes a long time to develop (Ross 1999), RA patients usually remain asymptomatic for a prolonged period of time before presenting with CV events or sudden cardiac death (Maradit-Kremers et al. 2005a; Stamatelopoulos et al. 2010). Thus, assessing the degree of arterial stiffness might prevent CV events by establishing early diagnosis and management. Arterial stiffness in RA has been assessed in epidemiological studies by measuring PWV. Moreover, the changes in the marker of arterial wave reflection (AIx) was examined among RA patients, but its clinical application in preventing CVD in RA is still under investigation.

Available evidence regarding the risk of arterial stiffness in RA is contradictory. There is good evidence that RA patients have higher arterial stiffness compared to healthy populations (Arosio et al. 2007; Avalos et al. 2007; Kim et al. 2012; Klocke et al. 2003; Pieringer et al. 2012; Pieringer et al. 2009; Wallberg-Jonsson et al. 2008; Wong et al. 2003; Yildiz et al. 2004). This is independent of the presence of traditional CV risk factors (Klocke et al. 2003; Pieringer et al. 2012; Pieringer et al. 2009). The case-control study by Klocke and colleagues (2003) showed that the mean AIx was significantly increased in RA cases compared to control (AIx 26.2% vs 18.9%, $p = 0.02$). Additionally, Pieringer et al. (2012) found even greater difference in AIx in RA patients compared to controls (30.5% vs 24%). This difference was comparable to that found among smokers, and diabetic patients (Stamatelopoulos et al. 2009), but other studies have reported contrasting results. Increased AIx among RA patients has been associated with one or more of the traditional CV risk factors. Hypertension (McVeigh et al. 1991), smoking (Mahmud & Feely 2003), T2D (Brooks, Molyneaux & Yue 2001), and hypercholesterolemia (Wilkinson et al. 2002) are the risk factors found to increase arterial stiffness. Similarly, a recent study showed that the risk of arterial stiffness among RA patients is similar to the normal population in the absence of traditional CV risk factors (Arida et al. 2015).

There were limited studies assessing arterial stiffness by PWV among RA patients; yet a small number of studies showed that PWV was higher in patients with RA (Kocabay, Hasdemir & Yildiz 2012; Yildiz et al. 2004). While some studies found that such a rise in PWV was independently associated with RA (Ikeda et al. 2011; Mulders et al. 2012), others claimed that it was dependent on traditional CV risk factors (Li et al. 2013). Li and colleagues (2013) showed that arterial stiffness measured by PWV was dependent on age and hypertriglyceridemia.

Even though the exact mechanism of increased arterial stiffness in RA patients is still unclear, chronic inflammation might partially contribute to it. The level of systemic inflammation measured by the acute-phase reactants CRP (Yasmin et al. 2004), and ESR (Crilly et al. 2009) has been correlated with higher arterial stiffness. Based on published estimators of CV risk, the study by Yasmin (2004) categorised the level of CRP into three groups: <1, 1–3, and >3 mg/L; the highest value of PWV was correlated with the highest category (>3 mg/L). Furthermore, PWV is directly related to the level of inflammation. The mean of aortic PWV was 7.0±2.4; 7.6±2.5; 8.2±2.8 m/second when CRP was <1, 1–3, and >3 mg/L, respectively. In fact, CRP has been found to be inversely related to the degree of arterial elasticity in RA patients (Wong, M. et al. 2003), which might explain the increased arterial stiffness. Similarly, there is a dose-response relationship between the level of cumulative inflammation measured by ESR and the degree of arterial stiffness assessed by AIx among the RA population (Crilly et al. 2009). AIx was increased by 0.51 (95% CI 0.13, 0.88) with every 100 increase in ESR-years; this finding was independent of the presence of traditional CV risk factors. Another study reported that high systemic inflammation measured by ESR is associated with increased PWV in RA patients, which can be reversed by effective anti-inflammatory treatment (Maki-Petaja et al. 2006).

1.3.6 Asymmetric dimethylarginine

Asymmetric dimethylarginine (ADMA) is a modified amino acid synthesised naturally in humans that regulates vascular tone indirectly. Recently, ADMA has received attention as it is a powerful endogenous inhibitor to the enzyme eNOS, which is responsible for NO synthesis (Wadham & Mangoni 2009). Increased plasma ADMA concentration resulted in a reduction in NO synthesis that contributes to arterial stiffness (Böger 2003; Böger 2003; Cooke 2005; Kielstein et al. 2006; Mangoni 2009; Zhang et al. 2008). This section will discuss the physiology and pathophysiology of ADMA, which contributes to CV risk.

1.3.6.1 Physiology of asymmetric dimethylarginine

1.3.6.1.1 Synthesis and degradation

ADMA is an amino acid circulating in plasma and excreted in urine (Cooke 2000; McDermott 1976). The formation of ADMA is based on the availability of $_{L}$-arginine, which is a protein residue. Protein arginine undergoes a methylation process (Clarke 1993; McBride & Silver 2001), where one or two methyl groups are added into guanidine nitrogen of the protein arginine. This methylation is activated by protein arginine methyl transferase enzymes (PRMT); there are two different types of PRMT enzymes involved in the methylation process (Vallance &

Leiper 2004). The first type of PRMT catalyses the formation of ADMA. The second type of PRMT helps in the formation of symmetric dimethyl arginine (SDMA) by methylating both guanidine nitrogens. This SDMA is the biologically inactive isomer of ADMA. In addition to methylation function of PRMT enzyme, both types of this enzyme are able to mono-methylate the protein arginine. The latter process leads to the formation of $N^{G}{}_{L}$-arginine (L-NMMA) (Clarke 1993; McBride & Silver 2001). Interestingly, ADMA and L-NMMA increase CV risk as they both inhibit eNOS (Vallance et al. 1992).

The majority of ADMA is extensively metabolised. The main metabolic pathway is via citrulline and dimethylamine reaction, which is catalysed by dimethylarginine dimethylaminohydrolases (DDAH) (Kimoto et al. 1995). Two isoforms of DDAH have been identified: DDAH-1 (identified on chromosome 1), and DDAH-2 (located on chromosome 6). Although both isoforms have the same activity, each one of them has a distinct tissue distribution. There are overlaps between the expression of DDAH-1 and DDAH-2 and neural NOS and endothelial eNOS, respectively (Leiper et al. 1999). While both forms of DDAH have been found in the tissue of the CV system, DDAH-2 has been identified in larger quantities. The increased level of ADMA might be theoretically reversed by arginine restoring NOS activity (Vallance & Leiper 2004). Another approach could be increasing the expression or activity of DDAH. In experimental models, oestrogens have been found to increase DDAH expression, which reduces the level of ADMA (Holden et al. 2003).

1.3.6.2 Pathophysiology

ADMA has been detected in the cardiovascular system and its bioavailability depends on type 1 PRMT enzyme (Vallance & Leiper 2004). This enzyme was found in cardiac smooth muscle as well as endothelial cells of the vascular system. The level of shear stress (Osanai et al. 2003) and oxidised LDL (Böger et al. 2000) have been found to regulate the expression of this enzyme in the endothelial cells. Thus, high levels of these regulators increase the expression of PRMT leading to synthesis of ADMA. Moreover, diseases affecting the kidneys, including renal failure, increase the concentration of circulating ADMA as methylarginines are mainly cleared by the kidneys (Vallance et al. 1992). In fact, the concentration of ADMA and SDMA were found to be high in uraemic patients and were correlated with the level of creatinine (MacAllister et al. 1996). The concentration of SDMA was, however, eightfold higher than ADMA in patients with renal failure corresponding to the level of creatinine concentration. This higher concentration of SDMA is not surprising; while almost all SDMA is removed by the renal excretion, both ADMA and L-NMMA undergo extensive metabolism by DDAH rather than renal excretion (Mathew et

al. 2015; Ogawa et al. 1987). High concentration of ADMA might be explained by the direct effect of NO. DDAH is directly inhibited by NO via S-nitrosation at the active site cysteine residue (Vallance & Leiper 2004). Subsequently, ADMA accumulates in higher concentration leading to eNOS inhibition (Wadham & Mangoni 2009). Thus, ADMA is considered as a powerful endogenous inhibitor of eNOS; and it is an indirect indicator of endothelial dysfunction (Böger 2004; Mangoni 2009).

1.3.6.3 The emerging role of asymmetric dimethylarginine in vascular diseases

High ADMA levels increase the risk of arterial stiffness and subsequent CV events. There is emerging evidence showing that subclinical atherosclerosis assessed by IMT and flow-mediated dilatation (FMD) is correlated with high concentration of ADMA (Miyazaki et al. 1999); this increased risk is more prominent with highest quartile for ADMA (> 0.62 micromol/L). Besides the increased risk of atherosclerosis, the literature shows growing evidence about the impact of high levels of circulating ADMA on the development of incident CV events (Valkonen et al. 2001). A recent meta-analysis of observational studies showed that the risk of combined CVD is 42% in participants with high ADMA (RR = 1.42, 95% CI 1.29, 1.56) (Willeit et al. 2015). Similar increased risk has been documented for coronary artery disease (RR = 1.39, 95% CI 1.19, 1.62) and stroke (RR = 1.60, 95% CI 1.33, 1.91). Conversely, the impact of SDMA concentration on CV risk was not significant (RR = 1.32, 95% CI 0.92 to 1.90). This lack of association might be explained by low statistical power as only a few studies were included in the meta-analysis.

1.3.6.3.1 L-arginine-nitric oxide pathway and endothelial dysfunction

The main precursor for the synthesis of NO is $_{L}$-arginine. Usually, eNOS enzyme converts $_{L}$-arginine into NO and L-citrulline in the endothelium (Böger 2003). In contrast, ADMA is an endogenous inhibitor of eNOS competing with arginine on the active site of this enzyme (Figure 1-20). The available literature suggests that ADMA plays a key role in regulating vascular tone, reducing the availability of NO by inhibiting eNOS enzyme, which contributes to vasoconstriction and subsequent endothelial dysfunction (Böger 2003; Cooke 2005; Kielstein et al. 2006; Mangoni 2009; Miyazaki et al. 1999; Zhang et al. 2008). There is some evidence that $_{L}$-arginine administration improves the endothelial function of the coronary arteries (Lerman et al. 1998). Additionally, low dose L-arginine supplementation improves not only vasodilatation, but also insulin resistance in diabetic and obese patients (Wascher et al. 1997). This enhancement in

the function of the endothelium could be explained by the ability of $_{L}$-arginine to stimulate NO production, improving vasodilatation.

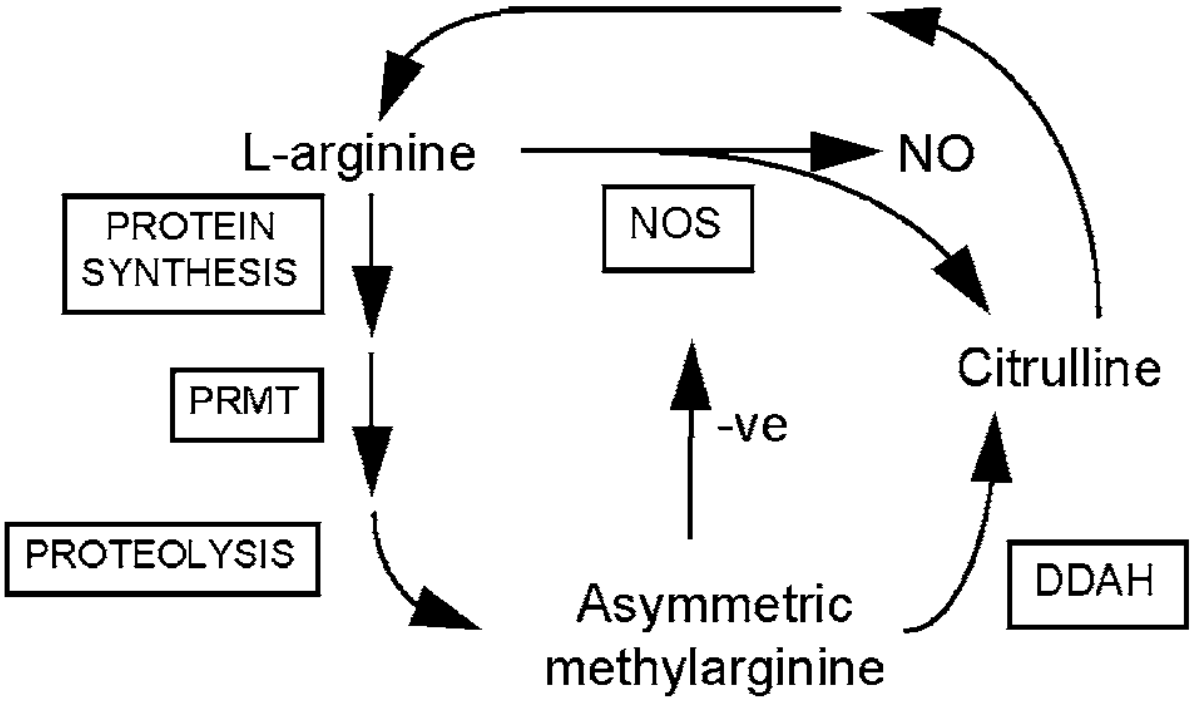


Figure 1-20 Asymmetric dimethylarginine and nitric oxide synthesis

From Anthony, Leiper & Vallance 2005, p. 7

Besides being a potent vasodilator, NO has the ability to inhibit inflammatory processes; the key processes inhibited by NO include platelet adhesion and aggregation, and monocytes adhesion (BogerBode-BogerSzuba et al. 1998). Another important function of NO is inhibiting LDL oxidation via reducing superoxide radicals (Hogg et al. 1993). ADMA thereby contributes to the process of developing CVD.

1.3.6.3.2 Asymmetric dimethylarginine and traditional cardiovascular risk factors

Increased level of ADMA has been found to be associated with traditional CV risk factors, such as hypertension, T2D, dyslipidaemia, and smoking.

First, high levels of ADMA are associated with hypertension (Goonasekera et al. 1997; Landim, Casella Filho & Chagas 2009; Leiper et al. 2007; Miyazaki et al. 1999). In healthy asymptomatic volunteers, the level of SBP and DBP were positively correlated with the level of ADMA ($r = 0.45$, $p < 0.0001$ and $r = 0.41$, $p < 0.0001$, respectively) (Miyazaki et al. 1999). In patients with

pulmonary hypertension induced by hypoxia, ADMA concentration has been shown to be increased, together with lower activity of DDAH (Arrigoni et al. 2003). This leads to eNOS inhibition and subsequent vasoconstriction. As the sensitivity to NOS inhibitors and L-NMMA is increased in the pulmonary arteries, the pulmonary BP increases rapidly (Petros et al. 1994). High concentration of ADMA has been associated not only with a prompt increase in BP, but also in systemic arterial resistance as a result of impairment of vascular tone. Another proposed effect of decreased level of NO and high concentration of ADMA is vascular inflammation (Böger et al. 2000), and LDL oxidisation (Hogg et al. 1993). These changes might contribute to arterial stiffness. In fact, silent brain infarcts identified by MRI were detected in patients with high levels of ADMA in the Framingham study (Pikula et al. 2009). Additionally, a meta-analysis of randomised controlled trials gives convincing evidence about the possibility of recuperating the function of the endothelium by administering an $_{L}$-arginine supplement (Bai et al. 2009), which subsequently reduces the BP (Dong et al. 2011).

Although the effect of T2D on the level of ADMA is still conflicting, there is little evidence showing that the level of ADMA is increased in diabetic patients (Landim, Casella Filho & Chagas 2009). There is some evidence pointing to insulin resistance as an explanatory factor. Concentration of ADMA has been positively correlated with insulin resistance (Dimitroulas et al. 2013; Stuhlinger et al. 2002). This insulin resistance increases the level of ADMA due to decreased NO bioavailability (Dimitroulas et al. 2013; Sydow, Mondon & Cooke 2005). In an experimental study, the level of ADMA was reduced when insulin was injected into diabetic rats (Xiong et al. 2003). Additionally, the level of ADMA was reduced by using metformin and the thiazolidinediones (Asagami et al. 2002). Recently, insulin sensitisers such as rosiglitazone have been recommended to modulate the NOS/DDAH pathway (Lai & Ghebremariam 2016). In fact, direct suppression of the activity of DDAH might be enhanced by glucose, leading to ADMA accumulation (Lin et al. 2002); however, the exact mechanism of diabetes and insulin resistance, and increased ADMA, is yet to be clarified.

While few studies have found a positive relationship between dyslipidaemia and ADMA (Böger 2003; Böger et al. 2000; Landim, Casella Filho & Chagas 2009), others have not (Miyazaki et al. 1999, Stuhlinger et al. 2001). Few experimental studies have found that ADMA was elevated with hypercholesterolemia (Böger et al. 1998), and with a cholesterol-enriched diet (Phivthong-ngam et al. 1998). In humans, however, the relationship between ADMA and hypercholesterolaemia is not yet established. Additionally, the level of ADMA increases in chronic mild to moderate hypertriglyceridemia leading to impaired vasodilatation measured by

FMD (Lundman et al. 2001; Stuhlinger et al. 2002). This impairment in vasodilatation is explained by reduced availability of NO as a result of increased level of ADMA leading to endothelial dysfunction. This indicates that ADMA is a novel marker of CV risk. Yet there is no clear evidence about the association between the ADMA and smoking. Even though smoking is an established risk factor for atherosclerosis, its association with ADMA concentration has not been found (Miyazaki et al. 1999); nevertheless, this study did not consider the duration and frequency of smoking. Despite the finding by Miyazaki et al. (1999), nicotine in the cigarettes has been found to impair the function of eNOS enzyme in synthesising NO (Chalon et al. 2000; Mayhan et al. 2009). The mechanism of such impairment is linked to the activation of NAD(P) H oxidase by nicotine, which contributes to superoxide anion production. This NAD(P) H oxidase is the enzyme available at endothelial cells and activated in oxidative stress; and its inhibition improves endothelial function (Hamilton et al. 2002).

1.3.6.4 Asymmetric dimethylarginine and endothelial dysfunction in rheumatoid arthritis

There is inconsistent evidence of the relationship between ADMA level and endothelial dysfunction among RA patients. Although a few authors have claimed that the level of ADMA has no effect on endothelial function (Sandoo et al. 2012), others found that ADMA is a surrogate marker for endothelial dysfunction predisposing to higher CV risk and arterial stiffness (Di Franco et al. 2012; Dimitroulas, Sandoo & Kitas 2012). Thus, it has been linked to the development of atherosclerosis among the RA population (Di Franco et al. 2012; Dimitroulas, Sandoo & Kitas 2012; Sandoo et al. 2011). Recently, the sensitivity and specificity of ADMA in detecting endothelial function in RA has been examined. ADMA is found comparable to RF in detecting the endothelial function in untreated rheumatoid patients (Spasovski et al. 2013). Sensitivity and specificity of ADMA is 57.14% and 88.57%, respectively. Interestingly, similar sensitivity (48.57%) and specificity (91.42%) are recorded for RF. Unlike ADMA, there is no evidence that SDMA modulates CV risk, both in the general population and in the RA population (Dimitroulas et al. 2015). The inconsistencies in available evidence regarding the relationship between ADMA level and the risk of endothelial dysfunction mandates further exploration.

One of the proposed effects of decreased level of NO and high concentration of ADMA is vascular inflammation (Böger et al. 2000; Sandoo et al. 2015), and LDL oxidisation (Hogg et al. 1993). Patients with RA are at higher risk of elevated level of ADMA and arterial stiffness as RA is a chronic inflammatory condition where the process of LDL oxidisation occurs in its pathway. Additionally, genetic variations in the enzymes involved in ADMA synthesis and metabolism have been investigated. For example, the association between DDAH genetic polymorphisms

and ADMA has been studied among diabetics and patients with kidney diseases (Andreozzi et al. 2012; Caplin et al. 2010; Lind et al. 2013; Sesti et al. 2013). The level of ADMA is correlated with DDAH SNPs in patients with T2D. Increased level of ADMA, which is linked to insulin resistance, has been found among diabetics (Toutouzas et al. 2008). Overexpression of *DDAH* gene enhances insulin sensitivity and improves hyperglycaemic status. This improvement in the insulin resistance decreases the ADMA accumulation and improves endothelial function; however, evidence of such association among RA patients is scarce. In fact, one study shows no significant impact of DDAH SNPs on the level of ADMA (Dimitroulas et al. 2014).

Another explanation of ADMA accumulation in RA is hyperhomocysteinemia, one of the CV risk factors in the general population (Boers 2000), and rheumatic diseases (Refai et al. 2002) including RA (Dimitroulas et al. 2016). In RA, the excess CV risk might be contributed to by an alteration in the metabolism of homocysteine by using DMARDs such as MTX. Folate deficiency has been found in patients with hyperhomocysteinemia; as MTX is an anti-folate drug, the homocysteine level is increased among RA patients on MTX via homocysteine–methionine pathway (van Ede et al. 2002); the action of DHFR enzyme is inhibited by MTX reducing the availability of folates which then impairs the metabolism of homocysteine. This leads to ADMA accumulation and subsequent premature atherosclerosis (Dayal & Lentz 2005). As homocysteine is formed as a result of methyl transfer reaction catalysed by the MTHFR enzyme, genetic variation in this enzyme might explain the observed relationship between ADMA and hyperhomocysteinemia. Genetic polymorphism of *MTHFR* C677T (rs1801133) has been examined in the RA population (Dimitroulas et al. 2016). This study shows that impaired activity of *MTHFR* gene is associated with increased levels of homocysteine and ADMA levels, which inhibit the production of NO. Similarly, *MTHFR* C677T variant has been found to increase homocysteine level among RA patients on MTX. This is because MTX supresses the folate level and *MTHFR* activity which leads to homocysteine accumulation; however, this study shows that the level of ADMA is reduced among RA patients using MTX. Patients using MTX showed a significantly lower ADMA level compared to patients not on MTX (β = -0.04, 95% CI -0.07, -0.01, p = 0.024). The mean level of ADMA was slightly reduced in RA patients with all *MTHFR* genotypes among MTX users compared to non-users; the mean ADMA level was 0.54 vs 0.57, 0.55 vs 0.56, and 0.58 vs 0.64 for the *MTHFR* 677CC, *MTHFR* 677CT, and *MTHFR* 677TT polymorphisms, respectively. Additionally, among all *MTHFR* gene variants, *MTHFR* C677T has been associated with increased risk of MTX toxicity (Caliz et al. 2012). This resulted from MTX accumulation as the activity of the MTHFR is reduced in the RA patients with this gene variant

(van der Put et al. 1998). These findings of increased MTX and decreased ADMA level might explain the reduced CV risk among RA patients using MTX.

1.3.6.4.1 Relationship between disease-modifying anti-rheumatic drugs and asymmetric dimethylarginine

Until recently, the evidence about the effect of different DMARDs on ADMA has been conflicting. Sandoo et al. (2012) claimed that there was no association between level of ADMA and endothelial dysfunction in RA patients; the same author found that the level of ADMA did not change using a TNF-α inhibitor drug among RA patients despite its anti-inflammatory effect evidenced by improved DAS28 score and reduced CRP (Sandoo et al. 2012). Alternatively, there is some evidence that a TNF-α inhibitor might improve endothelial function and subsequent arterial stiffness by reducing the level of ADMA in RA patients (Hurlimann et al. 2002; Spinelli et al. 2014; Turiel et al. 2010). In addition to the effect of TNF-α inhibitors on CV risk, MTX seems to have an effect on reducing the ADMA level in RA patients (Dimitroulas et al. 2016; Turiel et al. 2010). Such positive effect of MTX was, however, examined in a small population of patients diagnosed with early RA. Until recently, there has been no evidence to support the positive CV impact of MTX on RA patients through the ADMA pathway. Therefore, a larger prospective study would be more helpful in determining the temporal effect of MTX on ADMA and CV risk.

1.3.7 Food intake and cardiovascular risk

Although exposure to certain types of food has been linked to RA development, its effect on CV risk is still unclear. Dietary studies examined a variety of macronutrients (vegetables, fruits, and fish), and micronutrients (fatty acid, and L- ergothioneine antioxidants). There is good evidence showing that the risk of RA development was increased with lower ingestion of fruits and vegetables, and higher consumption of meat (Choi 2005) and sugary drinks (Hu et al. 2014). Conversely, evidence about the effect of consuming different food types on CV risk is emerging.

1.3.7.1 Vegetables and fruits

In the general population, there is good evidence stressing the importance of regular consumption of five serves (or 400g) of vegetables and fruits per day in preserving the CV wellbeing (World Health Organization 2003). A systematic review of cohort studies showed that regular consumption of vegetables and fruits reduce the CV morbidity (He et al. 2007). A reduction in the risk of stroke (He, Nowson & MacGregor 2006), and MI, which was independent of CV risk factors, was observed among fruit and vegetables consumers (Iqbal et al. 2008).

Recently, consumption of fruits and vegetables has been found as inversely related to the risk of all-cause mortality among the Australian population (Nguyen et al. 2016). Despite the published literature about the CV-protective effect of both fruits and vegetables, a systematic review showed that the protective effect of vegetables intake was stronger than that for fruit consumption (Mente et al. 2009).

The impact of vegetable and fruit intake on CV risk among RA patients is still in its infancy. There is some evidence of the beneficial effects of consuming such types of food on vascular function. Daily vegetable intake is independently associated with better vascular function (i.e. lower AIx) in RA (Crilly & McNeill 2012). One systematic review, however, recently published claims that there is no clear association between fruit and vegetable intake and vascular function improvement in RA (Blanch, Clifton & Keogh 2015). Yet it has been found that introducing a Mediterranean-type diet, which is high in fruit and vegetables, to RA patients improves not only disease activity, but also the CV profile (McKellar et al. 2007). Indeed, this study shows a significant reduction in SBP (about 4 mmHg) among RA patients following this dietary regimen. In summary, the association between fruits and vegetables and reduced BP and better vascular function needs more investigation.

The physiological explanation of the cardio-protective effects of fruits and vegetables has not yet been established, however, dietary inorganic nitrate is one of the factors involved (Kapil, Webb & Ahluwalia 2010). It has a role in maintaining arterial homeostasis and NO production. Leafy vegetables are enriched in nitrate (Chen et al. 2011; Menard et al. 2008; Ysart et al. 1999) but it was believed that conversion of dietary nitrate into NO was not possible. Nevertheless, Kapil, Webb and Ahluwalia (2010) have recently found that nitrate obtained from diet can be converted to the bioactive NO through ‘enterosalivary circulation’. Subsequent increase in the NO availability leads not only to vasodilation, but also BP reduction and vascular function improvement (Ignarro 2002; Ignarro & Napoli 2004). Other authors, however, suggest that the antioxidants in the fruits and vegetables exert more harm than benefits (Bjelakovic et al. 2007). This controversy in reported evidence highlights the importance of investigating the relationship between fruits and vegetables and cardiovascular risks in RA patients.

1.3.7.2 Omega-3 fatty acid

In the general population, the effect of certain food intake, such as fish oil, on BP has been examined. Fish oil consists of long-chain omega-3 fatty acids: EPA and DHA and there is good evidence of the its CV-protective effects (He et al. 2004a; He et al. 2004b; Maehre et al. 2015;

McLennan et al. 1996). The protective effects of omega-3 fatty acids include reducing the risk of developing traditional CV risk factors and CV events or CV death.

There is evidence that the risk of most of traditional CV risk factors is reduced by using omega-3 fatty acids. First, there is evidence showing that these fatty acids reduce BP (Geleijnse et al. 2002; Miller, Van Elswyk & Alexander 2014). Miller, Van Elswyk and Alexander (2014) found that the BP was reduced in hypertensive patients who took one of the two types of omega-3. Both SBP and DBP were lower by 1.52 mmHg (95% CI -2.25, -0.79), and 0.99 mmHg (95% CI -1.54, -0.44), respectively. One of the mechanisms of the observed reduction in BP was linked to the anti-inflammatory effects of these fatty acids (Ulu et al. 2014). This attenuation of inflammation was attributed to prostaglandins reduction and subsequent angiotensin-converting enzyme-2 upregulation (Ulu et al. 2013). One of the reported benefits of these fatty acids is reducing the level of plasma triglycerides and total cholesterol (O'Keefe & Harris 2000).

Compared with the general population, there is small emerging evidence showing that omega-3 fatty acids intake reduces the CV risk in the RA population (Cleland et al. 2006; Cleland, James & Proudman 2003), and it is inversely related to the incidence of MI (Verboom 2006), and stroke (He et al. 2004; Xun et al. 2012). Not only does fish oil intake prevent CVD, but it also protects from CVD death (He et al. 2004). There is a reduction of 7% in CV mortality risk with each 20 g increase in fish intake per day (RR = 0.93, 95% CI 0.87, 0.99, p-value = 0.03). The reduction in CV risk in RA has been explained by the effect of omega-3 on reducing the synthesis of pro-inflammatory eicosanoids (i.e. prostaglandin E_2 and leukotriene B_4) (Calder 2006; Smith et al. 1998). This leads to reduction in BP and lipid level. Another possible mechanism of observed lower CV risk could be related to the anti-thrombotic effect of omega-3 (Cleland et al. 2006; Kris-Etherton, Harris & Appel 2002) and, therefore, investigating the relationship between omega-3 fatty acids and arterial stiffness and BP among RA patients is importan, especially among patients on MTX.

1.3.7.3 Food intake and asymmetric dimethylarginine

Recent evidence shows that some food nutrients have an effect on the level of ADMA. In the general population there is inconsistent evidence about the level of ADMA and food intake. While high-fat meal consumption was associated with an elevation in the level of ADMA (Böger et al. 1998; Fard et al. 2000), ingesting carbohydrate (Paiva et al. 2004; van Hoorn et al. 2005) and tea and vegetables (Goralczyk et al. 2012) were associated with low levels of ADMA. Furthermore, a low protein diet and keto-amino acids among chronic kidney disease patients

have been linked to lower levels of ADMA (Teplan et al. 2008). A recent study showed that plant-derived $_{L}$-arginine is associated with lower levels of ADMA and subsequent CV protection (Bahadoran et al. 2016). Conversely, the level of ADMA has not been connected to dietary fibre (King & DeLegge 2009), soy isoflavones (Reimann et al. 2006), or fruit consumption (Goralczyk et al. 2012). There is evidence that ADMA might be influenced by ingesting dietary fibres (King, Egan & Geesey 2003), glucose and fatty acids (O'Keefe, J. H. & Bell 2007). The ingestion of a diet enriched with glucose and fatty acids causes oxidative stress as both nutrients resulted in a transient release of free radicals; this resulted in NO depletion and endothelial dysfunction, both of which are linked to the pathway of ADMA metabolism (O'Keefe & Bell 2007). Although fibre consumption reduces the risk of CVD (Hartley et al. 2016), it has shown no effect of the level of ADMA (King & DeLegge 2009; King et al. 2008); however, its ingestion reduces the oxidative stress (Ignarro, Balestrieri & Napoli 2007) which might reduce the level of ADMA and prevent CVD.

Similarly, evidence about the effect of dietary nutrients on the level of ADMA in RA patients is lacking. In fact, recent evidence shows that consuming omega-3 fatty acids, vitamins E and A, copper, and selenium showed the effects on ADMA levels (Kayacelebi et al. 2014; Willers 2011). Interestingly, these studies rejected the concept that ADMA is a surrogate marker for the excess CV risk documented in RA patients. There is evidence, however, linking high fibre consumption to lower risk of elevated CRP (OR = 0.64, 95% CI 0.43, 0.96) (King, Egan & Geesey 2003). As inflammation is one of the proposed pathways in increasing the ADMA and decreasing the NO (Böger et al. 2000; Sandoo et al. 2015), high fibre ingestion might play a key role in reducing inflammation and ADMA level in RA patients.

1.4 Review of the literature -Summary

RA increases the risk of CV morbidity and mortality. Meta-analysis published by our group showed that traditional CV risk factors exert additional impacts on developing CVD. In addition to the presence of traditional CV risk factors, the most common pathophysiological influence triggering the process of RA and atherosclerosis is inflammation. MTX is an anchor drug for RA that reduces inflammation. Additionally, MTX showed positive impacts on CV morbidity and mortality among the RA population. Even though MTX effects in reducing inflammation and preventing joint destruction among RA patients are well understood, the exact mechanism of this drug in reducing CV risk is still unknown. This raise an important question: “are the CV

protective effects of MTX related to its anti-inflammatory action or are they due to another mechanism?".

Although the main purpose of using MTX in the RA population is to reduce inflammation, there is emerging evidence highlighting the CV beneficial impact. Few observational studies have shown that MTX use is inversely related to BP (Ajeganova et al. 2013; Choi et al. 2002; Cuchacovich & Espinoza 2009; Hansel et al. 2003; Panoulas et al. 2007; Rho et al. 2009; van Halm et al. 2006). MTX reduces the BP despite it being an anti-folate drug reducing the level of folate and increasing the level of homocysteine. Hyperhomocysteinemia has been linked to endothelial dysfunction as it increases the BP and impairs endothelial vasodilatation. Additionally, hyperhomocysteinemia leads to ADMA accumulation, which has a direct effect on the endothelium by inhibiting eNOS reducing NO bioavailability. MTX seems to have an effect on reducing the ADMA level in RA patients (Dimitroulas et al. 2016; Turiel et al. 2010). This complex mechanism between MTX, ADMA, and NO might explain the cardio-protective effect of MTX.

Several limitations of the studies examined the impact of MTX on CV risks have been noted and interpretation of this data might be curtailed. First, peripheral BP was measured at clinic level and had not been integrated with either central BP or with 24-h ambulatory BP. Measuring central BP by either PWA or by ambulatory monitor gives a comprehensive assessment of aortic BP. Second, there is lack of BP comparison between RA patients on MTX and matched patients not on MTX. Third, there is no published data of markers of arterial dysfunction, including PWV and arterial stiffness. Moreover, there is a lack of data showing a comprehensive MTX exposure measured by the intracellular concentration of MTXPGs and adjusting for genetic polymorphisms of relevant transporters and enzymes modulating MTX pharmacokinetics. It is known that ADMA increases CV risk; however, until recently, there has been no evidence to support the positive CV impact of MTX on RA patients through the ADMA pathway. A larger prospective study would therefore be more helpful in determining the temporal effect of MTX on ADMA and CV risk.

CV risk and arterial stiffness can be influenced by dietary nutrient intake, such as omega-3 fatty acids, but evidence about the effects of food intake in the RA population is controversial. Determining and then adjusting the differences in nutrient intake between patients with RA allows better examination of the association between the use of MTX, BP, arterial stiffness and

inflammation. Additionally, establishing a causal effect between these food nutrients and CVD development can be achieved by following patients over time.

1.5 Project hypotheses and aims

1.5.1 Hypothesis

In patients with RA we speculate:

1. That MTX use is associated with a lower BP and lower markers of arterial function (PWV and AIx)
2. In patients on MTX, there is an inverse relationship between the dose and/or the concentration of MTX and BP
3. The effect of MTX on BP and arterial function is independent of inflammatory markers
4. The effect of MTX on BP and arterial function depends on the genetic polymorphisms of transporters and enzymes involved in MTX metabolism.

1.5.2 Aims

1. To determine, within the context of current clinical practice, the relationship between MTX dose and concentration, BP and vascular function among patients with RA in a cross-sectional manner and after 8 months of follow-up
2. To analyse the temporal effects of MTX, during the follow-up period, on BP and vascular function in a group of recently RA diagnosed patients commenced on MTX
3. To compare the BP and vascular function in patients on MTX versus patients not on MTX but on other anti-rheumatic medications
4. To study the relationship between MTX and several inflammatory markers
5. To determine whether any association between MTX and BP and vascular function is independent of inflammation.
6. To determine whether the relationship between MTX dose, concentration, BP and vascular function is affected by genetic polymorphisms of transporters and enzymes involved in MTX metabolism
7. To determine if the proposed CV-protective effect of MTX is related to ADMA plasma level.

In this project, a comprehensive approach was used that allowed us, in the first place, to explore the relationship between the use, dose and concentration of MTX, BP and arterial

function in RA. By measuring the intracellular MTX, we obtained an accurate measure of exposure versus plasma MTX concentrations. Additionally, examining the genetic polymorphisms of this anti-folate drug provided an insight into drug response based on inherited inter-individual differences. In this study, ADMA level was measured in patients' plasma. Assessing MTX polymorphism, measuring MTX level using the novel intracellular concentration, and measuring ADMA concentration helped us in exploring the relationship between MTX and CV risk and establishing a possible mechanism. Moreover, monitoring the BP over 24 hours is an accurate estimate of CV risk, especially among RA population. To our knowledge, this project is the first to use 24-h BP monitoring in RA patients and the first to measure intracellular MTX in the same population.

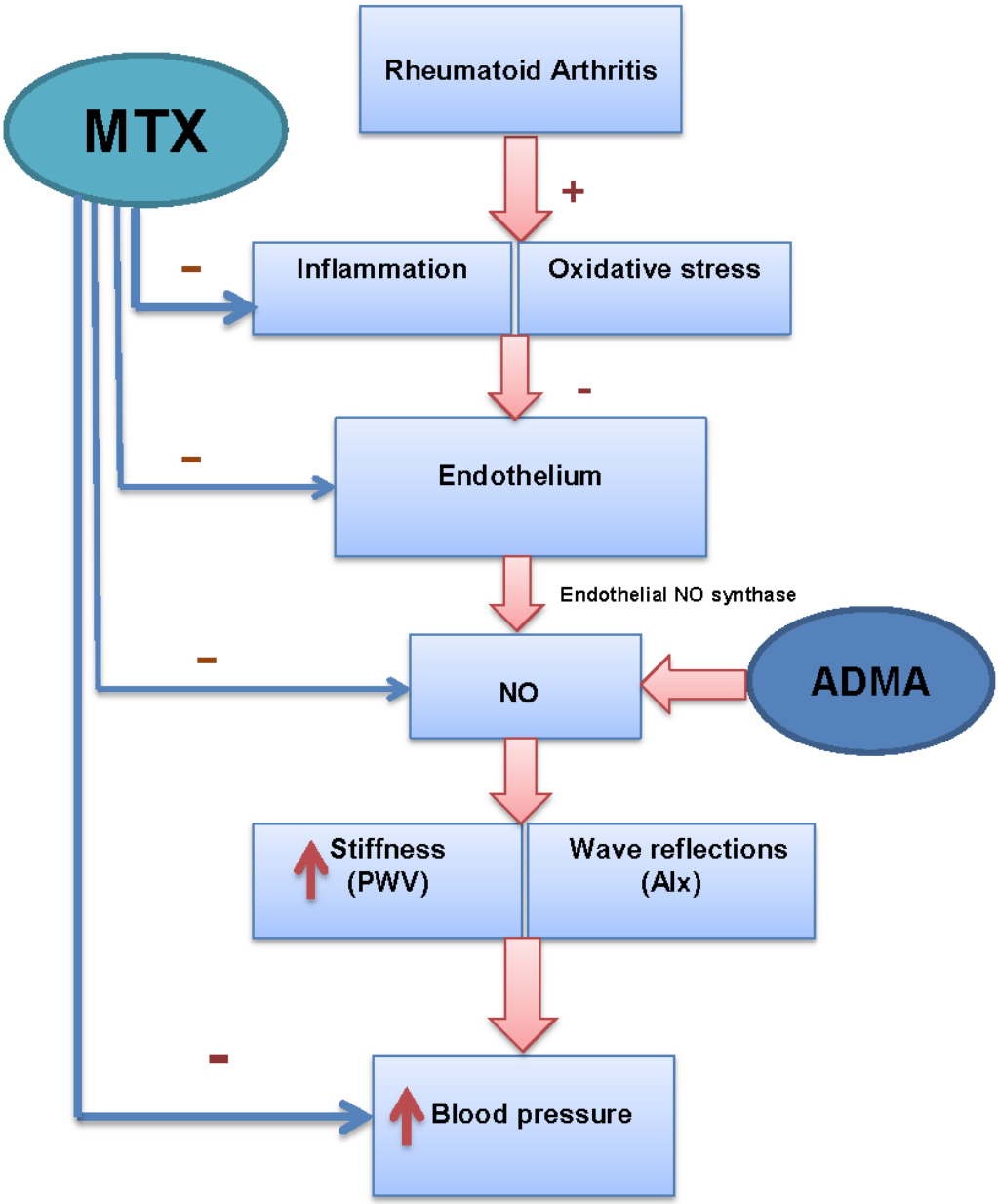


Figure 1-21 Proposed hypotheses

MTX = Methotrexate; ADMA = Asymmetric dimethylarginine; NO = Nitric oxide; eNOS = Endothelial NO synthase; PWV = Pulse Wave Velocity; AIx = Augmentation index; (-) inhibition; (+) stimulation

CHAPTER 2: METHODOLOGY

2.1 Introduction

Drawing from literature findings discussed in Chapter 1 this chapter will examine the relationship between the exposure to MTX and BP and arterial function in the RA population. RA is associated with high risk of CV morbidity and mortality with evidence supporting the protective effect of MTX still emerging. In determining CV risk measuring both peripheral and central BP are preferable in estimating patient's BP. Indeed, 24-h BP monitoring generally gives more accurate readings for the BP compared with a single clinic BP measurement. Therefore, measuring the clinic and 24-h peripheral BP, and central BP allow much richer comparisons of BP variability among RA patients. PWV is the gold standard method of assessing arterial stiffness, allowing further exploration of MTX and vascular function relationship in the RA population. Additionally, measuring concentration of MTXPGs inside the cells is a novel method providing another dimension in assessing the relationship between exposure to MTX and CV outcomes of interest. As ADMA has been considered a marker of endothelial dysfunction and its level might be decreased by MTX drug administration, its plasma concentration was measured in this study.

In this chapter, research methodology including study design and data collection and analyses will be presented. This will also include brief introductory background about central BP and PWA.

2.2 Study design and justifications

We used a quantitative observational approach in this research. In the first stage, a cross-sectional study was conducted to examine the BP and arterial markers in a) RA patients on MTX, b) RA patients not on MTX but on other DMARDs, and c) newly diagnosed RA patients on none of the DMARDs but suitable candidates for MTX. In the second stage, these participants were observed at 8 months follow-up thereby providing a repeated cross-sectional design, which allowed us to examine if there was any change in the associations between MTX usage and the BP level and/or arterial function. Thus, exposures and outcomes were assessed using the same methods of assessment (i.e. same questionnaires and 24-h BP monitoring) on the same RA population, but at different time points. Using this repeated cross-sectional design has an advantage in obtaining precise estimates because of an effectively larger sample size. Moreover, this study design is suitable because we wished to observe the change in the trend of the outcomes (BP and arterial function surrogate markers: AIx and PWV) over time.

The approach of selecting RA patients was based on identifying a single cohort of RA patients and then following them over time. These patients were further subdivided into different groups according to their exposures. The group classified as the exposed cohort was compared to the cohort that was classified as unexposed cohort “an internal comparison group”. Participants were classified according to their MTX exposure: 1) currently taking MTX for at least 8 weeks (MTX group); 2) on other DMARDs and either never used MTX (MTX naïve) or only used MTX for <1 year before using other DMARDs (non-MTX group); 3) newly diagnosed RA patients who were not commenced on any DMARDs (new RA group). This means that both exposed and unexposed cohorts were driven from the same underlying population (i.e. RA patients); the non-MTX cohort was used as a natural comparison group. This method has been used for decades in several studies: British doctors (Doll et al. 2004; Doll et al. 1994), US nurses (Belanger et al. 1978), and Framingham cohorts (Vasan et al. 2001). As the unexposed cohort was a natural comparison group, subjects in both exposed and unexposed cohorts were more likely to be comparable in terms of clinical and demographic characteristics.

2.3 Setting

Recruiting and following patients attending the rheumatology outpatient clinics at Flinders Medical Centre (FMC) and Repatriation General Hospital (RGH) took place between March 2014 and December 2015.

FMC is a major referral centre serving the southern suburbs, the northern parts of South Australia, interstate and regional areas. Being a major referral institution, FMC provides 350,000 outpatient consultations yearly. Rheumatology outpatient and inpatient services at FMC and the RGH are managed under one umbrella, which is the Rheumatology Unit. Weekly, there are approximately three new RA cases diagnosed by a rheumatologist in either location. Most patients with rheumatic joint pain are more likely to be referred by their local general practitioners to FMC or RGH. Thus, almost all new cases of RA are more likely to be initially diagnosed, managed and followed by a rheumatologist. Offering public and private medical services for most South Australian patients, the Rheumatology Unit is one of the suitable data sources as high participation rate is expected resulted in a better external validity.

2.4 Eligibility criteria

2.4.1 Inclusion criteria

Patients were included if:

1. they were aged 18 years or older

2. they had a diagnosis of RA made based on 1987 or 2010 American College of Rheumatology diagnostic criteria (Table 2-1), and the rheumatologist's diagnosis.

Table 2-1 The 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for rheumatoid arthritis

Classification criteria Score	
Target population (Who should be tested?): Patients who **1. have at least 1 joint with definite clinical synovitis (swelling)*** **2. with the synovitis not better explained by another disease†**	
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)‡**	
A. Joint involvement§	
1 large joint¶	**0**
2-10 large joints	**1**
1-3 small joints (with or without involvement of large joints)#	**2**
4-10 small joints (with or without involvement of large joints)	**3**
>10 joints (at least 1 small joint)**	**5**
B. Serology (at least 1 test result is needed for classification)††	
Negative RF and negative ACPA	**0**
Low-positive RF or low-positive ACPA	**2**
High-positive RF or high-positive ACPA	**3**
C. Acute phase reactants (at least 1 test result is needed for classification)‡‡	
Normal CRP and normal ESR	**0**
Abnormal CRP or abnormal ESR	**1**
D. Duration of symptoms§§	
<6 weeks	**0**
≥6 weeks	**1**

From Aletaha et al. 2010, p. 2574

* The criteria are aimed at classification of newly presenting patients. In addition, patients with erosive disease typical of rheumatoid arthritis (RA) with a history compatible with prior fulfilment of the 2010 criteria should be classified as having RA. Patients with longstanding disease, including those whose disease is inactive (with or without treatment) who, based on retrospectively available data, have previously fulfilled the 2010 criteria should be classified as having RA.

† Differential diagnoses vary among patients with different presentations, but may include conditions such as systemic lupus erythematosus, psoriatic arthritis, and gout. If it is unclear about the relevant differential diagnoses to consider, an expert rheumatologist should be consulted.

‡ Although patients with a score of 6/10 are not classifiable as having RA, their status can be reassessed and the criteria might be fulfilled cumulatively over time.

§ Joint involvement refers to any swollen or tender joint on examination, which may be confirmed by imaging evidence of synovitis. Distal interphalangeal joints, first carpometacarpal joints, and first metatarsophalangeal joints are excluded from assessment. Categories of joint distribution are classified according to the location and number of involved joints, with placement into the highest category possible based on the pattern of joint involvement.

¶"Large joints" refers to shoulders, elbows, hips, knees, and ankles.

"Small joints" refers to the metacarpophalangeal joints, proximal interphalangeal joints, second through fifth metatarsophalangeal joints, thumb interphalangeal joints, and wrists.

** In this category, at least 1 of the involved joints must be a small joint; the other joints can include any combination of large and additional small joints, as well as other joints not specifically listed elsewhere (e.g., temporomandibular, acromioclavicular, sternoclavicular, etc.).

†† Negative refers to IU values that are less than or equal to the upper limit of normal (ULN) for the laboratory and assay; low-positive refers to IU values that are higher than the ULN but ≤3 times the ULN for the laboratory and assay; high-positive refers to IU values that are >3 times the ULN for the laboratory and assay. Where rheumatoid factor (RF) information is only available as positive or negative, a positive result should be scored as low-positive for RF. ACPA = anti citrullinated protein antibody.

‡‡ Normal/abnormal is determined by local laboratory standards. CRP = C-reactive protein; ESR = erythrocyte sedimentation rate.

§ § Duration of symptoms refers to patient self-report of the duration of signs or symptoms of synovitis

(e.g., pain, swelling, tenderness) of joints that are clinically involved at the time of assessment, regardless of treatment status.

2.4.2 Exclusion criteria

Exclusion criteria were:

1. sustained atrial fibrillation, because conducting PWA is contraindicated in those patients as it gives unreliable analysis (Lantelme et al. 2002)

2. active cancers, as some anticancer drugs (including chemotherapy) induce endothelial dysfunction, which might affect PWA and BP (Mailloux et al. 2001; Stamatelopoulos et al. 2004)

3. CHF (left ventricular diastolic dysfunction)

4. cognitive impairment.

2.4.3 Withdrawal criteria

There were no strict withdrawal criteria unless the patients developed the conditions mentioned in the exclusion criteria or they withdrew their consent.

2.4.4 Attrition rate

The aim was to keep the percentage of loss of follow-up as low as possible and less than 20%. Minimum loss to follow-up was expected for several reasons. First, RA is a chronic disease characterised by remissions and relapses, which requires regular follow-up, and clinical and laboratory assessments. Additionally, information about any changes in MTX doses were documented in the patients' records and hospital database allowing a possible tracking procedure.

2.5 Participants

2.5.1 Methods of identifying the participants

Rheumatologists at FMC and RGH, who were part of one department of rheumatology, provided two lists of RA patients attending the outpatient clinics and with stable RA; one list included RA patients taking MTX and the other list for non-MTX RA patients on other anti-rheumatic medications. Additionally, a third list of new RA patients who were possible candidates for MTX was offered. The chief investigator (Dr Leena Baghdadi) was, however, attending the outpatient clinics, when needed for identifying potential subjects. After identifying potential participants, introductory letters explaining the research protocol (Appendix-2), including consent forms, (Appendix-3) were posted or given to the eligible participants. Eligibility of each patient was cross-checked by screening the patient's hospital records and by asking the patients about any history of active cancer or heart diseases (Figure 2-1). Patients who agreed to participate in the study were invited by phone to attend special sessions at the rheumatology department at the RGH.

Finding RA patients in the non-MTX cohort was somewht more difficult compared to the MTX cohort. Patients who attended the biologic clinics run under the rheumatology clinics were considered as a way to find more RA patients who were off MTX and whose inclusion would thereby increase the power of the study. We found, however, that RA patients taking different types of biologics were on MTX as well, and they needed to be part of the MTX cohort rather than in the control group. It is a common practice to commence RA patients on combined therapies (biologics and MTX), especially for those with uncontrolled disease activity. There is

evidence showing that a concomitant MTX drug added to biologic treatments improves the efficacy of biologics and gives longer persistence (Ruderman 2013). Further sorting of patients' medications history was therefore conducted to identify and allocate patients to be included to the non-MTX cohort. RA patients who were taking tocilizumab (actemra), an IL-6 receptor antibody, were sent information sheets and consent forms. This alternative approach was used as MTX is contraindicated in patients taking tocilizumab. This prohibited combination is due to possible drug interaction leading to a higher risk of developing several side effects such as liver toxicity (An et al. 2010; Campbell et al. 2011; Choy et al. 2005).

At the baseline visit, information about the study was explained to the patients, including indications of risks, any discomfort involved, anticipation of length of time, and the frequency of study visits. Any questions raised by the patients were answered in detail. Eight months after the baseline visit, all participants were invited by phone for the second session at the RGH (Figure 2-1). All patients were contacted between both visits to ensure the follow-up. The inclusion of newly diagnosed RA patients who were candidates for MTX provided an additional dimension as it allowed analysis of the short-term longitudinal changes in BP and arterial parameters after MTX was commenced (i.e. temporal effects of MTX). This was a subpilot study, within the main study, for further hypothesis generation as we speculated that there is a possible inverse relationship between the dose and/or the concentration of MTX and BP, and better PWA profile. This RA cohort was subsequently excluded as few eligible patients (only 6) were found.

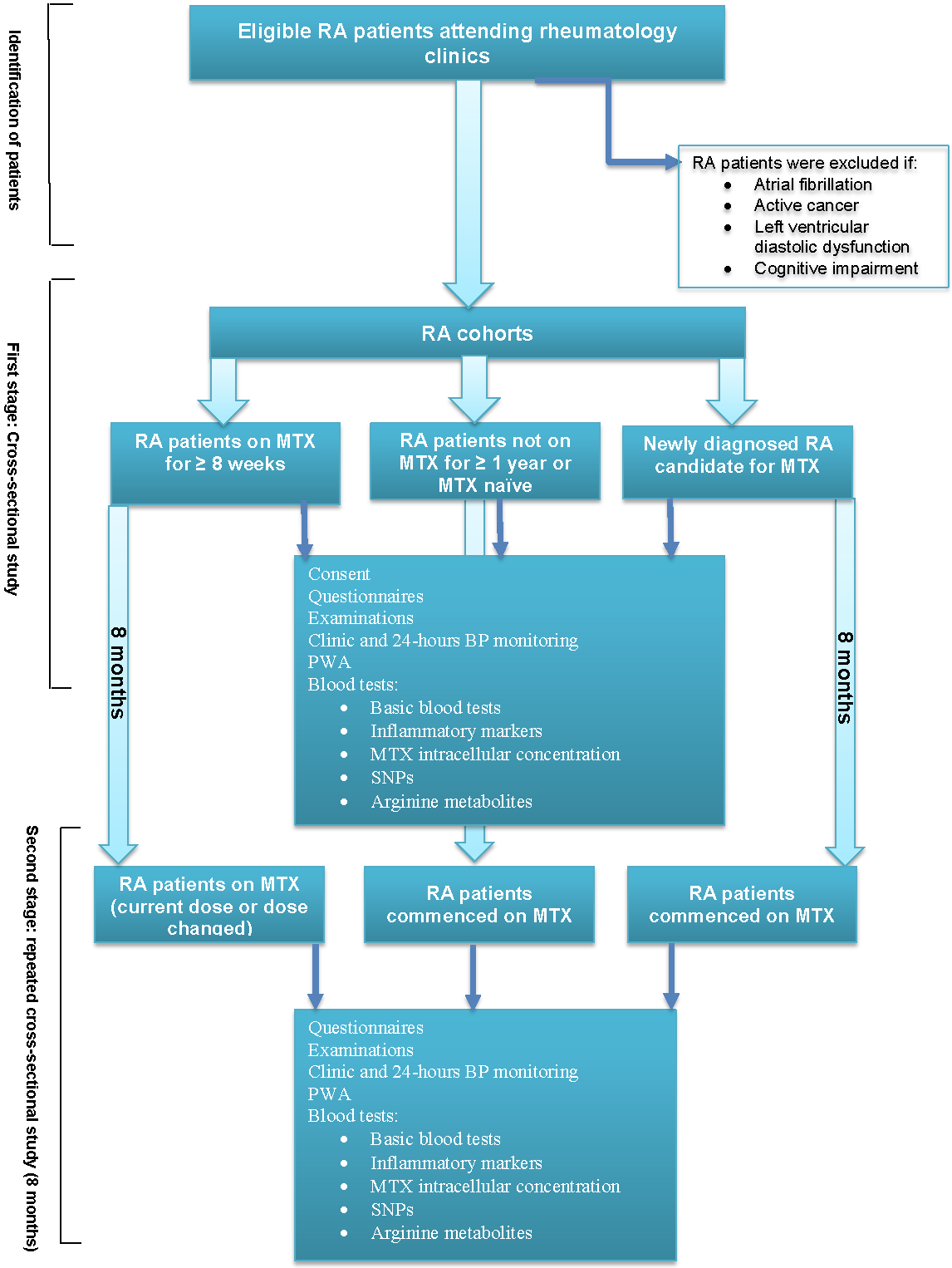


Figure 2-1 Research design and process of recruitment

A Gantt chart was drafted to enable an overview of the expected number of RA patients interviewed per week at baseline and follow-up visits (Appendix-4). About 3 to 4 patients were expected to be seen by the principal investigator weekly. Booking more than 4 patients per week was not practical as there were only 2 ambulatory BP monitors. Each interview session took about 40 minutes for each patient where history and examinations were obtained at both visits (Table 2-2). The first 5 minutes of the interview was allocated for answering the patient's questions and signing the consent forms while they were resting. This was followed by the following steps:

1. The first clinic BP reading was taken.
2. The patient's height and weight were recorded and the BMI was calculated.
3. While the patient was resting before taking the second BP reading (about 5 minutes), the Stanford HAQ was completed.
4. The second clinic BP reading was recorded.
5. Patient's joints were examined and DAS28 counts were calculated.
6. After another 5 minutes, the third clinic BP reading was taken and the last two clinic BP readings were averaged.
7. While resting, the patient was asked to complete the constructed questionnaire.
8. The PWA was conducted using the SphygmoCor where the averaged clinic BP reading was used for input.
9. While preparing the Mobil-O-Graph monitor, the patient was asked to fill in the food frequency questionnaire.
10. Blood was collected for MTXPGs, MTX pharmacogenetics, and arginine metabolites analysese.
11. The Mobil-O-Graph monitor was attached and tested.

Table 2-2 Information obtained from patients at each visits

	MTX participants	Non-MTX participants
History	1. Medical questionnaire 2. Medications history and, if required, from patients notes and hospital database. The information included type of medications, duration, dose changes, and drug combinations and shifting from one group of medications to another 3. Stanford Health HAQ 4. Food frequency questionnaire	1. Medical questionnaire 2. Medications history and if required from patients notes and hospital database. The information included type of medications, duration, dose changes, and drug combinations and shifting from one group of medications to another 3. Stanford HAQ 4. Food frequency questionnaire
Examinations	1. Weight and height were measured by the same researcher using the same scale. 2. BMI 3. DAS28 calculated with ESR 4. Heart rate 5. Averaged clinic BP (3 readings) 6. PWA (central BP and AIx) using SphygmoCor 7. 24-h BP monitoring including central BP, AIx, PWV) using Mobil-O-Graph PWA	1. Weight and height were measured by the same researcher using the same scale. 2. BMI 3. DAS28 calculated with ESR 4. Heart rate 5. Averaged clinic BP (3 readings) 6. PWA (central BP and AIx) 7. 24-h BP monitoring including central BP, AIx, PWV) using Mobil-O-Graph PWA
Blood tests	1. Basic biochemical tests including renal and liver function, random blood glucose and lipid and complete blood count 2. Autoantibodies (anti-CCP) 3. Inflammatory markers (ESR, and CRP) 4. Plasma for arginine metabolites 5. Whole blood for genetic SNPs of MTX 6. MTXPGs concentration in the RBCs and WBCs	1. Basic biochemical tests including renal and liver function, random blood glucose and lipid and complete blood count 2. Autoantibodies (anti-CCP) 3. Inflammatory markers (ESR, and CRP) 4. Plasma for arginine metabolites

2.6 Sample size

A sample size of 360 individuals was originally calculated based on the published literature (Cuchacovich & Espinoza 2009) to have 80% power (p-value of 0.05) to detect a significant difference of 3 mmHg in SBP between MTX and non-MTX groups in addition to a difference of 10 mmHg between MTX and newly diagnosed patients' group. This assumes a within-group

SBP SD of 20 mmHg. Three planned cohorts were formed based on the typical RA population's use of MTX (i.e. approximately distributed):

1. Taking MTX drug (N = 160 patients)
2. Not taking MTX drug (N = 160 patients)
3. Newly diagnosed with RA and not on MTX (N = 40)

Although the original aim was to recruit 360 RA participants, the total number was recalculated based on the BP obtained from the preliminary analysis after 6 months of the study commencement showing larger than previously expected differences between MTX and non-MTX patients. The final updated study sample included 86 RA patients (MTX group = 56 patients and non-MTX group = 30 patients). BP was taken three times during patients' visits according to the European Society of Hypertension (Mancia et al. 2007). The average of the last two readings were calculated for each patient and used to obtain the arithmetic mean of MTX and non-MTX cohorts. The final sample was recalculated to have greater than 80% power (p-value of 0.05) to detect a significant difference of 10 mmHg in SBP between MTX and non-MTX groups, which was the observed difference in SBP observed between the MTX and non-MTX cohort. This recalculation of the sample size was also necessary due to the limited time for the project and difficulties in finding eligible RA patients in the PhD timeframe.

2.7 Variables and instruments

This study was designed to assess the impact of MTX dose and its intracellular concentration on both peripheral and central BP, and on arterial function measured by AIx and PWV in patients with RA. Exposure to MTX was assessed by gathering information from the patients about the current dose taken, and from patients' medical records to confirm the exact duration of MTX doses. More accurately, exposure to MTX was evaluated by measuring its concentration inside the RBCs and WBCs. Clinic BP and 24-h BP were monitored by using "AND automatic BP monitor" and Mobil-O-Graph, respectively. PWA was conducted by using two validated instruments: SpygmoCor and Mobil-O-Graph.

Besides the main exposure and outcome variables, other variables which might influence the potential outcomes were considered (Table 2-2). RA activity was calculated using DAS28 score and based on ESR level; and the degree of disability was assessed by the Stanford HAQ. A

detailed demographic and medical history was obtained from all patients. Moreover, the presence of traditional CV risk factors including high BP, T2D, dyslipidaemia, smoking, level of physical activity, and obesity (BMI) was assessed to adjust for potential confounding. Past history of CVD namely, MI, angina pectoris, atrial fibrillation, stroke, and peripheral vascular disease was ascertained. A detailed medications history was gathered from patients, from medical records and from the hospital database. The dose and frequency of the following medications: anti-rheumatic, pain killers including NSAIDs, antihypertensive, lipid lowering, anti-diabetic, anti-angina, and heart failure treatments was obtained. Moreover, consuming supplements and vitamins were also considered, especially fish oil, vitamin D and folic acid. In addition, a food frequency questionnaire was used to gather dietary content information. All these variables were considered in the analyses of MTX exposure to prevent possible confounding effects.

2.7.1 Primary exposure

Participants were classified according to MTX exposure: currently taking MTX for ≥ 8 months, and not taking MTX for ≥ 1 year or MTX naïve (non-MTX) but taking other anti-rheumatic medications. A third cohort of RA patients was those who were not taking MTX nor any other medications. This group included RA patients who were MTX naïve (never treated) as well as those who had been exposed to MTX at some stage of their illness. Those started on MTX were considered on therapy until the follow-up visit. This approach has been previously used (Choi et al. 2002). After the follow-up visit, RA patients were reclassified into different cohorts if they were switched from MTX into other anti-rheumatic medications and vice versa.

MTX dose (mg) and MTXPGs intracellular concentration (nmol/L) were the main exposure variables used to examine the effect of MTX on BP and arterial function in RA patients. The principal investigator collected and prepared blood samples at the Rheumatology Research Laboratory at the RGH. Even though the level of MTX can be assessed in the plasma, measuring low levels of this element is impractical as it is rapidly cleared from the human body. Therefore, measuring RBC intracellular MTX polyglutamates (MXTPG-n) is more accurate.

2.7.2 Primary outcome variables

The main primary endpoints assessed in this study were BP (peripheral and central), AIx and PWV. The aim of the study was to define if there is an inverse relationship between MTX dose and/or concentration and BP and arterial function. Either a reduced AIx and/or PWV (PWV, $ms^{-}1$) reflected the improvement in arterial function/PWA.

2.7.2.1 Blood pressure

Until recently, it has been claimed that conventional brachial BP measurement is reflective of central BP (Lewington et al. 2002). This assumption was supported by observational findings showing that peripheral pressure determents (cardiac output and peripheral vascular resistance) can predict CV risks, morbidity and mortality. This supposition is implausible, however, as pressure in the central circuit depends on other parameters in addition to peripheral vascular resistance and cardiac output. These additional factors include arterial stiffness and reflective waves' time and magnitude (Izzo 2004; Mitchell et al. 2003; O'Rourke 1995; O'Rourke & Mancia 1999; Williams et al. 2006). Therefore, assessing both central and peripheral BP parameters is more accurate in understanding the relationship between BP and MTX exposure.

Even though conventional clinic sphygmomanometers are still being used in measuring BP, changing the clinical practice by using ambulatory BP monitors instead of a single reading has been noticed in recent years (Head et al. 2010; O'Brien et al. 2003). The interest in monitoring the BP over 24-h has been significantly increased in recent years to diagnose white coat syndrome, day and sleep variabilities, and hypertension—especially in RA patients with high CV risk profile. Therefore, the 24-h BP monitors were used in this study to explore the impact of MTX on BP in RA population. The main aim for measuring a patient's BP was to assess the relationship between the doses and intracellular concentration of MTX and peripheral and central BP.

2.7.2.1.1 Clinical (clinic) blood pressure measurement

Peripheral BP was clinically measured in a quiet environment at room temperature using the "AND automatic BP monitor" (model no.UA-767PC). This tool has been clinically validated against the BHS protocols (Palatini et al. 1998; Rogoza, Pavlova & Sergeeva 2000). The accuracy of this monitor was regularly checked and calibrated to avoid measurement bias. Likewise, the same trained investigator was measuring the BP for all RA patients and was following the ESH and European Society of Cardiology (ESC) guidelines for BP measurement (Liakos, Grassos & Babalis 2015; O'Brien et al. 2003). All patients were advised to sit and relax, at least for three to five minutes, before taking the first BP reading. Sitting in a comfortable position, the patient was instructed about the procedure of measuring the BP. The most important advice was neither the investigator nor the patient were able to talk while the BP cuff was inflated. Likewise, minimising the movement of the arm, where the cuff was placed, was recommended to obtain an accurate BP reading. After these explanations, the circumference of

the right arm of each patient was measured using a measure tape to select an appropriate BP cuff size. The BP monitor comes with three different cuff sizes: small (16–24 cm), medium (24–36 cm) and large (36–45 cm). After selecting the appropriate cuff size, the investigator attached the cuff above the elbow (for about 2–3 cm). This position ensures that the cuff was attached at the heart level. Ensuring a perfect fit can be done by sliding two fingers between the cuff and the arm. Additionally, the tubing attached to the cuff must be untwisted. After these premeasurement preparations, the first BP reading was taken and documented. Due to the variability associated with BP measurement, a single BP reading might be inaccurate or erroneous; therefore, repeated measures of BP are recommended by the ESH (O'Brien et al. 2003) to reduce the variability and improve reliability of BP measurement. Thus, the BP was taken 3 times with a 2-minute space between each BP measurement. The average of the last two BP measurements was calculated and used in analyses.

2.7.2.1.2 Central blood pressure assessed by the SphygmoCor

The gold standard method to measure the central BP is via invasive insertion of a catheter into the aorta during cardiac surgery (Tian & Chesler 2012), however, this invasive method is impractical and poses higher risk of complications. The clinical importance of measuring the central BP non-invasively has received the most attention in the last century. Waveform analysis by tonometric methods allows non-invasive recording of the central BP (Stoner, Young & Fryer 2012). SphygmoCor (version 7.1, AtCor Medical, Sydney, Australia) (Actor Medical 2011) was the instrument used to perform the PWA (Figure 2-2). This non-invasive tool has been clinically validated against other invasive techniques such as aortic catheterisation (Shapiro et al. 2008). Additionally, this technique was used in the Conduit Artery Function Evaluation (CAFE) study, which was a large observational study involving more than 2,000 participants (Williams et al. 2006). It uses a pin-like tonometer to capture the radial pressure waveforms by flattening without occluding the artery. It provides the systolic and diastolic pressures indicated by the maximum and minimum points of pressure wave, respectively. Then, the radial pressure waveform is mathematically transformed into a central waveform (ascending aortic) using a generalised transfer function. This waveform encloses the first and second systolic peaks (Figure 2-3).

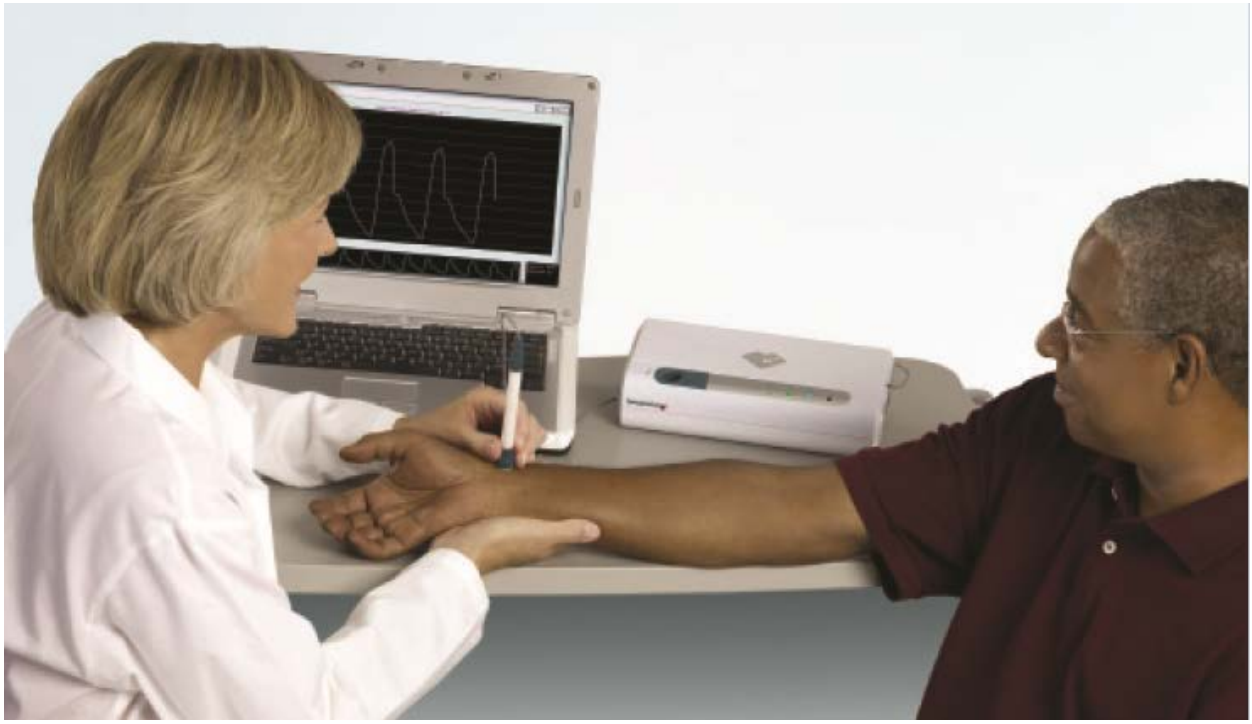


Figure 2-2 SphygmoCor (version 7.1, AtCor Medical, Sydney, Australia)

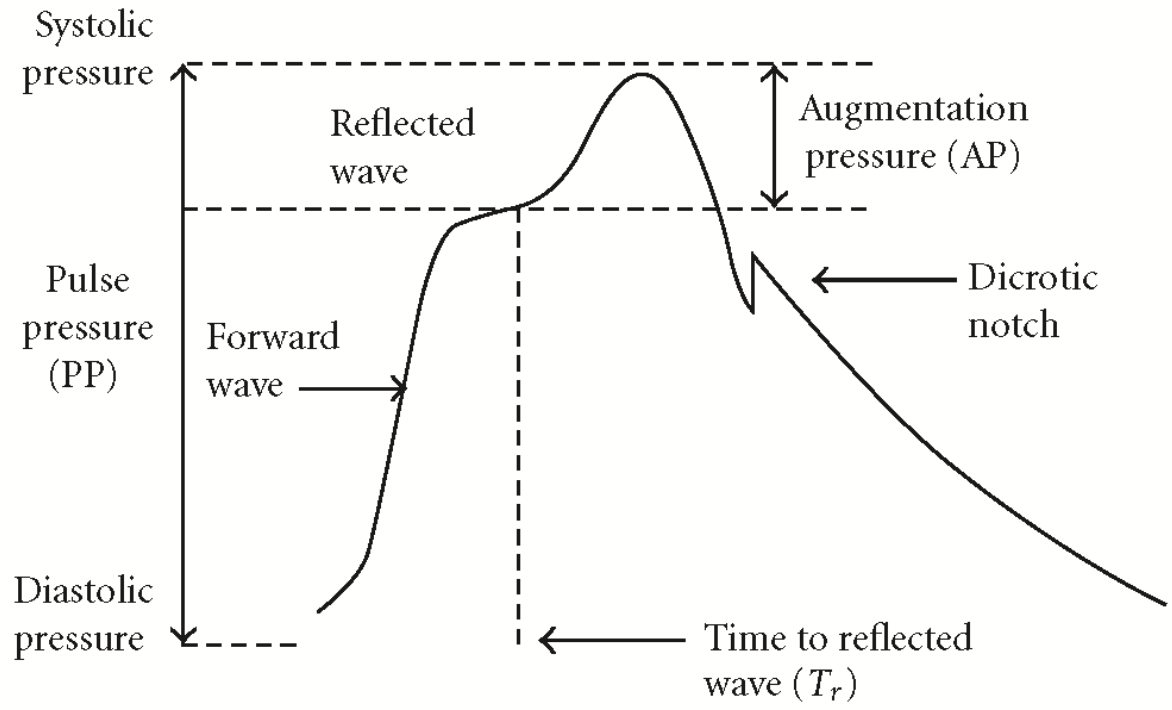


Figure 2-3 Central blood pressure waveform

From Stoner, Young & Fryer 2012, p. 2

At the clinic, the same trained researcher (the principal investigator) conducted the PWA measurements following a standard protocol. All patients were instructed to abstain from alcohol, and tobacco (for at least 12-h), and caffeine (for at least 4-h) prior to the measurement. Additionally, fasting for at least 6 hours prior to the measurement was advisable, however, if patients could not fast for any reason, a light meal before the measurement was allowed. Ensuring the stability of PWA measurement, the investigator conducted the analysis with the participant in the seated position and after resting for at least 10 minutes in a quiet room with a controlled temperature of 22–24 °C. The averaged peripheral BP calculated earlier from the last two clinic (office) BP readings obtained by the "AND monitor" was used in conducting the PWA. There was at least two minutes' space between measuring the office BP and performing the PWA to enable the artery to refill with blood. After entering the patient's personal details, the patient's medical information, height, weight and averaged BP were entered. Positioning and resting the patient and the examiner's hands were a vital step of the preparation stage to obtain accurate waveforms. To reach maximum stability, the patient's left wrist was supported by placing a pillow under it and the investigator's elbow and wrist were rested on the flat surface of examination table (Figure 2-2). After such support, the examiner dorsiflexed the patient wrist to expose the radial artery. Then, the maximum arterial pulsation was felt by the operator's index finger, where the tonometer of the SphygmoCor was placed. The tonometer was placed gently but firmly on the artery and adjusted medially and laterally until consistent uniform shapes of pulse waves were obtained and located at the centre of the screen. These consistent waveforms were captured after at least 11 seconds. After that, the readings were checked to be at acceptable quality limits. These quality control parameters include operator index, quality indices and the graph. First, the acceptable operator index must be ≥ 80. Second, the degree of variation or quality indices must be at acceptable limits composing ≥80 average pulse height, ≤5 pulse height variation, ≤5 diastolic variations and ≤4 shape variation (Figure 2-4). Third, the graph captured must have minimal variations between each waveform. If the quality of the acquired PWA was acceptable with less variabilities, another two readings were obtained. However, if the quality was unacceptable, the measurement was repeated until adequate reading was captured.

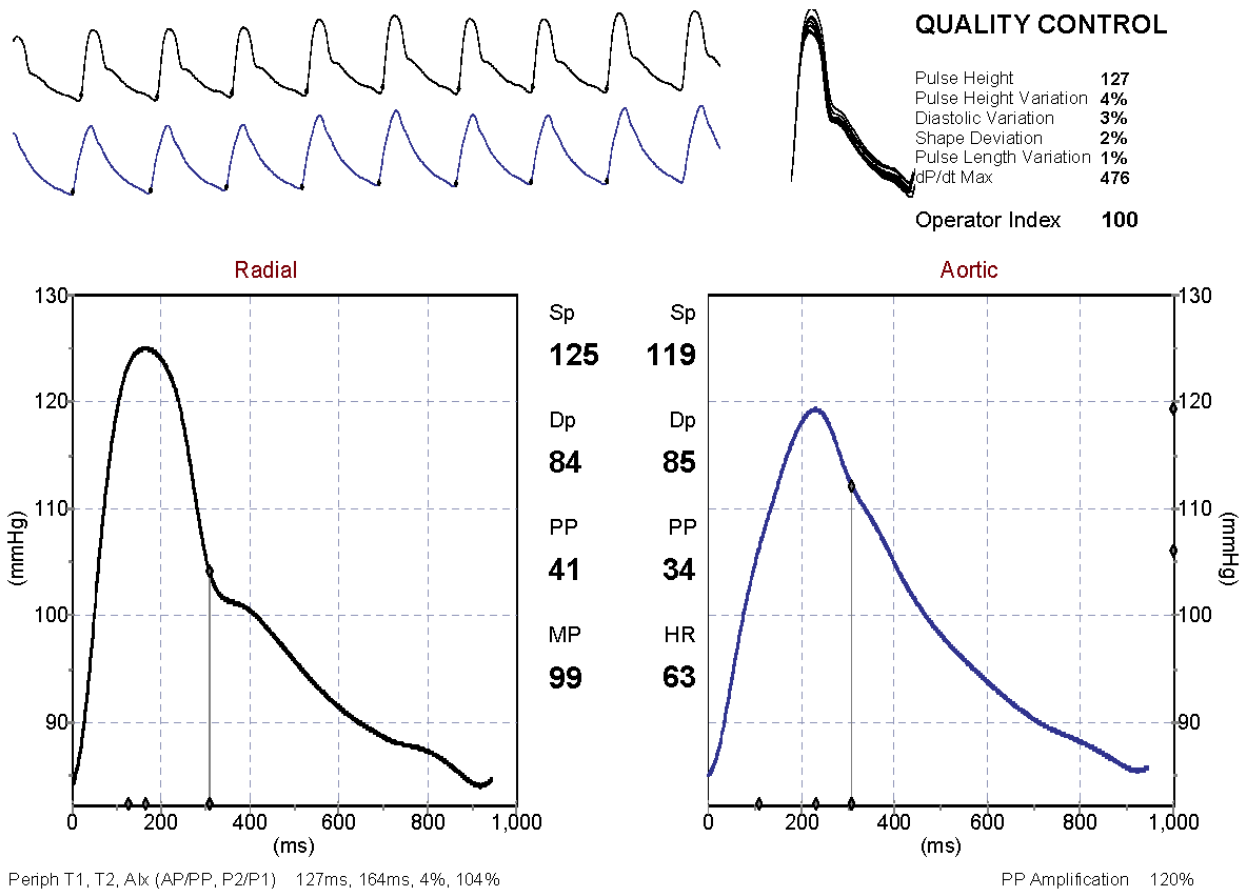


Figure 2-4 Patient report obtained from SphygmoCor conducted by the principal investigator showing radial and aortic waveforms and quality control parameters

2.7.2.1.3 Ambulatory blood pressure monitoring

In this study, 24-h, daytime, and night-time peripheral and central BP monitoring was conducted. It included wearing a portable BP monitor for 24-h. During that time, the monitor measured a series of BP readings during the day and night, recording any change in the BP with physical activity and sleep, respectively. Usually, the BP decreases during sleep and this phenomenon is called ‘BP dipping”. In this nocturnal dip, there is a fall in BP of >10% of awake-time BP (Stenehjem & Os 2004). This is followed by a surge in BP upon awaking, reaching its maximum. Hypertension and its associated end-organ damage such as CVD can be predicated if the BP remains consistently high during sleep. Therefore, monitoring the BP over 24-h is more

accurate in detecting CV risk, especially in the RA patients. Most available ambulatory BP monitors measure only the peripheral BP; however, in this study both peripheral and central BP were monitored over 24-h using Mobil-O-Graph PWA monitor (IEM, Stolberg, Germany) (Wei et al. 2010) (Figure 2-5). This is a new ambulatory oscillometric BP monitor approved by FDA and CE and it has been clinically validated for BP based on both the BHS protocol and the ESH international protocol (Franssen & Imholz 2010; Jones et al. 2000; Wassertheurer et al. 2010; Wassertheurer, Mayer & Breitenecker 2008; Wei et al. 2010; Westhoff et al. 2005). It has several advantages, one being saving time, as it is easy to use. Both brachial BP and brachial pressure waveforms are measured at the same time and then mathematically transformed into aortic BP and waveform. Compared to other ambulatory BP monitors, the Mobil-O-Graph has less discomfort, noise and disturbance during sleep (Westhoff et al. 2005). Another advantage of Mobil-O-Graph is that it gives reliable results independently from the user and provides high accuracy in repeated measurements. Moreover, there is paucity of studies using the 24-h BP monitoring among RA patients and thus incorporating this approach in this study was the first of its kind.

Figure 2-5 Mobil-O-Graph PWA Monitor (IEM, Stolberg, Germany)

In addition, studies have shown that the effectiveness of the Mobil-O-Graph is comparable to SphygmoCor (Weiss et al. 2012), which indicates that both monitors are able to measure central BP efficiently. Mobil-O-Graph has an advantage over SphygmoCor, however, as it records both

central and brachial BP concurrently. In addition, it is cheaper and more feasible than SphygmoCor. Table 2-3 compares the common features of both devices. The ability to measure both BPs is due to novel integrated mathematical calculations. This calculation is based on the ARCSolver method aiming to determine aortic BP, AIx and PWV using an oscillometric BP technique. ARCSolver software is implemented in Mobile-o-graph 24-h PWA monitor, which has a generalised transfer function. The exact generalised transfer function has been completely explained by a few studies (Wassertheurer et al. 2010; Wassertheurer, Mayer & Breitenecker 2008; Weber et al. 2011). In summary, this function is based on three levels of mathematical algorithm; two separate cycles are followed to obtain data for the algorithms. In the first cycle, SBP and DBP are measured using a conventional oscillometric method. After the first BP reading, the second cycle begins, where the pulse wave is recorded using a pressure sensor. The sensor measures the strength of the pulse signal during the continuous deflation (for about 10 seconds) of the brachial BP cuff from a highest SBP level to zero. The recorded signals are generated by a generalised transfer function. The aim of using this transfer function is to modify frequency range in the obtained signal and to catch the aortic pressure wave. Then, both AIx and PWV are calculated using mathematical equations. The AIx is calculated as the monitor is able to measure the difference between the DBP and SBP, while the calculation of the PWV is based on measuring the time between the first and second peak of the systolic pressure. Aortic PWV includes the distance from the ascending aorta, all the way to the bifurcation of the femoral arteries, and can therefore considered to be about equal to the carotid-femoral PWV.

Table 2-3 Comparison between SphygmoCor and Mobil-O-Graph Monitor

Feature	SphygmoCor	Mobil-O-Graph
Clinical validity	Yes	Yes
Cost	~ AU$ 25,000	~ AU$7,000
Feasibility in clinical practice	No	Yes
Required training skills	Yes	No
Easy to use	No	Yes
Operator-dependent	Yes	No
Obtain brachial and central BP	No	Yes
Validation analysis	Bland-Altman method	Bland-Altman method
Correlation with invasive cardiac catheterisation in assessing pressure augmentation	Significant (Ding et al. 2013)	Significant (Weber et al. 2011)
Correlation with invasive intra-aortic PWV	Acceptable (Weber et al. 2009)	Significant (Hametner et al. 2013)
Used in assessing clinical CV outcomes	Yes (Roman et al. 2007)	Yes (Koutroumbas et al. 2015; Zhang et al. 2015)

BP was monitored for 24-h by the Mobil-O-Graph Monitor according to a standard protocol. The BP was taken every half an hour during daytime (awake) and hourly during night-time (sleep). The trained primary researcher was responsible for attaching and instructing the patients about using the monitor to avoid any sort of measurement bias. First, the circumference of the upper arm of the non-dominant hand was measured by a measuring tape to select the correct cuff size. Then, the monitor was fitted about 2–3 cm above the elbow to be sure that the cuff was at the level of the heart. Then, tightness of the cuff around the arm was checked by passing two fingers under the cuff; if the cuff was perfectly fitted, it permitted passing two fingers, otherwise it

was too tight. Second, all participants were instructed to keep the arm with the fitted monitor still at the side of their body during inflation, but daily activities could be continued while wearing the monitor. Another important piece of advice was to ensure that tubing was not kinked while BP measurement was taking place. In case of inaccurate BP reading, the monitor was programmed to “beep” and the patient istructed that they should keep the monitored arm still and wait for a minute before taking another reading. Additionally, they were instructed that the day/night key should be pressed upon waking and before going to sleep (while in bed resting). It is advisable not to have a shower or bath while the monitor was attached. Patients were advised to keep a daily record of waking time and sleeping time to double check the day and sleep time and to have accurate analyses. Furthermore, they were instructed to keep a simple diary about their sensations, body posture and physical activity, and drinking alcohol or any type of caffeinated drink during each recording. Moreover, they were advised to keep a list of used medications during the BP monitoring as some medications might affect the BP reading. Finally, all patients were instructed to return the monitor the same time next day.

Turning off the BP monitor, the investigator downloaded the data and checked its quality. If the BP was recorded for more than a day, the first 24-h measurements were analysed. Moreover, 70% of obtained BP readings during the day or sleep time must be at least satisfactory. An algorithm, which is responsible for testing the quality of recorded signals in the cardiac cycle, was included in ARCSolver software of the BP monitor. This signal quality was rated from one to four (Figure 2-6). During signal acquirement, the quality was considered excellent or good if more than 80% or 50% of the recorded cardiac cycles was included, respectively. Grade three “poor quality” was considered if the cardiac cycle was included in less than 50%. Grade four “insufficient quality” represented missing results. Another way of examining the quality of the obtained 24-h BP results was to inspect the bar chart of the BP during daytime and sleep time for any missing readings (Figure 2-7).

		Total		Day		Night	
		Value	Goal	Value	Goal	Value	Goal
Time							
Start		23/03/2015 10:45		08:00		01:29	
End		24/03/2015 14:26		01:28		07:59	
Duration		27:13		21:12		06:31	
Measurements							
Total		41		35		6	
Valid		37		31		6	
Valid	%	90	>90	88		100	

Figure 2-6 24-h BP monitoring report produced by Mobil-O-Graph indicating the quality of the obtained readings

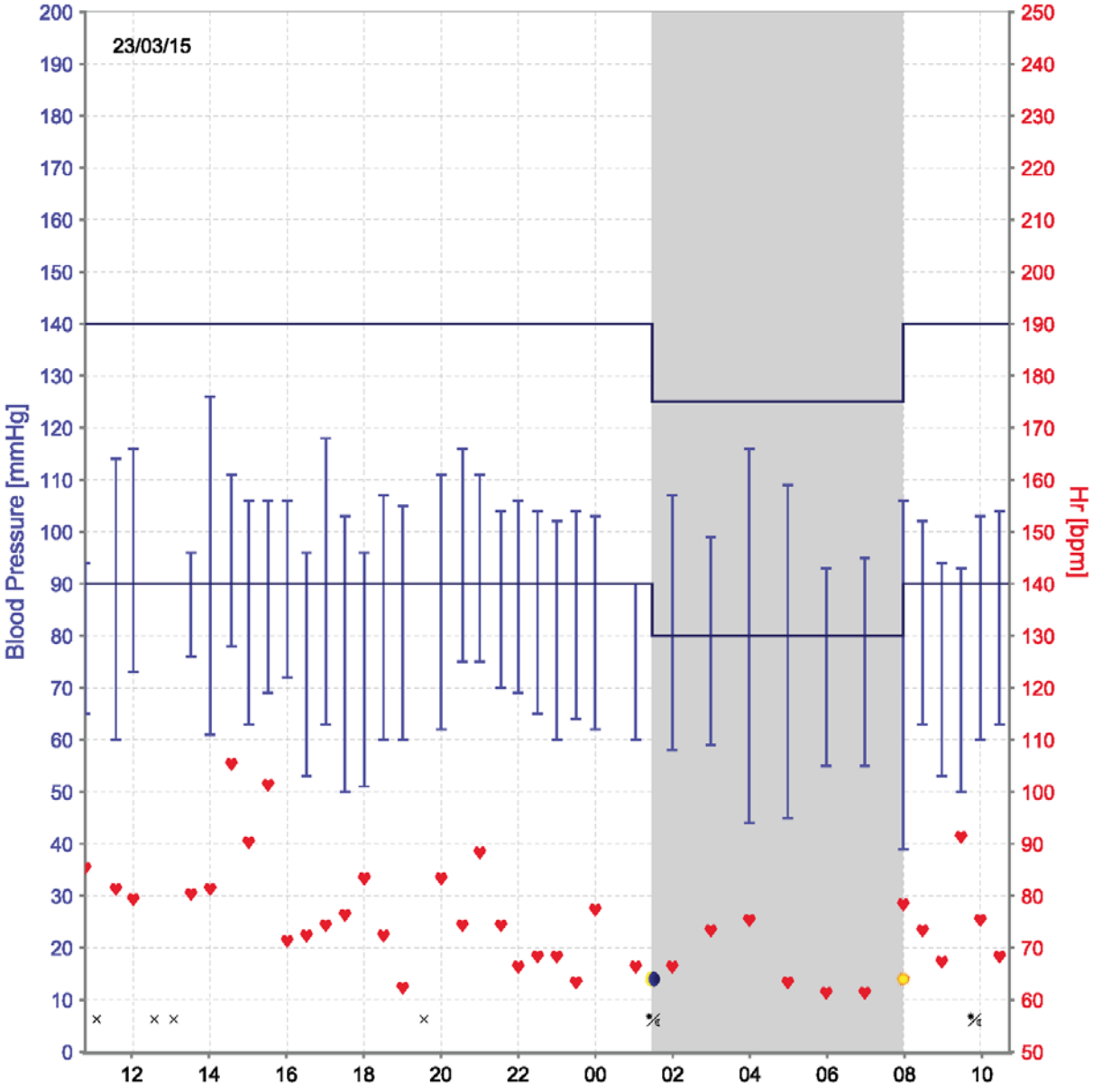


Figure 2-7 Bar chart of 24-h BP monitoring produced by Mobil-O-Graph showing the upper and lower values of BP during awake and sleep time

Fixed time periods for day and night were used for all patients to eliminate any differences between patients. Based on available literature, the daytime and night-time was set to be at 8:00 am and 12:00 am, respectively (Fagard et al. 1996; Ryu et al. 2015). This fixed time was allocated because patients usually forgot to push the key of day/night to change the pattern of BP reading, and overlooked noting their sleeping and rising times. Averaged 24-h, daytime, and night-time peripheral and central BP were calculated.

2.7.2.2 Arterial function/ PWA

Globally, CVD is one of the most common extra-articular complications among RA patients. As most CVD starts to develop in the early stages of RA, screening for early signs of CVD such as arterial stiffness might help in reducing CV morbidity and mortality. Although the gold standard method in assessing arterial function is via invasive cardiac catheterisation, non-invasive techniques has been recently introduced as the invasive method is not feasible. These include applanation tonometric and oscillometric methods.

2.7.2.2.1 Applanation tonometry

Currently, Sphygmocor (AtCorMedical, Sydney, Australia) is the gold standard non-invasive applanation tonometry enabling researchers to perform PWA. Quantitative analysis of arterial stiffness can be determined by conducting PWA, which has been widely used to assess the vascular structure and the tendency of arterial stiffness. It includes the estimate of the following markers: central SBP, central DBP, central pulse pressure (cPP), central AIx, and PWV. Arterial stiffness defined as reduction in dispensability, elasticity and compliance of the arterial system and can be measured locally, regionally and centrally (Stoner et al. 2012); local arterial stiffness assessment uses techniques to examine the change at a local area of the artery and is related to distending pressure. Conversely, regional and central arterial stiffness can be assessed by PWV and PWA, respectively.

Performing PWA using SphygmoCor enables researchers to obtain not only the central aortic BP, but also central aortic AIx and PWV. This assessment reflects the status of arterial stiffness and the effect of the reflected pressure wave. Normally, the aortic pressure wave generated by the heart, which is known as ‘forward wave’, travels through the blood vessels until it reaches a resistance in pressure. This usually occurs at the level of aortic bifurcation. As a consequence of that change in pressure, the wave is reflected back to the heart. This reflected wave is also known as ‘backward wave’. In normal individuals, this reflected wave arrives at the diastole while the heart is resting and helps in refilling the heart with blood. These two waves combine to produce pressure at any point in the arterial system, where the shape and the magnitude of this combined pressure depends on both PWV and on the strength of the reflective wave.

In arterial stiffness, however, the reflected wave arrives early to the heart during the systole while the heart is still contracting. In other words, it is directly related to arterial stiffness. As a result of early arrival of the backward wave, it is superimposed on the forward wave, generating an augmented pressure wave. This results in the generation of first (P1) and second systolic

peaks (P2) (Figure 2-8). The difference between the second and first systolic peak is known as AIx, which is a marker of arterial wave reflection (Avalos et al., 2007, van Varik et al., 2012). It is expressed as a percentage of PP. It is calculated as a ratio of AP produced by the reflected wave and PP (Aix = AP/PP). The PP is defined as the difference between the peak of systolic and diastolic BP (PP = SBP − DBP) (Wilkinson et al. 2002).

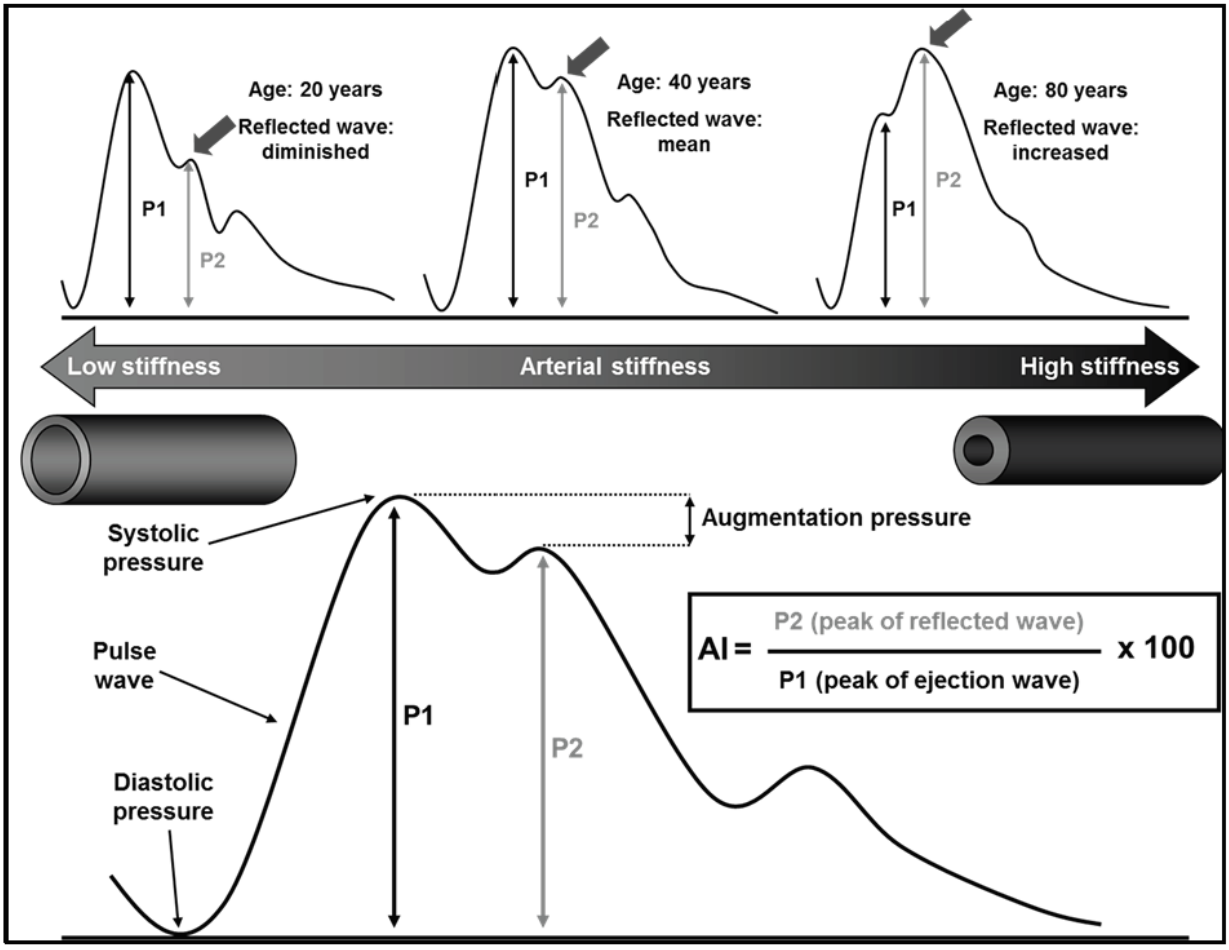


Figure 2-8 Arterial stiffness and pressure waveform

From Wilkinson et al. 2002, p. 1006

Again, PWV, which is a strong predictor of arterial stiffness, can be obtained by using the applanation tonometry technique. PWV is expressed as “the speed of travel of the pulse along an arterial segment” (PWV= distance+ t (m/s) (O'Rourke & Mancia 1999, p. 2). Using applanation tonometry, waveforms are obtained from two arterial locations; with the carotid and femoral arteries being the most common sites. Then, the distance between those arteries is usually recorded; followed by the measurements of the wave transit time between the two points being obtained to calculate the PWV. High PWV is a strong predictor of arterial stiffness

(Blacher et al. 1999). This method in obtaining the PWV was not used in this study; instead, the oscillometric technique was used.

2.7.2.2.2 Oscillometric technique

Currently, there are two validated non-invasive oscillometric devices: Mobil-O-Graph PWA (IEM, Stolberg, Germany) (Wei et al. 2010), and CardioMon (Vienna, Austria) (Wassertheurer et al. 2010). In this study, Mobil-O-Graph PWA was used to obtain 24-h AIx and PWV. The ARCSolver software is incorporated in this monitor, which has generalised transfer function to measure AIx and PWV by oscillometric way using a three-level mathematical algorithm (as described earlier).

In this study, PWA was obtained by applanation tonometric and oscillometric methods using SphygmoCor and ambulatory Mobil-O-Graph 24-h PWA monitor, respectively. There is recent evidence showing that results obtained by the conventional technique of examining the PWA (i.e. radial tonometry using SphygmoCor) are comparable to the results achieved by novel brachial oscillometry using Mobil-O-Graph 24h PWA Monitor (Luzardo et al. 2012). Although there was no difference between the two techniques in estimating cSBP, cDBP, cPP and cAIx, the estimate of PWV obtained by the brachial oscillometry method was slightly underestimated (Luzardo et al. 2012). Therefore, we assessed the arterial function using both SphygmoCor and ambulatory Mobil-O-Graph 24-h PWA monitor. To date, there is no published study measuring these vascular markers in the RA population and that compares the estimates obtained by both techniques.

2.7.3 Secondary variables

Most secondary outcomes were collected by using self-administered questionnaires. The aim was to examine the relationship between MTX dose and/or concentration and BP and arterial function. Another aim was to use this information in adjusting for demographic, clinical, biochemical, pharmacological, and nutritional differences between RA cohorts.

Generally, there were two types of self-administered questionnaires: paper and online form. Despite these surveys being self-administered, the principal researcher was available to answer any queries while the participants filled out the questionnaire. There are many advantages for collecting the data by this method; first, self-administered questionnaires have low cost and they are an easy way of collecting patients' information. Second, sensitive and personal information is effectively collected compared to other methods such as conducting interviews. Additionally,

handing questionnaires to patients is time-saving, especially if other outcome measures are being collected at the same time of the visit.

Three different questionnaires were given to all patients. Two of those questionnaires were fully validated: HAQ (Bruce & Fries 2003), and food frequency questionnaire (Hodge et al. 2000). The third questionnaire was drafted by the principal investigator to gather demographic and self-report medical information including CVD, CV risks and medications list. The latter questionnaire was tested prior to conducting the study; this testing was vital to ensure that questions were well-structured and clear, the flow of the questions was adequate, and the questions' sequence and spelling were correct.

These questionnaires took approximately 30 minutes to be completed and were provided at the initial and the follow-up visits. Although these questionnaires were used to gather information about secondary outcomes, the information provided was used in ensuring that confounding by induction was ruled out. For example, exposures to a certain group of micronutrients or macronutrients were adjusted in the BP regression analyses; this because ingestion of some food might have an impact on BP and arterial function and subsequently it might confound the results. This was also the case for the HAQ, DAS28 and information obtained from the constructed questionnaire, especially regarding CV risks and medications used by RA patients.

2.7.3.1 Quality of life questionnaire

To obtain an accurate assessment of the RA patient's quality of life, a valid, comprehensive, and reliable tool was required. The most frequently used questionnaire is the HAQ. As quality of life was not a primary outcome and it was used over short period in this project, using the HAQ was appropriate. HAQ has been clinically validated and tested on patients with a wide variety of rheumatic diseases, including RA (Bruce & Fries 2003; Felson et al. 1993). Even though it has been administered with patients who have diverse diseases, are from different cultures and languages, its reliability and validity is still robust. Moreover, it plays a major role in assessing the efficacy of therapeutic management and the risk of mortality in RA (Amjadi et al. 2009; Carter et al. 2007).

The short version of the HAQ was used in this project as it has been frequently used and cited (Appendix-5). This questionnaire is self-administered, requiring participants to answer series of questions relating to their abilities in using the usual tools over the last week before the visit. It takes approximately five minutes to complete. There are three core components of this

questionnaire including disability index (DI), pain visual analog scale (i.e. VAS), and patient global health VAS. First, the HAQ-DI consists of 20 items categorised into 8 groups. These categories include dressing and grooming, arising, eating, walking, hygiene, reach, grip, and common daily activities. There are at least two subcategory items under each category. For each item, the patient has to choose one of the four responses based on their own frame of reference. The responses range from 0 to 3, where zero indicates there is no difficulty and 3 represents that the patient is unable to do the task. Second, pain VAS assesses the RA pain and its severity over the past week. The length of the VAS line is 15 centimetres and the scale ranges between zero (*no pain*) and 100 (*severe pain*). The patient has to indicate the severity of their pain by placing a vertical line on this scale. Third, the patient global health VAS has a similar role where the horizontal line of the VAS ranges from zero (*very well*) and 100 (*very poor*).

To compute the results obtained, the principal investigator calculated the score of the HAQ. First, the highest scores given for each of the 8 categories were summed. If the patient indicated that they used one or more of the aids/devices or help from another, chosen aids in each category was considered in the calculation. For example, if one device was used to help in reach, one score was added to the category 'reach'. After adding all scores, the total score was divided by the number of categories answered. The obtained HAQ score usually ranges between 0 and 3, where 3 is the worst functioning. The score of HAQ was interpreted as the following: scores of 0 to 1 were generally considered to represent mild to moderate difficulty, scores 1 to 2 indicated moderate to severe disability, and scores 2 to 3 represented severe to very severe disability.

The VAS score for both pain and global health was also calculated. As the horizontal line of the VAS scoring system is in centimetres, it needs to be converted into the appropriate metric (e.g. mm). The distance between 0 cm and the mark placed by the patient on the VAS line was measured and multiplied by 0.2. The score obtained was ranged between zero and three. Pain intensity score was interpreted as no pain, mild, moderate and severe if the pain VAS score was 0,1, 2, and 3, respectively (Jensen, Chen & Brugger 2003). Similarly, patient global health VAS score ranged between zero (*very well*) and three (*very poor*).

2.7.3.2 Food frequency questionnaire

The electronic version of the Dietary Questionnaire for Epidemiological Studies Version 2 (DQES v2) was used (Appendix- 6). This questionnaire was developed in the late 1980s by the

Cancer Council of Victoria, Australia (Ireland et al. 1994), and it has been validated since then (Giovannelli et al. 2014; Hodge et al. 2000; Hodge et al. 2009; Ibiebele et al. 2009; Woods et al. 2002). It assesses regular habit of food intake over 12 months, which is more accurate in gathering dietary habit information compared with short-term food questionnaires. To assess macronutrients and micronutrients, the DQES included 74 food items divided into four groups. The first group includes cereal food, sweets and snacks; the second group consists of dairy products, meat, and fish. The third and fourth groups of questions include fruits, and vegetables, respectively; alcohol consumption was another group of diary habits assessed by the questionnaire. Answers for these questions were recorded on a frequency scale starting at '*never eat the food item'* and up to '*eat it daily'*. Additionally, the questionnaire includes questions to explore the frequency and habit of consuming a couple of food types—for example, milk, fruits, bread, egg, fat spread, sugar and vegetables. Moreover, four diagrams of common food types are included in the questionnaire to assess portion size and to calculate the 'portion size factor'. These food items include vegetables, potato, steak and meat or vegetable casserole. Alcohol consumption in this questionnaire assesses the overall quantity of alcohol consumed. Nutrients calculated from alcohol ingestion were separated from those nutrients obtained from food.

The online version of the DQES was used. Each patient was given an identification number (ID) and a link to access the questionnaire online. All questionnaires were checked before sending them for analyses at the Cancer Council, Victoria. The portion size was not dependant on age or sex of the patient. Daily intake of energy and nutrients was individualised by using the portion size factor, however, this was done by multiplying the frequency responses obtained for food items by the standard portion size. It was then adjusted at an individual level by adding the portion size factor.

2.7.3.3 Data collection questionnaire

A data collection questionnaire was structured and tested prior to use (Appendix-7). Each participant was handed this questionnaire during the baseline and follow-up visits, which usually took about 5 to 7 minutes to be completed. This questionnaire was designed to gather information about the following:

- **Demographic information:**
  This included patient's name, ID, contact information, age, gender, date of birth and ethnic background.

- **Socioeconomic status:**

  Patients were asked about their current work status, highest educational level, marital status, the level of health insurance cover, and approximate average annual income.

- **Cardiovascular history:**

  The questionnaire consisted of series of questions about past history of CVD and about CV risk factors. Patients self-reported history of any one CVD at any stage of their life. These CVDs include angina pectoris, MI, stroke, transient ischaemic attach (TIA), heart failure, deep vein thrombosis (DVT) and atrial fibrillation. To study the relationship between MTX exposure and CVD occurrence, a standard timeframe of commencing or stopping MTX was defined. In the MTX cohort, CVD were considered present if any of the listed CVDs occurred at least 6 weeks after commencing MTX. Likewise, patients were required to be off MTX for at least 1 year before the occurrence of any of the CVDs. Additionally, detailed history about exposure to traditional CV risk factors was ascertained. The questionnaire consists of series of questions about CV risk factors including history of hypertension (treated or not), T2D, dyslipidaemia, smoking status, and the level of physical activity. Each risk factor was defined based on published literature or on current and standard guidelines (Table 2-4).

Table 2-4 Traditional cardiovascular risk factors definition

CV risk factor	Standard definition
Treated hypertension	Physician's diagnosis of hypertension and patient takes antihypertensive medications
Untreated hypertension	Physician's diagnosis of hypertension, but no antihypertensive medications were commenced
T2D	Physician's diagnosis
Dyslipidaemia	Physician's diagnosis
Smoking	Self-reported smoking
Body weight	1. underweight <18.5 kg/m² 2. normal weight 18.5 to <25 kg/m² 3. overweight 25 to <30 kg/m² 4. obese ≥30 kg/m²
Physical activity	Self-reported regular physical activity questions and minutes spent per week for any physical activity

- **Medical history:**
  Besides the CV history, patients were asked to self-report their medical history. This included history of liver diseases, chronic renal diseases, and depression. Additionally, they were asked to specify any other diseases not listed in the questionnaire.
- **Medications history:**
  Detailed medications history was gathered from patients. Information about the use of traditional and biologic DMARDs was ascertained. This included detailed history of MTX use. Besides the DMARDs, medications which might have CV impacts were considered. These medications include anti-hypertensive drugs, lipid-lowering agents, anti-angina medications, heart failure drugs, antithrombotic therapies, prednisolone, and NSAIDs. Similarly, supplements such as any type of fish oil (omega-3), vitamin D and folic acid supplements were included in the questionnaire as they might affect the CV outcomes. Patients were asked to specify the dose, frequency and duration of each current medication.

2.7.3.4 DAS28

Assessing disease activity is crucial in managing RA. Although various assessment tools have been introduced worldwide, the DAS28 has been frequently used by rheumatologists (Anderson et al. 2012). Using the DAS28 in assessing RA activity is not only efficient, but also easy. In Australia, it is the most frequent tool used to assess RA activity based on acute phase reactants (Littlejohn et al. 2015). Although CRP can be used to calculate the DAS28, ESR is preferable and has been fully validated and used in the research field (Hensor et al. 2010). In this study, the DAS28 involving ESR was used (Appendix-8). The DAS28 account for the number of tender and swollen joints including shoulders, elbows, wrists, fingers, and knees. Additionally, the level of ESR, and the patient's global VAS were considered in the estimation of RA activity. The formula used for calculating the DAS28 was DAS28 = 0.56 * √(28TJC) + 0.28 * √(28SJC) + 0.70 * log [ESR] + 0.014 * VAS (Prevoo et al. 1995). The score obtained ranges from zero to ten. The disease activity score was classified into four grades: remission, low, moderate and high disease activity if the patient's DAS28 score was ≤ 2.6, 2.6 < DAS28 ≤ 3.2, 3.2 < DAS28 ≤ 5.1, and DAS28 >5.1, respectively.

2.7.3.5 Blood tests

Results of blood tests were taken from the hospital database and by collecting blood samples from patients. First, data about basic blood tests was gathered from the hospital database and documented in a patient's information sheet (Appendix-9). The main blood tests considered

included: complete blood count (WBC, RBCs, and haemoglobin), random biochemistry (glucose, total cholesterol, renal and liver function), inflammatory markers (ESR and CRP), and autoantibodies (Anti-ccp). A standardised laboratory method was used; latex-enhanced immunoturbidimetry on an automated Modular PPE Analyzer was used to analyse the serum CRP (Pepys & Hirschfield 2003). Similarly, a closed automated method, VES Matic Cube 80 (DIESSE S.p.A., Siena, Italy) was used to measure the ESR (Cerutti et al. 2011). If there were no recent results (≤3 months) however, for all blood tests but not Anti-ccp, these were requested for patients. An Anti-ccp value was taken from the patient database whenever it was done; however, it was requested that all patients in the follow-up visit have a recent figure for this antibody as its level changes with treatment. Second, blood samples were collected from patients to measure the level of arginine metabolites, omega-3 fatty acids, single-nucleotide polymorphisms of MTX, and intracellular MTXPGs concentration.

Blood samples

Venepuncture was performed under aseptic conditions where peripheral whole blood was collected. Patients were seated and instructed about the procedure of blood collection. After applying a tourniquet around the upper part of the arm, palpating the median cubital vein with the index finger was usually done and used to obtain the required amount of blood using 19-gauge needle and a plastic vacutainer.

Plastic vacutainer tubes

Two large (9 ml) lavender-top plastic tubes with spray-coated K2EDTA were used to collect the blood for MTX DNA, serum ADMA analysis and MTXPGs concentration. Other tubes were sometimes used if the patient had no recent blood tests for CRP, ESR and Anti-ccp. In this situation, a white-top tube with K2EDTA and gel for plasma separation was used for collecting blood for CRP and Anti-ccp. Small (4 ml) lavender-top plastic tubes with spray-coated K2EDTA were used for measuring ESR. Additionally, a grey-top tube with potassium oxalate/sodium fluoride was used to collect blood for blood glucose level; if blood was needed for total cholesterol level, a tube with either a green top with sodium heparin or a white top with K2EDTA and gel was filled.

2.7.3.5.1 Inflammatory markers

The aim was to examine the relationship between MTX dose and concentration and several inflammatory markers. Another aim was to find if the reduction in BP and improvement in arterial

function was independent of inflammation. The included inflammatory markers were ESR and CRP. The normal ESR level differs based on age and gender—for men, normal ESR level was defined as <15 mm/hr and <20 mm/hr for patients aged under 50 and above 50 years, respectively. The normal ESR value in women was defined as less than 20 mm/hr for those who were under 50 years of age, and less than 30 mm/hr for those above 50 years. Additionally, CRP was defined as normal if its plasma level was ≤ 10 mg/L (FT and III, 2009). In addition to ESR and CRP, the level of anti-CCP was recorded for RA patients. This marker plays a role not only in diagnosing RA, but also in monitoring the progression of the disease. The level of this marker has been found to change with changing doses of DMARDs or adding another class of medications (Zendman et al. 2006). Thus, the level of anti-CCP was collected at the baseline and then measured again after 8 months to examine if its level differed with changing medications.

2.7.3.5.2 Arginine metabolites

ADMA is found to be an indirect indicator of endothelial dysfunction (Mangoni 2009). It is a powerful endogenous inhibitor to the enzyme eNOS (Wadham & Mangoni 2009). Increased plasma ADMA concentration results in a reduction in NO synthesis that contributes to arterial dysfunction and arterial stiffness (Böger 2003; Cooke 2005; Kielstein et al. 2006; Mangoni 2009; Zhang et al. 2008). Therefore, the aim of measuring the level of ADMA was to examine the relationship between the level of ADMA and BP and arterial function.

Samples preparation

Blood was collected from each patient for ADMA analysis. After centrifuging the whole blood sample for 20 minutes, the retrieved plasma was transferred into 1.8 ml cryotube vial (Figure 2-9). Then it was stored at -80 °C until used for extraction of arginine and its metabolites. As ADMA is excreted by the kidney, patients' eGFR was documented for all patients and considered in quantifying the level of ADMA. Age and gender were additional factors considered in ADMA analyses. Blood samples were packed in containers filled with dry ice (solidified carbon dioxide) and were carefully sealed and labelled. Then, they were transferred to the

Clinical Pharmacology Department, Flinders University.

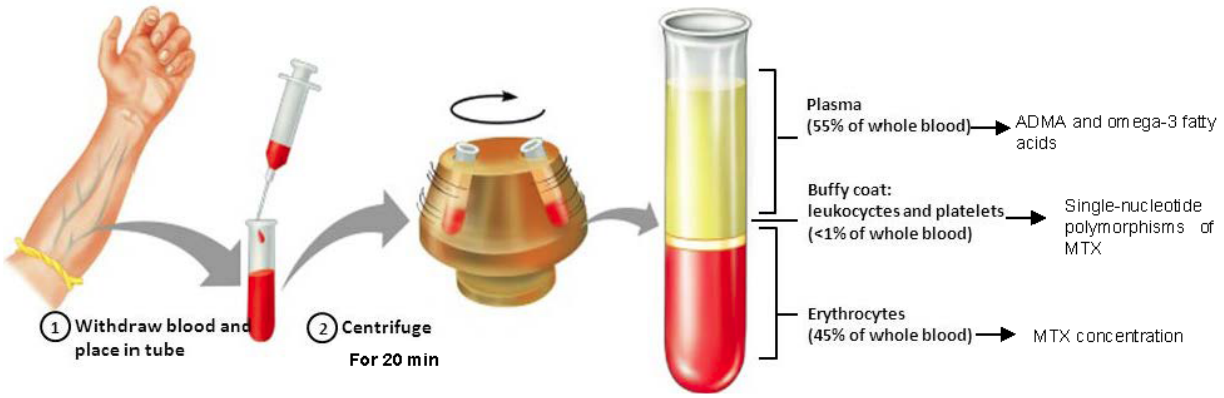


Figure 2-9 Blood elements used for analysing arginine metabolites, omega-3 fatty acids, single-nucleotide polymorphisms of MTX, and MTXPGs concentration

Protocol for analysing arginine metabolites

Ultra-performance liquid chromatography mass spectrometry (UPLC-MS) was used to quantify the arginine and its metabolites. Quantification of ADMA was conducted by Mr David Elliot at the Department of Clinical Pharmacology, Flinders University.

- **Extracting arginine and its metabolites from human plasma**

Plasma samples (50 µL) were prepared for analysis by UPLC-MS. Briefly, 250 µL of precipitation solution was added to 50 µL of sample and centrifuged for 5 minutes at 16000 g. Then 150 µL of the supernatant was placed under evaporation and the remaining solids were reconstituted with 150 µL reconstitution solution. Then 100 µL of the sample was placed in MS vials in a 96-well plate, where 2 µL of the sample was injected onto the UPLC column. Samples were prepared in 96-well format to facilitate high-throughput analysis.

- **UPLC Equipment**

Analysis of plasma samples was achieved by reversed-phase ultra-performance liquid chromatography (UPLC; separation) coupled to quadrupole time-of-flight mass spectrometry (Q-ToF-MS; detection). The UPLC system used a Waters Acquity series in-line vacuum degasser, binary solvent pump, sample manager, and temperature-controlled column oven (Waters, Sydney, Australia), the instrument was fitted with a Waters Acquity T3 HSS (1.8 µm particle size) analytical column (Waters, Sydney, Australia) measuring 2.1 mm (id) x 150 mm.

- **Chromatography Conditions**

The mobile phases comprise two solutions, which were mixed to generate the gradient shown in Figure 2-10. The solutions were: mobile phase A: 10 mM Ammonium Formate with 1% Methanol and mobile phase B: 10 mM Ammonium Formate with 90% methanol. Mobile phase solutions were filtered through a 0.45 µm membrane filter prior to use.

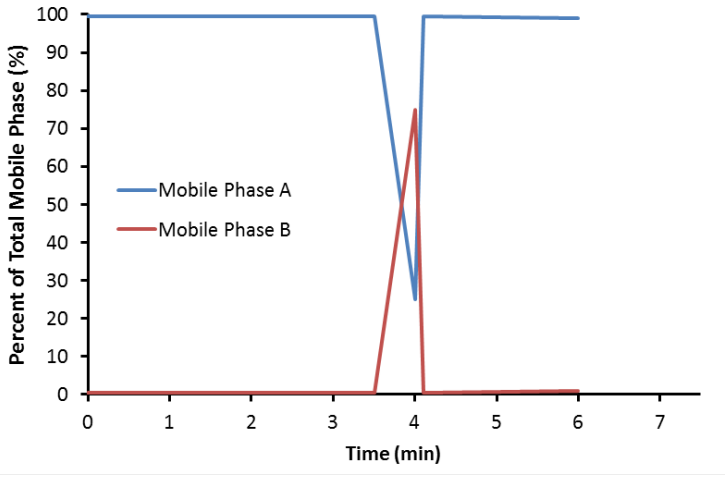


Figure 2-10 UPLC mobile phase gradient and composition

A range of different instrument settings for the UPLC were trialled until the optimal responses were obtained. The final mobile phase flow rate was set at 0.16 mL/min. Column temperature was maintained at 40 °C, while the sample compartment of the UPLC instrument was maintained at 15 °C. Column elutant was monitored by mass spectrometry using the conditions described below. Employing these chromatographic conditions, the retention times for each compound were as detailed in Table 2-5.

Table 2-5 Retention times for compounds of interest

Compound	Retention Time (min)
ARG	2.74
ADMA	2.81
SDMA	3.22
NMMA	2.69
HARG	2.59
CIT	2.92
ORN	2.32

- **Analytical conditions**

To create an optimal response in detecting each analyte, source conditions, TOF conditions and quadruple conditions were optimised for the panel of compounds. Electrospray ionisation (ESI) was chosen as the method to produce ions. The ESI source was operated in the positive mode, producing positively charged ions by the addition of H+ to compounds of interest (ESI+). As a result, each base compound was detected at an m/z one greater than the compounds mono-isotopic mass. After trialling various instrument settings, the key final MS conditions were as detailed in Table 2-6.

Table 2-6 Mass spectrometer instrument settings

Instrument Parameter	Setting
Capillary voltage (kV)	2.4
Sampling cone voltage (eV)	25
Extraction cone voltage (eV)	5.0
Ion guide	2.0
Source temperature (°c)	80
Desolvation temperature (°c)	180

Cone gas flow(L/Hr)	50
Desolvation gas flow (L/Hr)	550
LM resolution	4.7
HM resolution	15
Ion energy	1
Pre-filter	2
Collision energy: low energy (parent compound) mode (eV)	3
Collision energy: high energy (mass fragment) mode (eV)	gradient from 10 to 15
Collision Cell Entrance	2
Collision Exit	-10
Collision Gas Flow (mL/min))	0.3
Ion Guide Gas Flow (mL/min)	0
Detector voltage (eV)	1750
Pusher Interval (µS)	45
Pusher Width	4
Acceleration 1	55
Acceleration 2	200
Aperture width	80
Transport 1	70
Transport 2	70
Steering	0
Tube lens	75
Pusher	913
Pusher Offset	-1.50

Puller	675
Vacuum Lock (mbar)	x 10^{-3}

Employing these instrument settings, L-Arginine (ARG), ADMA and ornithine (ORN) were detected as the base compounds at m/z 175.09, 203.15 and 133.14, respectively, while SDMA, N^G-methyl-L-arginine (NMMA), L-homoarginine (HARG) and L-citrulline (CIT) were detected as mass fragments at m/z 172.13, 116.09, 130.11 and 159.09, respectively.

- **Calibration curves**

Calibration curves were generated using four different concentrations of each analyte. Calibration standards were prepared by dilution of authentic compounds in healthy human plasma samples, such that the final concentration ranges of analytes were as detailed in Table 2-7. Calibration standards also contained plasma, therefore preparation and chromatography conditions were treated as per patient plasma samples (detailed earlier) prior to injection on to the UPLC column. Plasma standard curves were constructed by plotting a set of the peak area ratio of compound over internal standard (minus that of the blank plasma) compared to spiked concentration samples. The plasma samples were expressed as the peak areas over internal standards and the unknown concentrations of compounds in human plasma were calculated from standard curves.

Table 2-7 Calibration ranges for compounds of interest

Compound	Calibration Range (μM)
ARG	10 – 500
ADMA	0.05 – 2
SDMA	0.05 – 2
NMMA	0.025 – 1
HARG	0.05 – 5
CIT	2.5 – 100
ORN	5 – 100

2.7.3.5.3 Single-nucleotide polymorphisms (SNPs) of MTX pathway genes

Despite the anti-inflammatory effect of MTX, some RA patients might not achieve adequate disease control even though they have been on treatment for a prolonged period. Thus, differences in drug responses to the same drug in different individuals could be partially inherited related to gene polymorphisms (Brinker & Ranganathan 2010). Therefore, the aim was to examine polymorphisms in the genes encoding enzymes responsible for MTX metabolism or MTX transmembrane transporters which might influence the efficacy of the drug. Additionally, examining the SNPs' variabilities among RA cohorts allows the establishment of a theoretical association with the CV effects of MTX. There are several SNPs affecting the efflux of MTX from target cells, including adenosine triphosphate-binding cassette transport proteins: ABCB1 3435C>T (rs1045642), ABCC2 1249G>A (rs2272397), ABCG2 421C>A (rs2231142). Moreover, there are SNPs affecting the de novo purine (i.e. adenosine) synthesis including adenosine monophosphate deaminase 1 (AMPD1 34C>T (rs17602729), and 5-aminoimidazole-4-carbox-amide ribonucleotide transformylase (ATIC 347C>G (rs2372536). Third, SNPs also might affect the bioactivation of folic acid including MTHFR 677C>T (rs1801133), MTHFR 1298 A>C (rs1801131), MTR 2756 A>G (rs1805087), MTRR 66A>G (rs1801394), and SHMT 1420C>T (rs1979277). Additionally, SNPs might impair the function of enzymes involved in adding and removing glutamic acid residues from MTX including γ-glutamyl hydrolase GGH 354G>T (rs719235) and GGH 16T>C (rs1800909). Finally, SNPs might affect the transportation of MTX from the extracellular to intracellular space by the reduced folate carrier SLC19A1 80G>A

(rs1051266). Due to budget limitations and short timeframe of the project, only SNPs of ABCG2 were analysed.

Sample preparation

Blood was collected from RA patients to prepare samples for analyses. Whole blood was allocated in two cryotube vials, each one was 1.8 ml. After centrifuging about 7–9 ml of blood at a high speed (2000 rpm) for 20 minutes, obtained plasma was removed. WBCs' buffy coat (white line above the RBCs), which is rich in MTX DNA, was removed using a 50Pcs plastic disposable 3 ml graduated transfer pipettes and it was transferred into a 1.8 cryotype and labelled (Figure 2-9). Blood samples (whole blood and buffy coat) were stored in an -80 °C freezer until use. WBCs count was obtained on the same day of preparing the samples by checking the patient's complete blood picture. Values relating to blood volume and WBCs were needed in analysing the buffy coat samples. Although there was a limitation in obtaining the exact count of WBCs on the first visit, the WBCs level was documented for all RA patients during the follow-up. Blood samples were packed in containers filled with dry ice (solidified carbon dioxide) and were carefully sealed and labelled. Then, they were transferred to the Clinical Pharmacology Department, University of South Australia.

Protocol of determination of MTX SNPs

The analyses of SNPs of MTX were conducted at Clinical Pharmacology Department, University of South Australia. Genomic DNA was extracted from patients' peripheral blood mononuclear cells and stored at -80 °C until retrieval. The concentration and purity of the samples was determined using a Nanodrop (Thermo Fisher Scientific, Waltham, MA, USA). DNA was plated into a 96-well master plate at 5 ng/µL and stored at -20 °C until required. The ABCG2 421C>A and ABCB1 3435C>T SNPs were determined by TaqMan® SNP genotyping assays using an ABI 7500 Fast Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA). Assays were carried out according to the manufacturer's instructions.

2.7.3.5.4 MTX intracellular concentration

The main aim was to measure intracellular MTXPGs concentration to explore the relationship between MTX and BP and arterial function in RA patients.

Preparing the samples

The level of MTXPGs inside red blood cells was examined following intracellular the MTXPGs measurement protocol. MXTPGs were measured by using ion-pairing liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique available at the Pharmacology Department, University of South Australia. It is a new validated method with high specificity (van Haandel et al. 2011). Before performing the LC-MS analysis, RBCs were processed and then stored. Between 4 ml and 9 ml of blood were obtained from RA patients. For most patients, about 9 ml of whole blood was centrifuged at a high speed (2000 rpm) for 20 minutes to pellet the RBCs. After centrifuging the blood, the separated plasma was removed and discarded. It is important to record the amount of the plasma removed as the same quantity will be replaced by saline at the washing stage. The remaining lower part of the RBCs was washed twice using sterile phosphate-buffered saline (i.e. isotonic solution) to minimise cell lysis. The RBCs were suspended in an equal volume of sterile normal saline (i.e. same amount of removed plasma). Then, it was mixed by gentle inversion and subjected to a low-speed centrifugation for 10 minutes. The supernatant was discarded and the wash procedure was repeated a second time. After the second wash, the supernatant was discarded and packed RBCs were removed by Gilson pipette (PIPETMAN classic P5000 1-5m) and transferred into 1.8 ml cryotype vial and labelled. If blood samples are stored in refrigerated conditions, the blood is usually stable for 48 hours. Therefore, blood samples (whole blood, buffy coat and packed RBCs) were stored at -80 °C freezer allowing a longer storage time. For the red cells, the results were provided as nmol/L packed RBCs, taking into account the blood volume of each patient. Five levels of MTXPGs, which are based on MTX chain-lengths, were examined (MTXPG1-5). While MTXPG5 has the longest chain, MTXPG1 contains the shortest one.

Protocol of MTX polyglutamates concentration determination

MTX-PGs are purified by solid phase extraction (Strata-X Strong Cation Exchange) prior to LC separation via HILIC LC (ZIC-HILIC Column, Merck Millipore) and analysis on a Shimazdu 8060 triple quadrupole MS/MS. This assay quantifies the five MTXPGs commonly seen in RA patients and has a limit of detection of <0.2 nmol/L packed RBCs.

Whole blood and plasma 5-MHTF were measured at each assessment visit according to a method previously published (van Haandel et al. 2012). The 50 µL of whole blood or plasma was diluted with 50 µL of 5% ascorbic acid solution containing 10 mM mercaptoethanol and 10

µL of internal standard (stable-isotope-labelled 5-MHTF) and incubated at 37 °C for 60 min. Then 60 µL of 10% trichloroacetic acid was added, the mixture vortexed and the clear supernatant was removed. The 5-MTHF was separated via reverse-phased LC using a Waters Acquity BEH C18 column (100X2.1mm), and detected with a Shimadzu 8060 Triple Quadrupole MS/MS (CIC).

For the RBC samples, 250 µL of packed red blood cells were lysed with 650 µL of Milli Q water, followed by the addition of 5 µL of 1000 nM D-MTX (internal standard solution containing 1000 nM each of stable-isotope-labelled MTX, MTX2, MTX3, MTX4, and MTX5). Stable-isotope-labelled internal standards ($^{13}C_5$, ^{15}N, i.e. +6Da), were purchased from Pepscan (Lelystad, The Netherlands). Proteins were precipitated with 0.1 mL 30% perchloric acid and vortex mixed. The samples were centrifuged at 2000 g for 10 minutes, after which 0.75 mL of the supernatant was removed. Then 2.25 mL of 0.1M of phosphate (pH 11) was added, and the sample was then adjusted to pH 5.5.

MTXPGs species were further purified by solid phase extraction (Strata-X-A Strong Anion SPE Tubes, Phenomenex™). The solid phase extraction (SPE) columns were prepared with 1.5 mL methanol, followed by 1.5 mL of 0.1M phosphate (pH 5.5). The RBC and PBMC samples were then loaded onto the SPE columns and allowed to filter through. The loaded columns were respectively washed with 1.5 mL of 0.1M phosphate (pH 5.5) and 1.5 mL methanol. Subsequently, all samples were eluted with 1.5 mL of 5% formic acid in methanol, and were evaporated to dryness using a vacuum centrifuge (Speedyvac).

The dried RBC samples were then reconstituted in 400 µL of mobile phase B and 10 µL was injected onto the column (HILIC HPLC column ZIC-HILIC, Merck Millipore™). Individual MTXPGs were separated with a reverse gradient—mobile phase A was composed of 40% Acetonitrile, 60% water, with 10mM ammonium bicarbonate and Mobile Phase B was made up of 75% Acetonitrile, 25% water, with 10 nM ammonium bicarbonate. Initially, 100% of mobile phase B was maintained for 1 minute, after which the percentage of mobile phase B was reduced to 0% via linear gradient at 4.5 minutes. This was maintained for 1.5 minutes, before returning to 100% of mobile phase B over 0.5 minutes, which was maintained for 2 minutes prior to injection of the next sample. Analytes were detected using positive ion mode on a Shimadzu 8060 tandem LC-MS/MS. The transitions for MTX species MTX1, MTX2, MTX3, MTX4, and MTX5 were 455.1/308.1 m/z, 2584.1/308.0 m/z, 3713.0/308.1 m/z, 842.2/308.1 m/z, and 970.9/308.1 m/z respectively, where m/z is the mass to charge ratio, and the area of each curve

was compared to the area of the corresponding internal standard (Figure 2-11). Calibration standard curves were included in the assay. They were prepared using 250 µL of packed red blood cells taken from a healthy volunteer who had never taken MTX, to which 5 µL of 1000 nM each stable-isotope-labelled MTX species and increasing amounts of MTX stock solution were added to prepare samples containing 0.2, 0.8, 3, 10, 40, and 100 nM/250 µL of packed RBC. 400 µL of water was then added, and the samples were prepared according to the aforementioned method for RBCs. Standard curve samples were injected in quadruplicate.

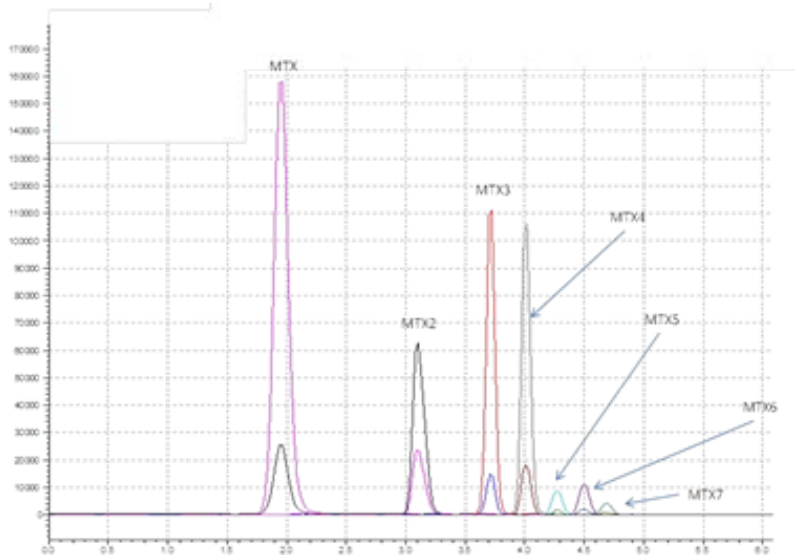


Figure 2-11 Example of chromatogram achieved from the LC-MS/MS procedure

The individual MTX species are present along with their corresponding deuterated internal strands.

2.7.4 Ethics and consents

The Southern Adelaide Clinical Human Research Ethics Committee approved our study (Appendix-10). Additionally, the study was registered in the Australian New Zealand Clinical Trials Registry with the registration number ACTRN12616001366448. All participants signed two consent forms at the beginning of the study (Appendix-3). One of them was to become part of the study, and the second one was for genetic analyses. These consent forms were written in plain language at the 5th to 6th grade reading level. Additionally, all participants were informed that their information will be confidential and their identity will be anonymous. Moreover, the participants' right to refuse or withdraw at any time of the study was highlighted. As this study was observational in nature, changing the clinical practice or causing major harms to the patients were not expected. Patients were informed, however, that few side effects from venepuncture were possible; these side effects include: pain, hematoma at the site of

venepuncture and, rarely, infection. They were also aware that discomfort at the site of the BP monitor's cuff might be experienced during the 24-h BP monitoring. Moreover, it was explained that minimal discomfort might be felt at the patient's wrist when applying the tonometer during PWA measurement using SphygmoCor. All patients gave their consent voluntarily. Before countersigning the consent forms, the principal investigator ensured that all queries raised by the participants were clearly answered.

2.7.5 Data storage

The data were stored in two formats: hardcopy data and electronic data. Anonymised data were stored in the principal investigator's personal password-protected USB drive, hard disk of a university laptop, and the university U drive. Patients' names and contact information were stored on Excel® sheets on the university drive, and linked to Oasis® database. Hard copies of data were stored in a locked cabinet in the student office located at the Discipline of General Practice, Flinders University. Data will be maintained for 5 years after the study completion. After that period, electronic files will be erased from the laptop and hard drive and any paper copies will be shredded

2.7.6 Statistical methods

2.7.6.1 Data entry

All data obtained from questionnaires, BP monitors, SphygmoCor, blood tests, and physical examinations were examined by the principal researcher before entering this data into Excel® 2013 and Stata® databases. All variables were coded and a code book was created (Appendix-11). Anonymisation of each patient's information was used to maintain their confidentiality. All patients were given a unique ID. This ID included the current exposure to MTX and the year of collecting the data. For the MTX cohort, the ID started from MTX001/14, MTX002/14, ……and so on; where MTX indicated that the patient number 1 and 2 was currently on MTX and data was collected in 2014. After entering the data into the databases, it was double-checked before running any analysis. Data were cleaned manually and statistically to detect and remove outliers and invalid information. Missing information, especially from the questionnaires, were ascertained again as much as possible by contacting the patients. About 70% of the 24-h BP monitoring data must be at least satisfactory; thus, if there was missing data that was ≥30%, the patient was asked to repeat the 24-h BP monitoring to have accurate data.

2.7.6.2 Special consideration

Clinic peripheral blood pressure data

Due to the variability associated with BP measurements, single BP reading might be inaccurate or erroneous. Therefore, repeated measures of BP improve reliability of BP results. Thus, the BP was taken three times where each BP measurement was spaced with at least 2 minutes. The average of the second and third BP measurements was calculated and used in the analyses.

Ambulatory blood pressure data

Each patient's dataset recorded in the device software was imported to the computer. Then, the raw dataset was exported as Excel® file. A common approach was used in assessing brachial BP readings (Protogerou et al. 2012). The following were annotated and recorded for brachial BP readings: (i) incorrect BP reading defined as SBP <60 or >250 mmHg, and DBP <30 or >150 mmHg; (ii) identical recordings defined as a duplicate reading of SBP and DBP; (iii) both sleep and awake BP readings were compared against the patient's logbook; (iv) errors between brachial BP and aortic BP defined as lower SBP or higher DBP observed in brachial BP readings compared to aortic readings; (v) invalid reading with poor quality was considered if the cardiac cycle was included in less than 50% pressure waveforms per recording meaning same amplitude and shape.

2.7.6.3 Data analysis

The Stata® software version 13 was used (StataCorp, Texas, USA). Statistical significance was defined as p-value< 0.05. Continuous variables were tested for normal distribution using the Kolmogorov-Smirnov test and then presented as the mean ± SD. Conversely, non-continuous variables were presented as frequencies and percentages. Correlation between methotrexate dose and blood pressure and arterial function was assessed by comparing Pearson correlation coefficients. Since all outcomes were linear continuous variables, linear regression was used to assess the effect of MTX exposure on the outcomes. Multiple linear regression analysis was used to adjust for a wide range of potential confounders. For the repeated cross-sectional study, linear mixed models were used to predict the individual variability in changes in BP with MTX treatment over time.

2.7.6.3.1 Confounders

Many confounding factors might be the reason behind changing the dose of MTX and BP or arterial dysfunction. Untreated hypertension, T2D, smoking, obesity, renal diseases and dyslipidaemia might be a part of the causal pathway of developing arterial stiffness (Glasser et al. 1997), and may be associated with exposure as well. RA patients who suffered from several uncontrolled comorbidities (severe cases with worse prognosis) might need higher doses of MTX. These latter associations may induce bias “by confounding”; thus, these factors were adjusted in the fitted regression model. Additionally, as ageing is a physiological marker for hypertension (Vokonas, Kannel & Cupples 1988), and arterial stiffness (Klocke et al. 2003), adjusting for the age of participants in the analysis was vital. Moreover, numerous drugs used commonly by RA patients were considered in the analyses as they might confound the results. These include medications for treating CVD (antianginal, antihypertensive drugs, aspirin, heart failure medications, vasoactive drugs and statins and other lipid-lowering therapy), for reducing inflammation such prednisolone, and for controlling pain such as NSAIDs. Additionally, inflammatory markers, disease activity, RA duration and duration of using MTX were adjusted in the analyses.

Factors potentially affecting the intracellular concentration of MTXPGs were adjusted in regression models. These include gender of the patients, disease activity, dose and duration of taking MTX, smoking history, the level of eGFR and anti-CCP or RA factors, BMI, and taking DMARDs, or prednisolone, or NSAIDs (Stamp et al. 2009).

There is some evidence that biologic DMARDs might affect BP and arterial stiffness. As tocilizumab (Kume et al. 2011; Provan et al. 2015) and TNFα (Kerekes et al. 2011; Maki-Petaja et al. 2006; Protogerou et al. 2011) have been associated with lower BP and PWV in the RA patients, these medications were adjusted in the regression models. In addition, food intake was included in the regression model to eliminate confounding effects of certain food elements such as natural anti-oxidants, folate, omega-3 fatty acids, alcohol and so forth.

2.7.6.3.2 Food variables

The means of macro and micronutrients were calculated. Multiple regression analyses were performed to examine the association between BP and food intake in the MTX and non-MTX cohort. Additionally, as various type of food might confound the effect of MTX on BP, it was adjusted in the analyses. The information about fruits and vegetables provided by the food questionnaire were based on pieces of fresh fruit and vegetables ingested per day. This

includes diced fruits (½ cup of diced fruit, berries or grapes was considered as one piece). Based on the published literature, participants' responses regarding daily consumption of vegetables were categorised into three groups: less than 3 pieces per day, between 3 and 5 pieces per day, and more than 5 pieces per day (He, Nowson & MacGregor 2006). Fruit consumption was categorised into two groups: less than 3 pieces per day, and 3 or more pieces per day.

CHAPTER 3: RESULTS

3.1 Introduction

As discussed in the first chapter, there is emerging evidence emphasising the positive effect of MTX on CV morbidity and mortality. There is, however, no published evidence investigating the effects of MTX on BP and arterial function in patients with RA independent of inflammation. Given the potential for MTX to decrease CV risk, a comparison between RA patients on MTX and those not taking this drug is required; thus, the main aim of this study is to explore the inverse relationship between the dose and concentration of MTX and the BP and/or arterial function in the RA population.

3.2 Recruitments of patients

Finding RA patients in the non-MTX cohort was rather difficult compared to the MTX cohort. RA patients were not taking MTX for several reasons, such as respiratory and interstitial lung infection, systemic infection, MTX side effects, liver diseases, booked for surgery, past history of cancer and fear of using MTX. Therefore, patients who attended the biologic clinics, run under the rheumatology clinics at the RGH, were considered to find more RA patients who were off MTX and to increase the power of the study. RA patients taking different types of biologics, however, were also taking MTX, and they should be included in the MTX cohort rather than the control group. Finding RA patients who were on combined therapies of biologics and MTX is a common practice. There is evidence showing that concomitant MTX drugs added to biologic treatments improves the efficacy of biologics and gives longer persistence (Ruderman 2013); therefore, a search was conducted for eligible patients based on their medication history. RA patients who were taking tocilizumab (actemra), an IL-6 receptor antibody, were sent information sheets. Using MTX is contraindicated in patients on tocilizumab as combining both drugs increases the risk of developing serious side effects, including liver toxicity (An et al. 2010; Campbell et al. 2011; Choy et al. 2005).

Figure 3-1 shows the total numbers of the RA patients originally found and the number of the RA patients that participated in the study. Overall, there were 385 RA patients identified. An invitation letter and information sheet were sent to all patients. RA patients were classified based on their exposure to the following cohorts: 265 RA patients were potential candidates for MTX cohort, 114 RA patients were eligible for the non-MTX cohort and six patients were possible candidates in the new patient cohort. All patients were contacted several times with a kind request for participation. They were contacted by mail (invitation letter) and then by

phone to attend two study sessions (at baseline and after 8 months) at the Department of Rheumatology at RGH. Each session took a maximum of one hour for each patient.

There were 89 RA patients included in the cross-sectional study and they were classified into MTX, non-MTX and new RA cohorts with 56, 30 and 3 RA patients, respectively. The new RA patients group was omitted from the study due to its small size, which was not sufficient for the sample power. There were several reasons for patients' refusal to participate in the study—these included being sick, suffering from heart diseases (especially angina) or psychiatric illness (e.g. depression), having transportation problems, being in a wheelchair, living in the country or far away, having personal issues, being a full-time carer for children or sick husband, having social phobia or needle phobia, working or studying full-time, unwilling to wear the 24-h BP monitor, or being sensitive to the BP cuff, having unstable RA, being on chemotherapy, and being uninterested. A few patients simply did not show up for sessions. Although the chef investigator explained the value of research in improving rheumatoid patients' health, a few potential participants were not convinced to participate. Most patients consented to participate in the study and a few agreed with excluding blood testing or wearing the 24-h BP monitor.

All participants recruited for the cross-sectional study were invited to attend the follow-up visit 8 months after the initial visit. Initially, the period between the baseline and follow-up was 12 months. The second visit (follow-up session) was changed from 12 months to 8 months to ensure that follow-up measures could be completed in the cohort within the PhD candidature period. A total of 52 patients in the MTX group agreed to participate, while only 28 non-MTX patients were happy to complete the study. There were 80 RA patients included in the repeated cross-sectional study. The percentage of patients lost to follow-up was below 20%; there were 7.1% and 6.7% lost to follow-up among RA patients in the MTX and non-MTX cohort, respectively. One patient pulled out from the study as she had multiple hospital appointments and was diagnosed with depression. Another patient was unable to attend the second visit as they lived 84 km away from Adelaide and worked full-time. A third patient moved away from Adelaide. The reasons for most patients who were unable to complete the study related to working commitments and travelling distance.

In the MTX cohort, 4 out of 52 patients were stopped taking MTX leading to a total of 48 in this cohort. In contrast, 4 out of 28 RA patients, who were off MTX, were commenced on MTX (n = 24).

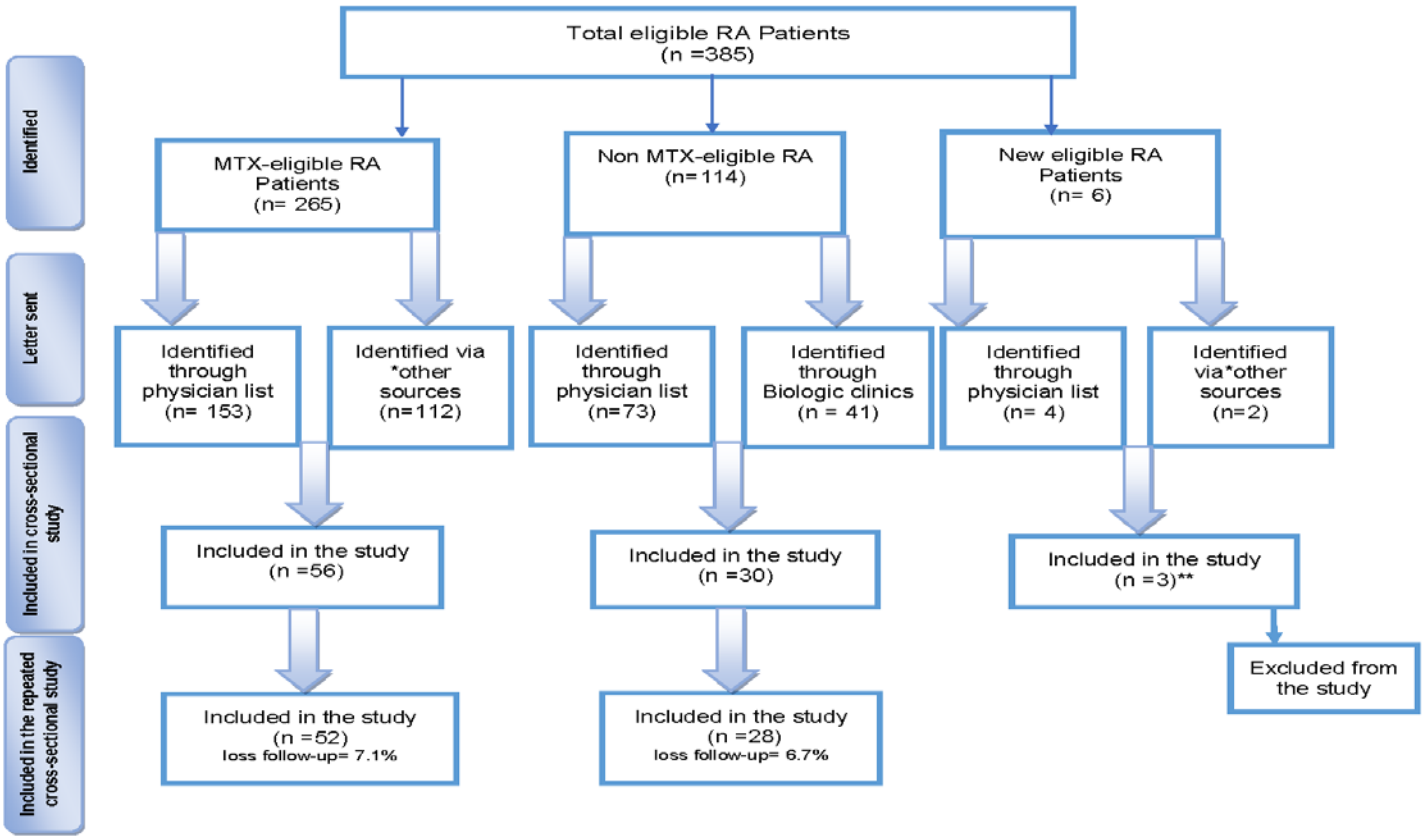


Figure 3-1 Numbers of identified and included rheumatoid arthritis patients

*other sources include biologics clinic and a word of mouth
** only one patient was a MTX naïve, second one was off MTX for 4 months and the third one was off MTX for 3 years
#follow-up was started in April 2015
$ Reasons for patient's refusal to participate in the study include being sick, suffering from heart diseases especially angina, having transportation problems, being in a wheelchair, living in the country or far away, having personal issues, having social phobia or haemaphobia, working or studying full-time, being a full-time carer for children or sick husband, unwilling to wear the 24-h BP monitor and having uncontrolled RA, being uninterested and not showing up.

3.3 Descriptive data at baseline

3.3.1 Baseline patient characteristics

Recruiting patients took place at the rheumatology clinics at the FMC and RGH between March 2014 and December 2015. There was a total of 86 RA patients that participated in the cross-sectional study. These patients had been assigned into two cohorts: MTX cohort (n = 56) and non-MTX cohort (n = 30). Table 3-1 summarises the baseline characteristics of both MTX and non-MTX cohorts. It can be clearly seen that most of the characteristics were comparable in MTX and non-MTX cohorts; patients' ages were similar in both cohorts, which was around 60 years. Although most of the RA participants were female, there was no significant difference between both cohorts; their prevalence was similarly distributed (i.e. around 70%).

Additionally, around 60% and 23% of RA patients were retired or working full-time, respectively. They had attended at least secondary school. Individuals reported earning bachelor and higher degrees; even though there was 18% of patients on MTX who had been awarded a bachelor degree, 10% of those who were not taking MTX had been awarded above bachelor degree. The majority of participants earned about $25,000 annually, but about 3% of the RA patients earned up to $124,000 yearly.

Table 3-1 Baseline demographics*

	MTX (n=56)	Non-MTX (n=30)	p-value
Age (years)	61±13	63 ±12	0.71
Gender (n)%			
Females	(39) 70	(23) 76	0.49
Males	(17) 30	(7) 24	0.62
Education (n) %			
Secondary school	(36) 64	(17) 57	0.54
Bachelor degree	(10)18	(4) 13	0.76
Above Bachelor degree	(2) 4	(3) 10	0.34
Work status (n)%			
Retired	(35) 63	(19) 64	0.54

	MTX (n=56)	Non-MTX (n=30)	p-value
Full-time	(13) 23	(7) 24	1.00
Annual income (n) %			
$0–$24,999	(29) 52	(15) 50	0.24
$25,000–$49,999	(9)16	(11) 37	0.59
$50,000–$74,999	(11) 20	(2) 7	0.13
$75,000–$99,999	(4) 7	(1) 3	0.65
$100,000–$124,999	(2) 4	(1) 3	1.00
$125,000–$149,999	(1) 2	(0) 0	1.00
Marital status (n)%			
Married	(39) 70	(16) 53	0.40
Divorced/separated	(5) 8	(6) 20	0.18
Widowed	(7)13	(5) 17	0.75
Not being married ever	(5) 9	(3) 10	0.87
Health insurance (n) %			
Insured	(35) 63	(14) 47	0.16
Not insured	(21) 38	(16) 53	0.24

*Data as mean±SD unless otherwise indicated

3.3.2 Rheumatoid arthritis-related clinical characteristics

Table 3-2 summarises the prevalence of the main clinical features of RA. The mean duration of RA was shorter in the MTX group compared to the non-MTX group, but it was not statistically significant (13.2±11 vs 16.6±10.8, p = 0.11). Additionally, there was no significant difference in the status of inflammation between the MTX and non-MTX groups. MTX duration was available for 43 RA patients (76.8%), this information was taken from the patients' medical notes, where commencing date of MTX is usually not documented by the treating physicians.

The mean of inflammatory markers, including CRP and ESR was similar. The mean CRP was 7.4±14.7 for the MTX cohort and 6.15±16.2 for the non-MTX cohort. Similarly, the level of anti-CCP was comparable at baseline. Moreover, the degree of disability from RA was

also comparable between the two cohorts as indicated by the Stanford HAQ. Additionally, there were no significant differences between groups in the pain (0.73±0.63 vs 0.84±0.63, p = 0.45) and global health scores (0.58±0.62 vs0.80±0.60, p = 0.12). Conversely, RA activity was slightly better controlled in the MTX participants compared with the non-MTX group as indicated by the DAS28 (2.7±1.1 vs 3.7±1.2, p < 0.001).

Table 3-2 Rheumatoid arthritis related clinical characteristics

	MTX (n = 56)	Non-MTX (n = 30)	p-value
RA duration (years)	13.2±11	16.6±10.8	0.11
****MTX duration (months)**	83.6±65.3	-	-
DAS28 score	2.7±1.1	3.7±1.2	<0.001
Inflammatory markers			
C-reactive protein (mg/L)	7.4±14.7	6.15±16.2	0.71
ESR	19.8±21	18.1±22.3	0.73
Anti-CCP	83.5±89.7	89.3±76.9	0.77
Stanford HAQ score	0.66±0.74	0.99±0.84	0.06
Pain visual analog score	0.73±0.63	0.84±0.63	0.45
Global health score	0.58±0.62	0.80±0.60	0.12

*Data as mean±SD unless otherwise indicated; DAS28: disease activity score on 28 joints; anti-CCP: anti-cyclic citrullinated peptide antibody; **MTX duration for n = 43

3.3.3 Prevalence of previous cardiovascular disease and other chronic diseases

History of the presence of traditional CV risk factors and previous CVD are shown in Table 3-3. The percentage of hypertension was similar in both cohorts; however, all hypertensive patients in the non-MTX group were on one or more anti-hypertensive medications. Moreover, current or past history of smoking was not statistically significant. Compared to RA patients on MTX, participants on other forms of DMARDs showed a higher percentage of traditional CV risk factors; these factors included dyslipidaemia and obesity recording (30% vs 27%) and (33% vs 30%), respectively, this was not statistically significant. Nevertheless, there was a significant trend toward higher prevalence for T2D among the non-MTX cohort compared to the MTX group (17% vs 5%, p = 0.09). Noticeably, the prevalence of previous

CVD and DVT were significantly higher among RA patients in the non-MTX group (p = 0.002, and p = 0.005 respectively). These CVD included MI, angina pectoris, congestive heart failure, transient ischaemic attack and stroke.

Besides previous CVD, the prevalence of RA-related conditions such as chronic renal and liver diseases and depression were compared in both groups. There was no significant difference in the distribution of chronic renal and liver diseases; however, there was a significantly higher percentage of depression among RA patients using DMARDS other than MTX (p = 0.009).

Table 3-3 Prevalence of traditional cardiovascular risk factors, cardiovascular disease and rheumatoid arthritis-related diseases in methotrexate cohort compared to non-methotrexate cohort

	MTX (n = 56)	Non-MTX (n = 30)	p-value
Traditional CV risks			
Hypertension (n) %	(21) 38	(10) 33	0.83
Treated hypertension	(16) 76	(10) 100	0.60
T2D (n) %	(3) 5	(5) 17	0.09
Smoking (n) %			
Current smokers	(9) 16.1	(3) 10	0.26
Ever smokers	(29) 51.8	(19) 63.3	0.30
Regular physical activity (n) %			
Yes	(44) 79	(20) 67	0.23
Type of activity (n)%			
Walk	(38) 73	(16) 53	0.47
Weight (kg)	76±16	80±18	0.45
BMI (kg/m²)	27±6	29±7	0.27
Obesity n (%)			
Obese (BMI≥30)	(17) 30.4	(10) 33.3	0.54

	MTX (n = 56)	Non-MTX (n = 30)	p-value
Traditional CV risks			
Dyslipidemia (n)%	(15) 27	(9) 30	0.75
Previous CVD and RA related diseases			
CVD (n)%			
Any CV disease	(1) 1.8	(7) 23	0.002
DVT	(0) 0	(4) 13	0.005
MI	(1) 1.8	(2) 7	0.24
CVA	(0) 0	(1) 3	0.17
Chronic renal diseases (n)%	(1) 1.8	(2) 6.7	0.24
Liver diseases (n)%	(1) 1.8	(1) 3.3	0.65
Depression (n)%	(7) 12.5	(11) 36.7	0.009

*Data as mean±SD unless otherwise indicated; CVD: cardiovascular diseases include myocardial infarction, angina pectoris, peripheral vascular diseases, transient ischaemic attack and stroke; DVT: deep vein thrombosis; T2D: type 2 diabetes mellitus

3.3.4 Prevalence of using medications

Table 3-4 shows the frequencies of using traditional and biologic DMARDs among RA patients who were on MTX compared with those who were not on MTX at the time of examination. It can be noticed that the percentage of using plaquenil was quite similar between both groups. Alternatively, more RA patients who were off MTX, were using abatacept, rituximab, tocilizumab, and enbrel. It should be highlighted that the observed significant higher percentage of using tocilizumab drug among the non-MTX group (23% vs 1.8%, p = 0.001) was due to intentional recruitment of RA patients on this drug to increase the number of patients in the non-MTX cohort.

Table 3-4 Traditional and biologic disease-modifying anti-rheumatic drugs used in both rheumatoid arthritis cohorts

Medications	MTX (n = 56)	Non-MTX (n = 30)	p-value
Plaquenil (n) %	(14) 25	(7) 23.3	0.86
Arava (n) %	(5) 8.9	0	0.09
Minocin (n) %	0	0	-
Sulfasalazin (n) %	(6) 10.7	(6) 20	0.24
Abatacept (n) %	(1) 1.8	(2) 6.7	0.24
Rituximab (n) %	0	(1) 3.3	0.17
Tocilizumab (n) %	(1) 1.8	(7) 23.3	0.001
Humira (n) %	(4) 7.1	(2) 6.7	0.93
Enbrel (n) %	(10) 17.9	(7) 23.3	0.54
Infliximab (n) %	0	0	-
Certolizumab pego (n) %	(1) 1.8	0	0.46
Golimumab (n) %	(35.4	0	0.20
Prednisolone (n) %	(18) 32.1	(12) 40	0.47
***Prednisolone daily dose (mg)**	5.00 [3.75, 10.00]	5.00 [5.00, 13.75]	0.28

*Data are medians [interquartile ranges]

Table 3-5 shows the frequencies of using medications, which might have an impact on BP and arterial function. These drugs included antihypertensive treatments, NSAIDs, prednisolone, fish oil and folic acid among RA patients. Clearly, the frequency of taking antihypertensive medications between both RA cohorts was comparable. The use of NSAIDs, such as ibuprofen and aspirin, and fish oil was not significantly different between both groups. Similarly, the use and the daily dose of prednisolone was not significantly different between MTX groups. Folic acid usage, as expected, was significantly higher among the MTX group ($p < 0.001$). In fact, the use of these medications was adjusted in the regression models.

Table 3-5 Frequencies of medications might affect the blood pressure in the rheumatoid arthritis cohorts

Medications	MTX (n = 56)	Non-MTX(n = 30)	p-value
Anti-hypertensive treatments (n) %	(16) 28.6	(7) 23.3	0.60
Ibuprofen (n)%	(5) 8.9	(2) 6.6	0.72
***Ibuprofen daily dose (mg)**	280±110	300±100	0.85
[#]Aspirin (n)%	(8)14.3	(4)13.3	0.90
Fish oil (n) %	(18) 32.1	(8) 26.7	0.60
Folic acid (n) %	(42) 75	(3) 10	<0.001

*Data are mean±SD; [#]daily dose 100 mg in all patients

3.3.5 Laboratory blood tests

The main blood tests examined were random blood sugar (mmol/L), total cholesterol (mmol/L), and estimated glomerular filtration rate (ml/min/1.73m^2) (Table 3-6). The level of both blood sugar and total cholesterol was significant lower among RA patients taking MTX (p = 0.004 andp = 0.009, respectively). Then again, the mean eGFR was similar in both MTX and non-MTX groups (77±15.5 vs 65.9±15.4, p = 0.77).

Table 3-6 Blood tests comparing the risk in rheumatoid arthritis cohorts

Blood test	MTX (n = 56)	Non-MTX(n = 30)	p-value
Random blood sugar (mmol/L)	4.9±0.79	5.6±1.5	0.004
Random total cholesterol (mmol/L)	4.9±0.98	5.6±1.3	0.009
eGFR (ml/min/1.73m^2)	77±15.5	65.9±15.4	0.77

Data are mean±SD

3.4 Primary outcome data at baseline

3.4.1 Relationship between methotrexate and blood pressure and arterial function

To examine the effect of MTX on BP and arterial function, the following assessments were performed in each patient: clinic BP, PWA including the measurement of AIx, central BP, and a 24-h BP monitoring including assessment of PWA (Table 3-7). It should be noted that

there were few patients on MTX (n = 2) and non-MTX (n = 4) who refused to wear the 24-h BP monitor. Data presented in Table 3-7 was adjusted for age; there was a trend toward lower peripheral BP among RA patients on MTX. In contrast, there was a significant lower central BP among MTX users compared to RA patients on other DMARDs. The reduction in SBP was about 7 mmHg. While there was about 3% difference in the AIx among MTX users compared with non-users (27.9±1.1 vs 30.7±1.5), this difference did not reach statistical significance (p = 0.15). Moreover, there were no significant differences between groups at baseline for ambulatory BP or arterial function (Table 3-7). There were also no significant differences between MTX cohorts in awake and asleep BP or arterial function (Table 3-8).

Table 3-7 Univariate associations between exposure to methotrexate and blood pressure and arterial function

	MTX (n = 56)	Non-MTX (n = 30)	p-value
Clinic peripheral SBP (mmHg)	124.3±2.0	130.6±2.7	0.06
Clinic peripheral DBP (mmHg)	73.01±1.5	79.5±2	0.01
Clinic central SBP (mmHg)	115.0±2.0	122.0±2.7	0.04
Clinic central DBP (mmHg)	75.1±1.5	80.3±2.0	0.04
24-h peripheral SBP (mmHg)	126.5±1.7	125.4±2.3	0.71
24-h peripheral DBP (mmHg)	74.5±1.0	73.1±1.5	0.45
24-h central SBP (mmHg)	116.4±1.6	116.7±2.3	0.91
24-h central DBP (mmHg)	76.5±1	75.2±1.4	0.45
Augmentation index @ 75bpm (%)	28.7±0.9	28.7±1.3	0.97
24-h Augmentation index @ 75bpm (%)	28.7±0.9	28.7±1.3	0.97
24-h PWV (m/s)	9.08±0.07	9.13±0.10	0.72

Data are age-adjusted mean±SD

Table 3-8 Mean 24-h, daytime, and night-time blood pressure and arterial function for methotrexate and non-methotrexate groups at baseline

	MTX (n = 56)	Non-MTX (n = 30)	p-value
24-h peripheral SBP (mmHg)			
Day	128.8±12.6	129.4±13.9	0.83
Nigh	117.9±17.2	120.5±16.5	0.52
24-h peripheral DBP (mmHg)			
Day	76.5±8.0	75.4±7.0	0.56
Night	68.1±10.3	68.0±7.5	0.95
24-h central SBP (mmHg)			
Day	118.5±11.3	118.8±11.9	0.90
Night	110.1±15.9	111.4±14.2	0.72
24-h central DBP (mmHg)			
Day	78.4±8.1	77.6±7.0	0.66
Night	69.6±10.1	69.0±8.3	0.80
24-h AIx@75 (%)			
Day	28.0±6.8	28.4±6.6	0.79
Night	30.5±9.1	30.0±10	0.79
24-h PWV			
Day	9.1±1.8	9.3±2.0	0.71
Night	8.9±1.9	9.0±2.1	0.73

Data are age-adjusted mean±SD

3.4.2 Methotrexate dose and blood pressure and arterial function

Table 3-9 shows that there was a negative correlation between SBP taken at the clinic and the dose of MTX (r = -0.10). Similarly, AIx and MTX dose were negatively correlated (r = -0.20). However, the associations between all CV outcomes and the dose of MTX were not statistically significant.

Table 3-9 Association between exposure to methotrexate dose and blood pressure/arterial function in rheumatoid arthritis

	correlation coefficient	regression coefficient	95% CI	p-value
Clinic peripheral SBP (mmHg)	-0.10	-0.21	-1.03, 0.61	0.61
Clinic peripheral DBP (mmHg)	0.10	0.21	-0.35, 0.78	0.45
Clinic central SBP (mmHg)	-0.1	-0.26	-1.1, 0.55	0.52
Clinic central DBP (mmHg)	0.10	0.22	-0.36, 0.79	0.45
24-h peripheral SBP (mmHg)	0.01	0.03	-0.65, 0.72	0.92
24-h peripheral DBP (mmHg)	-0.1	-0.12	-0.55, 0.31	0.58
24-h central SBP (mmHg)	0.12	0.27	-0.37, 0.92	0.40
24-h central DBP (mmHg)	-0.12	-0.19	-0.60, 0.23	0.37
Augmentation index @ 75bpm (%)	-0.20	-0.25	-0.69, 0.2	0.27
24-h Augmentation index @ 75bpm (%)	-0.12	-0.23	-0.58, 0.12	0.20
24-h PWV (m/s)	-0.13	-0.05	-0.15, 0.06	0.36

3.4.3 Inflammation and methotrexate cardiovascular protective effect

The impact of inflammation on the effect of MTX on SBP was examined in Table 3-10. Inflammatory markers and disease activity were adjusted in the regression model to examine if this relationship between MTX and BP is independent of inflammation. There were, however, no significant associations between inflammatory markers (ESR and CRP) or DAS28 and clinic SBP (p = 0.92, 0.96 and 0.83), respectively. In particular, CRP levels were similar in MTX and non-MTX patients; and when the scattered plot was examined, there was no significant correlation between the level of CRP and SPB neither in MTX nor in non-MTX group (p = 0.60 and 0.24, respectively) (Figure 3-2).

Table 3-10 Unadjusted and adjusted effects of methotrexate dose on clinic peripheral systolic blood pressure

	regression coefficient	standard error	p-value	95% CI
Unadjusted	-0.21	±0.41	0.61	-1.03, 0.61
Adjusted for:				
ESR	-0.04	±0.43	0.92	-0.90, 0.82
CRP	-0.02	±0.39	0.96	-0.80, 0.76
DAS28	-0.09	±0.41	0.83	-0.92, 0.73

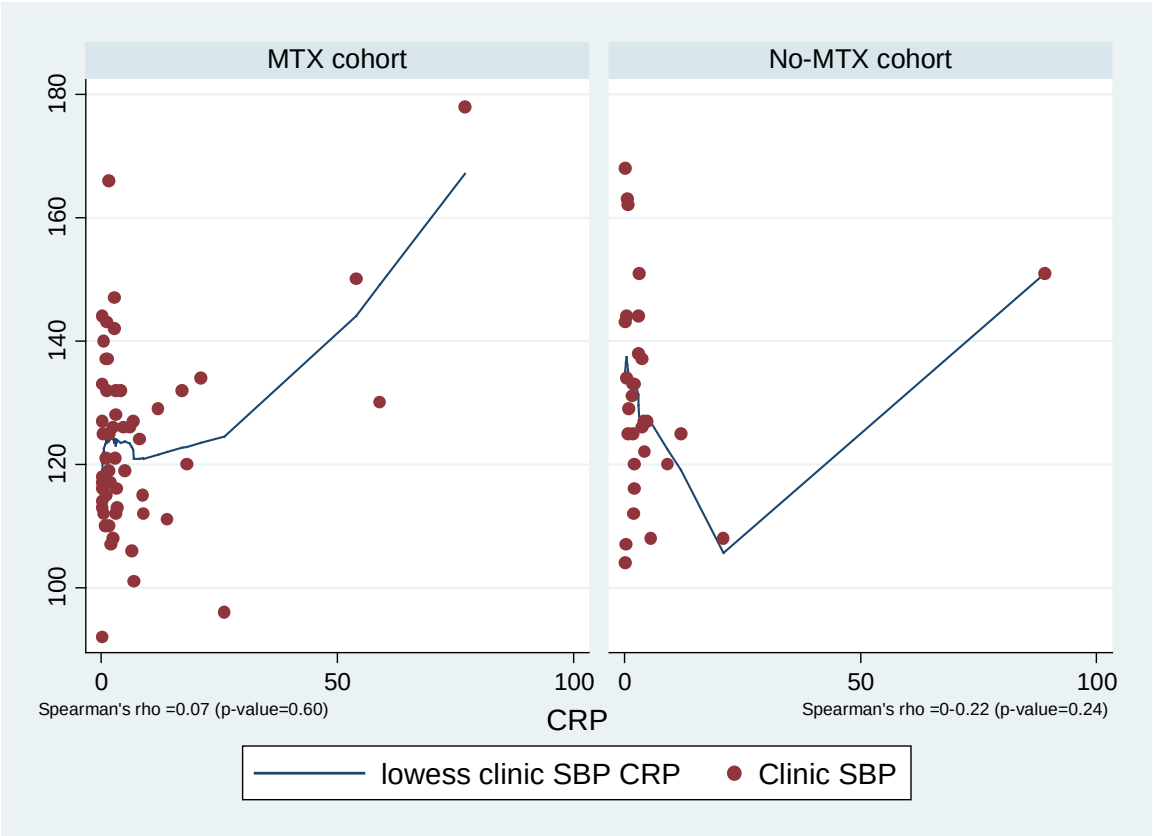


Figure 3-2 Association between clinic peripheral systolic blood pressure and inflammation

3.4.4 Methotrexate polyglutamates concentration and blood pressure and arterial function

3.4.4.1 Association between methotrexate dose and concentrations

Correlation between MTX and different chains of intracellular MTX polyglutamates (MTXPG1-5) was examined in Table 3-11. It can be noticed that there was a week negative correlation between MTX dose and the shortest chain MTX polyglutamates (i.e. MTXPG1), but it was not statistically significant (r = -0.01, p = 0.90). The MTX dose and combined MTX polyglutamates was positively correlated (r = 0.18, p = 0.04). Conversely, there was an inverse relationship between the dose of MTX and the duration of taking this drug, even though it did not reach the statistical significance; additionally, there was a significant negative correlation between the dose of MTX and the RA activity (r = -0.29, p = 0.03).

Table 3-11 Association between methotrexate dose and MTXPGs concentrations

	MTX dose	MTXPG1	MTXPG2	MTXPG3	MTXPG4	MTXPG5	Total MTXPG
MTX dose (mg/week)	1.00						
MTXPG1 (nmol/L)	-0.01	1.00					
MTXPG2 (nmol/L)	0.04	0.78*	1.00				
MTXPG3 (nmol/L)	0.29*	0.46*	0.60	1.00			
MTXPG4 (nmol/L)	0.21	0.22	0.28	0.88*	1.00		
MTXPG5 (nmol/L)	0.12	0.05	0.05	0.67*	0.95*	1.00	
Total MTXPGs (nmol/L)	0.18*	0.70*	0.75*	0.94*	0.82*	0.66*	1.00
MTX duration (months)	-0.07	0.36	0.26	-0.10	-0.19	-0.16	-0.03
DAS28	-0.29*	0.08	0.05	0.06	0.09	0.04	0.09

*These correlations were statistically significant

3.4.4.2 Effects of methotrexate polyglutamates concentrations on blood pressure and arterial function

As the dose tends to get up-titrated if RA is active or if MTX is taken for a longer duration, disease activity and RA duration were adjusted for when assessing the association between BP and arterial function and MTX concentration. Table 3-14 looks at the relationship between total MTX concentration and BP/arterial function. Multiple regression analyses

including several factors potentially affecting the intracellular concentration of MTXPGs were conducted. These confounders include gender, smoking history, the level of eGFR and anti-CCP or RA factors, BMI, and taking DMARDs, or prednisolone, or NSAIDs (Stamp et al. 2009). While BP and markers of arterial function were inversely related to the level of MTXPGs, this relationship was not statistically significant.

Table 3-12 Effects of methotrexate polyglutamates concentration (nmol/L) on cardiovascular outcomes at baseline (n = 56)

CV outcomes	β	95% CI	p-value
Clinic peripheral SBP (mmHg)	-0.001	-0.09, 0.09	0.99
Clinic peripheral DBP (mmHg)	-0.01	-0.07, 0.06	0.74
Clinic central SBP (mmHg)	-0.01	-0.10, 0.08	0.87
Clinic central DBP (mmHg)	-0.02	0.08, 0.05	0.64
24-h peripheral SBP (mmHg)	-0.01	-0.06, 0.08	0.78
24-h peripheral DBP (mmHg)	-0.04	-0.08, 0.01	0.08
24-h central SBP (mmHg)	-0.01	-0.07, 0.09	0.80
24-h central DBP (mmHg)	-0.04	-0.08, 0.01	0.12
Augmentation index @ 75bpm (%)	-0.01	-0.06, 0.03	0.54
24-h Augmentation index @ 75bpm (%)	-0.02	-0.08, 0.05	0.64
24-h PWV (m/s)	-0.002	-0.002, 0.005	0.29

Model adjusted for age, gender, BMI, anti-CCP, DAS28, prednisolone, NSAIDs and MTX duration

3.4.5 Genetic polymorphisms of transporters involved in methotrexate metabolism

SNPs in the genes encoding enzymes responsible for MTX metabolism or MTX transmembrane transporters that might influence the efficacy of the drug were examined. There are several SNPs affecting the efflux of MTX from target cells; however, only SNPs of *ABCG2* were analysed in this study due to budget limitations and short timeframe of the PhD project.

3.4.5.1 ABCG2

ABCG2 (rs2231142) is one of the SNPs of MTX. Table 3-13 shows the prevalence of homozygous (CC) and heterozygous (AC) genotypes of this SNP. The majority of RA patients presented with 'wild type' or normal gene (CC) recoding about 80% of the MTX cohort. On the contrary, less than 20% of the RA patients on MTX tested positive for the mutant genes (i.e. AC).

Table 3-13 Prevalence of homozygous and heterozygous genotypes of methotrexate *ABCG2* SNP

Genotypes	Frequency (n)	Percentage (%)
Homozygous (CC)	46	82.14
Heterozygous (AC)	10	17.86
Total	56	100

Table 3-14 compares the effects of normal and mutant genes of MTX on BP and arterial function and on the accumulation of MTX inside the cells. The most striking feature is that RA patients with AC variant showed lower BP, and better arterial function. Both peripheral and central SBP were significantly lower recording about 12 mmHg reduction ($p = 0.03$ and 0.02, respectively). Similarly, markers of arterial function were better among those with AC allele; compared to CC genotype, RA with AC allele showed a difference of approximately 5% in AIx and 2 m/s in PWV. Another important effect of AC genotype was related to intracellular MTXPGs concentration. This mutant gene was associated with higher intracellular accumulation of MTXPGs compared with normal homozygous gene (CC) (132.7 vs 115.8). Yet, it did not reach statistical significant. There was, however, a moderate positive correlation between clinic SBP and the level of MTXPGs in CC genotypes of *ABCG2* SNP (Figure 3-3).

Table 3-14 Comparison between CC and AC genotyping of methotrexate ABCG2 SNP

	CC genotype (n = 46)	AC genotype (n = 10)	*P*-value
MTX dose (mg/week)	14.5±5.0	16.0±6.0	0.57
MTXPGs (nmol/L packed RBCs)	115.8±63	132.7±73.1	0.46
C-reactive protein (mg/L)	8.4±16.1	3.1±2.4	0.31
DAS28 score	2.8±1.1	2.1±0.7	0.06
Clinic peripheral SBP (mmHg)	126.2±15.4	114.3±12.1	0.03
Clinic peripheral DBP (mmHg)	73.6±10.8	70.6±10.2	0.43
Clinic central SBP (mmHg)	117±14.7	104.7±14.4	0.02
Clinic central DBP (mmHg)	75.7±11	72.7±10.1	0.43
24-h peripheral SBP (mmHg)	127.8±12.6	120.3±11.6	0.09
24-h peripheral DBP (mmHg)	74.8±8.1	72.7±7.7	0.44
24-h central SBP (mmHg)	117.2±11.8	110.3±11.4	0.08
24-h central DBP (mmHg)	77.03±8.03	74.1±6.3	0.30
Augmentation index (%)	30.7±9.1	23.6±14	0.04
Augmentation index @ 75bpm (%)	28.9±7.1	23.03±12.30	0.04
24-h Augmentation index @ 75bpm (%)	29.1±6.9	26.7±5.5	0.30
24-h PWV (m/s)	9.3±1.8	7.6±2.1	0.01
ADMA µmol/l	4.8±0.66	4.7±0.90	0.68
SDMA µmol/l	4.3±1.4	3.1±1.2	0.02
CIT µmol/l	351.8±103.4	367.4±120.6	0.68

HMA µmol/l	14.8±5.6	15.7±4.5	0.67

CC = normal gene; AC = mutant allele; RBCs = red blood cells; HMA = N(G)-hydroxymethyl-arginine

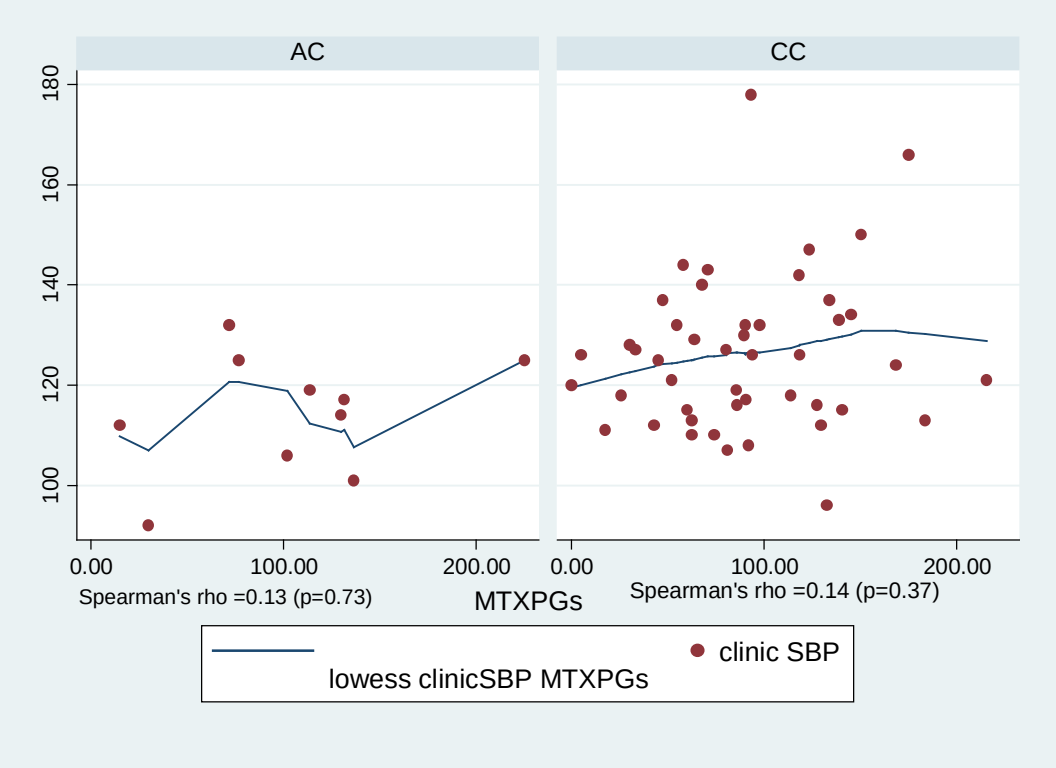


Figure 3-3 Association between clinic peripheral systolic blood pressure and methotrexate polyglutamates concentration in methotrexate ABCG2 genotypes

3.4.6 Effect of arginine metabolites on cardiovascular risk and methotrexate exposure

3.4.6.1 Relationship between methotrexate exposure and arginine metabolites

Table 3-15 summarises the mean plasma levels of arginine metabolites in the RA patients based on their exposure to MTX. Noticeably, there was a significant slight increase in the ADMA concentration among the non-MTX cohort after adjusting for age. Moreover, there was a significant increase in the level of CIT in the same RA cohort. There was a trend of negative association between ADMA level and the exposure to MTX (p = 0.08) (Table 3-16). Other common confounders, which might bias this association toward the null, were considered; these include age, disease activity, eGFR, smoking, T2D, dyslipidaemia, anti-hypertensive and anti-diabetic medications, and nutritional intake (protein and alcohol consumption per day). While the negative relationship between MTX exposure and ADMA concentration was maintained, it did not reach statistical significance (p = 0.80). The effect of

each confounder on the ADMA concentration was separately studied (Appendix-12), where no significant association was reported.

Table 3-15 Plasma levels of arginine metabolites in rheumatoid patients based on their exposure to methotrexate at baseline

	MTX (n = 56)	Non-MTX (n = 30)	p-value
ADMA µmol/l	4.8± 0.1	5.1± 0.1	0.05
SDMA µmol/l	4.1± 0.2	4.1± 0.3	0.98
CIT µmol/l	357.1± 14.0	433.2± 19.5	0.002
HMA µmol/l	15± 0.88	17.2± 1.2	0.14

Mean± SD are age adjusted

Table 3-16 Association between methotrexate exposure and asymmetric dimethylarginine level (n = 86)

ADMA		
	β (95% CI)	**p-value**
Unadjusted	-0.02 (-0.05, 0.003)	0.08
***Adjusted**	-0.006 (-0.05, 0.04)	0.80

*Model adjusted for age, DAS28, eGFR, smoking, T2D, dyslipidaemia, medications and nutritional intake

3.4.6.2 Asymmetric dimethylarginine and cardiovascular outcomes

Table 3-17 depicts the association between BP/ arterial function and ADMA level in the MTX and non-MTX RA patients. The regression model was adjusted for traditional CV risk factors. Among RA patients on MTX, there was a negative association between the level of ADMA and SBP measured at the clinic and over 24-h, even though it did not reach the statistical significance. Conversley, the level of ADMA was positively correlated to the level of SBP and the markers of arterial function among RA patients not using MTX. Interestingly, 24-h peripheral and central SBP were significantly increased with increasing the level of ADMA among the non-MTX cohort (β = 14.6, p = 0.004 and β = 9.5, p = 0.01, respectively). Moreover, there was a significant positive association between the exposure to ADMA and markers of arterial function. There was a significant increase in the 24-h AIx (around 6%, p = 0.03), and in the 24-h PWV (around 2 m/s, p = 0.005).

Table 3-17 Association between cardiovascular outcomes and asymmetric dimethylarginine level (0.1µmol) in methotrexate and non-methotrexate cohort*

CV outcome	MTX (n = 56)		Non-MTX (n = 30)	
	β (95% CI)	p-value	β (95% CI)	p-value
Clinic peripheral SBP (mmHg)	-0.53 (-9.3, 8.2)	0.90	8.2 (-5.0, 21.4)	0.20
Clinic peripheral DBP (mmHg)				
Clinic central SBP (mmHg)	-1.6 (-9.9, 6.7)	0.70	8.1 (-5.3, 21.5)	0.22
Clinic central DBP (mmHg)				
24-h peripheral SBP (mmHg)	-4.0 (-11.4, 3.4)	0.28	14.6 (5.5, 23.8)	0.004
24-h peripheral DBP (mmHg)				
24-h central SBP (mmHg)	-4.04 (-11, 2.9)	0.24	9.5 (1.9, 17.2)	0.01
24-h central DBP (mmHg)				
Augmentation index @ 75bpm (%)	-1.7 (-5.8, 2.4)	0.41	2.6 (-3.2, 8.4)	0.37
24-h Augmentation index @ 75bpm (%)	0.74 (-2.2, 3.7)	0.61	7.0 (0.50, 13.4)	0.03
24-h PWV (m/s)	0.23 (-0.77, 1.2)	0.64	2.3 (0.81, 3.8)	0.005

*Model adjusted for CV risk factors

3.5 Secondary outcome data at baseline

3.5.1 Methotrexate exposure and rheumatoid arthritis activity and quality of life

Table 3-19 shows the effect of exposure to different doses of MTX on RA activity and quality of life assessed by DAS28 and the Stanford HAQ, respectively. The most prominent finding was a statistically significant inverse relationship between MTX dose and RA activity. With every 1 mg increase in MTX dose, the score of DAS28 was expected to decrease by 0.06 (95% CI -0.23, -0.006, p = 0.03); after considering main predictors of disease activity (i.e. age, HAQ and rheumatoid factor), there was a significant negative relationship between the exposure to MTX and the DAS28 (β = -0.76, 95% CI -1.2, -0.35, p < 0.001). Likewise, there was inverse relationship between patients' quality of life represented by less pain and better global health and MTX dose. MTXPGs concentration was not, however, associated with low disease activity (Table 3-20). Moreover, there was a statistically significant direct association

between MTXPGs concentration and quality of life measured by HAQ. This association was still positive after adjusting for the duration of using MTX.

Table 3-18 The effect of methotrexate dose exposure on disease activity and quality of life among rheumatoid patients

	β	95% CI	p-value
DAS28	-0.06	-0.23, -0.006	0.03
***Adjusted DAS28**	-0.76	-1.2, -0.35	<0.001
Quality of life			
Stanford HAQ score	-0.002	-0.04, 0.04	0.94
Pain visual analog score	-0.008	-0.04, 0.03	0.63
Global health score	-0.001	-0.03, 0.03	0.95

*Model adjusted for predictors of disease activity including age, HAQ and RF

Table 3-19 The effect of methotrexate polyglutamates concentration on the status of disease activity and quality of life among rheumatoid arthritis patients

	β	95% CI	p-value
DAS28	0.001	-0.003, 0.01	0.53
Quality of life			
Stanford HAQ score	0.004	0.002, 0.007	0.003
Pain visual analog score	-0.001	-0.003, 0.002	0.67
Global health score	0.001	-0.002, 0.004	0.49

Model adjusted for age, BMI and RF

3.5.2 Food intake

3.5.2.1 Macronutrients and micronutrients intake

As diet is considered an inevitable universal exposure, food intake in the RA patients might impact the risk of arterial stiffness and subsequent CVD occurrence. The prevalence of the main nutrients intake for the MTX and non-MTX groups are summarised in Table 3-20. There was no significant difference in micronutrients intake between both groups, except for vitamin D and folic acid supplements. RA patients using MTX consumed more vitamin D and folic acid compared to those not on MTX (57% vs 27 %, $p = 0.007$) and (77% vs 10%, $p < 0.001$), respectively; however, the level of folate was not statistically different between both RA cohorts ($p = 0.28$). Similarly, there was no significant difference between both RA

cohorts in the prevalence of macronutrients intake except for the beef and egg consumption. The prevalence of eating 1–2 eggs per week was significantly higher in the MTX cohort (p = 0.02). Additionally, there was a borderline significantly greater beef ingestion among MTX users compared to non-MTX group (p = 0.06).

Table 3-20 Prevalence of macronutrients micronutrients intake among rheumatoid arthritis cohorts

Diet	MTX (n = 56)	Non-MTX (n = 30)	p-value
Fish oil supplements, n (%)	18 (32)	8 (27)	0.60
Fish (g/day)	25.9 (±49.1)	12.8 (±16.2)	0.16
Vitamin D supplements, n (%)	32 (57)	8 (27)	0.007
Folic acid supplements, n (%)	43 (77)	3 (10)	<0.001
Folate (ug/day)	229.4 (±106.4)	206.1(±68.4)	0.28
Energy (kJ/day)	6171±2643	6392±3389	0.74
Total fat (g/day)	60.3±30.3	65.1±40.3	0.53
Saturated fat (g/day)	24.6±13.4	27.6±19.4	0.40
Polyunsaturated fat (g/day)	9.1±5.4	8.3±4.4	0.49
Monounsaturated fat (g/day)	21.2±11.0	23.5±14.6	0.43
Protein (g/day)	77.8±36.3	77.8±44.6	0.99
Eggs 1–2 eggs/week (n)%	(26) 46.4	(11) 36.7	0.02
Beef steak twice/week (n)%	(20) 35.7	(5) 16.7	0.06
Sugars (g/day)	78.4±35.0	74.3±37.5	0.61
Fibre (g/day)	18.0±8.4	17.1±6.7	0.63
Alcohol (g/day)	8.9 ±16.1	10.2 ±16.5	0.72

*Values are mean±SD unless indicated otherwise

The association between MTX exposure and BP and arterial function was examined after adjusting for micronutrients and macronutrients to eliminate confounding effect (Table 3-21). The association between MTX and BP/arterial function was retained. While there was a

distinct trend toward significant lower clinic peripheral SBP among MTX users (β = -7.3, 95% CI -0.63, 15.1, p = 0.07), there was a significant decrease in the clinic central SBP (β = -8.2, 95% CI 0.42, 16.1, p = 0.04). Additionally, there was a trend toward significant lower AIx in the MTX group (β = -4.0, 95% CI -0.37, 8.4, p = 0.07).

Table 3-21 Differences in blood pressure and arterial function in methotrexate and non-methotrexate cohorts at baseline (n=86)

CV outcomes	β (95% CI)	p-value
Clinic peripheral SBP (mmHg)	-7.3 (-0.63, 15.1)	0.07
Clinic peripheral DBP (mmHg)	-7.8 (2.3, 13.2)	0.01
Clinic central SBP (mmHg)	-8.2 (0.42, 16.1)	0.04
Clinic central DBP (mmHg)	-6.7 (1.3, 12.0)	0.02
24-h peripheral SBP (mmHg)	-0.74 (-6.0, 7.5)	0.83
24-h peripheral DBP (mmHg)	-0.71 (-4.7, 3.2)	0.72
24-h central SBP (mmHg)	-2.3 (-3.8, 8.5)	0.45
24-h central DBP (mmHg)	-0.56 (-4.4, 3.2)	0.77
Augmentation index @ 75bpm (%)	-4.0 (-0.37, 8.4)	0.07
24-h Augmentation index @ 75bpm (%)	-1.7 (-1.7, 5.1)	0.32
24-h PWV (m/s)	-0.21 (-0.76, 1.2)	0.66

Model adjusted for macronutrients and micronutrients

3.5.2.2 Effect of fruits and vegetables consumption on blood pressure and arterial function

A one-way ANOVA was conducted to determine if BP and arterial function were different for MTX and non-MTX groups with different level of consuming fruits and vegetables per day. Participants were classified into two groups (<3 and ≥ 3), and three groups (<3, 3–5, and ≥5) based on the number of pieces of fruit or vegetables consumed per day, respectively. There was a statistically significant difference between higher daily intake of vegetables and 24-h peripheral SBP (β = -1.93, p = 0.04), 24-h central SBP (β = -2.20, p = 0.02), and 24-h PWV (β = -0.42, p = 0.03) among RA patients who were on MTX. Thus, the association between MTX and CV outcomes were re-examined after adjusting for fruits and vegetables consumption (Table 3-22). Both clinic peripheral and central BP remained significantly

decreased among RA patients on MTX. Therefore, lower BP observed among MTX users was potentially from the exposure to MTX rather than the between-groups differences in consuming fruits and vegetables.

Table 3-22 Differences in blood pressure and arterial function for rheumatoid arthritis cohorts after adjusting for fruit and vegetable consumption at baseline (n = 86)

CV outcome	β (95% CI)	p-value
Clinic peripheral SBP (mmHg)	-7.0 (-0.24, 14.2)	0.05
Clinic peripheral DBP (mmHg)	-6.4 (1.4, 11.4)	0.01
Clinic central SBP (mmHg)	-7.8 (0.67, 15.0)	0.03
Clinic central DBP (mmHg)	-5.1 (0.14, 10.1)	0.04
24-h peripheral SBP (mmHg)	-0.61 (-6.8, 5.5)	0.84
24-h peripheral DBP (mmHg)	-1.2 (-4.9, 2.4)	0.50
24-h central SBP (mmHg)	-0.65 (-4.9, 6.2)	0.82
24-h central DBP (mmHg)	-1.2 (-4.7, 2.3)	0.49
Augmentation index @ 75bpm (%)	-3.3 (-0.68, 7.3)	0.10
24-h Augmentation index @ 75bpm (%)	-0.30 (-2.9, 3.5)	0.86
24-h PWV (m/s)	-0.23 (-0.68, 1.1)	0.62

3.6 Repeated cross-sectional study analyses (follow-up)

3.6.1 Descriptive statistics

Table 3-23 and Figure 3-4 show age-adjusted means for the BP and markers of arterial function measured at follow-up. Among RA patients in the MTX cohort, but not the control group, there was a significantly lower clinic peripheral and central BP (126.2/73 vs 133.7/78.4 mmHg, respectively). After adjusting for potential confounders (i.e. visit, age, gender, BMI and DAS28), differences in BP and arterial function between visits were lower in the MTX compared to non-MTX group (Figure 3-5).

Table 3-23 Age-adjusted means of blood pressure, arterial function and methylarginines for MTX groups at follow-up

	MTX (n = 47)	Non-MTX (n = 24)	p-value
Clinic peripheral SBP (mmHg)	126.2±2.2	133.7±3.0	0.05
Clinic peripheral DBP (mmHg)	73.0±1.5	78.4±2.1	0.04
Clinic central SBP (mmHg)	116.4±2.0	124.4±2.7	0.02
Clinic central DBP (mmHg)	74.7±1.5	79.5±2.1	0.07
24-h peripheral SBP (mmHg)	107.1±2.3	108.7±3.2	0. 69
24-h peripheral DBP (mmHg)	62.6±1.3	62.2±1.8	0.88
24-h central SBP (mmHg)	113.0±1.8	117.4±2.6	0.17
24-h central DBP (mmHg)	73.7±1.1	74.4±1.6	0.74
Augmentation index (%)	28.6±1.1	30.0±1.6	0.48
24-h Augmentation index @ 75bpm (%)	28.5±1.2	30.8±1.7	0.29
24-h PWV (m/sec)	9.1±0.14	9.4±0.2	0.18
ADMA (µmol/L)	0.54±0.01	0.55±0.01	0.97
SDMA µmol/l	0.37± 0.02	0.39± 0.02	0.65
CIT µmol/l	37.4± 1.7	41.3± 2.4	0.18
HMA µmol/l	1.8± 0.11	1.7± 0.16	0.82

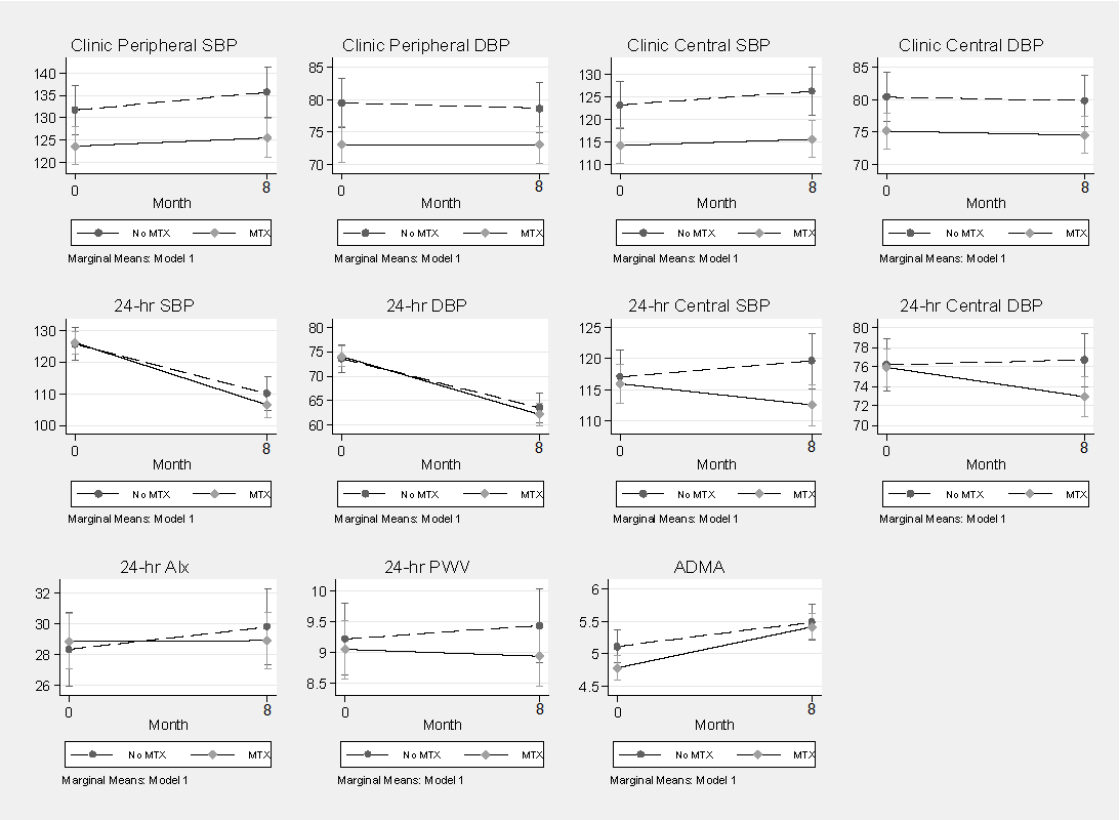


Figure 3-4 Comparison between methotrexate and non-methotrexate age-adjusted means for cardiovascular outcomes at baseline and follow-up

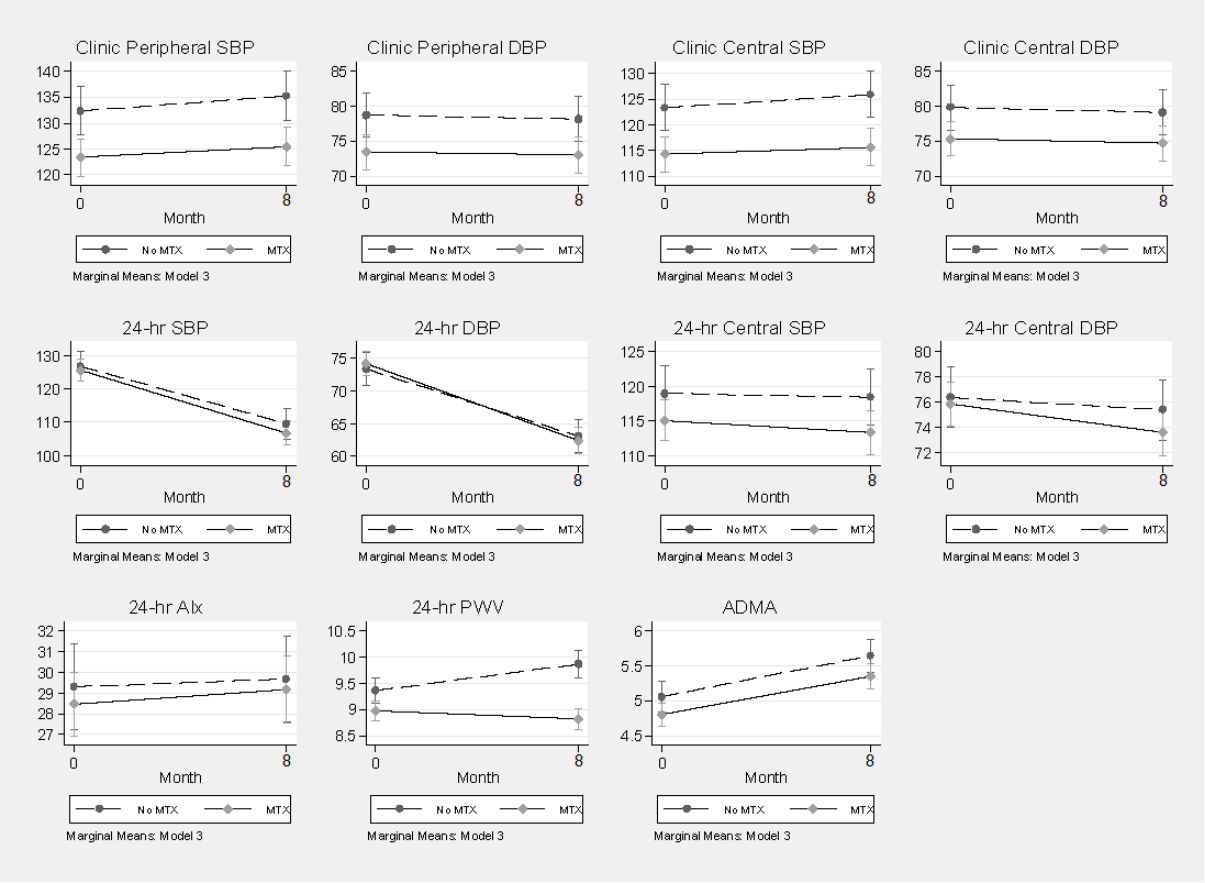


Figure 3-5 Comparison between methotrexate and non-methotrexate adjusted mean for visit, age, gender, BMI and DAS28 for cardiovascular outcomes at baseline and follow-up

3.6.2 Diurnal and nocturnal blood pressure and arterial function

Monitoring RA patients for 24-h showed a non-significant trend toward lower peripheral SBP during awake time among MTX users (125.0 mmHg vs131.2, p = 0.08) (Table 3-24). In contrast, the awake central SBP was significantly lower among MTX users (114.1 vs121.0, p = 0.03). Interestingly, while RA patients on MTX showed lower BP and markers of arterial function with time, those not on MTX showed increase in all CV outcomes (Figure 3-6 to Figure 3-8).

Table 3-24 Mean 24-h, daytime, and night-time blood pressure and arterial function for methotrexate groups at follow-up including subjects who switched treatment

	MTX (n = 44)	Non-MTX (n = 22)	p-value
24-h peripheral SBP (mmHg)			
Day	125.0±14.0	131.2±13.3	0.08
Nigh	117.1±16.4	120.1±19.1	0.50
24-h peripheral DBP (mmHg)			
Day	73.4±8.1	75.1±5.2	0.37
Night	67.2±10.1	67.4±6.8	0.93
24-h central SBP (mmHg)			
Day	114.1±12.8	121.0±11.4	0.03
Night	108.0±15.0	112.5±17.0	0.27
24-h central DBP (mmHg)			
Day	75.2±8.5	77.2±5.3	0.30
Night	68.5±10.4	68.7±7.4	0.93
24-h AIx@75 (%)			
Day	28.3±6.0	30.2±2.0	0.32
Night	30.4±10.2	31.9±11.0	0.58
24-h PWV			
Day	9.0±2.0	10.0±2.2	0.08
Night	8.8±2.0	9.7±8.7	0.12

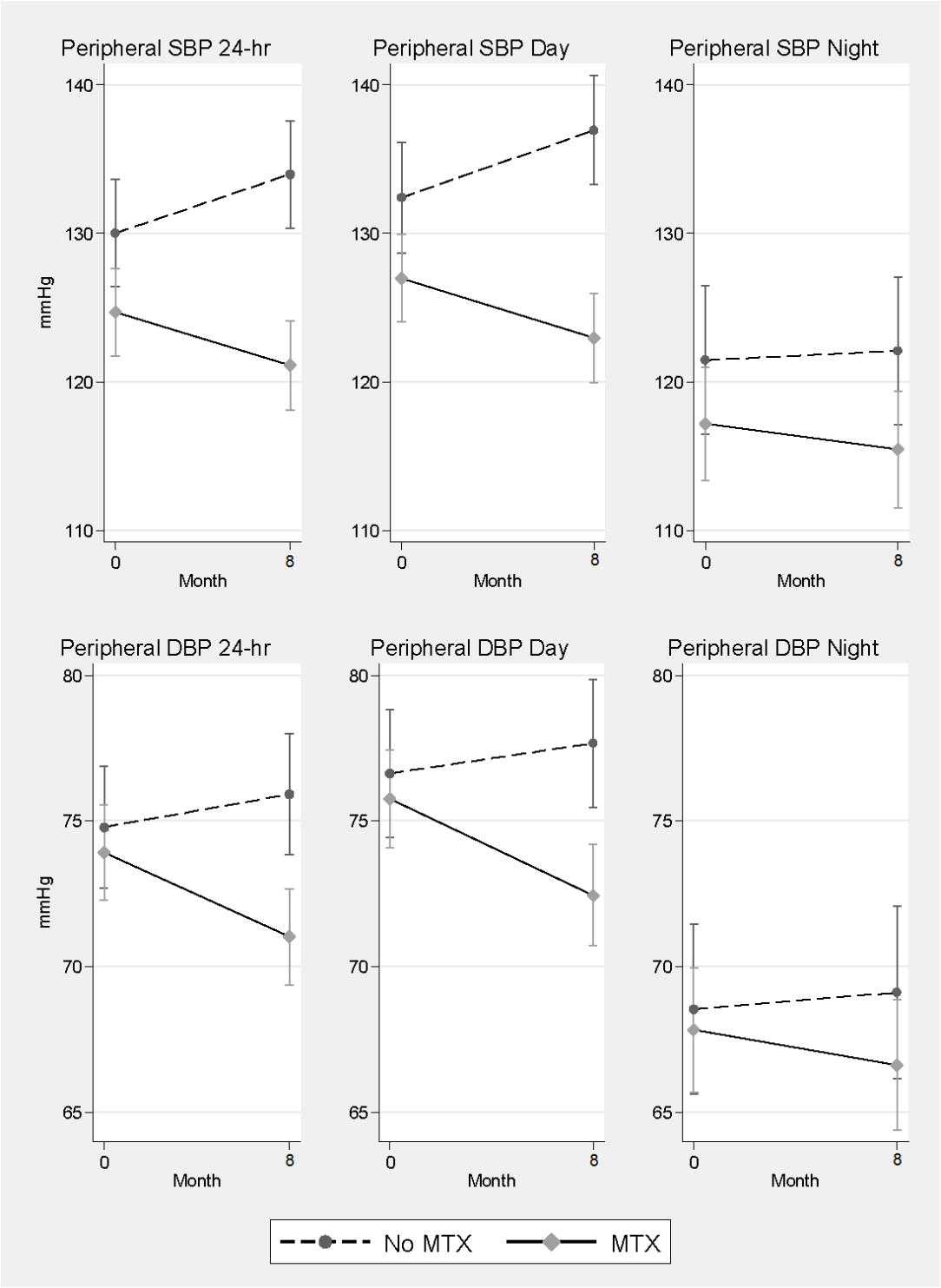


Figure 3-6 Baseline and follow-up changes in 24-h peripheral blood pressure for methotrexate and non-methotrexate rheumatoid arthritis patients

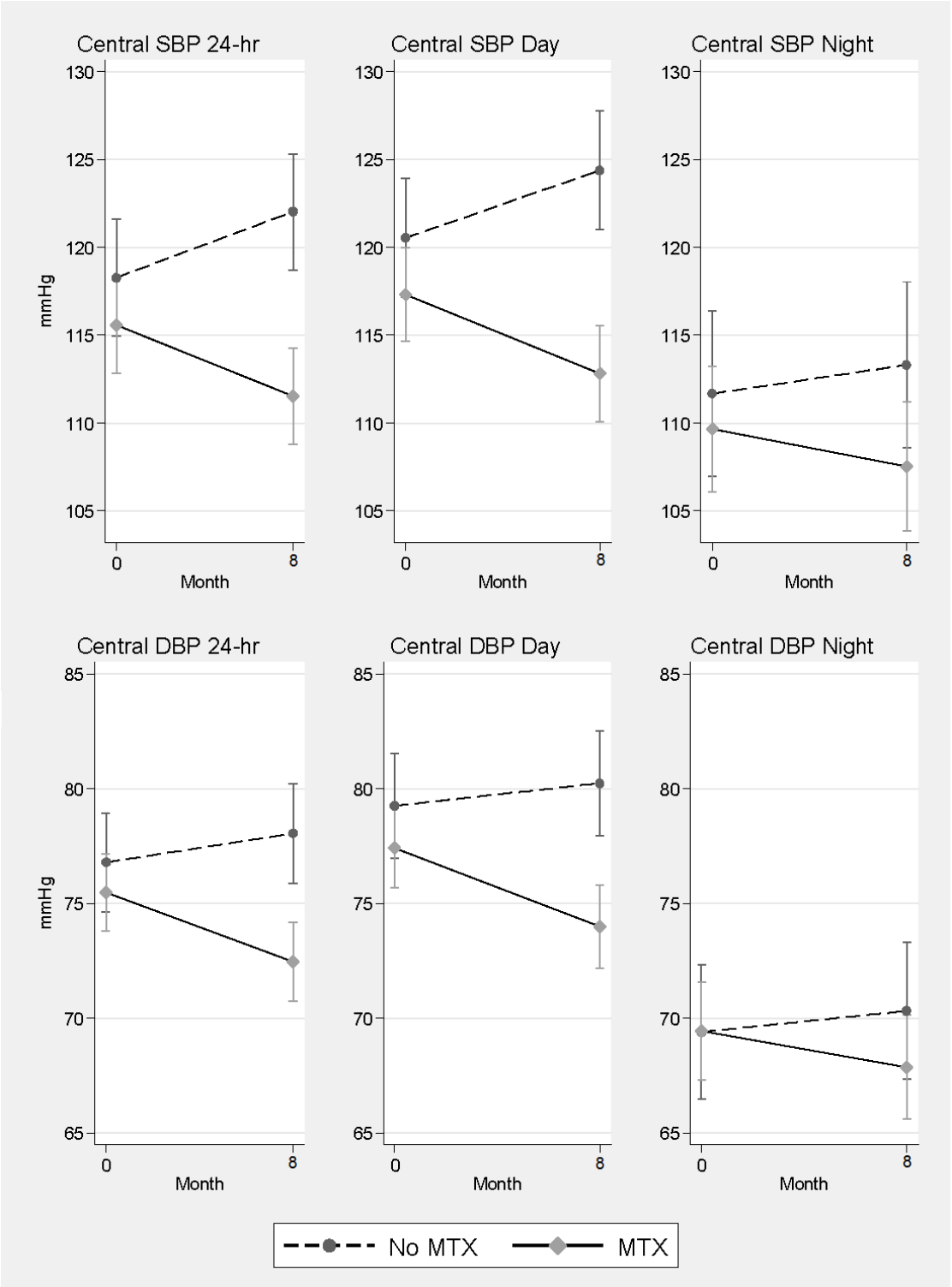


Figure 3-7 Baseline and follow-up changes in 24-h central blood pressure for methotrexate and non-methotrexate rheumatoid arthritis patients

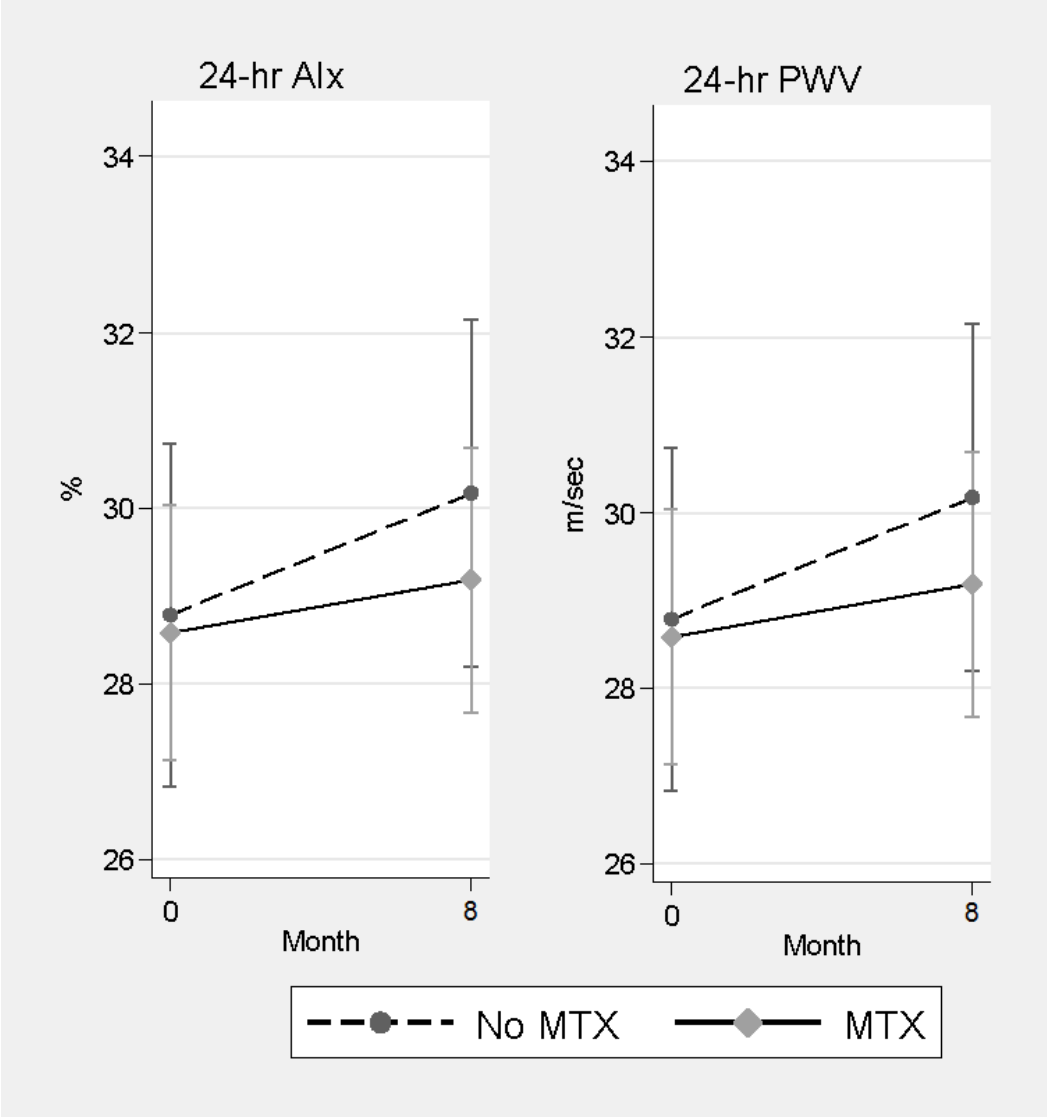


Figure 3-8 Baseline and follow-up changes in 24-h augmentation index and pulse wave velocity for methotrexate and non-methotrexate rheumatoid arthritis patients

Figure 3-9 and 3-10 show the hourly changes in ambulatory BP at baseline and at 8 months. The difference in the change between the MTX groups was more related to daytime BP than night-time BP. Peripheral and central SBP and DBP were significantly lower for MTX than non-MTX groups at baseline; and this drop was significantly greater for MTX than non-MTX graoups at 8 months. Additionally, both markers of arterial function showed a similar pattern (Figure 3-11 and 3-12).

Figure 3-9 Baseline and follow-up hourly changes in ambulatory peripheral blood pressure for methotrexate and non-methotrexate rheumatoid arthritis patients

Figure 3-10 Baseline and follow-up hourly changes in ambulatory peripheral blood pressure for methotrexate and non-methotrexate rheumatoid arthritis patients

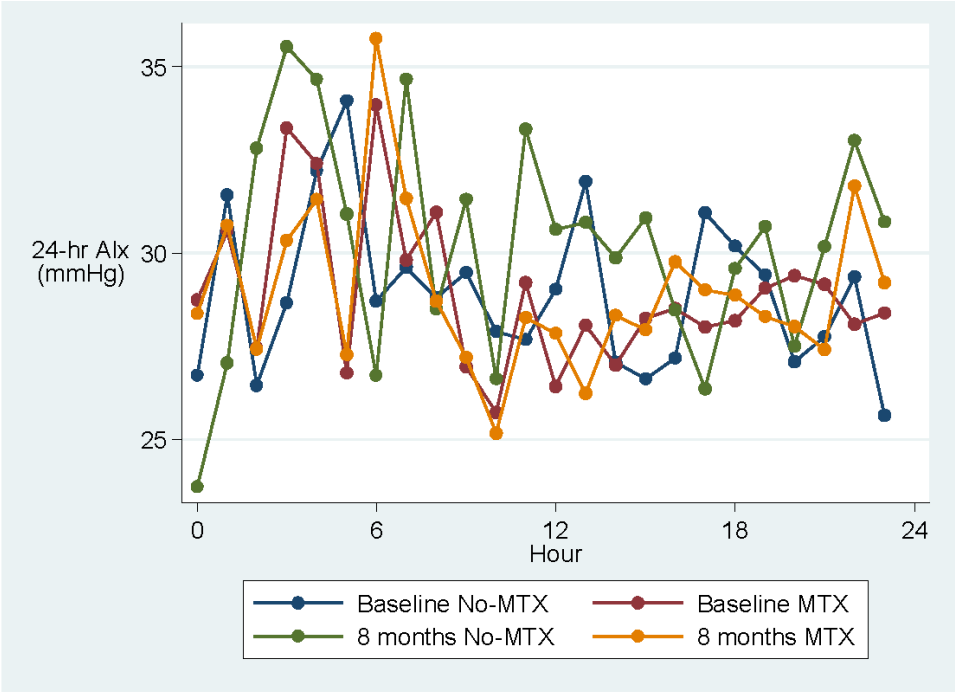


Figure 3-11 Baseline and follow-up hourly changes in ambulatory augmentation index for methotrexate and non-methotrexate rheumatoid arthritis patients

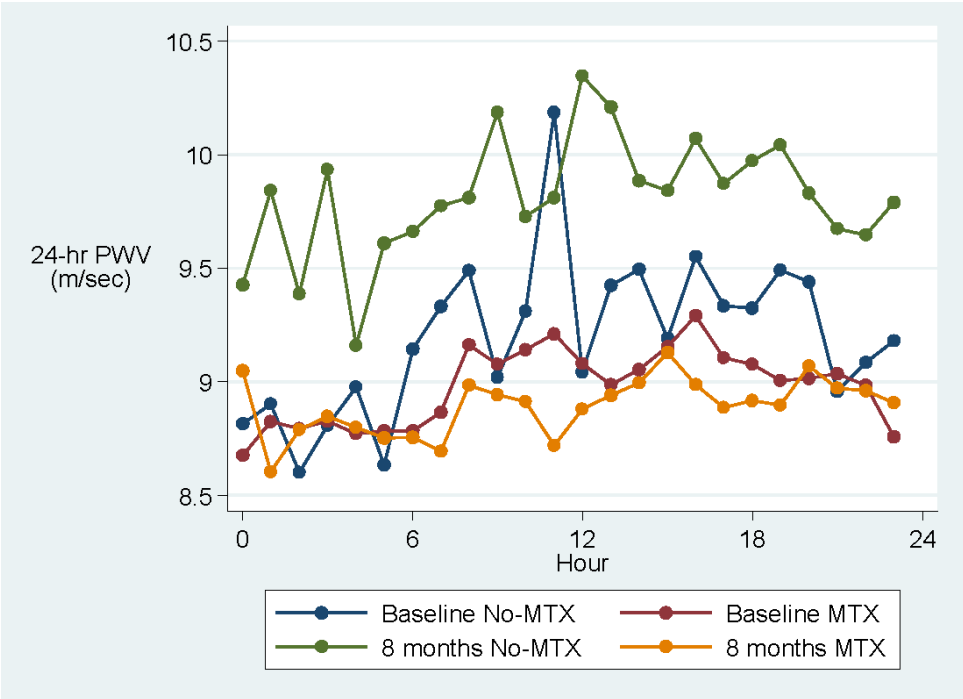


Figure 3-12 Baseline and follow-up hourly changes in ambulatory pulse wave velocity for methotrexate and non-methotrexate rheumatoid arthritis patients

3.6.3 Longitudinal differences in cardiovascular risk for methotrexate and non-methotrexate users

3.6.3.1 Blood pressure, arterial function and plasma asymmetric dimethylarginine concentrations

A linear mixed effect model was used to examine the changes in BP and arterial function on the second visit. After adjusting for clinical, demographic, pharmacological, nutritional and biochemical confounders, differences in BP between MTX and non-MTX groups were similar across the baseline and follow-up visits. Both clinic peripheral and central SBP were significantly lower among MTX users (β = -7.7, 95% CI -13.2, -2.3, p = 0.006) and (β = -7.8, 95% CI (-13.1, -2.6, p = 0.003), respectively (Table 3-25).

In addition, the 24-h central SBP increased in those not using MTX while those using MTX showed lower SBP. Adjusted regression model showed that there was a trend of lower 24-h central SBP for MTX users at the second visit (β = -4.2, 95% CI -8.7, 0.4, p = 0.07) (Table 3-25). In contrast, there were no significant between-group differences in either AIx or plasma ADMA concentrations. Moreover, there was no significant difference in MTXPGs concentration between visits (Appendix 12).

RA patients who switched treatments between the baseline and the 8-month assessments (n = 8) were considered; from the linear mixed effects model, the within- vs between-subject effects of MTX on BP, AIx, PWV and plasma ADMA concentrations were compared in Table 3-26. This comparison showed that the within-subject effects, although not significantly different, were generally larger and beneficial for RA patients on MTX treatment than the between-subject effects for MTX.

Ambulatory means for peripheral and central BP, AIx and PWV were further examined during daytime and night-time (Table 3-27). The following were adjusted in the model—age, gender, visit, MTX treatment, BMI, DAS28 and folic acid use, and hour of measurement—patients treated with MTX showed significant lower average 24-h and daytime, but not night-time, peripheral and central SBP and DBP and PWV compared to RA patients not using MTX.

Table 3-25 Differences in blood pressure, augmentation index, pulse wave velocity and asymmetric dimethylarginine concentrations for methotrexate and non-methotrexate users including subjects who switched treatment (n = 86)

	Model 1		Model 2		Model 3	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Clinic peripheral SBP (mmHg)	-9.0 (-14.7, -3.3)	0.002	-8.1 (-13.4, -2.8)	0.003	-7.7 (-13.2, -2.3)	0.006
Clinic peripheral DBP (mmHg)	-6.0 (-9.8, -2.3)	0.002	-5.9 (-9.5, -2.3)	0.001	-6.1 (-9.8, -2.4)	0.001
Clinic central SBP (mmHg)	-9.8 (-15.3, -4.3)	<0.001	-8.1 (-13.1, -3.0)	0.002	-7.8 (-13.1, -2.6)	0.003
Clinic central DBP (mmHg)	-5.3 (-9.0, -1.5)	0.006	-5.1 (-8.7, -1.5)	0.006	-5.4 (-9.1, -1.6)	0.005
24-h peripheral SBP (mmHg)	-1.4 (-6.7, 3.9)	0.60	-1.1 (-6.2, 4.1)	0.69	-2.0 (-7.4, 3.3)	0.46
24-h peripheral DBP (mmHg)	-0.4 (-3.4, 2.6)	0.81	-0.5 (-3.4, 2.5)	0.76	-1.3 (-4.4, 1.7)	0.38
24-h central SBP (mmHg)	-3.8 (-8.3, 0.6)	0.09	-4.0 (-8.4, 0.4)	0.07	-4.2 (-8.7, 0.4)	0.07
24-h central DBP (mmHg)	-1.9 (-4.6, 0.9)	0.18	-1.8 (-4.5, 0.9)	0.18	-1.8 (-4.5, 1.0)	0.20
Augmentation index (%)	-0.1 (-2.6, 2.4)	0.93	0.4 (-1.9, 2.7)	0.71	0.6 (-1.8, 3.0)	0.65
24-h AIx@75 (%)	-0.1 (-2.6, 2.4)	0.93	0.8 (-1.5, 3.0)	0.50	1.1 (-1.2, 3.4)	0.36
24-h PWV (m/sec)	-0.3 (-0.9, 0.2)	0.25	-0.2 (-0.5, 0.1)	0.14	-0.2 (-0.5, 0.1)	0.12
ADMA (µmol/L)	-0.21 (-0.47, 0.06)	0.12	-0.18 (-0.43, 0.07)	0.15	-0.19 (-0.45, 0.07)	0.15
SDMA (µmol/l)	0.14 (-0.48, 0.76)	0.65	0.16 (-0.43, 0.74)	0.60	0.09 (-0.53, 0.70)	0.78

	Model 1		Model 2		Model 3	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
CIT (µmol/l)	-42.0 (-93.2, 9.2)	0.11	-38.7 (-88.3, 11)	0.13	-57.7 (-109.0, -6.3)	0.03
HMA (µmol/l)	-1.5 (-4.5, 1.4)	0.31	-1.2 (-4.3, 1.8)	0.43	-1.5 (-4.6, 1.6)	0.35

Model 1 = Unadjusted; Model 2 = Adjusted for visit, age, gender and BMI; Model 3 = Adjusted for visit, age, gender, and BMI. DAS28 was adjusted because its level was different between groups at baseline where SBP and DBP remain significantly different.

Table 3-26 Within and between-subject effects of methotrexate on blood pressure, augmentation index, pulse wave velocity and asymmetric dimethylarginine concentrations

	Within-subject effect	Between-subject effect	Difference between effects	P-value for the difference
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**p-value**
Clinic peripheral SBP (mmHg)	-13.3 (-24.5, -2.1)	-5.9 (-12.2, 0.37)	7.4 (-5.6, 20.3)	0.26
Clinic peripheral DBP (mmHg)	-7.2 (-15.8, 1.5)	-5.9 (-10.0, -1.7)	1.3 (-8.3, 10.9)	0.79
Clinic central SBP (mmHg)	-13.2 (-24.3, -2.1)	-6.3 (-12.3, -0.32)	6.9 (-5.8, 19.6)	0.29
Clinic central DBP (mmHg)	-7.6 (-16.3, 1.1)	-4.9 (-9.0, -0.68)	2.7 (-7.0, 12.5)	0.58
24-h peripheral SBP (mmHg)	-7.5 (-18.6, 3.6)	-0.31 (-6.5, 5.9)	7.2 (-5.7, 20.0)	0.28
24-h peripheral DBP (mmHg)	-4.7 (-11.0, 1.5)	-0.25 (-3.7, 3.2)	4.5 (-2.7, 11.7)	0.22
24-h central SBP (mmHg)	-9.0 (-17.5, -0.50)	-2.1 (-7.6, 3.3)	6.8 (-3.4, 17.1)	0.19
24-h central DBP (mmHg)	-6.6 (-11.8, -1.3)	0.07 (-3.2, 3.3)	6.6 (0.42, 12.8)	0.04
Augmentation index (%)	0.93 (-3.5, 5.4)	0.40 (-2.5, 3.3)	-0.53 (-5.9, 4.8)	0.85
24-h AIx@75 (%)	-0.39 (-1.1, 0.33)	-0.20 (-0.53, 0.13)	0.19 (-0.61, 0.98)	0.65
24-h PWV (m/sec)	-0.03 (-0.63, 0.58)	-0.22 (-0.51, 0.06)	-0.19 (-0.87, 0.48)	0.57
ADMA (µmol/L)	-13.3 (-24.5, -2.1)	-5.9 (-12.2, 0.37)	7.4 (-5.6, 20.3)	0.26

Table 3-27 Adjusted[1] mean (95% CI) 24-h, daytime, and night-time blood pressure and arterial function for MTX and non-MTX groups at baseline and 8 months

	Baseline				8 months					
	No-MTX (n=26)	MTX (n=54)	Adjusted Δ	p-value	No-MTX (n=26)	MTX (n=54)	Adjusted Δ	p-value	MTX x Visit interaction	p-value for MTX x Visit interaction
Peripheral SBP										
Day	**132 (128, 136)**	**127 (124, 130)**	**-6.8 (-11, -2.8)**	**<0.001**	**136 (132, 140)**	**123 (120, 126)**	**-13 (-17, -9)**	**<0.001**	**-6 (-8.7, -3.4)**	**<0.001**
Night	121 (116, 126)	117 (113, 121)	-4.3 (-9.9, 1.3)	0.13	121 (116, 126)	116 (112, 120)	-3.5 (-8.9, 2)	0.21	0.8 (-3, 4.7)	0.67
24-h	**129 (125, 133)**	**125 (122, 128)**	**-6.5 (-10, -3)**	**<0.001**	**133 (129, 137)**	**121 (118, 124)**	**-11 (-15, -7.7)**	**<0.001**	**-4.6 (-6.9, -2.3)**	**<0.001**
Peripheral DBP										
Day	**77 (75, 79)**	**76 (74, 77)**	**-2.9 (-5.5, -0.3)**	**0.03**	**78 (75, 80)**	**72 (70, 74)**	**-5.7 (-8.2, -3.2)**	**<0.001**	**-2.8 (-4.6, -1.0)**	**0.003**
Night	68 (65, 71)	68 (66, 70)	-1.9 (-5.6, 1.7)	0.30	69 (66, 72)	67 (65, 69)	-1.6 (-5.2, 2.1)	0.40	0.4 (-2.5, 3.2)	0.81
24-h	**75 (73, 77)**	**74 (72, 75)**	**-2.8 (-5.2, -0.5)**	**0.02**	**76 (73, 78)**	**71 (69, 73)**	**-5.1 (-7.3, -2.8)**	**<0.001**	**-2.1 (-3.8,-0.6)**	**0.006**
Central SBP										
Day	**120 (117, 124)**	**117 (115, 120)**	**-4.2 (-7.8, -0.6)**	**0.02**	**124 (120, 127)**	**113 (110, 116)**	**-10 (-14, -6.7)**	**<0.001**	**-6.1 (-8.5, -3.7)**	**<0.001**
Night	112 (107, 117)	110 (106, 113)	-2.7 (-8.1, 2.7)	0.33	113 (108, 118)	108 (104, 112)	-3.7 (-9, 1.6))	0.18	-1.0 (-4.9, 3.0)	0.64
24-h	**118 ((115, 122)**	**116 (113, 118)**	**-3.7 (-7, -0.4)**	**0.03**	**121, (118, 125)**	**112 (109, 115)**	**-8.8 (-12, -5.5)**	**<0.001**	**-5.1 (-7.2, -2.9)**	**<0.001**
Central DBP										
Day	**79 (77, 82)**	**77 (76, 79)**	**-3.4 (-5.9, -0.8)**	**0.01**	**80 (78, 83)**	**74 (72, 76)**	**-6.2 (-8.7, -3.7)**	**<0.001**	**-2.8 (-4.6, -1.1)**	**0.002**
Night	69 (66, 72)	69 (67, 71)	-1.5 (-5.1, 2.2)	0.43	70 (67, 73)	68 (66, 70)	-1.7 (-5.3, 1.9)	0.35	-0.25 (-3.2, 2.7)	0.87
24-h	**77 (75, 79)**	**75 (74, 77)**	**-3 (-5.3, -0.8)**	**0.009**	**78 (76, 80)**	**73 (71, 74)**	**-5.4 (-7.7, -3.1)**	**<0.001**	**-2.4 (-3.9, -0.8)**	**0.003**
PWV										
Day	**9.3 (9.1,9.45)**	**9.0 (8.98,9.1)**	**-0.21 (-0.35,-0.07)**	**0.003**	**10.0 (9.8,10.2)**	**8.8 (8. 7, 8.9)**	**-0.43(-0.56,-0.29)**	**<0.001**	**-0.2(-0.3,-0.13)**	**<0.001**
Night	8.9 (8.7, 9.2)	8.8 (8.6, 8.9)	-0.15(-0.35, 0.06)	0.16	9.7 (9.5, 9.9)	8.6 (8.5, 8.8)	-0.19 (-0.38,0.01)	0.06	-0.04(-0.2, 0.1)	0.57
24-h	**9.2 (9.0, 9.4)**	**9.0 (8.8, 9.1)**	**-0.19 (-0.32,-0.07)**	**0.002**	**9.9 (9.8, 10.0)**	**8.8 (8.6, 8.9)**	**-0.37(-0.49,-0.25)**	**<0.001**	**-0.18(-0.3,-0.1)**	**<0.001**
AIx @75 bpm										
Day	28.7 (26.7, 30.7)	27.9 (26.5, 29.4)	0.7 (-1.9, 3.2)	0.60	30.1 ((28.0,32.2)	28.3 (26.7,29.8)	-1.1 (-3.7, 1.5)	0.40	-1.8 (-3.8, 0.2)	0.09
Night	29.3 (26.1, 32.5)	30.3 (28.1, 32.5)	3.12 (-1.18, 7.41)	0.16	31.4 (28.0, 34.7)	31.5(29.1, 33.9)	2.76(-1.7,7.2)	0.22	-0.35(-4.4, 3.7)	0.87
24-h	28.8 (26.8, 30.8)	28.5 (27.1, 30.0)	1.35(-1.09,3.8)	0.28	30.3 (28.3, 32.4)	29.1 (27.6,30.6)	0.07(-2.4,2.5)	0.96	-1.28(-3.15,0.6)	0.18

[1]Adjusted for visit, methotrexate use, hour, age, gender, BMI, DAS28 and folic acid use

3.6.3.2 Impacts of genetic polymorphisms of transporters involved in methotrexate metabolism

Table 3-28 shows the differences in blood pressure, arterial function and ADMA concentrations between the CC and AC genotypes of the MTX ABCG2. There were no significant differences between both genotypes when visit, age, gender, BMI and disease activity were adjusted in the mixed-effect model.

Table 3-28 Differences in blood pressure, augmentation index, pulse wave velocity and asymmetric dimethylarginine concentrations between the CC and AC polymorphisms of the methotrexate ABCG2 (n = 86)

	Model 1		Model 2		Model 3	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Clinic peripheral SBP (mmHg)	6.3 (-3.02, 15.7)	0.18	5.7 (-3.7, 15.1)	0.23	5.8 (-3.7, 15.4)	0.23
Clinic peripheral DBP (mmHg)	1.9 (-4.5, 8.3)	0.55	1.7 (-4.7, 8.05)	0.61	1.4 (-5.03, 7.7)	0.68
Clinic central SBP (mmHg)	7.4 (-1.5, 16.4)	0.10	7.0 (-2.0, 16.0)	0.13	7.02 (-2.1, 16.1)	0.13
Clinic central DBP (mmHg)	2.6 (-3.7, 9.0)	0.42	2.3 (-4.0, 8.7)	0.47	1.9 (-4.4, 8.2)	0.56
Augmentation index @75 (%)	-1.03 (-6.4, 4.4)	0.71	-0.92 (-6.3, 4.4)	0.74	-0.81 (-6.2, 4.6)	0.77
Augmentation index (%)	2.2 (-4.0, 8.4)	0.49	2.5 (-3.6, 8.7)	0.42	2.4 (-3.8, 8.7)	0.45
24-h peripheral SBP (mmHg)	7.7 (-1.8, 17.2)	0.11	7.1 (-2.3, 16.4)	0.14	7.2 (-2.2, 16.6)	0.13
24-h peripheral DBP (mmHg)	3.9 (-1.2, 9.1)	1.4	3.5 (-1.6, 8.7)	0.18	3.4 (-1.8, 8.6)	0.20
24-h central SBP (mmHg)	4.04 (-2.9, 11.0)	0.25	3.1 (-3.7, 9.8)	0.37	3.4 (-3.3, 10.0)	0.32

	Model 1		Model 2		Model 3	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
24-h central DBP (mmHg)	1.9 (-2.2, 6.0)	0.37	1.4 (-2.7, 5.4)	0.52	1.6 (-2.5, 5.6)	0.45
24-h Augmentation index @75 (%)	1.3 (-2.5, 5.2)	0.50	1.3 (-2.5, 5.1)	0.50	1.6 (-2.2, 5.4)	0.42
24-h PWV (m/sec)	0.02 (-0.71, 0.75)	0.97	-0.1 (-0.76, 0.63)	0.85	-0.1 (-76, 0.65)	0.89
ADMA (µmol/l)	-0.14 (-0.70, 0.42)	0.62	-0.15 (-0.70, 0.41)	0.61	-0.17 (-0.72, 0.38)	0.55
SDMA (µmol/l)	-0.84 (-2.1, 0.44)	0.20	-0.87 (-2.1, 0.37)	0.17	-0.91 (-2.2, 0.34)	0.15
CIT (µmol/l)	97.8 (8.4, 187.3)	0.03	69.04 (7.1, 185.0)	0.03	86.7 (-1.2, 174.6)	0.05
HMA (µmol/l)	0.66 (-4.3, 5.7)	0.80	0.88 (-4.1, 5.9)	0.73	1.2 (-3.8, 6.2)	0.63
MTXPGs (nmol/L)	26.3 (-11.8, 64.4)	0.18	27.7 (-10.6, 65.9)	0.16	27.9 (-11.2, 67.0)	0.16

Model 1 = Unadjusted; Model 2 = Adjusted for visit, age, gender and BMI; Model 3 = Adjusted for visit, age, gender, BMI and DAS28

3.7 Summary of the main results

This study provides the first evidence of an association between MTX treatment and lower clinic and 24-hr peripheral and central BP and PWV in RA patients. After adjusting for visit, age, gender, body mass index, folic acid use and disease activity, the MTX group had significantly lower clinic peripheral SBP (-7.7 mmHg, 95% CI -13.2 to -2.3, p = 0.006) and DBP (-6.1 mmHg, 95% CI -9.8 to -2.4, p = 0.001), and central SBP (-7.8 mmHg, 95% CI -13.1 to -2.6, p = 0.003) and DBP (-5.4 mmHg, 95% CI -9.1 to -1.6, p = 0.005) vs the non-MTX group. Furthermore, the MTX group had significantly lower 24-h peripheral and central BP and PWV compared to the non-MTX group. In contrast, there were no significant between-group differences in AIx. There was an inverse relationship between peripheral and central SBP and intracellular MTXPGs concentrations. Similarly, both AIx and PWV were inversely related to MTXPGs concentrations. About 20% of RA patients on MTX were heterozygous for the MTX transporter ABCG2 (AC genotype). This group of patients showed significantly lower BP, AIx and PWV, and higher concentrations of MTXPGs vs MTX patients with homozygous normal genotype. Additionally, this study showed a trend towards lower concentrations of plasma ADMA, an endogenous NO synthase inhibitor, a marker of endothelial function and cardiovascular risk, in RA patients treated with MTX.

Traditional risk factors are also involved in the pathophysiology of CVD in RA. A recent systematic review and meta-analysis indicates that despite the increased CV risk associated with RA in general and the use of anti-inflammatory drugs in this population, traditional CV risk factors such as hypertension, T2D, smoking, hypercholesterolaemia and obesity, still independently increase the risk of CV morbidity in this patient population, and the magnitude of this increase appears similar to that observed in the general population (Baghdadi et al. 2015). This suggests that a careful diagnosis and management of CV risk factors should be considered equally important to the management of the symptoms of RA in order to mitigate the risk of CV morbidity and mortality among these patients.

CHAPTER 4: DISCUSSION

4.1 Summary of key findings

This is the first study to examine the relationship between MTX use, dose and intracellular concentration, and BP and markers of arterial function in patients with RA. It included a cross-sectional study followed by a repeated cross-sectional analysis to examine the longitudinal effect of MTX on BP and arterial function. These analyses demonstrated that exposure to MTX was associated with significant differences in BP and markers of arterial function vs treatment with other DMARDs. In the cross-sectional study the mean clinic and ambulatory peripheral and central BP and PWV, the gold standard marker of arterial stiffness (i.e. PWV) were significantly lower in RA patients treated with MTX versus those treated with other DMARDs (non-MTX group, $p < 0.01$ for all comparisons). In contrast, there were no significant between-group differences in the marker of arterial wave reflection (AIx). Exposure to MTX was inversely related to the level of peripheral and central BP, which was negatively correlated to the dose of MTX; however, the relationship between MTX dose and BP was statistically significant. There was about 7 mmHg reduction in both peripheral and central SBP in MTX users. These associations were independent of inflammation and disease activity as there was no significant association reported between BP and markers of arterial function and the level of CRP, ESR and DAS28 ($p = 0.92$, 0.96 and 0.83), respectively. More importantly, there was an inverse relationship between peripheral and central SBP and intracellular MTXPGs concentrations. Similarly, both AIx and PWV were inversely related to MTXPGs concentrations. About 20% of RA patients on MTX were heterozygous for the MTX transporter *ABCG2* (AC genotype). This group of patients showed significant lower BP, AIx and PWV, and higher concentrations of MTXPGs versus MTX patients with homozygous normal genotype. Additionally, this study showed a trend towards lower concentrations of plasma ADMA, an endogenous NO synthase inhibitor, marker of endothelial function and cardiovascular risk, in RA patients treated with MTX. Moreover, ADMA concentrations were positively correlated with BP and both markers of arterial function among non-MTX RA patients. Most importantly, after adjusting for clinical, demographic, pharmacological, nutritional and biochemical confounders, differences in BP between MTX and non-MTX groups were similar for each outcome across the baseline and follow-up visits; clinic and central BP were significantly lower among MTX users. While ambulatory BP and PWV were reduced in the MTX group, the ambulatory central BP increased in those not using MTX. The association between exposure to MTX and ambulatory BP and PWV reduction was driven by significant daytime, but not night-time, differences in these parameters.

4.2 Comparison of the findings to available literature

4.2.1 Blood pressure

The finding of lower BP in the MTX cohort is supported by observational studies suggesting a lower SBP in MTX users (Ajeganova et al. 2013; Choi et al. 2002; Cuchacovich & Espinoza 2009; Hansel et al. 2003; Panoulas et al. 2007; Rho et al. 2009; van Halm et al. 2006). Most of these studies, however, had limitations that hinder making definitive conclusions about MTX treatment and BP associations. The prime aim for most of these studies was to examine the effect of MTX on CV morbidity (Ajeganova et al. 2013; Hansel et al. 2003; Panoulas et al. 2007; van Halm et al. 2006), or mortality (Ajeganova et al. 2013; Choi et al. 2002); however, the effect of MTX on BP in the RA patients was not independently studied. Indeed, in most of these published studies the prevalence of hypertension among MTX users was reported, instead of the actual values of the BP. Moreover, no information was given in these studies about the methods and protocols used for BP assessment; which might significantly affect BP measurement (Gabb et al. 2016). Additionally, hypertension was diagnosed based on BP reading taken at the clinic or home, but not by monitoring the BP for 24-h or by measuring central BP.

The study by van Halm et al. (2006) reported a significant higher percentage of hypertension among non-MTX users compared to MTX users (39% vs 12%, $p < 0.001$) (van Halm et al. 2006). This study used a case-control design, which might be at risk of selection bias where more severe cases of RA, who were more likely to be on aggressive DMARDs regimen, were recruited. Another possible type of bias might be related to confounding by indication. The selection of severe cases of RA with high inflammatory burden in this study was indicated by the presence of RA factors and radiographic joints erosions. These markers were associated with higher CV risk. Similarly, the cross-sectional study by Panoulas et al. (2007) reported the prevalence of hypertension in RA. While the main finding of Panoulas and colleagues was high prevalence of hypertension in the RA population, RA patients using MTX drugs showed lower percentage of hypertension compared to other medications (60% vs 87%). In fact, this observed reduction in the prevalence of hypertension might be underestimated as 31% of RA patients on MTX were using prednisolone, which might be associated with slight increase in the BP (Goodwin & Geller 2012). Moreover, this study reported that the majority of the RA patients were on angiotensin receptor blockers and calcium channel blockers. Both antihypertensive drugs (Erne et al. 1984) and prednisolone (Reid et al. 1982) are known to reduce the level of cellular Ca^{+2}. This reduction in intracellular Ca^{+2} leads to vasorelaxation and subsequent fall in

the BP; however, the RA patients taking these medications were found to have uncontrolled hypertension. This indicates that there is another mechanism of the reported lower percentage of high BP in patients taking MTX. It is possible that MTX exerts a direct effect on BP. Although the cohort study by Choi and colleagues (2002) reported a lower percentage of hypertension among MTX users compared to non-users (13% vs 15%), this result might be underestimated. This is because RA patients were commenced on prednisone before visiting the rheumatology clinic and some of them were using prednisone intermittently, which might have affected BP per se.

Another possible bias threatening the validity of these studies is indication bias from the pattern of using MTX drugs; where the choice of given MTX drug is subconsciously related to the outcome of interest. Another important factor influencing the effect of MTX is the duration of using MTX. This is because the true effect of MTX is not reflected by the current use of the drug. Indeed, MTX duration was not considered in all of these observational studies. In addition to not reporting the duration of using MTX, MTX was given in different doses (Choi et al. 2002, van Halm et al. 2006). In fact, the study by Choi et al. (2002) censored any patient receving a MTX dose of ≥20 mg per week. High doses of MTX were associated with lower risk of CVD (Kisiel et al. 2015). The latter case-control study found that RA patients receiving a dose of ≥ 20 mg of MTX per week reported a lower risk of atherosclerosis measured by carotid and femoral IMT. Therefore, published studies considering low dose of MTX might underestimate the CV beneficial effect of MTX.

In contrast to previous studies, the cross-sectional study by Rho et al. (2009b) examined the effect of different DMARDs on the level of BP. Although the major finding of this study pointed to the CV beneficial effect of antimalarial drugs, the effect of MTX on BP might be overlooked for several reasons. Among RA patients using MTX, there was about 7 mmHg and 4 mmHg reduction in the mean SBP and DBP, respectively; almost three-quarters of the RA population were on MTX. In addition, the study lacks any statistical adjustment for potential confounders. This could explain the absence of association between the exposure to different DMARDs, including MTX and BP. Another possible explanation might be related to the BP recording technique. While the medical assessment of RA patients was described in detail (Chung et al. 2005), there was no information about the method used to measure the BP. Furthermore, no published studies assessed BP by 24-h monitoring, which is more accurate in diagnosing hypertension and predicting CV outcomes than clinic BP; especially in patients who are at

higher risk of developing CVD as it is able to examine the pattern of nocturnal BP (Boggia et al. 2007; Ohkubo et al. 2002; Verdecchia et al. 1994).

The finding of lower BP in MTX patients shed new light on the impact of MTX in lowering BP in the RA population. Researchers have long assumed that biologics have stronger effects on lowering CV risk, including high BP, compared to MTX (Solomon et al. 2013). Although the sample size of the cohort study by Solomon et al. (2013) was large, it was exposed to several sources of biases. First, the protective effect of TNF-α blocker was overestimated due to depletion of susceptible bias. Very severe cases of RA are more vulnerable to developing CV complications and to receive TNF-α blockers (del Rincon et al. 2005). The severity of RA was not controlled in the analysis. Another source of selection bias in that cohort is exposure misclassification bias. During the first 6 months of the study, about 1 in 10 patients were switched to one of the DMARDs. This change in the treatment plan exposed the study to participant's being misclassified into an incorrect cohort. Thus, it is possible that the observed lower BP was due to the effect of MTX. Additionally, it is possible that the effect of MTX was confounded by the use of other medications known to elevate the BP. While a randomised double-blind placebo-controlled trial found that combining MTX with glucocorticoid or cyclosporine showed better outcomes (e.g. rapid control to disease activity) compared to MTX monotherapy, RA patients on the MTX monotherapy showed lower BP after one year compared to those on combination therapy (Hetland et al. 2006). Indeed, the percentage of those with high BP (>140/90 mmHg) in the MTX monotherapy group was half that reported for the combined therapy, despite comparable BP at baseline (130/80 mmHg). The actual effect of MTX in lowering the BP was underestimated as both glucocorticoid and cyclosporine are known to increase the BP (Goodwin & Geller 2012); thus, exposure to those treatments might have biased the effect of MTX toward the null. Furthermore, it might take up to 6 months after the initial dose for the maximum anti-inflammatory and immunosuppressive effects of MTX to occur (Kremer & Lee 1986; Weinblatt et al. 1988); the date of commencing MTX in the study by Hetland et al. (2006) was not reported. This might explain the reduction in BP with time. Similarly, a double-blind control trial reported that BP was lower among RA patients using MTX alone compared to combination therapies (van der Heijde et al. 2006). Although the mean BP was not reported in the study, the percentage of developing hypertension over 2 years was the lowest among MTX monotherapy (5%); compared to MTX monotherapy, MTX and etanercept combination therapy and etanercept monotherapy showed a significant higher percentage of hypertension (9% and 13%, $p < 0.01$), respectively. Moreover, the AMBITION study found that

tocilizumab monotherapy was superior to MTX monotherapy in terms of improving the signs and symptoms of RA; however, the percentage of hypertension among RA patients using tocilizumab monotherapy was more than twice the percentage of those on MTX alone (5.6% vs 2.1%) (Jones et al. 2010). This reduction in BP could be explained by prior exposure to MTX even before commencing the study. Most of these studies showed the effect of MTX on the BP indirectly by reporting the prevalence of hypertension as one of the baseline or follow-up clinical characteristics.

In this current study, there was also a significant reduction of about 7 mmHg in central SBP among MTX users versus non-users. In the repeated cross-sectional study, the 24-h central BP increased in those not using MTX while those using MTX showed lower BP. To our knowledge, this study is the first to explore the positive effect of MTX on central BP in the RA population.

One possible explanation of the lack of published studies about the effect of MTX on central BP is publication bias (Siddiqi 2011). In this type of bias, the beneficial effects of MTX on central BP might be overlooked for several reasons; first, MTX is an old drug introduced in 1950. Conversely, new biologics were introduced recently and attracted most clinical researchers to compare the effects of different biologic agents on the central BP and markers of arterial stiffness (Kume et al. 2011; Maki-Petaja et al. 2012). This draws the attention away from the effect of MTX monotherapy. Second, scientific studies failed to report positive results on reduced central BP among MTX users. These null results give the researchers a false impression about the favourable effect of MTX, which subsequently influences their clinical decisions. Finally, most of the published studies compared the effect of different types of biologics to DMARDs; where the effect of MTX was underestimated due to the modification of other drugs. For example, one study compared the effect of anti-TNFα and DMARDs on central BP and arterial stiffness parameters (Maki-Petaja et al. 2012). The main finding was pointing to the beneficial effect of anti-TNFα; however, interpretation of the result should be done with caution. All RA patients in the DMARDs group were also taking steroids. Indeed, MTX was the most studied DMARDs, which was combined with prednisolone in most subjects. As prednisolone is known to increase BP, the true effect of MTX cannot be isolated.

The finding of a significant greater reduction in 24-h peripheral and central BP for MTX users emphasises the potential CV protective effects of MTX in the RA population. In the cross-sectional study, the mean ambulatory peripheral and central BP were significantly reduced among MTX RA patients (about 7 mmHg reduction). This reduction was sustained among MTX

users during the second visit. The association between MTX treatment and lower 24-h BP was driven by significant daytime, but not night-time, differences in these parameters.

Until recently, no study has investigated the relationship between MTX use and 24-h BP in the RA population. One case-control study analysed the diurnal BP variability according to exposure to various medications: NSAIDs, prednisone and MTX (Rihacek et al. 2009). It included RA patients who were hypertensive and newly diagnosed with high BP. While RA patients on NSAIDs (38%) and prednisone (37%) were found to be non-dippers, especially for SBP, 47% of RA patients using MTX were excessive dippers for SBP. Patients showing a dipping pattern have lower systemic vascular resistance and higher vascular compliance versus non-dippers (Cavelaars et al. 2004). As the effect of MTX was studied among hypertensive RA patients, it is possible that the actual protective impact of MTX was underestimated. Unfortunately, information about controlling for using antihypertension medications was not available as the paper was published in language other than English. Although hypertension is associated with high cardiac afterload due to the increase in systemic vascular resistance and the decrease in arterial compliance (Chemla et al. 2003; Segers, Stergiopulos & Westerhof 2000), the finding by Rihacek and colleagues (2000) indicates that MTX exerts a positive effect on CV risk, as indicated by lower ambulatory SBP.

Compared to all the published literature about the association between MTX and BP, this study examined the effect of MTX on BP in a repeated cross-sectional study. The approach of selecting RA patients was based on identifying a single cohort of RA patients where the unexposed cohort was an internal comparison group, and then followed them over time. As the unexposed cohort was a natural comparison group, subjects in both exposed and unexposed cohorts were more likely to be comparable in terms of clinical and demographic characteristics. In addition, the repeated cross-sectional design allows obtaining precise estimates due to its larger sample size. More importantly, this study assessed not only clinic, but also central and ambulatory BP. The effect of MTX dose and its intracellular concentration on BP was then examined. Moreover, several potential confounders were adjusted in the regression models.

4.2.2 Arterial function

4.2.2.1 AIx

No study has previously investigated the direct effect of MTX monotherapy on AIx. Although in this study there were no significant between-group differences in AIx, the mean AIx was lower in RA patients on MTX therapy. Although determinants of AIx in physiological and

pathophysiological states have not been fully identified, there is evidence that the cardiac and arterial factors might determine AIx (Avolio, Butlin & Xu 2012; Davies et al. 2010; Sakurai et al. 2007). Cardiac blood flow, cardiac contractility, arterial compliance and resistance are common examples. It is therefore possible that some of these factors might have counterbalanced the MTX-mediated effects of BP on AIx. Once again, the lack of studies about the relationship between MTX and AIx could be explained by publication bias (Siddiqi 2011); where more attention was given to the effect of the newer class of DMARDs (biologics) instead of MTX (Cypiene et al. 2007; Kume et al. 2011; Maki-Petaja et al. 2012). Further research is warranted to investigate the possible effects of MTX on AIx in RA patients and other study populations.

4.2.2.2 PWV

In this study, PWV was assessed using the Mobil-O-Graph. PWV was significantly lower in MTX users. The association between MTX treatment and lower 24-h PWV was driven by significant daytime, but not night-time, differences in this parameter. Although the PWV was slightly lower in MTX RA patients compared to non-MTX (9 m/s vs 9.3 m/s), it is a novel finding, as there is no study to date that has examined such a relationship.

Similar to this finding, there is good evidence showing that anti-TNFα treatment is associated with better PWV in RA both in short- (Angel et al. 2010; Cypiene et al. 2007; Maki-Petaja et al. 2012; Provan et al. 2015) and long-term studies (Wong et al. 2009). The unavailability of studies examining the association between MTX and PWV might be explained by several reasons. Most of the published studies focused on new DMARDs such as anti-TNFα, leading to publication bias. Additionally, patients' medications, including DMARDs, have not been considered in the analyses when evaluating the risk of arterial stiffness by measuring PWV in the RA population (Kocabay, Hasdemir & Yildiz 2012; Li et al. 2013); thus, the effect of MTX might be overlooked. Another possible explanation for the lack of scientific evidence is related to the recent introduction of non-invasive methods for assessing PWV and the relatively high cost of available applanation tonometry methods. The gold standard method is to assess the shape and velocity of pressure waves via inserting a catheter in the aorta during cardiac surgery (Tian & Chesler 2012); however, this was replaced by the non-invasive applanation tonometry, such as SphygmoCor, in last century. This technique is new; it is possible that researchers were more eager to discover the effect of biologics. Moreover, the high cost of this machine and the high skill required to use it deterred researchers from examining the effect of an old DMARD such as MTX on PWV.

The finding of better PWV among MTX users might be related to the ADMA plasma concentrations. RA patients treated with MTX in this study showed a trend towards lower concentrations of plasma ADMA, which is an endogenous NO synthase inhibitor and marker of endothelial function and cardiovascular risk. The observed improvement in the PWV among the MTX group might be influenced by the reduction in the ADMA concentrations. While there is inconsistent evidence of the relationship between ADMA level and endothelial dysfunction among RA patients (Sandoo et al. 2012), there is good evidence showing that ADMA is a surrogate marker for endothelial dysfunction predisposing to higher CV risk and arterial stiffness (Di Franco et al. 2012; Dimitroulas, Sandoo & Kitas 2012). In fact, high plasma ADMA concentration has been linked to arterial stiffness in the RA population (Di Franco et al. 2012; Dimitroulas, Sandoo & Kitas 2012; Sandoo et al. 2011). Recently, the sensitivity and specificity of ADMA in detecting endothelial function in RA has been examined. ADMA is found comparable to RF in detecting the endothelial function in untreated rheumatoid patients (Spasovski et al. 2013). Sensitivity and specificity of ADMA is 57.14% and 88.57%, respectively. Interestingly, similar sensitivity (48.57%) and specificity (91.42%) are recorded for RF.

4.2.3 Cardiovascular disease in rheumatoid arthritis

4.2.3.1 Methotrexate and traditional cardiovascular risk factors in rheumatoid arthritis

Although there were inconsistencies in the literature reporting the impact of traditional CV risk factors in RA patients on CV morbidity, our meta-analysis provided evidence for a significant negative impact of hypertension, T2D, smoking, hypercholesterolaemia and obesity in this population, with the magnitude of effects similar to that for the general population (Baghdadi et al. 2015). In the cross-sectional study, the prevalence of T2D, dyslipidaemia and obesity was higher among RA patients not using MTX compared to the RA participants on MTX. Although the prevalence of these factors did not reach the statistical significance, the mean level of random plasma sugar and total cholesterol was significantly lower among RA patients taking MTX ($p = 0.004$ and $p = 0.009$, respectively). The relationship between MTX exposure and lower risk of developing T2D and hypercholesterolaemia mandates further exploration in future studies.

4.2.3.2 Methotrexate and previous cardiovascular disease

The lower frequency of previous CV events in MTX users is in accordance with previous CV studies (Kisiel et al. 2015; Mason & Libby 2015; Ogdie et al. 2015; Roubille et al. 2015b). Noticeably in this study, the combined frequency of previous CV events (MI, angina pectoris,

congestive heart failure, transient ischaemic attack and stroke), and DVT were significantly higher among RA patients in the non-MTX group compared with the MTX cohort (23% vs 1.8%, p = 0.002, and 13% vs 0%, p = 0.005, respectively). It has been found that patients with inflammatory diseases such as RA are more likely to develop CVD during earlier stages of the disease (Choi et al. 2013; Zoller et al. 2012). This is more likely to threaten the validity of the study if the induction time, which is the time between the onset of RA and the incidence of developing CVD, is short (Choi et al. 2014). This phenomenon is called differential depletion of susceptibility bias. In this study, however, this type of selection bias was avoided. A thorough examination of patients' medical records about the exact date of RA diagnosis, MTX commencement and previous CV events development was ensured. In addition, information was gathered from patients for further verification. The occurrence of the past CV events was after the diagnosis of RA and before commencing the patients on the initial dose of MTX. The mean duration of RA was at least 16 years for all RA participants, which indicates that the induction time is long enough for the CVD to develop. In fact, MTX seems to exert an independent effect in protecting RA patients from developing CV outcomes. In the meta-analysis published by our group (Baghdadi et al. 2015), the importance of MTX was emphasised by results obtained from the subgroup analysis; among studies assessing hypertensive RA patients treated with MTX alone, the risk of combined CV morbidity was slightly lower than all studies. Thus, MTX has a protective effect in this population compared to other DMARDs.

A recent study conducted in the Sub-Saharan Africans claimed that RA patients have no excess CV risk compared to the general population (Singwe-Ngandeu et al. 2016); however, this finding should be interpreted with caution. First, the study was conducted in a Sub-Saharan urban central African hospital. The results obtained from this study cannot be generalised to other populations as it was conducted in Africa where CV risk differs between white and black Africans with RA (Solomon et al. 2014). Second, the sample size was small (i.e. 50 RA patients). Additionally, information about CV events was based on recalling past personal history of CV events, which might cause differences in the accuracy of retrieving the information; this is a source of systematic error known as recall bias. Moreover, the study might be exposed to measurement bias. Measuring and analysing protocols and techniques for biochemistry blood tests might differ between laboratories as each laboratory has a different approach to analysing blood samples. Many potential confounders in the study by Singwe-Ngandeu et al. (2016) were not adjusted. For example, the majority of rheumatoid patients were

females (78%); women are usually at lower risk of CVD compared to men due to the protective effects of female sex hormones (Skafar et al. 1997). There is evidence that oestrogen has positive effects on arterial function (Teede 2007), and atherosclerosis (Vaidya et al. 2015). Additionally, the risk of CVD and arterial stiffness vary during different phases of the menstrual cycle. A longitudinal study found that AIx reduces during the luteal phase and starts to increase at the beginning of each menstrual cycle (Robb et al. 2009). Hormonal changes that occur during the luteal phases of the menstrual cycle might confound the lower CV risk reported by Singwe-Ngandeu et al. (2016).

This current study found that the prevalence of T2D, dyslipidaemia, and obesity were lower in RA patients on MTX compared to non-MTX group. Additionally, the mean level of random blood sugar and total cholesterol were significantly lower among RA patients taking MTX (4.9±0.79 vs 5.6±1.5 mmol/L, p = 0.004) and (4.9±0.98 vs 5.6±1.3 mmol/L, p = 0.009), respectively. These findings agree with the results obtained by the meta-analysis published by our group showing that the risk of combined CV morbidity was lower among hypertensive patients using MTX (RR 2.18, 95% CI 0.50, 3.86). Furthermore, the AMBITION study comparing the use of MTX and tocilizumab monotherapy in an RA population found that dyslipidaemia was more common among non-MTX users; the level of total cholesterol, and low-density lipoprotein were elevated in RA patients on tocilizumab monotherapy compared to MTX monotherapy (13.2% vs 0.4%) and (3.1% vs 0%), respectively (Jones et al. 2010). The study by Singwe-Ngandeu et al. (2016) used the WHO risk charts for the African region to calculate the CV risk. The risk was lower in RA patients compared with non-RA patients (78.8% vs 83.3%). This finding might be explained by using MTX as half of the RA population were on MTX; in fact, the risk of dyslipidaemia was lower in the RA population (OR 0.7, 95% CI 0.32, 1.54).

4.2.4 Methotrexate dose and intracellular concentration

To the best of our knowledge, this study is the first to examine the effect of MTX dose and RBCs' MTXPGs concentrations on BP and arterial function in RA. There was a highly positive correlation between MTX dose and MTXPGs concentrations. There was, however, a weak negative correlation between MTX dose and the shortest chain MTXPGs (i.e. MTXPG1) which might be explained by the rate of MTXPGs clearance from the body; from a clinical perspective, about 6–8 weeks are needed to observe a disease response in RA patients after commencing MTX (Kremer & Lee 1986; Weinblatt et al. 1988). In fact, the maximum response is usually achieved after 6 months. This highlights the important role of long chains of MTXPGs in clinical

response; where longer chains usually take a longer time to reach steady state and be detected compared to shorter chains. Another explanation is related to the lifespan of RBCs. The lifespan of the RBCs in human is about 120 days. Once the MTX enter the RBCs, small proportion of it does not efflux but remains inside the RBCs for the whole lifespan (Dalrymple et al. 2008). It has been suggested that the persisted proportion of RBCs' MTXPGs consisted of the longest chains (i.e. $MTXPGs_{3-5}$). While the shortest MTXPGs chains ($MTXPG_1$ and $MTXPGs_2$) are initially eliminated, the longest chains are slowly eliminated based on the RBCs' lifespan. Another explanation of low MTXPGs concentration could be due to the dilution effect of releasing new RBCs into the circulation as these new RBCs have no MTXPGs inside them (Schroder, Fogh & Herlin 1988).

In contrast, the current study reported that there was an inverse relationship between the dose of MTX and treatment duration and RA activity. In clinical practice, the dose of MTX is usually increased if RA is active or if MTX is taken for a long duration. Therefore, adjusting for RA activity and MTX duration was considered when assessing the association between exposure to MTX and BP and arterial function. After controlling for biological, pharmacological, biochemical and medical confounders, this study found that both clinic and central SBP were negatively correlated to the MTXPGs concentration ($\beta = -0.02$ and -0.03, respectively). Additionally, both AIx and PWV were negatively correlated with the MTXPGs concentration; however, these relationships did reach statistical significance.

The finding of an association between MTX treatment and lower BP values is supported by the work of Choi and colleagues (2013), who reported in a conference abstract that RA patients receiving high doses of MTX have a lower prevalence of hypertension ($p < 0.01$). This finding might have been explained by the positive effects of high-dose MTX on intracellular MTXPGs concentrations; however, these patients were relatively young, with particularly aggressive RA. Unfortunately, the lack of additional study details, due to the abstract format of the report, curtail data interpretation. There are no other published studies examining the relationship between the dose of MTX and BP or AIx or PWV among the RA population. In fact, most published evidence was investigating the effect of MTX dose on atherosclerosis and the findings were contradictory. While few studies showed no such relationship (Giles et al. 2011; Greenberg et al. 2011), a recent case-control study reported that weekly high dose of MTX (≥20 mg) had a beneficial effect on the development of atherosclerotic plaques in RA patients compared to controls (Kisiel et al. 2015). Another case-control study reported that MTX showed a beneficial effect on carotid IMT compared to other DMARDs (0.64 ± 0.14 mm vs 0.77 ± 0.23 mm, $p < 0.05$)

(Kim et al. 2015). The MTX effect was negatively correlated with its dosage (β = -0.03, $p < 0.01$). This discrepancy in the reported evidence might be related to using different MTX doses as the prescribed dose of MTX in RA has increased from 5 mg to 30 mg per week in the last 30 years (Kisiel et al. 2015). In the study by Giles et al. (2011), for example, recruiting RA patients was conducted between 2004 and 2006. In contrast, the study by Greenberg et al. (2011), which included a large data from the consortium of rheumatology researchers of North America (CORRONA) registry, recruited patients between 2001 and 2006. Additionally, there was no reported information about the dose of MTX and regularity of treatment in these studies, which might affect the actual relationship between MTX and atherosclerosis.

4.3 Pathophysiological explanations

4.3.1 Vascular tone regulators

The finding of lower BP and improvement in the marker of arterial stiffness (PWV) in the RA population using MTX might be explained by several mechanisms. One of the proposed mechanisms of MTX in lowering the BP and improving the arterial function in RA patients is via increase the availability of NO in the endothelial cells (Figure 4-1). As soon as MTX enters the cell, it is converted from inactive mono-glutamate form to the biologically active polyglutamate form (i.e. MTXPGs) by the FPGS enzyme. Polymorphism in the gene *ABCG2* is associated with higher concentrations of MTXPGS (this will be discussed later). MTXPGs inhibit aminoimidazole carboxamide ribonucleotide transformylase (ATIC) (Cutolo et al. 2001). This inhibition leads to the accumulation of the substrate aminoimidazole carboxamide ribonucleotide (AICAR) and its metabolites; consequently, this accumulation inhibits adenosine deaminase and adenosine monophosphate deaminase (Chan & Cronstein 2013). As a result of reducing the catabolism of adenosine and its metabolites, its concentration is increased both directly and indirectly from AMP dephosphorylation. Although the main function of adenosine is tissue protection, there is good evidence showing that adenosine has a simultaneous action on the peripheral vascular system and central nervous system to reduce the BP (Koupenova, Johnston-Cox & Ravid 2012). It has a direct effect on blood vessels by causing vasodilation or via increasing the level of NO (Li et al. 1998). Adenosine acts on the vascular endothelium via its receptors A1 and A2A and stimulates the release of PG, which enhances the release of NO. Concurrently, adenosine acts centrally on the brain nucleus tractus solitarii (NTS), where adenosine activates eNOS through A2A receptors. As a result, the level of NO is increased and thus, adenosine has a key role in modulating the BP and arterial function. There is good evidence supporting this finding

which showed that pharmacological inhibition of the adenosine receptors was associated with an increase in the BP (Daniels et al. 1998; Smits et al. 1987), and markers of arterial function especially PWV (Mahmud & Feely 2001).

Second, RA is a chronic disease associated with shear stress (Sandoo et al. 2010), which is a potent pulling friction force applied on the wall of the blood vessels by the blood flow in the arterial lumen. This type of stress stimulates arterial dilation (Davies 2009) via 'capacitative Ca^{2+} entry'. In the endothelial cells, this shear stress stimulates the Ca^{+2} to diffuse inside the endothelial cells increasing its concentration. This process initiates eNOS phosphorylation where $_{L}$-arginine is converted into NO, as a result, the level of NO is increased; however, this study suggests that MTX exerts a direct beneficial effect on vascular endothelium. The laminar shear stress associated with longstanding RA was suggested to activate AMP-activated kinase (AMPK) (Fisslthaler & Fleming 2009), which contributes to vascular protection. AMPK is a universal signalling kinase which plays an important role in endothelial NO synthesis (Morrow et al. 2003). A recent study showed that MTX directly activates the AMPK leading to more synthesis of endothelial NO (Thornton et al. 2016). In fact, the same study suggested that the MTXPGs concentration might mediate the activation of AMPK. While this finding provides a new mechanistic explanation of the reduced CV risk in MTX users, the exact mechanism of activating AMPK is still unknown.

The increased level of NO has the ability to relax smooth muscle cells in conductance arteries and consequently, the force exerted on the vessels' walls (i.e. shear stress) is reduced. Subsequently, this leads to a reduction in the peripheral vascular resistance, which reduces the wave reflection (Wilkinson et al. 2002). This also leads to a reduction in arterial stiffness and pulse pressure and thus BP is reduced. Along with our explanations, other authors have hypothesised that enhancing the production of NO among patients with heart failure not only increases the vasodilation of blood vessels, but also maintains the vessels' blood flow (Irace et al. 2004). The generated hypothesis by Irace and colleagues (2004) was based on two published studies showing that TNF-α inhibition might stimulate the synthesis and release of NO from endothelial cells among patients with heart failure (Fichtlscherer et al. 2001; Hurlimann et al. 2002). Indeed, adding MTX to TNF-α inhibitors (infliximab) synergises the response to the biologic agent in patients with RA as MTX reduces the development of anti-infliximab antibodies (Ruderman 2013). In fact, a randomised control trial showed a relatively low prevalence of hypertension (3%) in patients treated with TNF-α inhibitors and MTX (Maini et al. 1998). It is possible that the reduced prevalence of hypertension is due to the effect of MTX and the

concomitant increase in NO; time-dependent bias might be the explanation for that result. This type of bias is uncommon in randomised control trials as treatments for study groups are usually started together sometime after the start of the trial (Beyersmann et al. 2008). This was not the case in the trial by Maini et al. (1998); half of the cases were commenced on MTX for ≥3 years; however, infliximab exposure occurred sometime after the start of the study. This difference in commencing treatments misclassifies time of developing hypertension; where the effect of MTX was started prior to the initiation of the study.

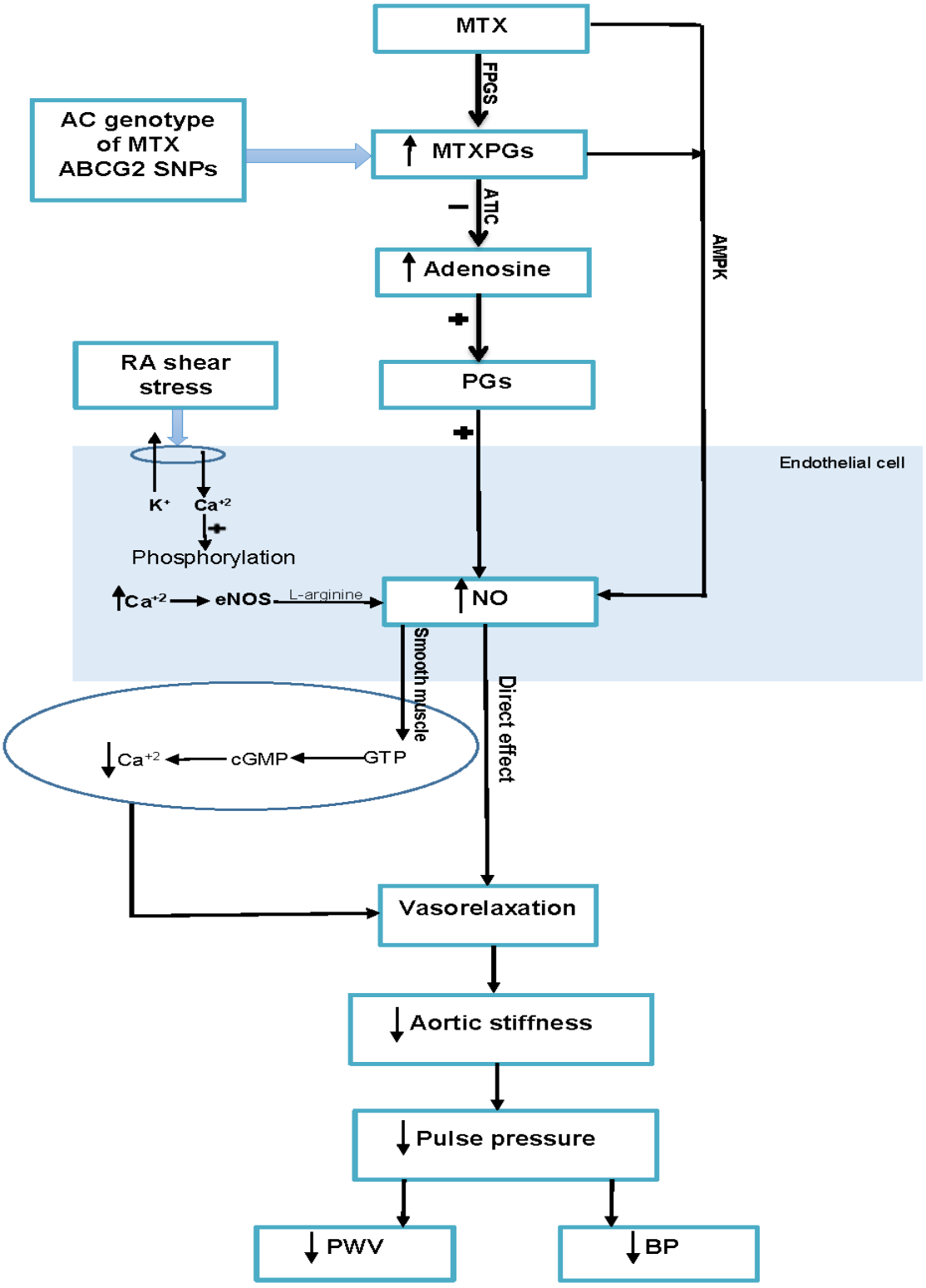


Figure 4-1 Potential mechanisms explaining the effects of methotrexate on blood pressure and arterial function in rheumatoid arthritis

4.3.2 Inflammation and folic acid

RA is a chronic inflammatory condition characterised by an activation of several cytokines and inflammatory cells such as T cells, TNF-α, IL-6, and IL-18; this inhibits the function of NO. As RA is associated with increased oxidant stress, it stimulates NADPH oxidase which then increases the production of vascular reactive oxygen species (ROS) (Griendling, Sorescu & Ushio-Fukai 2000; Lassegue & Clempus 2003). This increased level of ROS causes eNOS uncoupling, where the production of NO is reduced (Haruna et al. 2006). This reduction in NO leads to vasoconstriction and consequent endothelial dysfunction. Many mechanisms of MTX in reducing the CV morbidity and mortality have been postulated (Choi et al. 2002; Prasad et al. 2015; Wessels, Huizinga & Guchelaar 2008). One of these mechanisms was related to reduced inflammation (Choi et al. 2002); however, other mechanisms might be involved. MTX is an anti-inflammatory drug acting on the folate pathway and it is associated with lower concentrations of folic acid. This is because MTX is an anti-folate drug inhibiting DHFR enzyme, which reduces folate production. Folic acid has been found to reduce the development of CV outcomes such as stroke (Wang et al. 2007; Yang et al. 2012). Folic acid was also found to reduce BP and improve endothelial function in other populations such as diabetics (Mangoni et al. 2005), smokers (Mangoni et al. 2002), and patients with chronic heart failure (Paul et al. 2010). In this current study, the association between MTX, BP and arterial function was independent of inflammation, disease activity and exposure to folic acid. Notably, CRP concentrations were similar in MTX and non-MTX patients; furthermore, there were no significant correlations between CRP and SPB in both the MTX and the non-MTX groups ($p = 0.60$ and 0.24, respectively).

There is good evidence showing that the use of folic acid reduces the BP and improve arterial function in patients with T2D (Mangoni et al. 2005), in patients with chronic heart failure (Paul et al. 2010), and in smokers (Mangoni et al. 2002). Although the prevalence of consuming folic acid supplement in this study was significantly higher among MTX users compared with non-users ($p < 0.001$), its use was not independently associated with BP or markers of arterial function. The structural similarities, especially in pteridine nucleus, between MTX, active forms of folic acid and tetrahydrobiopterin (BH_4) might explain the mediating effect of MTX on BP and arterial function in RA. First, BH_4 is a critical cofactor for eNOS, which catalyses the electronic transfer from NADPH to $_L$-arginine to generate NO and citrulline (Hyndman et al. 2002). Second, the structure of the active form folic acid, which is known as 5-methyltetrahydrofolate (5-MTHF), is similar to the BH_4 (Hyndman et al. 2002). 5-MTHF also has the ability to attach to the active

site of the eNOS enzyme mimicking the presentation and the interactions of the BH_4 cofactor (Hyndman et al. 2002). Therefore, eNOS-mediated NO synthesis can be restored by 5-MTHF, which has been found to improve endothelial function in inflammatory diseases, such as RA that is associated with high oxidative stress (Haruna et al. 2006; Hyndman et al. 2002). This increase in NO synthesis might reduce the BP and improve the arterial function in RA (Figure 4-1), however, similar effect of the MTX on the eNOS enzyme is still to be explored.

4.3.3 Disease activity

In this study, RA activity was reduced among MTX users. The mean activity score measured by DAS28 was significantly reduced in the MTX group (2.7±1.1 vs 3.7±1.2, $p < 0.001$); after considering main predictors of disease activity (i.e. age, HAQ and rheumatoid factor), there was a significant negative relationship between the exposure to MTX and the DAS28 ($\beta = -0.76$, 95% CI -1.2, -0.35, $p < 0.001$), and this was significantly related to MTX dose ($p = 0.03$). The finding of reduced RA activity among MTX compared to non-MTX patients agrees with many published studies (Arts et al. 2014; Ma et al. 2010; Verstappen et al. 2007). These studies showed that better remission of RA is achieved by using MTX, especially if it was commenced at the early stage of RA. Few authors argue that the RA duration might increase the CV risk as a result of cumulative inflammation associated with longer disease duration. There is evidence, however, that it is the RA activity, but not RA duration, which influences the risk of CVD (Arts et al. 2014). High disease activity (i.e. DAS28 >5.1) was associated with the lowest CVD survival rate. Additionally, it showed that the risk of developing CVD is lower among RA patients who have ever used MTX independent of disease duration (HR = -0.34, $p = 0.07$). The finding from the study by Arts et al. (2014) is reliable as it had a large sample size and a long duration of follow-up (about 25 years), which was long enough for the CVD to develop. This finding is consistent with the finding of lower BP and PWV among MTX users despite their RA duration. Furthermore, the current study found that there was an inverse relationship between the dose of MTX and the duration of taking this drug, as well as the RA activity. One of the explanations is related to the disease duration and activity, where the dose tends to get up-titrated if disease is active or if MTX is taken for a longer duration (Hernandez-Baldizon 2012). Although this study reported statistically significant inverse relationship between MTX dose and RA activity, no relationship was documented between MTXPGs concentration and the disease's activity. While this finding is in accordance with the result reported by Stamp et al. (2010), a recent published work by the pharmacology group at the University of South Australia identified some evidence suggesting that the concentration of MTXPGs inside RBCs is associated with reduced RA

disease activity (Mohamed et al. 2015). The finding of lower BP and PWV in the MTX group was significant after considering the RA duration and activity.

4.3.4 Arginine metabolites

There remains inconsistent evidence of the relationship between the level of plasma ADMA and BP/arterial function in the RA population. Moreover, there is no evidence of the CV impact of MTX on the RA patients through the ADMA pathway. In this cross-section study the mean concentration of plasma ADMA was significantly lower in the MTX group (a difference of 0.19 µmol/L). There was a trend towards negative association between the level of ADMA and SBP measured at the clinic and over 24-h. Interestingly, the level of ADMA was positively correlated with BP and the markers of arterial function among RA patients not using MTX.

The difference in ADMA level between MTX groups is likely to be biologically and clinically relevant because smaller differences in ADMA concentrations (0.12 µmol/L) between healthy controls and patients with chronic heart failure hav been previously reported (Paul et al. 2010). The reduction in ADMA concentration in the MTX group could be explained by DDAH enzyme overexpression or increased activity (Figure 4-2). This enzyme metabolises ADMA into citrulline and dimethylamine by hydrolysing methylated arginines (Wadham & Mangoni 2009). Thus, the activity of DDAH modulates the intracellular concentration of ADMA. For example, high activity or overexpression of DDAH leads to increased ADMA metabolism and subsequent reduction in its concentration; as a result, eNOS is stimulated to synthesise NO, which improves endothelial function. Therefore, DDAH has been considered as a strong pathophysiological regulator of ADMA level and its associated vascular damage (Tommasi et al. 2015; Wadham & Mangoni 2009). The activity of DDAH can be influenced by the level of ROS; more specifically, its activity is inversely related to the level of ROS (Palm et al. 2007; Wadham & Mangoni 2009). RA is an inflammatory condition associated with high infiltration of inflammatory cytokines (Dayer & Demczuk 1984); of all cytokines, IL-6 has been found in high concentration in the synoviocytes and linked to RA pathogenesis (Sung et al. 2000). Indeed, the study by Sung and colleagues (2000) was the first to report that ROS is induced by IL-6 in the RA patients. Additionally, the same study reported a protective effect of low-dose MTX on the ROS. Even though MTX has been found to induce oxidative stress (Herman, Zurgil & Deutsch 2005), Sung and colleagues (2000) found that MTX (1 µg/ml of MTX) suppresses the IL-6 induced production of ROS. This inhibition in the ROS generation might increase the activity of DDAH and the subsequent decrease in the ADMA concentration. As ADMA is an endogenous competitive inhibitor of NOS,

the reduction in the ADMA concentration leads to NO synthesis via endothelial NOS (i.e. L-arginine-NO pathway). Consequently, NO-associated vasodilation reduces the BP and improves the endothelial function (Figure 4-2). The mediating effect of MTX on the synthesis of ROS via increasing the activity of DDAH needs to be tested in further experimental studies.

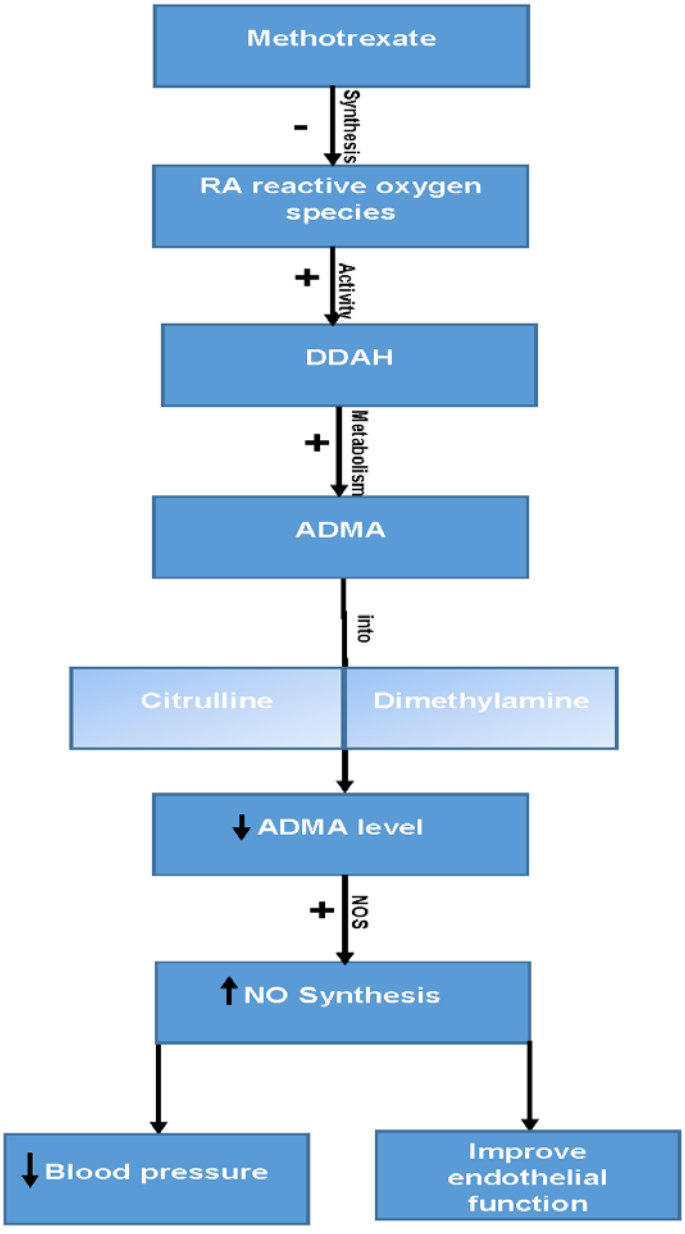


Figure 4-2 Proposed effects of methotrexate on dimethylarginine dimethylaminohydrolases enzyme and asymmetric dimethylarginine

DDAH = dimethylarginine dimethylaminohydrolases enzyme; NOS = endothelial NO synthase; NO = nitric oxide

4.4 Pharmacogenetics explanations

4.4.1.1 ABCG2 SNPs

In RA patients with the AC variant both peripheral and central SBP were significantly lower (about 12 mmHg reduction, p = 0.03 and 0.02, respectively). Similarly, compared to CC genotype, RA with AC allele showed a difference of approximately 5% in AIx and 2 m/s in PWV. Another important effect of AC genotype was related to intracellular MTXPGs concentration. This mutant allele was associated with higher intracellular accumulation of MTXPGs compared with normal homozygous gene (CC) (132.7 vs 115.8). Thus, the finding of lower BP and PWV among MTX users might be due, at least in part, to SNP of MTX ABCG2 (rs2231142). About 20% of the RA patients on MTX cohort tested positive for the mutant genes.

The availability of ABCG2 transporter is associated with reduced response to DMARDs (van der Heijden et al. 2009). Additionally, ABCG2 plays a role in eliminating MTX and its metabolites in the absence of ABCC2 (Vlaming et al. 2009). Most importantly, ABCG2 transports not only MTX, but also its plolyglutamates, specifically polyglutamate 2 and 3 (Volk & Schneider 2003). Although a few studies have claimed that this form of SNPs of ABCG2 had no impact on clinical response to MTX (Kato et al. 2012; Stamp et al. 2010), the work by Zhang et al. (2013) has proven otherwise. In the latter study, A homozygotes have reduced function of the ABCG2 transporter (i.e. reduce the efflux capability of MTX) (Zhang et al. 2013). This results in greater MTX accumulation and better MTX response (Warren et al. 2008). In agreement with the latter study, this study found that the mutant allele of ABCG2 (AC) is associated with higher intracellular accumulation of MTXPGs. The AC allele is associated with reduced function of the MTX transporter, hence greater MTXPGs accumulation. This would explain the greater concentration of MTXPGs, which reduces the BP and improves arterial function via NO pathway (Figure 4-1). This explains the observed BP lowering effect of MTX in the RA population.

4.5 Other possible explanations

4.5.1 Random error (chance)

The observational nature of these studies makes the findings susceptible to the effects of random error (i.e. chance). The effect of chance might be introduced from insufficient power of the study; due to recruiting a lower than anticipated number of the RA patients in the non-MTX cohort. This was inevitable as most patients were on combined DMARDs including MTX; however, the repeated cross-sectional study design allowed collecting repeated measures for

all outcomes on interest on the same RA population. This approach increased not only the power of the study, but also the precision of the MTX and CV outcomes association. Furthermore, the percentage of loss to follow-up in the MTX and non-MTX cohorts was low (7.1% and 6.7%, respectively). This may help in excluding the possibility of chance.

4.5.2 Confounding

The findings about BP and arterial function in this study might be partially or totally due to factors other than the exposure to MTX (confounding). The differences in BP and arterial function between MTX and non-MTX cohorts are, however,unlikely to be biased for at least three reasons: 1) potential clinical, demographic, pharmacological, nutritional and biochemical confounders were controlled in the regression models; 2) RA patients in the comparison groups were matched for the common confounders such as age, gender and clinical characteristics; and 3) the power of the study was increased by obtaining repeated measures on the same RA population which controls for confounding without affecting the precision of the estimate.

4.5.3 Medications

Medications used by the RA patients might influence the BP and arterial function. While some medications might decrease the BP—such as a few traditional DMARDs, vasoactive medications, anti-hypertensive drugs, folic acid supplements, and biologic DMARDs such as TNF-α inhibitors—others might increase the BP (NSAIDs, and corticosteroids). In this study, however, use of medications was accounted for in the regression models and the difference between exposure groups remained significant.

The use of tocilizumab, a biologic DMARD, was significantly more frequent in the non-MTX group compared to MTX group (23.3% vs 1.8%, $p = 0.001$). Previous studies showed that tocilizumab reduced PWV, but not AIx, when compared to treatment with other biologics (Kume et al. 2011; Provan et al. 2015). A controlled trial also showed that tocilizumab reduced AIx (Kume et al. 2011). Even though tocilizumab usage was adjusted in the regression model, its effect might be incompletely controlled leading to the so-called “residual confounding bias” (Lash & Fink 2003; Psaty et al. 1999). This bias might affect the estimate of the MTX-CV outcome association. The difference in the BP between MTX and non-MTX RA cohorts might be underestimated; as more patients in the non-MTX group were using tocilizumab the BP and markers of arterial function might be lower than the actual level. In addition to tocilizumab, sulfasalazine is another traditional DMARD which has been linked to reduce CV risk in the RA population (Choi et al. 2002; van Halm et al. 2006). Even though these researchers might argue

that sulfasalazine reduces CV risk in the RA population, the prevalence of sulfasalazine use among the non-MTX cohort of this study was double the percentage reported for MTX group (10.7% vs 20%, p = 0.24).

Corticosteroids, such as prednisolone, are another important drug class which might affect the CV risk. High doses of corticosteroids were found to predispose RA patients to atherosclerosis (del Rincon et al. 2004). This effect was inversely related to the time of exposure. While high doses of corticosteroids were associated with increased CV risk, the effect of low doses of this drug on CV risk in RA patients is still controversial (Conn 2001; Girod & Brotman 2004; Pieringer & Pichler 2011; Raynauld 1997; Saag 2001). Most epidemiological studies indicated that there was no association between low dose of corticosteroids and the CV risk might be confounded by indication bias (Wei & Walker 2004). In addition, most of these studies had a small sample size and the duration of the study was insufficient for CV events to develop (Capell et al. 2004; Svensson et al. 2005).

4.6 Impact of dietary intake

As diet is considered as inevitable universal exposure, manipulating the food intake of the RA patients might reduce inflammation, the risk of arterial stiffness and subsequent CVD occurrence. Until recently, there has been little evidence about the effect of different dietary nutrients on the CV risk among the RA population.

4.6.1 Fruit and vegetables

There is an emerging evidence showing that fruit and vegetables have positive effects on arterial function in the RA population (Crilly & McNeill 2012) similar to that found in the general population (He et al. 2007; He et al. 2006; Iqbal et al. 2008). In addition, a recent study showed that consumption of fruits and vegetables was inversely related to the risk of all-cause mortality among the Australian population (Nguyen et al. 2016). The cross-sectional study by Crilly and McNeill (2012) reported lower AIx among RA patients who were eating vegetables and fruits daily compared to those having a less than daily consumption; the mean difference in AIx was -3.2% (95% CI -6.4, -0.1, p = 0.05). One of the suggested explanations was that vegetables consumption improves arterial function via increasing the availability of NO. Vegetables, especially leafy vegetables, are enriched with nitrate (70% of nitrate dietary sources) (Crilly & McNeill 2012; Kapil, Webb & Ahluwalia 2010). This dietary nitrate is then converted into NO through ‘enterosalivary circulation’ leading to increased level of NO. As increased production of NO is associated with an improvement in the endothelial function, the arterial resistance in the

peripheral arteries is expected to reduce, which decreases the magnitude of the reflected wave. This increase in NO could be due to the exposure to MTX. Most RA patients reported to have lower AIx were currently taking DMARDs (75% vs 29%), but unfortunately, no information about specific DMARDs was reported in the study. Moreover, these patients showed lower BP (124.5/81.2 mmHg vs 126/83.3 mmHg). Although age was adjusted when the relationship between AIx and vegetables intake was examined, other potential confounders, mainly patients' medications, were not controlled. Although the association between MTX exposure and AIx might be confounded by the vegetables intake, the association between MTX and BP was significant after controlling for the fruit and vegetables consumption.

4.6.2 Beef meat and eggs intake

In this study, the prevalence of eating 1–2 eggs per week was significantly higher for MTX cohort ($p = 0.02$). The same RA cohort also showed a borderline significantly greater beef ingestion per week compared to non-MTX group ($p = 0.06$). Consuming a diet enriched with protein has shown an antihypertensive effect (Vasdev & Gill 2008); the Dietary Approaches to Stop Hypertension (DASH) diet is an example of protein-rich diet (Appel et al. 1997; Svetkey et al. 1999). It was suggested that the protein in such types of diet is a prime source of arginine, which has the ability to increase the level of NO and improve the endothelial function (Vasdev & Gill 2008). Thus, arginine in meat has been considered as an antihypertensive nutrient. Evidence about this effect among the RA population is lacking. A recent conference abstract of a study conducted in the Ukraine showed that arginine not only reduced the BP, but also improved endothelial function in the RA population (Sirenko, Kuryata & Lysunets 2015); however, there was no information about the magnitude of the BP reduction. This finding should be interpreted with caution as half of the RA patients, who were on arginine, were on standard treatment for RA. The standard treatment in the Ukraine is defined as taking MTX combined with folic acid for no less than 6 months (Smyrnova 2015). Indeed, it was reported in the abstract that the majority of RA patients were on DMARDs for an average duration of 4.5 years. Therefore, the reduced BP observed in the study by Sirenko et al. (2015) could be due to the use of MTX, where arginine might be a confounder. Unfortunately, the full paper was not available for thorough evaluation. In this study, BP remained significantly lower in the MTX cohort after adjusting for the protein intake.

4.7 Study strengths and limitations

4.7.1 Strengths

4.7.1.1 Cross-sectional study

A quantitative observational approach using cross-sectional design was conducted. In this study, 89 RA patients were recruited and followed over time. This stage took about 2 years to be completed. It is an appropriate sample size to have 80% power (p-value of 0.05) and to detect a significant difference of 10 mmHg in SBP between MTX and non-MTX groups. These patients were selected from the rheumatology outpatient clinics at FMC and RGH. The Rheumatology Unit is a suitable data source as high participation rate was expected and resulted in a better external validity. FMC is a major referral centre serving the southern suburbs, the northern parts of South Australia, interstate and regional areas. Being a major referral institution, FMC provides 350,000 outpatient consultations yearly. Rheumatology outpatient and inpatient services at FMC and the RGH are under one umbrella, which is the Rheumatology Unit. Weekly, there are approximately three new RA cases diagnosed by a rheumatologist in either location. Most patients with rheumatic joint pain are more likely to be referred by their local general practitioners to FMC or RGH. Thus, almost all new cases of RA are more likely to be initially diagnosed, managed and followed by a rheumatologist. As this unit is offering public and private medical services for most South Australian patients, our patients are representative of the Australian population (i.e. external validity). Another strength is that RA groups were comparable in terms of clinical and demographic characteristics; because both exposed and unexposed cohorts were driven from the same subgroup of the population. The non-MTX cohort was used as the natural comparison group “an internal comparison group”. This way of selecting a comparison group reduces the possibility of selection bias as the outcomes for both groups were unknown at the time of recruitment.

4.7.1.2 Repeated cross-sectional study

Repeated cross-sectional design has an advantage in obtaining precise estimates due to having a larger sample size. Moreover, this design is suitable for the project because the changes in trends of the outcomes (BP, AIx and PWV) were observed over time. Using this study design enabled us to examine the effect of time on the outcomes. The same investigator that collected the data at baseline was also responsible for the data collection at follow-up. Moreover, the interview and medical examinations were conducted at the same sitting using the same instruments and techniques. This approach reduces the chance of measurement bias. Additionally, the possibility of observer bias was considered; data were collected, entered and

analysed by a single investigator. To have accurate results, statistical tests and analyses were double-checked by an expert biostatistician. Regardless of missing data, mixed-effect modelling used in the follow-up analysis enabled us to use and examine all variables, and this model does not need the assumption of sphericity (the variances of the differences between independent variables are equal). More importantly, the rate of loss to follow-up in this study was not serious (about 7%). This indicates that bias threatening the internal validity is minimal.

4.7.1.3 Ambulatory blood pressure monitoring

In addition to the previous strengths, this study is the first to monitor the BP, AIx and PWV over 24-h in the RA population. It is considered a better prognostic tool in detecting CV risk, which is associated with elevated nocturnal BP (Boggia et al. 2007). Another advantage of monitoring the BP is to eliminate white coat syndrome and to detect BP variabilities during daily activity and RA progression, especially with increasing the dose of MTX or switching to other DMARDs. This study used the Mobil-O-Graph monitor to record peripheral and central BP, and markers of arterial function for 24-h. It is clinically validated and easy to use. One of the advantages of using this monitor is that it records both brachial BP and brachial pressure waveforms and then transforms these waveforms to aortic BP. Studies have shown that the effectiveness of Mobil-O-Graph is comparable to SphygmoCor (Weiss et al. 2012) indicating that both monitors are able to measure central BP efficiently. Moreover, it gives reliable BP readings independent of the users and provides high accuracy in repeated measurements. To our knowledge, this is the first study using Mobil-O-Graph to examine the effect of MTX on BP and arterial function in the RA population.

4.7.1.4 Methotrexate duration and concentration

Most previous studies have reported the current use of MTX without reporting the actual duration of use (Choi et al. 2002; Kisiel et al. 2015), however, this way of examining the effect of MTX does not reflect its true effect. This is because the duration of using MTX is more important for several reasons; first, about 6–8 weeks is needed to initiate the therapeutic response and 6 months is required to reach the maximum effect. Moreover, the duration of MTX is significant in RA, especially if the disease is active. Usually, the dose tends to get up-titrated if the disease is active or if MTX is taken for longer duration. Compared to other observational studies, this study adjusted for the duration of using MTX and for the MTXPGs concentration. Moreover, several factors that might affect the MTXPGs concentration such as increased age, higher dose and longer duration of using MTX and using prednisolone were controlled in this study (Stamp et al. 2009).

4.7.1.5 Pharmacogenetics of methotrexate

One of the important strengths of this study is that it involved data about MTX transporter polymorphisms. Genetic variations in the ABCG2 transporter were examined. This gave us an advantage to understand the pharmacogenetics of MTX and its relation to the observed lower BP in the RA. Genetic variation of the ABCG2 and its relationship to the MTXPGs concentration assisted us to establish the mechanism of MTX in reducing the BP and improving arterial function in the RA patients. MTX pharmacogenetics provide key mechanistic insights to the epidemiological evidence supporting the CV-protective effect of MTX. Genetic polymorphisms can provide a personalised approach to determine the accurate dose of MTX and to help in monitoring the RA patients. If pharmacogenetic testing is implemented in the clinical practice, it could be used as a guide to identify not only MTX toxicity, but also MTX response rate. To date, this is the first study to explore the effect of the MTX transporter SNPs on the BP and arterial function in the RA population.

4.7.1.6 Asymmetric dimethylarginine

For the first time, ADMA was measured among RA patients who were on MTX compared to RA patients not using MTX. Repeated measures of ADMA was also obtained after 8 months of follow-up, which helped in assessing the effect over time. In particular, the effect of commencing the RA patients on MTX, or changing the dose of MTX on the level of plasma ADMA. Measuring ADMA gives an indirect assessment of eNOS activity and, therefore, it provides another novelty of this study in understanding the exact MTX pathway in lowering the BP and in improving the arterial function in the RA population.

4.7.2 Limitations

4.7.2.1 Cross-sectional study

Although this study provides, for the first time, evidence about the CV-protective effects of MTX on BP and arterial function in the RA population, there were a few limitations. In this study, controlling for observer bias was somehow difficult as the observer was hardly blinded to the exposure to MTX or physical signs and symptoms and therefore, complete avoidance of this type of bias was not possible. Selecting RA participants and gathering information from all participants were done by the principal investigator; who was the only researcher recruiting the potential participants. In practice, complete elimination of such bias is impossible in most observational studies (Mann 2003). All information about exposure and outcomes, however, were obtained the same way for both exposed (MTX) and unexposed (non-MTX) cohorts, which

assists in reducing the chance of observer bias (Grimes & Schulz 2002). Recall bias is another possible source of bias as participants were asked to answer series of questions provided in the food frequency questionnaire about their dietary habits (Subar et al. 2001). This questionnaire assessed their food intake over 12 months and it is widely used and has been specifically designed for the Australian population and has been extensively validated (Collins et al. 2014; Giovannelli et al. 2014; Ibiebele et al. 2009; Ireland et al. 1994; Woods et al. 2002). Additionally, confounding by indication is possible. This occurs when participants with more severe or active RA usually receive more aggressive treatments. In this case, it is possible that the dose of MTX was high among patients with lower BP, however, this study examined the relationship of MTX dose and the concentration of MTXPGs. The latter is more accurate as it is independent of the dose of MTX and the RA activity. Moreover, positive CV effects of MTX after adjusting for the disease severity were reported in this study. Another limitation of cross-sectional design is knowing the route of taking MTX and the patient's adherence to the drug. Compared to randomised clinical trials, observation studies reported lower therapy adherence rate in the routine practice among the RA population (Panoulas et al. 2007; Treharne et al. 2005). The possibility of this phenomenon to threaten the validity of these findings is, however, minimal as detailed history about taking MTX drug was reported in this study. Additionally, the exact concentration of MTX (MTXPGs) was measured in this study, which gives an accurate estimate of the effect of MTX on BP and arterial function regardless of patient's adherence.

4.7.2.2 Medications

In this study, withholding any medications prior to assessing the BP and the arterial function was not required. Many DMARDs, vasoactive medications and antihypertensive drugs used by the RA patients might influence the BP and arterial function. For example, TNF-α inhibitors taken by RA patients have been found to improve endothelial function (Hurlimann et al. 2002; Komai et al. 2007; Maki-Petaja et al. 2006) and PWV (Kerekes et al. 2011; Maki-Petaja et al. 2006; Protogerou et al. 2011). This biologic drug has a long half-life (up to 10 days) and thus it would need a considerable time, if stopped, to be virtually eliminated from the body (Maini 1998). Similarly, there are various medications which increase the BP and worsen the arterial function with NSAIDs and corticosteroids being the most common examples. Examining the BP and arterial function while RA patients continued their regular medications might give an accurate reflection of the condition of the arteries in a naturalistic sitting. Furthermore, all medications were adjusted in the regression models. In particular, BP and PWV remained significantly lower among MTX users.

4.7.2.3 Timeframe

Another limitation is the duration of MTX treatment. After the initial visit, few RA patients (n = 4) were commenced on MTX, where the duration of MTX ranged between 3 to 8 months. Examining the effect of MTX taken for longer time (≥1 year) would add more dimension to understanding its mechanism in reducing CV risk, especially on arterial stiffness, in the RA population. Two Mobil-O-Graph monitors were available. As every patient was wearing the monitor for 24-h, it was time-consuming and it affected the rate of patients booking for the interview. The investigator interviewed a maximum of two patients per day. Moreover, the rheumatology clinics are usually fully booked for most days of the week, which also affected the rate of interviewing and examining the RA participants. The researcher was aware of the importance of extending the follow-up period and that it would be more ideal in understanding causality between MTX and BP. The importance of including new RA patients who were MTX naïve was also acknowledged to understand the exact mechanism of MTX in reducing CV risk and establish causality. Initially, three RA patients were recruited; however, due to the time limit and difficulty in including new patients in the study, this cohort was excluded from the analyses.

4.7.2.4 Generalisability

Findings from this study could be generalisable to the Australian population. As previously discussed, RA patients were recruited from the Rheumatology Unit at the FMC and RGH. Genetic factor must be considered, however, as there is ethnic variation in the distribution of genotypes for ABCG2 SNPs.

CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

5.1 Conclusions

Excess mortality in the RA population is due to CVD and this is expected to increase. This upsurge is linked to untreated CV risk such as hypertension. Hypertension and arterial dysfunction play a key role in the pathophysiology of atherosclerosis and subsequent development of CVD, which is the leading cause of death in the RA and the general population. Although MTX is mainly used to reduce inflammation in RA patients, there is emerging evidence documented by observational studies highlighting the CV-beneficial impact of MTX. The results of this study showed the first evidence of associations between MTX use and both lower BP (peripheral, central and 24-h BP) and PWV in the RA population. The association between exposure to MTX and ambulatory BP and PWV reduction was driven by significant daytime, but not night-time, differences in these parameters. This study also provides key mechanistic insights to the emerging epidemiological evidence of a specific cardioprotective effect of MTX that was independent any differences in markers of inflammation. This study found a trend towards lower ADMA concentration among MTX users. DDAH can be considered as a strong pathophysiological regulator of ADMA level and its associated vascular damage, where it might be a potential therapeutic target for CVD. More interestingly, the effect of MTX on BP and arterial function can be influenced, at least in part, by genetic polymorphisms of transporters and enzymes involved in the MTX metabolism. The polymorphisms in the *ABCG2* gene was studied. Compared to RA patients with normal gene, those with AC variant showed lower BP and better arterial function. This mutant allele was also associated with higher intracellular accumulation of MTXPGs compared with normal homozygous gene. Unfortunately, this genetic tool of testing individual variability for MTX response is still underutilised in clinical practice. Personalised MTX treatment to achieve better BP and arterial function control may confer CV and survival benefits in the RA population.

5.2 Implications and future directions

This study has several implications for changing the future of clinical practice, in particular CV risk management. The reported differences, in this study, in the BP and markers of arterial function between MTX and other DMADRs would translate into a significant, clinically meaningful, reduction in CV risk in the RA population. This would reduce the burden of CVD, and associated health care costs, not only in Australia, but also worldwide. Possibly, this substantial change in the CV management might involve other populations such as patients with CVD.

The current study highlights that more attention must be given by health care providers to patients with RA. In particular, a different approach is needed which considers the high risk of CV morbidity and mortality in this population. Early detection and aggressive management of CVD is mandatory to reduce the risk of CV morbidly and mortality. Thus, screening all RA patients at the primary care level for CV risk should receive the highest priority. Once high-risk patients are identified, prompt management is advisable. Despite the increased CV risk in the RA population, which is similar to those with T2D, additional screening for such risks has not been applied in most countries (Monk et al. 2013). Additionally, this study highlighted the importance of identifying and differentiating between RA patients using MTX and those not using MTX. This might guide health care professionals—including rheumatologists, cardiologists and general practitioners—to identify RA populations at higher CV risk, which might facilitate targeted CVD screening and assist early detection and management. Furthermore, for RA patients who are already suffering from one or more of the CV risk factors, both RA and CV risk factors might be approached in an integrative manner or might be managed using a different approach addressing the needs of each patient. The findings might aid policy makers to implement or reform policies of improving patients' health; for example, targeted CV risk screening could be implemented for the RA population, which might help in reducing the CV mortality among this population.

Additionally, the finding of better arterial function (improved PWV) and lower BP in RA patients with MTX *ABCG2* polymorphism introduces the role of genetic polymorphisms in modulating the effects of MTX on these CV markers. Due to inter-individual variability, genetic polymorphisms might also provide essential information for a personalised approach to initiate the dose of MTX and subsequent monitoring. This MTX personalised approach could then be used to examine the BP and arterial function effects of MTX on other high-risk patients with diseases other than RA—for example, designing randomised control trials for high-risk cardiac patients using MTX. Moreover, this genetic polymorphism could provide vital information in the field of molecular biology and genetic engineering. In particular, changing the sequence of the DNA intentionally is a known technique to introduce a mutation to a particular gene; it is also called site-specific mutagenesis. Researchers could use this finding in constructing novel MTX analogues which are characterised by a combined anti-inflammatory and BP/arterial function effects.

Furthermore, the CV-protective effects of MTX has a pharmacological attraction. MTX could be reused for managing CV risk in not only RA patients but also the general population. As inflammation plays a key role in the process of atherosclerosis and in the development of CVD,

this novel strategy could reduce the CV burden, because it combines the well-established anti-inflammatory and immunomodulating effects of MTX with the novel additional effects in lowering the BP and improving the arterial function. Circulating MTX has relatively short half-life and is associated with undesirable side effects, which negatively impacts on the patient's adherence and leads to withdrawal. The finding of intracellular accumulation of MTXPGs, with subsequent longer duration of action and few dosing (i.e. once a week) could, however, improve patients' compliance and adherence to MTX.

The effect of MTX on the BP and arterial function was studied over a relatively long period (baseline and at 8 months). This allowed us to observe changes in both CV markers over time and to closely monitor any changes in the dose of MTX or treatment plan. Accordingly, we can be confident that the findings of improved BP and PWV represent the effects of MTX. To draw conclusions about causal relationship between the exposure to MTX and BP/arterial function, however, a prospective longitudinal study is needed. In the future, the novelty of these results will provide new opportunities for epidemiological researchers to design randomised control trials for thorough understanding of the relationship between MTX and BP. Moreover, recruiting newly diagnosed RA patients, who are eligible candidates to commence MTX, gives a new insight into the effects of MTX on the BP and arterial function; this will allow in-depth exploration of the temporal relationships between MTX, BP, and arterial function. Then, causality between MTX and BP/arterial function could be assessed longitudinally.

Moreover, additional most-likely mechanistic explanations for the decrease in BP and arterial function, such as factors involved in the MTX pathway, could be further explored. For example, plasma concentration of adenosine and the enzyme activity of endothelial NOS could be studied in future research. Another future recommendation is to study the polymorphisms of other genes involved in the MTX pathway to understand mechanisms of reducing the BP.

The beneficial CV effects of MTX might be one of the preventive ways to curb the rapid escalation in the rate of CV morbidity and mortality in the RA population. CV prevention, such as improving the management of CVD, has been proven to be effective; despite the important role of CVD prevention, more attention was given to the diagnosis and treatment of CVD while limited budgets were allocated for the prevention interventions (O'Kelly et al. 2011). In 2016, the Heart Foundation proposed a program to prevent CVD advocating for increasing the health care budget for CVD prevention (The Heart Foundation & The National Stroke Foundation 2016). It has also been lobbying for increases to CV research funding. Thus, the findings reported by this

study give a huge opportunity for researchers to design an interventional study to examine CV effects of MTX and to provide conclusions about using MTX in preventing CVD in the RA population.

APPENDICES

Appendix-1 Publications

Systemic review and meta-analysis

PLOS | ONE

RESEARCH ARTICLE

The Impact of Traditional Cardiovascular Risk Factors on Cardiovascular Outcomes in Patients with Rheumatoid Arthritis: A Systematic Review and Meta-Analysis

Leena R. Baghdadi[1,2,4], Richard J. Woodman[2], E. Michael Shanahan[3], Arduino A. Mangoni[4]*

1 Department of Family and Community Medicine, King Saud University, Riyadh, Saudi Arabia, Department of Clinical Pharmacology, School of Medicine, Flinders University, Adelaide, Australia, 2 Flinders Centre for Epidemiology and Biostatistics, School of Medicine, Flinders University, Adelaide, Australia, 3 Department of Rheumatology, School of Medicine, Flinders University, Adelaide, Australia, 4 Department of Clinical Pharmacology, School of Medicine, Flinders University, Adelaide, Australia

* arduino.mangoni@flinders.edu.au

OPEN ACCESS

Citation: Baghdadi LR, Woodman RJ, Shanahan EM, Mangoni AA (2015) The Impact of Traditional Cardiovascular Risk Factors on Cardiovascular Outcomes in Patients with Rheumatoid Arthritis: A Systematic Review and Meta-Analysis. PLoS ONE 10(2): e0117952. doi:10.1371/journal.pone.0117952

Academic Editor: Gianpaolo Reboldi, University of Perugia, ITALY

Received: October 1, 2014

Accepted: January 3, 2015

Published: February 17, 2015

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: The authors have no support or funding to report.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Background

Rheumatoid arthritis (RA) is known to increase the risk of cardiovascular (CV) disease. However, the individual impact of traditional CV risk factors in RA is unknown.

Objective

To assess the strength of the association between individual CV risk factors and rate of either myocardial infarction (MI), combined CV morbidity (MI, angina pectoris, heart failure, stroke, and peripheral arterial disease (PAD)) or CV mortality in RA patients.

Methods

RA studies reporting traditional CV risk factors [hypertension, type 2 diabetes (T2D), smoking, hypercholesterolaemia, obesity, and physical inactivity] as exposures and MI, CV morbidity (MI, angina, heart failure, stroke, and PAD combined) or CV mortality alone as outcomes were searched until March 2013 using MEDLINE, Scopus and Cochrane. Meta-analyses combined relative risk (RR) estimates from each study where either the RR and 95% confidence intervals or where raw counts were available.

Results

Ten studies reporting sufficient data for inclusion into meta-analyses were identified. Relevant data was available for each risk factor and MI and CV morbidity but no studies reported on CV mortality. Risk of MI increased in RA patients with hypertension (RR 1.84, 95% CI 1.38, 2.46) and T2D (RR 1.89, 95% CI 1.36, 2.63). CV morbidity increased with hypertension (RR 2.24, 95% CI 1.42, 3.06), T2D (RR 1.94, 95% CI 1.58, 2.30), smoking (RR

1.50, 95% CI 1.15, 1.84), hypercholesterolaemia (RR 1.73, 95% CI 1.03, 2.44) and obesity (RR 1.16, 95% CI 1.03, 1.29) but not with physical inactivity (RR 1.00, 95% CI 0.71, 1.29).

Conclusion

Hypertension, T2D, smoking, hypercholesterolaemia and obesity increased CV risk in patients with RA. These results highlight the importance of managing CV risk factors in RA, similarly to non-RA patients.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease complicated by progressive joint destruction [1]. RA is often characterized by extra-articular manifestations mainly affecting cardiovascular, immune, respiratory, and renal systems. One of the most common and, indeed, serious complications is cardiovascular disease (CVD). A 48% and 60% excess risk of CV morbidity [2,3] and mortality [4] respectively, have been reported in RA patients. The association between RA and CVD has been linked to a pro-inflammatory state [5–8]. However, this does not seem to be the only mechanism involved as an increased CV risk is still present despite the availability of well-established anti-inflammatory medications in this patient group [9].

Traditional risk factors such as hypertension, type 2 diabetes (T2D), smoking, hypercholesterolaemia, obesity and physical inactivity is likely to explain at least some of the excess CV risk in RA patients, similarly to what has been extensively reported in the general population [10–14]. However, whether the relative impact of individual CV risk factors on CV risk, beyond that caused by RA alone, is similar to the general population is still unclear. Studies on hypertension, T2D, smoking, hypercholesterolaemia, obesity and physical inactivity have provided conflicting results. Few studies have found an increased CV risk in hypertensive RA patients in contrast to the general population [12,15–18]. There is no conclusive evidence on the presence, and magnitude, of an association between T2D and CV morbidity in RA. While some authors argue that the increased CV risk observed in RA patients is unrelated to the presence of T2D [11,18,19], others have documented that its presence increases CV risk [12,14,16,20,21]. Similarly, although smoking is well established as a CV risk factor, its potential impact in RA patients remains unclear [2,12,14,20,22–24] with reports of weak [25] or non-significant associations between smoking and CV risk in RA [12,18]. The impact of hypercholesterolaemia [12,14,16,26–32], obesity [15,33–35] and physical inactivity [13,36] on CV morbidity in RA have been contrasting with some decreases in risk with the presence of a risk factor; a phenomenon known as 'risk factor paradox'. These paradoxes may arise due to several different forms of selection bias which can occur particularly in rheumatic disease research and have been termed 'index event bias' or 'collider stratification bias'[37–40] and occur as a result of conditioning on a common effect (the index event). As a result, instead of reducing bias due to confounding, a spurious (perhaps negative) association may be induced. This usually occurs when multiple independent risk factors lead to both the development of the disease itself (the "index event" i.e. RA) and the disease sequelae (CV morbidity or mortality). In addition to index event bias, differential loss to follow-up, differential depletion of susceptible participantsand immortal time bias (differential misclassification of pre-exposure periods) are all forms of selection bias threatening the internal validity of the findings reported in rheumatic diseases research.

These issues notwithstanding, the aim of this systematic review and meta-analysis was to investigate the relative impact of individual traditional CV risk factors on CV morbidity and mortality among patients with RA.

Material and Methods

Searching methodology

A literature search was conducted for articles on the impact of traditional CV risk factors on CV morbidity and mortality among patients with RA. Pre-Medline, Medline, Scopus and Cochrane databases were searched until March 2013; the Premedline and Medline databases were searched using PubMed. Articles were identified by using controlled vocabulary terms (MeSH terms) as well as keywords (S3 Materials). Hand searching the citation lists of relevant articles was performed to look for additional papers.

Study selection and patient outcomes

The exposure of interest was the presence or absence of risk factors in RA patients. The outcomes of interest were MI, combined CV morbidity (incidence of combined CV morbidity including MI, angina pectoris, heart failure, stroke, and peripheral arterial disease), and CV mortality.

Studies were included for inclusion in the meta-analyses if: (i) the diagnosis of RA in adult patients ($\geq$ 18 years) was made according to current guidelines or by a rheumatologist; (ii) traditional CV risk factors (hypertension, T2D, smoking, hypercholesterolaemia, obesity, and physical inactivity) were assessed; (iii) the assessed outcomes included either myocardial infarction (MI), combined CV morbidities and/or CV mortality; (iv) either the raw count data or the estimated relative risk (RR) and 95% confidence interval of risk factors on CV morbidity were reported. Relevant studies were excluded if (i) the above inclusion criteria were not fulfilled; (ii) no information about clinical CV morbidity was available; (iii) no information about the effect of CV risk factors on clinical CV morbidity was available; (iv) the required data was not available.

Data extraction

Data from each study was summarised in terms of: study design, participant characteristics, the assessed CV risk factors and outcomes, study quality score (Qi), and a summary of estimated effects.

Quality scores of included studies

The different methodological approaches used across the studies required that the differences in study quality were accounted for. A reproducible and effective checklist is a feasible way to assess the quality of studies included in the meta-analysis, distinguishing between those with higher precision and reduced bias. Therefore, a generic checklist based on published studies [41,42] was used to assess the quality of selected studies and to calculate the study quality score (Qi). This checklist consisted of 14 questions evaluating internal validity, external validity and statistical analysis. Points awarded for each question were added to calculate the Qi score; high or low quality score was defined as Qi score of $\geq$10 or $\leq$ 9, respectively. Some of the questions were tailored to meet the study requirements (S1 Table). The balancing of key prognostic indicators affecting CV morbidity across exposure groups was considered in the checklist when creating a prognostic score (question 9 of checklist in S1 Table). The prognostic items included age, sex, hypertension, body mass index (BMI), diabetes, hypercholesterolaemia, smoking,

family history of CVD, physical inactivity, duration of RA, and medications (folic acid, corticosteroids, and anti-rheumatic medications). A prognostic score of 1 was given to studies that balanced five or more of these items across comparison groups; a score of 0.5 was given to studies that balanced three or four items; a score of 0 was given if none, one or two of these items were balanced, or not documented in the study.

Statistical analysis

Meta-analyses were performed to assess the association between exposure to CV risk factors and MI, and between exposure to CV risk factors and combined CV morbidity (MI, angina pectoris, heart failure, stroke, and peripheral arterial disease). For MI, where raw counts for exposed/non-exposed and event/non-event groups were available we used MetaXL software version 1.2 (www.epigear.com, Brisbane, Queensland, Australia). For combined CV morbidity, we first used raw counts where available to calculate effect sizes (RR) and confidence intervals (CI) using MetaXL and then combined these with studies that reported effect sizes (RR) and CI only using the user-written "metan" command for STATA software (version 13.0, StataCorp LD, College Station, Texas, USA).

Statistical methods for testing heterogeneity such as Q-statistic and its variants have low statistical power. Therefore vigilance, common sense and prior biological knowledge are required when synthesizing the results of different studies [43]. Statistical heterogeneity was anticipated across different study groups if tau-squared was >0 and/or the Q-statistic was significant at a $p < 0.1$ [43].

A sub-group analysis was performed for the effect of hypertension on combined CV morbidities to examine the possible modifying effects of various patient characteristics including age, disease duration, year of publication, and type of treatments. Subgroups were defined by the mean age of RA patients (≤55 years or >55 years), mean disease duration (<5 years or ≥ 5years), year of publication (before or in 2007 or after 2007), and type of current treatment (using methotrexate alone or methotrexate with other disease modifying anti-rheumatic drugs).

No formal funnel plot analysis was conducted as there were less than ten studies included in each meta-analysis [44].

Results

Search and screening

There were 10,812 studies identified through database search. After removing duplicates, there were 10,200 records published between 1947 and March 2013. The abstracts of these studies were screened and 10,091 reports were excluded, leaving 109 records (Fig. 1). After evaluating the full-text documents of these 109 records, 99 studies were excluded for the following reasons (S2 Table): 21 did not fulfil the inclusion criteria, 44 had no information on clinical CV morbidity, 25 had no information about CV risk factors, three publications were multiple reports on the same sample population [24,45,46] and six studies had no required data [11,13,14,16,20,35]. Authors of these six studies were contacted several times with a request for relevant information. Authors of one study refused to participate, two authors were unable to provide the requested data and three did not respond, despite several attempts.

Therefore, a total of 10 published studies meeting the inclusion criteria contributed to the various meta-analyses [10,12,15,17,19,21,25,36,47,48]. Of these, raw counts for exposed/non-exposed groups and event/non-event groups were extractable from only five studies [10,15,21,36,47] and provided data on a total of 4,388 RA patients exposed to the following CV risk factors: hypertension (n = 1,879), T2D (n = 453) and smoking (n = 2,056). The remaining

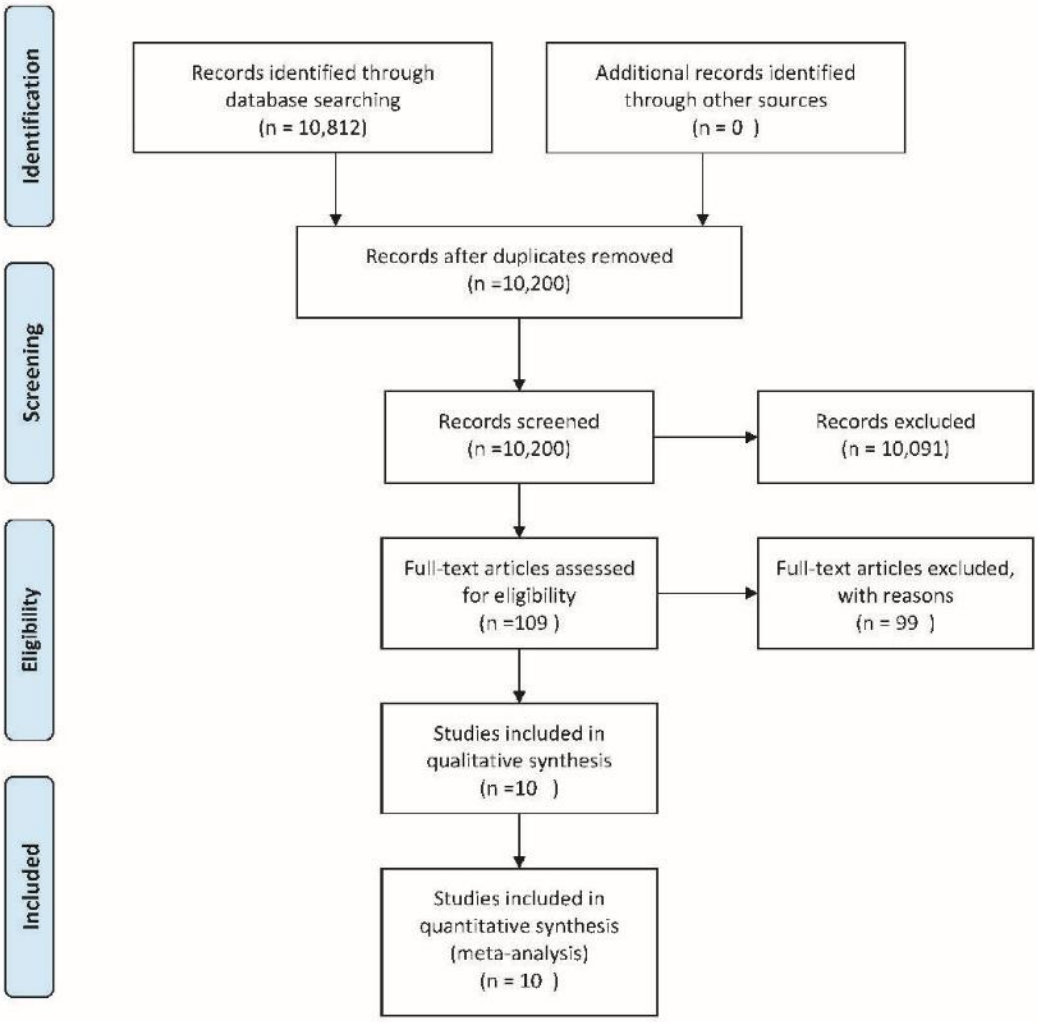


Fig 1. Flow diagram of study selection.

doi:10.1371/journal.pone.0117952.g001

five studies, however, reported estimated relative risks (RR) and 95% CI for combined CV morbidity and all risk factors [12,17,19,25,48].

Characteristics of studies and subjects

Table 1 describes specific information from the final 10 studies meeting the inclusion criteria and included in the meta-analyses. There was considerable variation in study design, age, RA duration, methodological quality and ascertainment of exposure and outcomes across the

Table 1. Studies meeting the inclusion criteria and included in the quantitative meta-analysis (n = 10).

Reference	N	Design	Participants	Risk factors assessed	CV outcomes	Risk factors associated with CV risks (estimated effect size)	Quality score (Qi) out of 14
[47]	RA with MI = 41; RA without MI = 181	Nested case-control study	RA diagnosed according to the 1987 revised ACR criteria; random selection of controls from the PCS was done; Mean age: 67.5±10 years; Mean RA duration: 4.3±8.5 years	*Hypertension, *T2D, *Smoking status, Dyslipidaemia, Obesity	CV morbidity: either MI or unstable angina	**OR of MI** ǂ Hypertension: OR 2.41 (95% CI 1.14, 5.11) ǂ T2D: OR 1.29 (95% CI 0.40, 4.14) ǂ Smoking: OR 1.46 (95% CI 0.72, 2.97) HDL: OR 0.50 (95% CI 0.09, 2.71) Obesity: OR 1.09 (95% CI 0.99, 1.20)	11
[21]	Total RA = 369	RCS	No diagnostic criteria for RA; Mean age: 57±14 years; Mean RA duration: 17±11 years	*Hypertension, *T2D	CV morbidity: coronary heart disease, MI and CHF	**OR of MI** ǂ Hypertension: OR 2.48 (95% CI 1.31, 4.70) ǂ T2D: OR 3.75 (95% CI 1.50, 9.35)	8.5
[10]	Total RA = 239 (Female = 196; Male = 43)	RCS	RA diagnosed according to the 1987 revised ACR criteria; Mean age: 56.3±15.7 years; Mean RA duration: 11.6±8.8 year	*Hypertension, *T2D, *Smoking, Hypercholesterolemia, Body weight	CV morbidity: MI and stroke; MI as separate CV outcome and as combined CV event; MI; Stroke. CV mortality: CV death due to MI, stroke or CHF	**RR of combined CV morbidity** Hypertension: RR 4.3 (95% CI 1.4, 13.2) ǂ T2D: RR 2.62 (95% CI 0.83, 8.28) ǂ Smoking: RR 0.75 (95% CI 0.18, 3.13 Hypercholesterolemia: RR 6.0 (95% CI 1.80, 20.70) # Body weight: -	7
[36]	Total RA = 4,363 (Female = 3,403; Male = 960)	Cross-sectional	RA diagnosed according to the 1987 revised ACR criteria; Mean age: 57±1 years; Mean RA duration: 11±9 years	*Hypertension, *T2D, *Smoking status, Hypercholesterolaemia, Obesity, Physical activity	CV morbidity: MI, angina, coronary disease and stroke	**HR of combined CV morbidity** Hypertension: HR 2.97 (95% CI 2.31, 3.83) T2D: HR 2.09 (95% CI 1.50, 2.92) Smoking: HR 1.60 (95% CI 1.25, 2.04) Hypercholesterolaemia: HR 3.19 (95% CI 2.47, 4.13) Obesity: HR 1.34 (95% CI 0.96, 1.86) Physical inactivity: HR 1.00 (95% CI 0.75, 1.33)	10.5

(Continued)

Table 1. (*Continued*)

Reference	N	Design	Participants	Risk factors assessed	CV outcomes	Risk factors associated with CV risks (estimated effect size)	Quality score (Qi) out of 14
[15]	Total RA = 325 (Female = 250; Male = 75)	RCS	RA diagnosed according to the 1987 revised ACR criteria; Mean male age: 56±15 years; Mean female age: 50±15 years; Mean RA duration: 2 years	*Hypertension, T2D, Smoking, Hypercholesterolaemia, Obesity, Physical activity	CV morbidity: MI, angina pectoris, coronary disease, and stroke; CV mortality: coronary heart disease death	**HR of combined CV morbidity** Hypertension: HR 3.76 (95% CI 0.99, 15.06) T2D: HR 1.09 (95% CI 0.20, 5.92) Smoking: HR 2.02 (95% CI 0.35, 7.69) Hypercholesterolaemia: HR 1.03 (95% CI 0.22, 4.75) Obesity: HR 0.71 (95% CI 0.13, 3.85) Physical inactivity: HR 2.53 (95% CI 0.31, 20.56)	10
[19]	Total RA = 234; Non-RA = 5,158	RCS	RA diagnosed according to 1987 ACR criteria; O 'RALE cohort was used; matched non-RA: SAHS cohort; Median age: 56 years (ranged 22–80)	Hypertension, T2D, Smoking, Hypercholesterolaemia, BMI	CV morbidity: MI or stroke or other arterial occlusive events or arterial revascularization procedures; CV mortality: CV deaths	**IRR of combined CV morbidity** Systolic blood pressure (per 15 mm Hg): IRR 1.18 (95% CI 1.03, 1.33) T2D: IRR 2.28 (95% CI 1.65, 3.12) Smoking: IRR 1.37 (95% CI 1.01, 1.83) Hypercholesterolaemia: IRR 1.35 (95% CI 1.01, 1.82) BMI (per 5 kg/m²): IRR 1.13 (95% CI 0.99, 1.28)	11
[25]	Total RA = 603; Non-RA = 603	RCS	RA diagnosed by 1987 ACR criteria; matched non-RA cohort; randomly selected; Mean age: 58 years	Hypertension, T2D, Smoking, Hypercholesterolaemia, Obesity	CV morbidity: MI, CHF; CV mortality: CV death	**HR of combined CV morbidity** Hypertension: HR 1.97 (95% CI 1.24, 3.11) T2D: HR 1.62 (95% CI 1.17, 2.24) * Smoking: HR 1.32 (95% CI 0.97, 1.81) Hypercholesterolaemia: HR 0.92 (95% CI 0.67, 1.26) Obesity: HR = 1.27 (95% CI 0.93, 1.74)	9.5

(*Continued*)

Table 1. (*Continued*)

Reference	N	Design	Participants	Risk factors assessed	CV outcomes	Risk factors associated with CV risks (estimated effect size)	Quality score (Qi) out of 14
[12]	Total at entry = 700 (Male = 219; Female = 481);Total at end = 422 (Male = 141; Female = 301)	PCS	Early RA diagnosed by ARA criteria; patients records and self-reported questionnaire on co-morbidity and local rheumatologist follow up assessment were used; Mean age: 55.2±14.3 years; Mean disease duration: 3.3 months	Hypertension, T2D, Smoking, Dyslipidaemia, Obesity	CV morbidity: MI, stroke and peripheral vascular disease, Stroke/TIA, DVT/ PE; CV mortality: Fatal CV events	**HR of combined CV morbidity** Hypertension: HR 4.07 (95% CI 2.31, 7.16) T2D: HR 2.89 (95% CI 1.30, 6.45) # Smoking: - Triglycerides level: HR 1.92 (95% CI 1.46, 2.52) # Obesity: -	11
[48]	Total RA = 211 (Male = 85; Female = 126)	RCS	Seropositive RA according to the 1958 revised diagnostic criteria for rheumatoid arthritis; Mean age for women: 50.6 years; Mean age for men: 53.7 years; RA duration <1 year; Patients selected from the only reference centre for rheumatology	Hypertension, T2D, Smoking	CV morbidity: MI, peripheral vascular disease and stroke, First CV event, Stroke/TIA, DVT/PE; CV mortality: Fatal CV events	**RR of combined CV morbidity** Hypertension: RR 2.48 (95% CI 1.48, 4.17) # T2D: - # Smoking: -	10
[17]	Total RA = 606 (Male = 194; Female = 412)	RCS	Seropositive RA, classification number 71238 according to 8th edition, ICD-8, Swedish National Board of Health and Welfare, 1968; 1987 ARA criteria for RA; Mean age for women: 54 years; Mean age for men: 56 years; Mean RA duration: 12.5 years; patients selected from the only reference centre for rheumatology	Hypertension, T2D	CV morbidity: MI, peripheral vascular disease and stroke, DVT/PE, Cerebrovascular lesion/TIA; other CV events: peripheral arterial embolus and dissecting aorta aneurism; CV mortality: Fatal CV events	**RR of combined CV morbidity** Hypertension: RR 1.76 (95% CI 1.32, 3.35) # T2D: -	9.5

*Risk factors included in the meta-analysis because raw data was available

ǂ Calculated effect estimate (OR = ratio of odds of exposure among cases to odds of exposure among controls; RR = ratio of the probability of CV events in exposed group to the probability of the event in non-exposed group)

Effect estimate could not be calculated as raw data was not available

ACR = American College of Rheumatology, RA = Rheumatoid Arthritis, ARA = American Rheumatism Association, RCS = Retrospective Cohort Study, PCS = Prospective Cohort Study, BMI = Body Mass Index, T2D = Type 2 Diabetes, CV = Cardiovascular, QUEST-RA = Questionnaires in Standard Monitoring of Patients with Rheumatoid Arthritis, MI = Myocardial infarction, CHF = Congestive Heart Failure, ICD-8 = International Classification of Diseases, eight revision, HR = Hazard ratio IRR = Incidence Rate Ratio, OR = Odd Ratio, RR = Relative Risk, CI = Confidence Interval; Qi = Study Quality Score

doi:10.1371/journal.pone.0117952.t001

10 studies. One study had a nested case-control design, seven were retrospective cohorts, one prospective cohorts and one was cross-sectional studies. Mean age of RA patients ranged from 50 [15] to 67 years [47]. Mean RA duration ranged from ≤ 1 year [12,48] to 17 years [21]. Importantly, methodological quality varied among studies with scores ranging from 7 [10] to 11[12,19,47]. Each study reported on at least one CV risk factor (hypertension, T2D, smoking, hypercholesterolaemia, obesity and physical inactivity) but no standard criteria were used when ascertaining exposure to CV risks factors [10] or in the selection of RA patients [21]. Nevertheless, all studies suggested an increased CV morbidity (MI, angina, heart failure, stroke, and PAD combined) with different risk factors (Table 1). Similarly, there was a trend towards increased CV mortality among RA patients in the majority of studies [10,12,15,17,19,25,48].

Meta-analyses

No information was available on CV mortality, therefore only MI and combined CV morbidity (MI, angina pectoris, heart failure, stroke and peripheral arterial disease) were considered in the analyses (S3 Table)[10,12,15,17,19,21,25,36,47,48]. Two studies investigated MI as a separate CV outcome [21,47], one study described separate results for MI and for combined CV morbidity [10] and the remaining studies presented data for combined CV morbidity (S3 Table).

Risk of myocardial infarction

Hypertension. Three out of 10 studies [10,21,47] showed an increased risk of MI in hypertensive RA patients. The RR was 1.84 (95% CI 1.38, 2.46) implying an 84% higher risk of MI among RA patients with hypertension compared with non-hypertensive RA patients (Fig. 2).

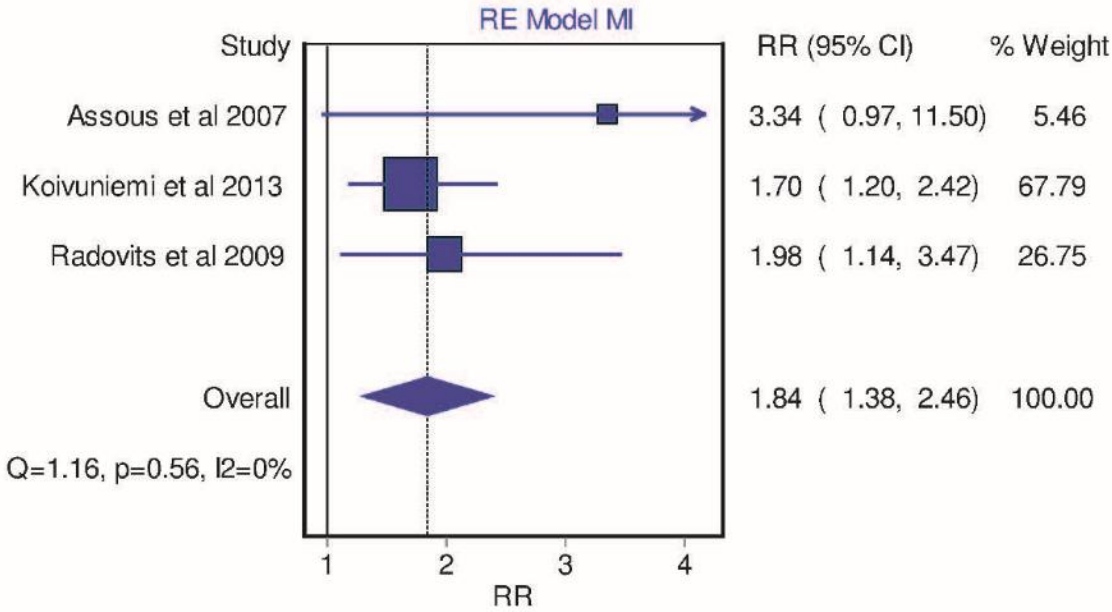


Fig 2. Forest plot depicting the relative risk of MI in hypertensive RA patients versus those without hypertension using random effect model.

doi:10.1371/journal.pone.0117952.g002

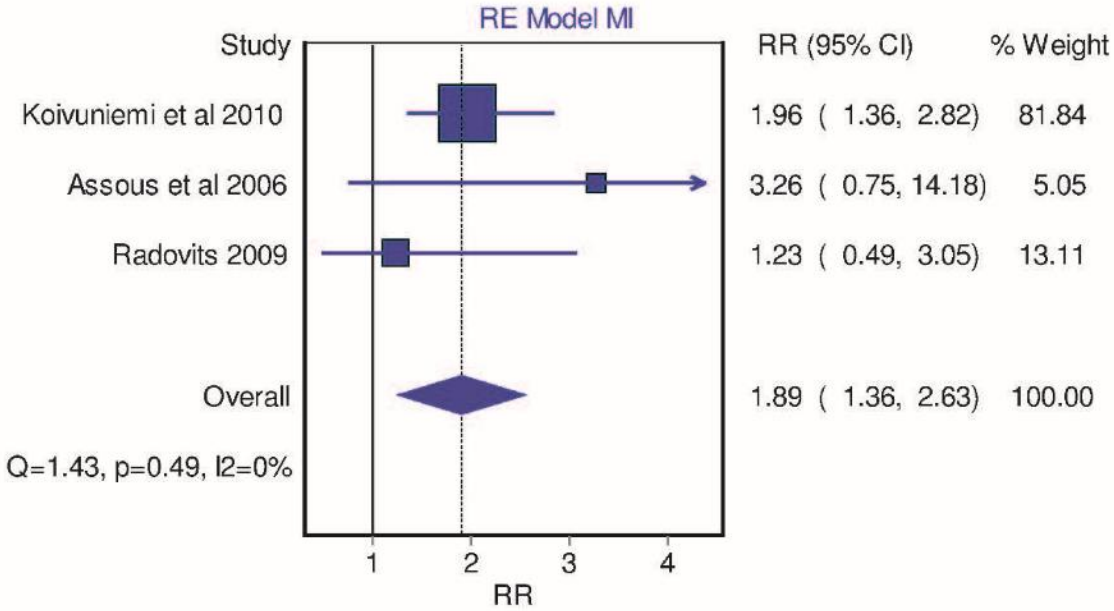


Fig 3. Forest plot depicting the relative risk of MI in diabetic RA patients versus those without T2D using random effect model.

doi:10.1371/journal.pone.0117952.g003

Diabetes. Three out of 10 studies [10,21,47] showed an increased risk of MI in RA patients with T2D, leading to a combined RR of 1.89 (95% CI 1.36, 2.63) (Fig. 3).

Risk of combined CV morbidity

Hypertension. Eight of the 10 studies [10,12,15,17,19,25,36,48] that assessed the risk of combined CV morbidity with hypertension showed an overall RR of 2.24 (95% CI 1.42, 3.06), implying that hypertensive RA patients are 2 times more likely to experience combined CV morbidity compared with non-hypertensive patients (S1 Fig.).

Diabetes. Five of the 10 studies [10,12,19,25,36] that assessed the risk of combined CV morbidity showed an excess risk in RA patients with T2D. Overall, RA patients with T2D were almost 2 times more likely to experience an event compared with non-diabetic patients (RR 1.94, 95% CI 1.58, 2.30) (S2 Fig.).

Smoking. Data from four out of 10 studies [10,19,25,36] examined the effect of smoking on the risk of CV morbidity. Overall, the RR was 1.50 (95% CI 1.15, 1.84) indicating a 50% increased risk of a CV event in smokers with RA compared to non-smoking RA patients (S3 Fig.).

Hypercholesterolaemia. Six out of 10 studies [10,12,15,19,25,36] that assessed the risk of combined CV morbidity showed an excess risk in RA patients with hypercholesterolaemia. Overall, RA patients with hypercholesterolaemia had a 73% increase in the incidence of combined CV morbidity compared with patients without this risk factor (RR 1.73, 95% CI 1.03, 2.44) (S4 Fig.)

Obesity. Data from four of 10 studies [15,19,25,36] that assessed the risk of combined CV morbidity with obesity showed an increased risk of combined CV morbidity, and an overall RR of 1.16 (95% CI 1.03, 1.29), implying that obese RA patients had a 16% increase in the incidence of combined CV morbidity compared with non-obese RA patients (S5 Fig.).

Physical inactivity. The two studies [15,36] that assessed the risk of combined CV morbidity showed no significant association between physical inactivity and combined CV morbidity in RA patients. Overall, the risk of combined CV morbidity in RA patients was similar in physically inactive and physically active RA patients (RR 1.00, 95% CI 0.71, 1.29) (S6 Fig.)

Sub-group analysis

Table 2 shows the results of the sub-group analysis of hypertensive patients for combined CV morbidity. Hypertensive RA patients tended to have a higher risk of combined CV morbidity if: they were older (≥ 55years); duration of RA was shorter (< 5years); they were reported in studies published after 2007; and they were on combined anti-rheumatic medications especially if they were on biologics therapy. However, the trend of having higher risk of combined CV morbidity in hypertensive RA patients was retained.

Discussion

Although there were inconsistencies in the literature reporting the impact of traditional CV risk factors in RA patients on MI and CV morbidity, this meta-analysis provides evidence for a significant negative impact of hypertension, T2D, smoking, hypercholesterolaemia and obesity in this population, with the magnitude of effects similar to that for the general population.

The role of traditional CV risk factors in the general population is well established [49–52]. Hypertension is a well-known modifiable risk factor [50] and the risk of CV morbidity can be reduced with a modest reduction in blood pressure. In the general population, an international case-control study estimated that people with hypertension were 91% more likely to develop MI (OR 1.91, 99% CI 1.74, 2.10) [50]. Hypertension is not only highly prevalent among

Table 2. Sensitivity analyses of RA patients with hypertension and cardiovascular outcomes.

Parameters and Combined CV morbidity [RR RE model (95% CI)]
Mean age of subjects (years)
<55 (n = 3): 2.04 (95% CI 1.24, 2.85)
>55 (n = 5): 2.35 (95% CI 1.20, 3.49)
Mean duration of RA (years)
<5 (n = 3): 2.88 (95% CI 1.72, 4.04)
>5 (n = 3): 2.48 (95% CI 2.03, 3.21)
Year of publication
<2007 (n = 4): 1.61 (95% CI 0.91, 2.32)
>2007(n = 4): 2.68 (95% CI 1.90, 3.47)
Type of treatments
MTX alone (n = 4): 2.18 (95% CI 0.50, 3.86)
Combined treatments (n = 4): 2.31 (95% CI 1.45, 3.16)
With biologics (n = 2): 2.99 (95% CI 2.23, 3.74)
Without biologics (n = 2): 1.43 (95% CI 0.71, 2.15)

n = number of studies involved in the analysis
CV: cardiovascular

doi:10.1371/journal.pone.0117952.t002

patients with RA [53,54], but also an important predictor of CV events within this population [10,12,48,55]. This meta-analysis showed greater risk of both MI [10,21,47] and combined CV morbidity rates in RA patients with hypertension [10,12,15,17,19,25,36,48]. Compared with the general population, there was a similar increase in MI risk among RA patients with hypertension (RR 1.84, 95% CI 1.38, 2.46) (S4 Table). In line with our findings, it was recently reported that a systolic blood pressure increase of 20 mmHg in RA patients would result in 1,572 additional ischaemic heart disease events yearly (95% CI 1,024, 2,120) [56].

T2D is also a well-known risk for cardiovascular diseases. In the general population, T2D confers a 2–4 fold excess risk of CV morbidity [50,57–59]. Although the relationship between T2D and CV morbidities in RA has been questioned [2,18,19,25], results from five studies in this meta-analysis [10,12,19,25,36] showed that T2D increases CV risk in RA patients similarly to that in the general population. This is consistent with recent evidence from a prospective cohort study showing that T2D increased the incidence of combined CV morbidity compared with non-diabetic patients (HR 2.89, 95% CI 1.29, 6.45) [12]. The impact of T2D on MI risk tended to be meaningfully higher in the general population (OR 2.37, 99% CI 2.07, 2.71) than in RA patients (RR 1.89, 95% CI 1.36, 2.63) (S4 Table).

Although the prevalence of smoking in RA is well established [9], its impact on CV morbidity in RA have not yet been identified despite the established relationship in the general population [25,50]. In the INTERHEART study, current or former smokers in the general population were 2.3 times more likely to develop MI than non-smokers [50]. Interestingly, in RA patients, it has been claimed that there might be a weaker association between smoking and CV disease risk [25]; however, this result might be biased given the inherent risk of under reporting [60]. Most importantly, this smoking paradox might be caused by index event bias in which smoking is associated with the development of the disease itself (RA 'index event') and the disease sequelae (CV events). By conditioning on the index event of RA [40] (i.e. stratifying RA and non-RA patients) this may lead to a spurious association (reduced effect estimate) between the risk factor (smoking) and CV morbidities due to unmeasured or unknown confounders that are associated with both the index event and the events downstream of RA. Another explanation of the putative CV protective effects of smoking among RA patients is depletion of susceptible participant bias in which RA patients who smoke may die earlier but not from the outcome of interest. In a study showing a protective effect of smoking on CV events [25], RA patients who were more susceptible to CV complications related to smoking tended to die earlier of non-CV event outcomes. Although the effect of hypercholesterolemia on CV morbidity has not been well described, it was associated with higher combined CV morbidity among RA patients in this meta-analysis (RR 1.73, 95% CI 1.03, 2.44). On the other hand, the impact of body weight on CV morbidity showed a paradoxical relationship. Even though few authors argued that rheumatoid cachexia was associated with worse CV morbidity [33], others found no such association [34]. On the contrary, obesity was associated with increased CV events [19,25,35,36]. Our finding that obese RA patients had a 94% increase risk of combined CV morbidity supports this evidence. Compared to the general population, RA patients are usually less active [61]. As physical exercise improves both quality of life and physical function [62], encouraging RA patients to be more active has been suggested to be part of routine clinical care [63,64]. However, the two studies [15,36] in this meta-analysis found no significant association between physical inactivity and combined CV morbidity. This result should be interpreted with caution as one of the two studies in the meta-analysis had a cross-sectional design with a relatively short follow-up period [36]. Moreover, information about physical inactivity was based on self-report questionnaires.

Managing CV risk in RA patients is an emerging concept although little evidence exists regarding the efficacy and safety of specific treatment strategies [65]. It was traditionally assumed

that the RA-associated pro-inflammatory state independently increased CV risk [5–8]. However, studies supporting this concept had a relative short follow-up period [5] and were often observational with a cross-sectional design. Assessing inflammatory markers at a single time point does not capture the cumulative burden of inflammation over time. Additionally, CV risk is still high despite the wide spread prescribing of anti-inflammatory medications in this population [9]. Therefore, it appears that other factors, e.g. traditional CV risk factors, might still have a role in this context.

Several factors potentially impacting on the pooled results were examined in sub-group analysis. A relatively higher risk of CV morbidity was observed in patients aged ≥55 years (RR 2.35 compared to those <55 years of age RR 2.04). This is consistent with the results of a recent prospective cohort study, showing that older RA patients had rapid disease progression and higher CV morbidity [66]. Interestingly, studies published after 2007 showed a higher impact of hypertension on CV morbidity (RR 2.68 compared to those studies published before or in 2007 RR 1.61). RA participants included in studies published after 2007 were followed between 2006 and 2008, which is known as Global Financial Crisis period. This economic crisis was linked to higher mortality rate in the general population [67]. It is possible that the observed high CV morbidity in studies published after 2007 was due to the impact of hypertension associated with financial stress; this imply that increased CV morbidity might be explained by the impact of CV risk factors. The widespread use of biologics in the last ten years is also a possible contributor to the variability in the observed effects of hypertension on CV morbidity. Although the use of biologics may more effectively reduce blood pressure among RA patients [68] our sub-group analysis of biologic versus non-biologic therapy on CV morbidity shows an increased risk amongst those using biologics. Clearly, further research is needed to corroborate these findings. Additionally, a trend towards increased CV risk was documented in patients with shorter disease duration (<5 years). Although this is in contrast with a report suggesting a higher CV risk with longer disease duration [65], recent evidence supports the concept of higher risk in the first few years of RA diagnosis [69]. It is possible that most RA patients are not appropriately treated with MTX during this critical period, i.e. within two years of diagnosis [66]. The importance of MTX was emphasized by results obtained from the sub group analysis; amongst studies assessing hypertensive RA patients treated with MTX alone, the risk of combined CV morbidity was slightly lower than all studies. Thus, MTX might have a protective effect in this population compared to other treatments for RA. Notably, hypertensive RA patients on treatment combination with biologics were approximately two times more likely as those patients on treatment combination without biologics to develop CV outcomes. Although some claim that biologics treatment, particularly tumour necrosis factor (TNF) blockers, reduces the risk of first CV event in RA patients [70], others have found no such association [71–75]. The reported protective effect of TNF blocker [70] may be biased as several important risk factors were not controlled for in this study. Furthermore, the protective effect of biologics might be explained by immortal time bias in which patients receiving biologics might have differentially had a shorter exposure period and longer pre-exposure assigned them than their control group counterparts. RA patients are initially started on non-biologics drugs, and therefore pre-exposure and follow-up time might be differentially classified between biologics and non-biologics group. Moreover, RA patients with complications such as infections may stop medication, causing differential loss to follow-up where selection bias occurs despite effective control for potential confounders [40]. Once again, further larger studies are required to investigate the impact of different RA treatment strategies on hypertension and cardiovascular risk in this group.

This systematic review and meta-analysis has some limitations. Studies included had different participant's age at enrolment, RA duration and treatments type. These factors might be a

potential confounders producing biased effect estimate. However, sub-group analysis was conducted and the trend of having higher risk of combined CV morbidity in RA patients was retained.

In conclusion, our meta-analysis indicates that despite the increased CV risk associated with RA in general, traditional CV risk factors such as hypertension, T2D, smoking, hypercholesterolaemia and obesity, independently increase the risk of CV morbidity in this patient population, and the magnitude of this increase appears similar to that observed in the general population. This suggests that a careful diagnosis and management of CV risk factors should be considered as important as the management of the symptoms of RA in mitigating the risk of CV morbidity and mortality amongst these patients.

Supporting Information

S1 Fig. Forest plot depicting the relative risk of combined CV events in hypertensive RA patients versus those without hypertension using random effect model.
(TIFF)

S2 Fig. Forest plot depicting the relative risk of combined CV events in diabetic RA patients versus those without T2D using random effect model.
(TIFF)

S3 Fig. Forest plot depicting the relative risk of combined CV events in RA patients who smoke versus non-smokers using random effect model.
(TIFF)

S4 Fig. Forest plot depicting the relative risk of combined CV events in RA patients with hypercholesterolaemia versus those without hypercholesterolaemia using random effect model.
(TIFF)

S5 Fig. Forest plot depicting the relative risk of combined CV events in obese RA patients versus s those without obesity using random effect model.
(TIFF)

S6 Fig. Forest plot depicting the relative risk of combined CV events in physical inactive RA patients versus physical active using random effect model.
(TIFF)

S1 Materials. PRISMA 2009 flow diagram.
(PDF)

S2 Materials. PRISMA 2009 checklist.
(DOC)

S3 Materials. Appendix 1.
(DOCX)

S1 Table. Checklist for assessing quality of included studies in the meta-analysis.
(DOCX)

S2 Table. Excluded studies and reasons of exclusion.
(DOCX)

S3 Table. Exposures and outcomes in studies included in the meta-analysis.
(DOCX)

S4 Table. Effect estimates of myocardial infarction in RA patients compared to general population.
(DOCX)

Author Contributions

Conceived and designed the experiments: LB RW AAM. Performed the experiments: LB. Analyzed the data: LB RW. Wrote the paper: LB RW EMS AAM. Data interpretation: RW EMS AAM.

References

1. Firestein GS (2003) Evolving concepts of rheumatoid arthritis. Nature 423: 356–361. PMID: 12748655
2. Solomon DH, Karlson EW, Rimm EB, Cannuscio CC, Mandl LA, et al. (2003) Cardiovascular morbidity and mortality in women diagnosed with rheumatoid arthritis. Circulation 107: 1303–1307. PMID: 12628952
3. Avina-Zubieta JA, Thomas J, Sadatsafavi M, Lehman AJ, Lacaille D (2012) Risk of incident cardiovascular events in patients with rheumatoid arthritis: a meta-analysis of observational studies. Ann Rheum Dis 71: 1524–1529. doi: 10.1136/annrheumdis-2011-200726 PMID: 22425941
4. Meune C, Touze E, Trinquart L, Allanore Y (2009) Trends in cardiovascular mortality in patients with rheumatoid arthritis over 50 years: a systematic review and meta-analysis of cohort studies. Rheumatology (Oxford) 48: 1309–1313. doi: 10.1093/rheumatology/kep252 PMID: 19696061
5. Del Rincon I, Williams K, Stern MP, Freeman GL, O'Leary DH, et al. (2003) Association between carotid atherosclerosis and markers of inflammation in rheumatoid arthritis patients and healthy subjects. Arthritis Rheum 48: 1833–1840. PMID: 12847676
6. Maki-Petaja KM, Hall FC, Booth AD, Wallace SM, Yasmin, et al. (2006) Rheumatoid arthritis is associated with increased aortic pulse-wave velocity, which is reduced by anti-tumor necrosis factor-alpha therapy. Circulation 114: 1185–1192. PMID: 16952987
7. Kerekes G, Szekanecz Z, Der H, Sandor Z, Lakos G, et al. (2008) Endothelial dysfunction and atherosclerosis in rheumatoid arthritis: a multiparametric analysis using imaging techniques and laboratory markers of inflammation and autoimmunity. J Rheumatol 35: 398–406. PMID: 18203326
8. Rho YH, Chung CP, Oeser A, Solus J, Asanuma Y, et al. (2009) Inflammatory mediators and premature coronary atherosclerosis in rheumatoid arthritis. Arthritis Rheum 61: 1580–1585. doi: 10.1002/art.25009 PMID: 19877084
9. Gabriel SE, Crowson CS (2012) Risk factors for cardiovascular disease in rheumatoid arthritis. Curr Opin Rheumatol 24: 171–176. doi: 10.1097/BOR.0b013e32834ff2fd PMID: 22249350
10. Assous N, Touze E, Meune C, Kahan A, Allanore Y (2007) Cardiovascular disease in rheumatoid arthritis: single-center hospital-based cohort study in France. Joint Bone Spine 74: 66–72. PMID: 17174586
11. Brady SR, de Courten B, Reid CM, Cicuttini FM, de Courten MP, et al. (2009) The role of traditional cardiovascular risk factors among patients with rheumatoid arthritis. J Rheumatol 36: 34–40. doi: 10.3899/jrheum.080404 PMID: 19012364
12. Innala L, Moller B, Ljung L, Magnusson S, Smedby T, et al. (2011) Cardiovascular events in early RA are a result of inflammatory burden and traditional risk factors: a five year prospective study. Arthritis Res Ther 13: R131. doi: 10.1186/ar3442 PMID: 21843325
13. Metsios GS, Stavropoulos-Kalinoglou A, Panoulas VF, Wilson M, Nevill AM, et al. (2009) Association of physical inactivity with increased cardiovascular risk in patients with rheumatoid arthritis. Eur J Cardiovasc Prev Rehabil 16: 188–194. doi: 10.1097/HJR.0b013e3283271ceb PMID: 19238083
14. Mohammad A, Hartery K, Bond U, Phelan M (2010) Increased occurrence of cardiovascular events and comorbidities in a general rheumatology cohort. Irish Journal of Medical Science 179: 273–276. doi: 10.1007/s11845-010-0475-5 PMID: 20186515
15. Serelis J, Panagiotakos DB, Mavrommati M, Skopouli FN (2011) Cardiovascular disease is related to hypertension in patients with rheumatoid arthritis: a greek cohort study. J Rheumatol 38: 236–241. doi: 10.3899/jrheum.100564 PMID: 21078723
16. Kremers HM, Crowson CS, Therneau TM, Roger VL, Gabriel SE (2008) High ten-year risk of cardiovascular disease in newly diagnosed rheumatoid arthritis patients: a population-based cohort study. Arthritis Rheum 58: 2268–2274. doi: 10.1002/art.23650 PMID: 18668561

17. Wallberg-Jonsson S, Ohman ML, Dahlqvist SR (1997) Cardiovascular morbidity and mortality in patients with seropositive rheumatoid arthritis in Northern Sweden. J Rheumatol 24: 445–451. PMID: 9058647

18. Boyer JF, Gourraud PA, Cantagrel A, Davignon JL, Constantin A (2011) Traditional cardiovascular risk factors in rheumatoid arthritis: a meta-analysis. Joint Bone Spine 78: 179–183. doi: 10.1016/j.jbspin.2010.07.016 PMID: 20851020

19. del Rincon ID, Williams K, Stern MP, Freeman GL, Escalante A (2001) High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. Arthritis Rheum 44: 2737–2745. PMID: 11762933

20. Nadareishvili Z, Michaud K, Hallenbeck JM, Wolfe F (2008) Cardiovascular, rheumatologic, and pharmacologic predictors of stroke in patients with rheumatoid arthritis: a nested, case-control study. Arthritis Rheum 59: 1090–1096. doi: 10.1002/art.23935 PMID: 18668583

21. Koivuniemi R, Paimela L, Suomalainen R, Leirisalo-Repo M (2013) Cardiovascular diseases in patients with rheumatoid arthritis. Scand J Rheumatol 42: 131–135. doi: 10.3109/03009742.2012.723747 PMID: 23244227

22. Wolfe F (2000) The effect of smoking on clinical, laboratory, and radiographic status in rheumatoid arthritis. J Rheumatol 27: 630–637. PMID: 10743800

23. McEntegart A, Capell HA, Creran D, Rumley A, Woodward M, et al. (2001) Cardiovascular risk factors, including thrombotic variables, in a population with rheumatoid arthritis. Rheumatology 40: 640–644. PMID: 11426020

24. Maradit-Kremers H, Nicola PJ, Crowson CS, Ballman KV, Gabriel SE (2005) Cardiovascular death in rheumatoid arthritis: a population-based study. Arthritis Rheum 52: 722–732. PMID: 15751097

25. Gonzalez A, Maradit Kremers H, Crowson CS, Ballman KV, Roger VL, et al. (2008) Do cardiovascular risk factors confer the same risk for cardiovascular outcomes in rheumatoid arthritis patients as in non-rheumatoid arthritis patients? Ann Rheum Dis 67: 64–69. PMID: 17517756

26. Hurt-Camejo E, Paredes S, Masana L, Camejo G, Sartipy P, et al. (2001) Elevated levels of small, low-density lipoprotein with high affinity for arterial matrix components in patients with rheumatoid arthritis: possible contribution of phospholipase A2 to this atherogenic profile. Arthritis Rheum 44: 2761–2767. PMID: 11762936

27. Choi HK, Seeger JD (2005) Lipid profiles among US elderly with untreated rheumatoid arthritis—the Third National Health and Nutrition Examination Survey. J Rheumatol 32: 2311–2316. PMID: 16331755

28. Park YB, Lee SK, Lee WK, Suh CH, Lee CW, et al. (1999) Lipid profiles in untreated patients with rheumatoid arthritis. J Rheumatol 26: 1701–1704. PMID: 10451065

29. Heldenberg D, Caspi D, Levtov O, Werbin B, Fishel B, et al. (1983) Serum lipids and lipoprotein concentrations in women with rheumatoid arthritis. Clin Rheumatol 2: 387–391. PMID: 6678199

30. Semb AG, Kvien TK, Aastveit AH, Jungner I, Pedersen TR, et al. (2010) Lipids, myocardial infarction and ischaemic stroke in patients with rheumatoid arthritis in the Apolipoprotein-related Mortality RISk (AMORIS) Study. Ann Rheum Dis 69: 1996–2001. doi: 10.1136/ard.2009.126128 PMID: 20551156

31. Steiner G, Urowitz MB (2009) Lipid profiles in patients with rheumatoid arthritis: mechanisms and the impact of treatment. Semin Arthritis Rheum 38: 372–381. doi: 10.1016/j.semarthrit.2008.01.015 PMID: 18395771

32. Lakatos J, Harsagyi A (1988) Serum total, HDL, LDL cholesterol, and triglyceride levels in patients with rheumatoid arthritis. Clin Biochem 21: 93–96. PMID: 3390902

33. Kremers HM, Nicola PJ, Crowson CS, Ballman KV, Gabriel SE (2004) Prognostic importance of low body mass index in relation to cardiovascular mortality in rheumatoid arthritis. Arthritis Rheum 50: 3450–3457. PMID: 15529378

34. Metsios GS, Stavropoulos-Kalinoglou A, Panoulas VF, Sandoo A, Toms TE, et al. (2009) Rheumatoid cachexia and cardiovascular disease. Clinical and Experimental Rheumatology 27: 985–988. PMID: 20149317

35. Ajeganova S, Andersson ML, Hafström I (2013) Association of obesity with worse disease severity in rheumatoid arthritis as well as with comorbidities: A long-term followup from disease onset. Arthritis Care and Research 65: 78–87. doi: 10.1002/acr.21710 PMID: 22514159

36. Naranjo A, Sokka T, Descalzo MA, Calvo-Alen J, Horslev-Petersen K, et al. (2008) Cardiovascular disease in patients with rheumatoid arthritis: results from the QUEST-RA study. Arthritis Res Ther 10: R30. doi: 10.1186/ar2383 PMID: 18325087

37. Dahabreh IJ, Kent DM (2011) Index event bias as an explanation for the paradoxes of recurrence risk research. JAMA 305: 822–823. doi: 10.1001/jama.2011.163 PMID: 21343582

38. Hernandez-Diaz S, Schisterman EF, Hernan MA (2006) The birth weight "paradox" uncovered? Am J Epidemiol 164: 1115–1120. Epub 2006 Aug 1124. PMID: 16931543

39. VanderWeele TJ, Mumford SL, Schisterman EF (2012) Conditioning on intermediates in perinatal epidemiology. Epidemiology 23: 1–9. doi: 10.1097/EDE.0b013e31823aca5d PMID: 22157298

40. Choi HK, Nguyen US, Niu J, Danaei G, Zhang Y (2014) Selection bias in rheumatic disease research. Nat Rev Rheumatol 10: 403–412. Epub 2014 Apr 1031. doi: 10.1038/nrrheum.2014.36 PMID: 24686510

41. Doi SA, Thalib L (2008) A quality-effects model for meta-analysis. Epidemiology 19: 94–100. PMID: 18090860

42. Baghdadi LR, Abu Hashim H, Amer SA, Palomba S, Falbo A, et al. (2012) Impact of obesity on reproductive outcomes after ovarian ablative therapy in PCOS: a collaborative meta-analysis. Reprod Biomed Online 25: 227–241. doi: 10.1016/j.rbmo.2012.05.010 PMID: 22809865

43. Takkouche B, Cadarso-Suarez C, Spiegelman D (1999) Evaluation of old and new tests of heterogeneity in epidemiologic meta-analysis. Am J Epidemiol 150: 206–215. PMID: 10412966

44. Higgins J, Green S (2011) Cochrane Handbook for Systematic Reviews of Interventions. 5.1.0 [updated March 2011]: 151–152.

45. Kremers HM, Nicola PJ, Crowson CS, Ballman KV, Gabriel SE (2004) Prognostic importance of low body mass index in relation to cardiovascular mortality in rheumatoid arthritis. Arthritis Rheum 50: 3450–3457. PMID: 15529378

46. Maradit-Kremers H, Crowson CS, Nicola PJ, Ballman KV, Roger VL, et al. (2005) Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a population-based cohort study. Arthritis Rheum 52: 402–411. PMID: 15693010

47. Radovits BJ, Popa-Diaconu DA, Popa C, Eijsbouts A, Laan RF, et al. (2009) Disease activity as a risk factor for myocardial infarction in rheumatoid arthritis. Ann Rheum Dis 68: 1271–1276. doi: 10.1136/ard.2008.089862 PMID: 18701555

48. Wallberg-Jonsson S, Johansson H, Ohman ML, Rantapaa-Dahlqvist S (1999) Extent of inflammation predicts cardiovascular disease and overall mortality in seropositive rheumatoid arthritis. A retrospective cohort study from disease onset. J Rheumatol 26: 2562–2571. PMID: 10606363

49. Greenland P, Knoll MD, Stamler J, Neaton JD, Dyer AR, et al. (2003) Major risk factors as antecedents of fatal and nonfatal coronary heart disease events. JAMA 290: 891–897. PMID: 12928465

50. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, et al. (2004) Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 364: 937–952. PMID: 15364185

51. Pais PS (2006) Early intervention and prevention of myocardial infarction. J Hypertens Suppl 24: S25–30. PMID: 16601557

52. Thom T, Haase N, Rosamond W, Howard VJ, Rumsfeld J, et al. (2006) Heart disease and stroke statistics—2006 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 113: e85–151. PMID: 16407573

53. Panoulas VF, Douglas KM, Milionis HJ, Stavropoulos-Kalinglou A, Nightingale P, et al. (2007) Prevalence and associations of hypertension and its control in patients with rheumatoid arthritis. Rheumatology (Oxford) 46: 1477–1482. PMID: 17704521

54. Panoulas VF, Metsios GS, Pace AV, John H, Treharne GJ, et al. (2008) Hypertension in rheumatoid arthritis. Rheumatology (Oxford) 47: 1286–1298. doi: 10.1093/rheumatology/ken159 PMID: 18467370

55. Wolfe F, Freundlich B, Straus WL (2003) Increase in cardiovascular and cerebrovascular disease prevalence in rheumatoid arthritis. J Rheumatol 30: 36–40. PMID: 12508387

56. Singh G, Miller JD, Huse DM, Pettitt D, D'Agostino RB, et al. (2003) Consequences of increased systolic blood pressure in patients with osteoarthritis and rheumatoid arthritis. J Rheumatol 30: 714–719. PMID: 12672188

57. Koskinen P, Manttari M, Manninen V, Huttunen JK, Heinonen OP, et al. (1992) Coronary heart disease incidence in NIDDM patients in the Helsinki Heart Study. Diabetes Care 15: 820–825. PMID: 1516498

58. Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, et al. (2010) Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. Lancet 375: 2215–2222. doi: 10.1016/S0140-6736(10)60484-9 PMID: 20609967

59. Barrett-Connor EL, Cohn BA, Wingard DL, Edelstein SL (1991) Why is diabetes mellitus a stronger risk factor for fatal ischemic heart disease in women than in men? The Rancho Bernardo Study. JAMA 265: 627–631. PMID: 1987413

60. Lewis SJ, Cherry NM, Mc LNR, Barber PV, Wilde K, et al. (2003) Cotinine levels and self-reported smoking status in patients attending a bronchoscopy clinic. Biomarkers 8: 218–228. PMID: 12944174

61. Ekdahl C, Broman G (1992) Muscle strength, endurance, and aerobic capacity in rheumatoid arthritis: a comparative study with healthy subjects. Ann Rheum Dis 51: 35–40. PMID: 1540034

62. de Jong Z, Munneke M, Zwinderman AH, Kroon HM, Ronday KH, et al. (2004) Long term high intensity exercise and damage of small joints in rheumatoid arthritis. Ann Rheum Dis 63: 1399–1405. PMID: 15479889

63. Metsios GS, Stavropoulos-Kalinoglou A, Veldhuijzen van Zanten JJ, Treharne GJ, Panoulas VF, et al. (2008) Rheumatoid arthritis, cardiovascular disease and physical exercise: a systematic review. Rheumatology (Oxford) 47: 239–248. Epub 2007 Nov 2028. PMID: 18045810

64. Eurenius E, Stenstrom CH (2005) Physical activity, physical fitness, and general health perception among individuals with rheumatoid arthritis. Arthritis Rheum 53: 48–55. PMID: 15696555

65. Peters MJ, Symmons DP, McCarey D, Dijkmans BA, Nicola P, et al. (2010) EULAR evidence-based recommendations for cardiovascular risk management in patients with rheumatoid arthritis and other forms of inflammatory arthritis. Ann Rheum Dis 69: 325–331. Epub 112009 Sep 113622. doi: 10.1136/ard.2009.113696 PMID: 19773290

66. Ajeganova S, Andersson ML, Frostegard J, Hafstrom I (2013) Disease Factors in Early Rheumatoid Arthritis Are Associated with Differential Risks for Cardiovascular Events and Mortality Depending on Age at Onset: A 10-year Observational Cohort Study. J Rheumatol 15: 15.

67. Stuckler D, Meissner CM, King LP (2008) Can a bank crisis break your heart? Global Health 4: 1. doi: 10.1186/1744-8603-4-1 PMID: 18197979

68. Klarenbeek NB, van der Kooij SM, Huizinga TJ, Goekoop-Ruiterman YP, Hulsmans HM, et al. (2010) Blood pressure changes in patients with recent-onset rheumatoid arthritis treated with four different treatment strategies: a post hoc analysis from the BeSt trial. Ann Rheum Dis 69: 1342–1345. doi: 10.1136/ard.2009.124180 PMID: 20472597

69. Arts EE, Fransen J, den Broeder AA, Popa CD, van Riel PL (2014) The effect of disease duration and disease activity on the risk of cardiovascular disease in rheumatoid arthritis patients. Ann Rheum Dis 23: 2013–204531.

70. Jacobsson LT, Turesson C, Gulfe A, Kapetanovic MC, Petersson IF, et al. (2005) Treatment with tumor necrosis factor blockers is associated with a lower incidence of first cardiovascular events in patients with rheumatoid arthritis. J Rheumatol 32: 1213–1218. PMID: 15996054

71. Suissa S, Bernatsky S, Hudson M (2006) Antirheumatic drug use and the risk of acute myocardial infarction. Arthritis Rheum 55: 531–536. PMID: 16874796

72. Davis JM, Maradit Kremers H, Crowson CS, Nicola PJ, Ballman KV, et al. (2007) Glucocorticoids and cardiovascular events in rheumatoid arthritis: A population-based cohort study. Arthritis & Rheumatism 56: 820–830. doi: 10.3892/ijmm.2015.2079 PMID: 25626121

73. Greenberg J (2006) Association of duration of TNF antagonist treatment with reduction in cardiovascular outcomes in RA patients. Arthritis and rheumatism 54. PMID: 17274110

74. Carmona L, Descalzo MA, Perez-Pampin E, Ruiz-Montesinos D, Erra A, et al. (2007) All-cause and cause-specific mortality in rheumatoid arthritis are not greater than expected when treated with tumour necrosis factor antagonists. Ann Rheum Dis 66: 880–885. Epub 2007 Feb 2026. PMID: 17324968

75. Dixon WG, Watson KD, Lunt M, Hyrich KL, Silman AJ, et al. (2007) Reduction in the incidence of myocardial infarction in patients with rheumatoid arthritis who respond to anti-tumor necrosis factor alpha therapy: results from the British Society for Rheumatology Biologics Register. Arthritis Rheum 56: 2905–2912. PMID: 17763428

Published conference abstract

ABSTRACT NUMBER: 473

Methotrexate, Blood Pressure and Arterial Wave Reflection in Rheumatoid Arthritis

Leena R Baghdadi[1,2,3], Richard J Woodman[2], **E Michael Shanahan**[4] and Arduino A Mangoni[3], [1]Department of Family and Community Medicine, King Saud University, Riyadh, Saudi Arabia, [2]Flinders Centre for Epidemiology and Biostatistics, Flinders University, Adelaide, Australia, [3]Department of Clinical Pharmacology, Flinders University, Adelaide, Australia, [4]Department of Rheumatology, Flinders University, Adelaide, Australia

Meeting: 2015 ACR/ARHP Annual Meeting

Date of first publication: September 29, 2015

Keywords: Atherosclerosis, Cardiovascular disease, methotrexate (MTX) and rheumatoid arthritis (RA)

SESSION INFORMATION

Date: Sunday, November 8, 2015

Session Type: ACR Poster Session A

Session Title: Rheumatoid Arthritis - Clinical Aspects Poster I

Session Time: 9:00AM-11:00AM

Background/Purpose: Methotrexate (MTX) use has been associated with reduced cardiovascular morbidity and mortality in patients with rheumatoid arthritis (RA). Although MTX has anti-inflammatory effects, there is little evidence to support additional salutary effects on markers of cardiovascular risk such as blood pressure (BP) and arterial wave reflection (augmentation index, AIx), an indirect marker of arterial stiffness. Observational studies have suggested a lower systolic BP (SBP) in MTX users, approximately 3 mmHg, but it is unknown whether this is independent of anti-inflammatory effects. Thus, the purpose is to investigate associations between MTX, SBP and AIx in RA patients.

Methods: Using a cross-sectional study design, a total of 86 patients with RA (diagnosed using the 2010 American College of Rheumatology criteria) were recruited from rheumatology outpatient clinics at Flinders Medical Centre and Repatriation General Hospital, Adelaide, Australia. Participants were classified according to MTX exposure: MTX and non-MTX (defined as off MTX for >1 year or MTX naïve).

Results: There were 56 MTX (70% female; mean age 61 years) and 30 non-MTX patients (76% female; mean age 63 years). After adjusting for age, mean ±SD clinical SBP was significantly lower in MTX patients (124±1.9 mmHg, $p<0.001$) compared to non-MTX patients (131±2.9 mmHg, $p<0.001$). There were similar differences for central SBP 115 ±1.9 vs. 122 ±3 mmHg, $p<0.001$). MTX use was also associated with lower AIx (28%, 95% CI= 25.7, 29.9 vs. 31%, 95% CI= 27.5, 34.5, $p<0.001$). Changes in the global inflammatory marker (ESR) and disease activity score (DAS28) and anti-CCP were not significantly associated with clinical SBP ($p=0.80$, 0.79 and 0.65) or AIx ($p=0.86$, 0.76 and 0.051), respectively. Changes in the short-term inflammatory marker CRP were associated with SBP ($\beta=0.42\pm0.17$, $p=0.02$), but not AIx ($\beta=0.86\pm0.1$, $p=0.37$).

Conclusion: Our findings show that the use of MTX is associated with lower systolic blood pressure, which is associated with reduced inflammation. The lower arterial wave reflection in MTX patients was independent of changes in inflammation.

Disclosure: L. R. Baghdadi, None; **R. J. Woodman**, None; **E. M. Shanahan**, None; **A. A. Mangoni**, None.

To cite this abstract in AMA style:

Baghdadi LR, Woodman RJ, Shanahan EM, Mangoni AA. Methotrexate, Blood Pressure and Arterial Wave Reflection in Rheumatoid Arthritis [abstract]. *Arthritis Rheumatol.* 2015; 67 (suppl 10). http://acrabstracts.org/abstract/methotrexate-blood-pressure-and-arterial-wave-reflection-in-rheumatoid-arthritis/. Accessed October 24, 2016.

Appendix-2 Studies' information sheets

Participant information sheet

Thank you for considering your participation in the project titled "The relationship between methotrexate, blood pressure and arterial markers in rheumatoid arthritis". This is a research project and your involvement is entirely your decision. If you do not wish to participate, your medical care will not be affected in any way.

Who is organising and funding the study?

Dr Leena Baghdadi, who is a physician at King Saud University in Saudi Arabia and a PhD student at Flinders University in Australia, is conducting this project. She is being supervised by Professor Arduino Mangoni, Associate Professor Richard Woodman and Associate Professor Michael Shanahan from Flinders University.

What is the study about?

The main aim of this study is to examine whether taking methotrexate drug can improve blood pressure and vascular function in rheumatoid arthritis. Methotrexate is a rheumatic drug that has the ability to reduce the inflammation associated with rheumatoid arthritis. In this study, we will examine if methotrexate has the ability to reduce the level of blood pressure and to improve vascular function.

Can Methotrexate drug reduce blood pressure?

A few small studies have shown that methotrexate drug can improve blood pressure. Those rheumatoid patients who are on methotrexate therapy had a reduction of three (3) mmHg in their blood pressure on average.

Things required from participantsYou will be asked to attend the clinic up to two (2) times over twelve (12) months. Each visit will take about one and a half hours (90 minutes) for

clinical assessments and about forty-five (45) minutes for the questionnaire and will involve performing a few procedures (see the next question). The research team will need access to your hospital notes so that we can view your medical and drugs history, and blood tests.

What will happen during each visit?

There will be an imbursement of $20 per patient per visit (travel cost). In each visit the following will be done:

Completing three (3) questionnaires asking you about some general information, your medication, your dietary habit and your quality of life. The questionnaires should take approximately forty-five (45) minutes to complete

Measuring your weight and height

Measuring your blood pressure

Counting your heart rate

Drawing blood to examine the level of some important markers; the amount of the blood is about one to two (1-2) teaspoons

Examining your central blood pressure:

Prior to a central blood pressure measurement, the following protocol is recommended:

No alcohol intake for 12 hours prior to measurement

No caffeine or tobacco use for four hours prior to measurement

Fasting for 12 hours is recommended, but a light meal (particularly in diabetics) is permissible, since patients should be comfortable and not be overly hungry

Drinking water is permissible

During central blood pressure measurement, the following should be done:

You will be sitting beside a table with your arm on the table and your palm facing upwards

You are required to have a rest for 10 minutes in the seated position

A cuff blood pressure will be taken

Before taking central pressure readings, you will have a rest for 1-2 minute and then a small probe, like a pencil, will be placed on your wrist (radial artery) to start capturing the data

Attaching a 24 hours blood pressure monitor, the following instruction should be followed:

You should have a shower before the appointment

You have to wear loose clothing

Blood pressure cuff is attached to a machine and you have to keep your arm still during inflation and deflation

Daily activity can be continued while wearing the monitor

The cuff will inflate every 30 minutes during predicted waking hours (daytime) and hourly during sleep (night-time)

You cannot see each blood pressure reading during the 24 hours monitoring

The monitor will “beep” if the reading is not recorded correctly due to arm movement and the reading will be retaken after one minute

You have to push Day/Night button as you are going to bed and when you get up in the morning

You are NOT allowed to do the following during the 24 hours monitoring:

Shower/ bath

Go swimming

To get monitor wet at any stage whilst wearing

You should return the monitor at the same time next day

During the 24 hours use of the monitor, you should keep a record of the time of:

any emotional feelings

the use of any medication

whether you were drinking coffee or tea or any drink containing caffeine when the 24 hour monitor automatically commenced the recording of blood pressure.

You should keep a record of the name of anti-rheumatic medications, current dose taken and if there are any changes in the dose of medications or shifting from one to another class of drugs. This should take approximately two (2) minutes

What are the benefits anticipated from this study?

You might not receive any direct benefit from participating in this study. However, the information collected from this study may help in improving blood pressure control among rheumatoid patients in the future and might advance our understanding of the relationship between blood pressure and methotrexate in rheumatoid arthritis patients.

What will happen to me at the end of the study?

Your access to normal medical care of your treating doctor will continue.

Are there risks involved in taking part in the study?

It is highly unlikely that you will experience any major risks. However, slight discomfort might be expected. During withdrawing blood sample, you might suffer from pain, or skin bruises at the site of needle puncture, and rarely infection or faint. Secondly, discomfort at the site of the blood pressure monitor's cuff might be experienced during the inflation. Lastly, minimal discomfort might be felt at your wrist while measuring your central pressure. If you experience any of these events, appropriate support will be offered.

You may feel some distress from participation in this study. If this occurs you may withdraw from this study if you wish and your care will not be affected in any way. By participating in this study you do not give up any of your legal rights.

What happens to the information I give?

All records containing personal information will remain confidential and no information which could lead to your identification will be released, except as required by law. You will be given a unique identification (ID) number which will be used on your questionnaires and measurements records. The questionnaires that you fill in will not have details of your name and address. The data from all the patients participating in the study will be presented as group data. In accordance with the usual practice, results of this study become the property of Flinders University. The researcher is intending to submit the results as part of PhD thesis and therefore it is intended to submit the results for publication in scientific journals and at conferences. No one will be able to identify you from the results. It is possible that the results may not be published for scientific or other reasons. Records and data about your participation in this study may be used for study purposes, to obtain regulatory approval for the study drug, or for further analyses in the future. All such records and your right to them will be protected in accordance with Australian law.

What happens to the blood collected and the results obtained?

All blood samples will be labelled with an ID number and a log of these will be kept in a separate secure location. Blood will be collected, stored, used and disposed of in accordance with the hospital policy. All blood samples will be labelled with an ID number and a log of these will be kept in a separate secure location. The amount of collected blood will be about one to two (1-2) teaspoons and it will be stored at -20°C at the Repatriation General Hospital laboratory until use.

All information will remain confidential and no information which could lead to your identification will be released, except as required by law.

We would like to stress that your participation in this study is not obligatory and you are, of course, free to discontinue participation at any time or to decline to answer particular questions.

Am I going to be informed of the results of the study?

All participants will be informed of the results of the study.

Who is to be contacted for further information about participation and the project?

Professor Arduino Mangoni Head of Clinical Pharmacology Department- Clinical Pharmacologist and General Physician, Flinders University and Flinders Medical Centre, Australia (Email: arduino.mangoni@flinders.edu.au or telephone +61 8 8204 7495).

What should I do if I have any Complaints?

This study has been reviewed by the Southern Adelaide Clinical Human Research Ethics Committee (Project number 76.14). If you wish to discuss the study with someone not directly involved, in particular in relation to policies, your rights as a participant, or should you

wish to make a confidential complaint, you may contact the Executive Officer, SAC HREC at the Flinders Medical Centre (8204 6453) or emailresearch.ethics@health.sa.gov.au.

Participant Information Sheet for Genetic Research and Tissue Banking

Project Title

The relationship between methotrexate (MTX), blood pressure and arterial markers in rheumatoid arthritis single-nucleotide polymorphisms (SNPs) of 10 methotrexate pathway genes

Researchers

You have already agreed to take part in a research project to test the effect of drug called Methotrexate on the blood pressure and arterial markers. This drug has been around for many years for the treatment of rheumatoid arthritis and other inflammatory disorders. This form is an invitation to take part in genetic testing to see how the methotrexate works. You will also be asked to donate a blood sample for future testing. This part of the research is optional. You can take part in the main project without agreeing this study if you do not want to participate. This will make no difference to your participation in the main research project or your treatment for rheumatoid arthritis.

This project is carried out under the guidelines set out by the National Statement on Ethical Conduct in Human Research and there is certain information we are required to give you. If you do not understand this explanation, please talk to the research staff. You may want to discuss this information and your involvement with family, friends or your usual doctor. Once you understand the research and agree to take part, you will need to sign a Consent Form. If you sign, you are saying that you understand and agree to participate in this part of the research. You will be given a copy of this information sheet and your signed Consent Form for your records.

If you agree to participate in this research project, we will take a blood sample of 4-5mL which is about one teaspoon during special sessions at the Repatriation General Hospital.

What is genetic research?

The cells of your body contain deoxyribonucleic acid, DNA for short. DNA is passed down from your parents. It carries the genes that determine physical features such as the colour of your hair and eyes. Differences in our genes help explain why we all look different. The DNA in most cells in your body is the same. The instructions for physical features are contained in your DNA.

This project will study the differences in participants' DNA to better to understand the relationship between methotrexate drug, blood pressure and vascular function.

What is pharmacogenetic testing?

Pharmacogenetic testing helps to understand how people react to a drug and how the drug works. It is not a test to diagnose a person for a genetic disease or risk of disease, and it is not the kind of test that would tell about relationships in a family. It is not directly used in medical care. The test is used to study how drugs work in different groups of people, and whether DNA may be involved in those differences.

What will the sample be used for?

If you agree to take part in the DNA testing, you are giving permission for the research team to use your samples unless you change your mind and withdraw them before the completion of the project.

The samples collected for this study will be enough both for the immediate study in relation to methotrexate polymorphism, and for storage of a sample for future research. There are different parts to this testing, and you can agree to any or all of them, by marking that you 'agree' or 'disagree' on the consent form before you sign it. Some of this research is for this specific project, and some involves storing your sample for research in the future, as we find out more about methotrexate polymorphism.

Type 1: Testing DNA in relation to methotrexate polymorphism for immediate study

In this part of the project, we will test DNA from your blood for single-nucleotide polymorphisms (SNPs) of 10 methotrexate pathway genes. No other testing will be done on your sample in this part of the research. Some information from your medical history may be provided in coded form, so that the results of the testing can be looked at in relation to your progress with methotrexate response. No information that could identify you will be given to anyone outside of the local research team, except for particular inspection and quality control purposes as explained below. Once this testing is complete, any remaining sample will be destroyed.

Type 2: Storage of DNA and general clinical information for future research

In this part of the project, you may agree to have us store the DNA from your blood to test for other genes that may be discovered in the future that might affect MTX response in rheumatoid arthritis. Only those genes will be tested. No other testing will be done on your sample. For this part of the testing the 'code key' that would be used to link your results with your clinical data will be removed so that you cannot be identified with the results. Some general clinical information (e.g. your age, sex, and severity of rheumatoid arthritis) will be stored anonymously with the sample. The sample will remain in storage for future research on single-nucleotide polymorphisms (SNPs) of other newly-discovered methotrexate pathway genes until it has been used up.

Type 3: Storage of blood sample / DNA / general clinical information for future research

In this part of the project, you may agree to part of your sample and some general clinical information being stored for any kind of future research by the research team. If you agree to this part of the research, the part of your sample which is not needed for this research project and some general clinical information will be stored at the laboratory of pharmacology department at the Flinders University for use in future research. Unless at some later time you change your mind and withdraw your consent, your material and information will continue to be used for research by Prof. Arduino Mangoni and his research team until is it used up. For this part of the research, the 'code key' that is used to link your results to you will be removed, so you cannot be directly identified with your results. All research for which your sample will be used will have been approved by an ethics committee in Australia, but it will not necessarily relate to blood pressure and vascular function conditions.

Confidentiality

These tests will be done at the pharmacology laboratory at University of South Australia (UniSa), Adelaide.

For types 1 tests your stored blood sample will be labelled only with a code number. Your trial doctor will send your samples to the pharmacology laboratory at UniSa for testing, but will not include the code key that links your identity with your sample. The code key is a list of codes and the participants to whom they have been assigned. This information will stay with the local research team.

If you agree to types 2 or 3 tests your code number will be replaced by a new code number which does not correspond to anything in the research project, so the samples will effectively be

anonymous. While genetic samples are never completely anonymous because they contain your unique DNA, once the identifying code has been changed, the sample can only be identified if there is another sample of your DNA to compare it with.

In each of these cases, general information about your age, sex and state of health will be kept with the information about your sample. This information will not be specific enough to identify you.

Information collected as part of the research study will be accessed only by staff members of the hospital who would normally have access to the records, and no information that could identify you will be passed on to anyone who is not either an employee of Southern Adelaide Health Service or a member of the research team.

Your trial doctor will keep your signed consent forms. These will be kept separate from your medical record. Representatives of the research team, SA Health, or the ethics committee, may at times access the records at your local research site to ensure that the research is being done correctly. Your results and samples will be kept securely at all times. Anyone who has access to your identified records is bound by law and by professional codes of conduct to keep your information confidential.

Results from this research will be published in various ways, including conference papers and journal articles, and if you agree to types 2 or 3 tests may in future be used in the development of treatments for rheumatoid arthritis including new drugs. They will not be published in a form that could identify you.

Your results may be sent to research partners in countries other than Australia. Some of these countries may not have the same levels of privacy protection as in Australia, but the researchers and Flinders University will ensure that your information is protected. Unless the law requires it, your information will not be given to employers, insurance companies or the public.

What if I change my mind later?

If you change your mind, you may ask for your samples to be destroyed. This can be done at any time. Once the research has been completed on types 1 tests, and the samples fortypes 2 and 3 tests have received new codes, there will be no way to tell which results are yours. If you withdraw your

samples, the results of any tests done so far will remain as part of the research, to ensure that the results are as useful as possible. What are the benefits?

There is unlikely to be any benefit to you from taking part in this testing, but it may help other people with rheumatoid arthritis later on. The results of the project are only for research, and cannot be used directly to assist your medical treatment.

What are the risks?

The risks are the same as for any standard blood sampling. There may be some pain and bruising, and (very rarely) infection. Some people faint when blood is drawn.

Taking part in DNA testing is not expected to affect your employment or health / life insurance as results will not be given to any third parties. Your results cannot be directly used to make a diagnosis about your health. However, if you ask for your test results, it is possible that you could later find out information about your health (or your relatives' health) that you (or they) did not want to know.

Compensation

If you suffer injury as a result of participation in this research project, compensation might be paid without litigation. However, such compensation is not automatic and you may have to take legal action to determine whether you should be paid.

What are the implications for me and my family?

Although this study is not intended to provide information about your individual health, it is possible that we might later find out information about risks of disease or other matters that might be relevant to you or members of your family. It is up to you whether you want to be contacted about this kind of information. Please think about this and indicate on the consent form whether you do or do not wish to be contacted if we later discover information that might be relevant to you.

Even if you do not wish to be notified, if information arises that could be important for you or your family, we may be obligated to contact you to confirm whether you wish to know or not.

If the research reveals that a member of your family may be at risk of a serious illness for which treatment is available, or likely to become available they can be contacted without your agreement, but only if an ethics committee has considered the evidence and approved the contact.

Other members of your family will only be contacted about the research project if you agree, and you will have the opportunity to contact them first. They will not be given results of your tests unless you authorise it.

There is no possibility that the research could give us unexpected information about your parentage or blood relationships to other family members.

How will my samples be stored?

All samples will be stored in a secure facility. Only authorised people from the research team will be able to access them.

Will I get my DNA test results?

The tests will be performed in a research laboratory, and they are not designed to give information about a particular person's health. Also, research laboratories are not set up to provide health information or counselling. For these reasons, you will not be given your DNA results, unless you specifically request them, or unless information arises that is of relevance to you or your family. You may ask your research doctor (in writing) for copies of your test results. If you ask for your genetic results it is possible that they may be included in your medical file. You will not be able to access results from types 2 and 3 testing, as these will not be linked with your identity.

Will I be paid for taking part?

You will not be paid for taking part in genetic testing or for the use of your samples or information, even if the results of the study are profitable for the research team. The results of the testing and any information that comes out of it are owned by the research team and their organisation.

For further information

If you would like more information about this study, now or later, you can contact: Prof. Arduino Mangoni on Tel: +61 8 8204 7495.

This study has been reviewed and approved by the Southern Adelaide Clinical Human Research Ethics Committee (Project number 76.14). Should you wish to discuss the study with someone not directly involved, in particular in relation to matters concerning policies, your rights as a participant, or should you wish to make a complaint, you may contact the Executive Officer on (08) 8204 6453 or email research.ethics@health.sa.gov.au

Appendix-3 Consent forms

CONSENT TO PARTICIPATION IN RESEARCH

I, ……give consent to my involvement in the research project: The relationship between methotrexate, blood pressure and arterial markers in rheumatoid arthritis.

I acknowledge the nature, purpose, and contemplated effects of the research project, especially as far as they affect me, have been fully explained to my satisfaction by

……
…… and my consent is given voluntarily.

I acknowledge that the detail(s) of the following have been explained to me, including indications of risks; any discomfort involved; anticipation of length of time; and the frequency with which they will be performed:

1. Accessing hospital notes to view my medical and drugs history, and blood tests

2. Taking medical and medication history, performing medical examinations and completing three questionnaires

3. Drawing blood to examine the level of some important markers

4. Examining my central blood pressure by using non-invasive technique (SphygmoCor)

5. Attaching a 24 hours blood pressure monitor (Mobil-O-Graph)

I have understood and am satisfied with the explanations that I have been given.

I have been provided with a written information sheet.

I understand that my involvement in this research project may not be of any direct benefit to me and that I may withdraw my consent at any stage without affecting my rights or the responsibilities of the researchers in any respect.

I declare that I am over the age of 18 years.

I acknowledge that I have been informed that should I receive an injury as a result of taking part in this study, I may need to start legal action to determine whether I should be paid.

Signature of Research Participant:
……Date:……..

I, ……have described to
……..

The research project and nature and effects of procedure(s) involved. In my opinion he/she understands the explanation and has freely given his/her consent.

Signature: ……..
Date: ……

Status in
Project:……
…..

Consent to participation in research

I, ...

(first or given names) (last name)

give consent to my involvement in Methotrexate polymorphism sub-study of the research project titled “The relationship between methotrexate, blood pressure and arterial markers in rheumatoid arthritis”

I acknowledge the nature, purpose and contemplated effects of the research project, especially as far as they affect me, have been fully explained to my satisfaction by

.....

(first or given name) (last name)

and my consent is given voluntarily.

I acknowledge that the details of the following have been explained to me, including indications of risks; any discomfort involved; anticipation of length of time; and the frequency with which they will be performed. I agree to the following procedures:

That a blood sample for genetic testing be taken and the following tests done as agreed below.

[Type 1]

I agree / do not agree to testing DNA from my blood sample blood for genes related to Methotrexate drug.

[Type 2]

I agree / do not agree that a stored blood sample may be used in future genetic research in relation to Methotrexate drug.

[Type 3]

I agree / do not agree that a stored blood sample may be used in any future genetic research approved by an appropriate ethics committee.

If the research discovers information relevant to me or my family, I agree / do not agree to be notified about the information.

I have understood and am satisfied with the explanations that I have been given.

I have been provided with a written information sheet.

I understand that my involvement in this research project may not be of any direct benefit to me and that I may withdraw my consent at any stage before the completion of the research project without affecting my rights or the responsibilities of the researchers in any respect.

I declare that I am over the age of 18 years.

I acknowledge that I have been informed that should I receive an injury as a result of taking part in this study, I may need to start legal action to determine whether I should be paid.

Signature of Research Participant:.....

Date:....

Research Team Member:

I,..... have described to .....

the research project and nature and effects of procedure(s) involved. In my opinion he/she understands the explanation and has freely given his/her consent.

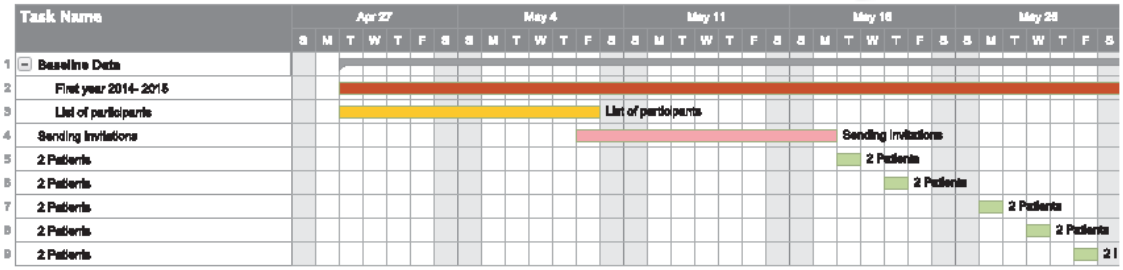
Signature:.....

Date:....

Status in project:.....

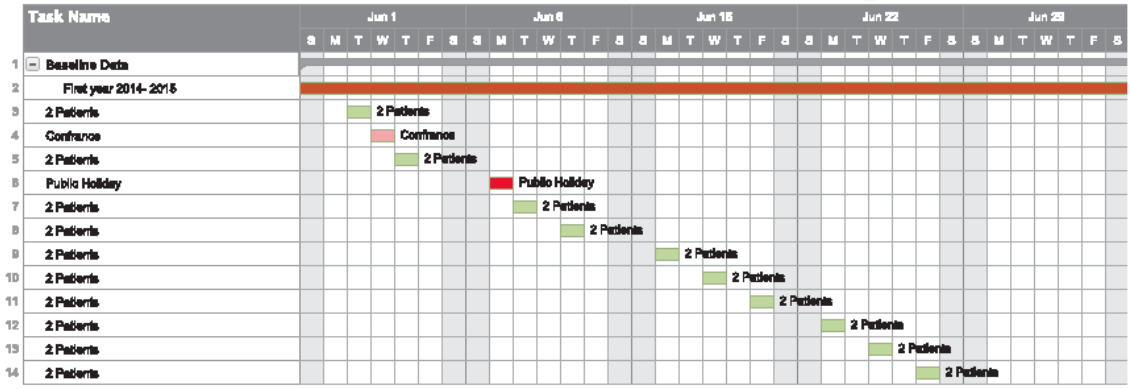
Appendix-4 Gantt charts for expected rate of patients' recruitments

May

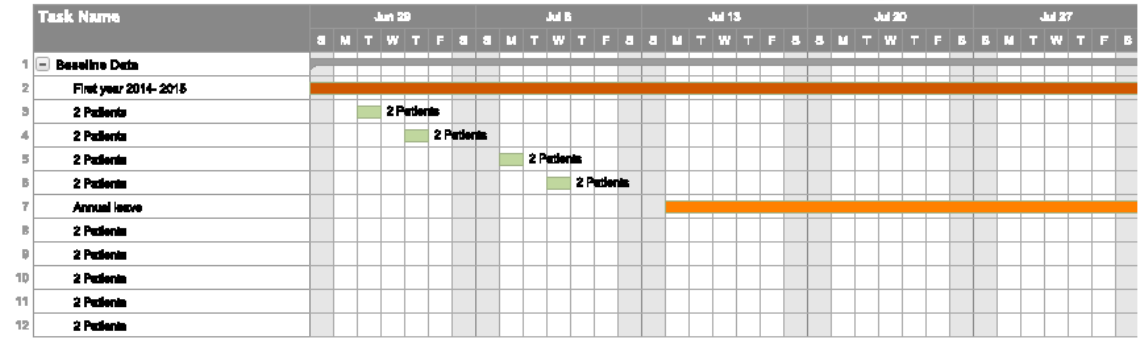


Exported on May 6, 2014 5:30:21 PM PDT

June

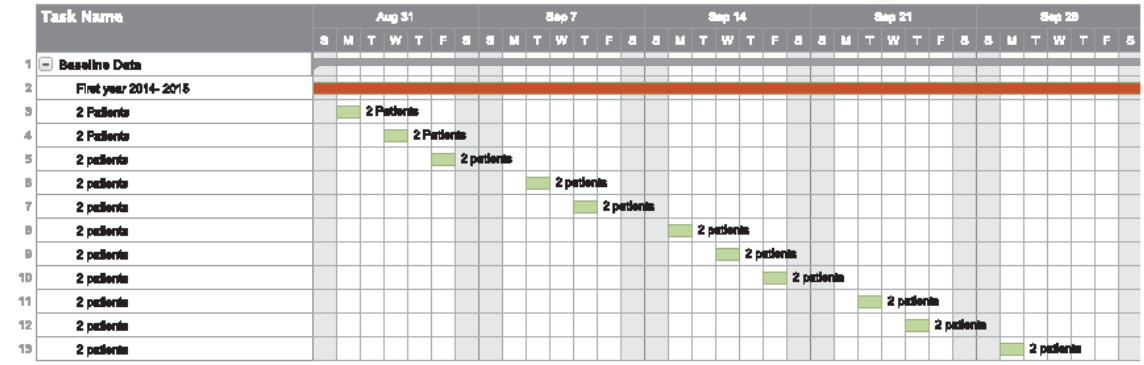

Task Name
Jun 1
Jun 8
Jun 15
Jun 22
Jun 29
S M T W T F S S M T W T F S S M T W T F S S M T W T F S S M T W T F S
1 Baseline Data
2 First year 2014- 2015
3 2 Patients
4 Confrance
5 2 Patients
6 Public Holiday
7 2 Patients
8 2 Patients
9 2 Patients
10 2 Patients
11 2 Patients
12 2 Patients
13 2 Patients
14 2 Patients

July-Aug



Exported on April 23, 2014 7:51:16 PM PDT

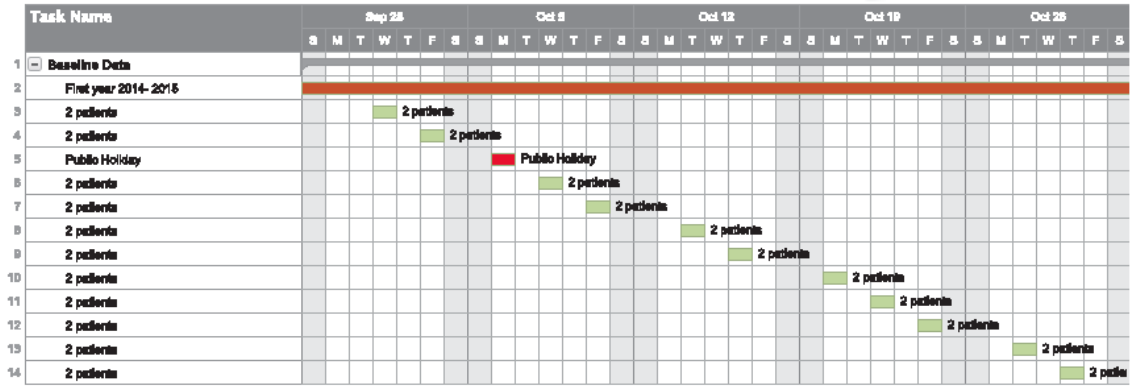
Sept



Exported on April 23, 2014 9:40:05 PM PDT

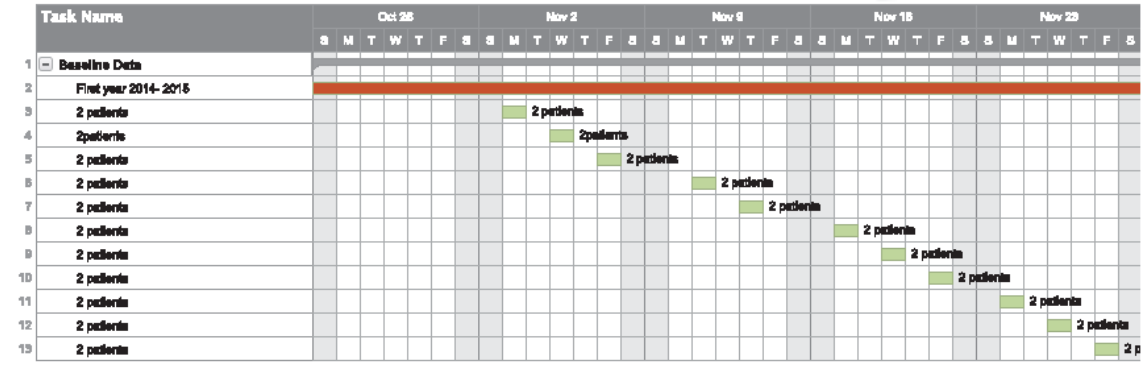
Oct

smartsheet



Exported on April 23, 2014 8:17:59 PM PDT

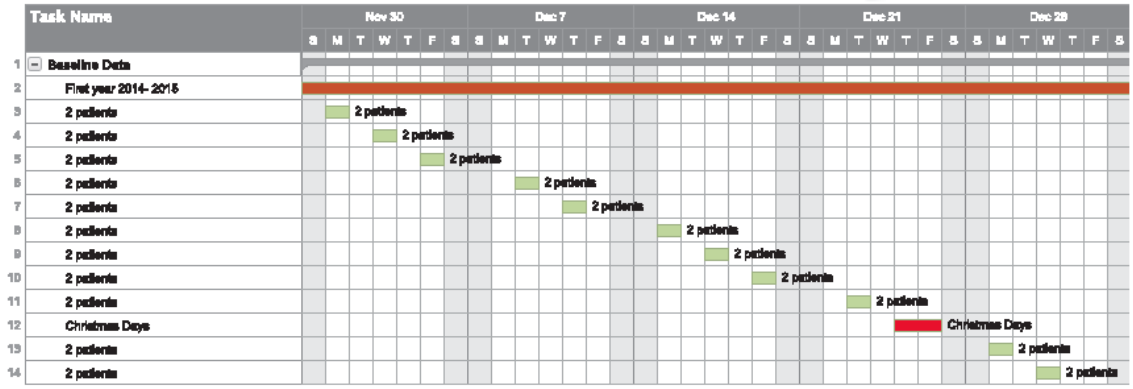
Nov



Exported on April 23, 2014 8:21:43 PM PDT

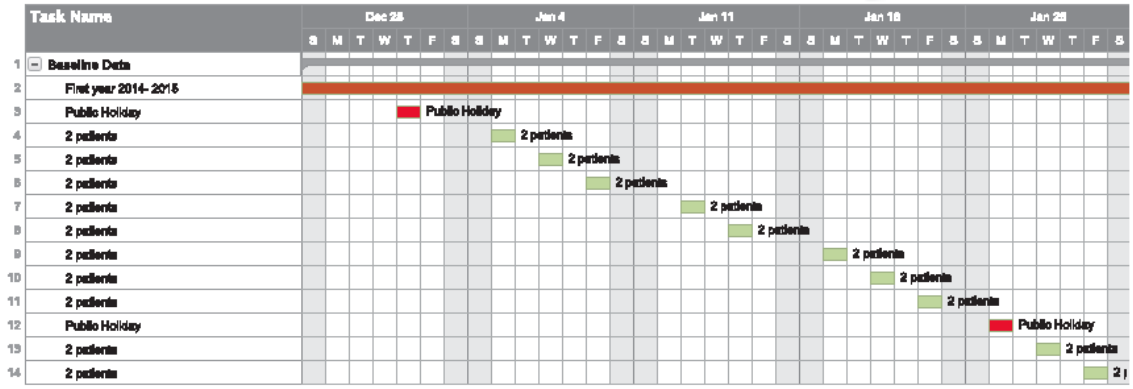
Dec

smartsheet



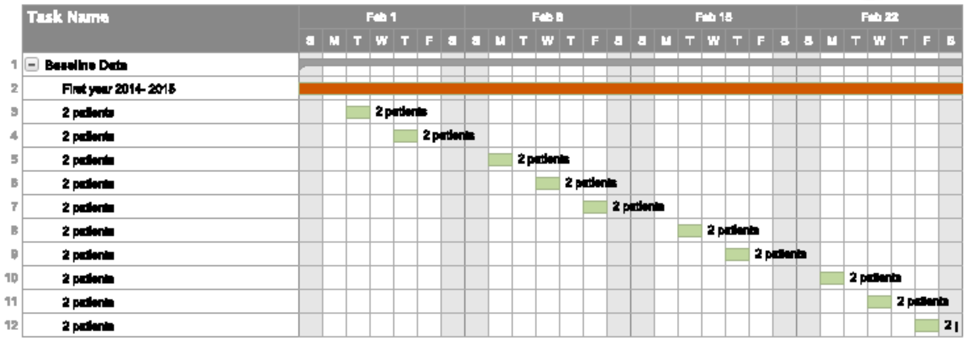
Exported on April 23, 2014 9:18:11 PM PDT

Jan



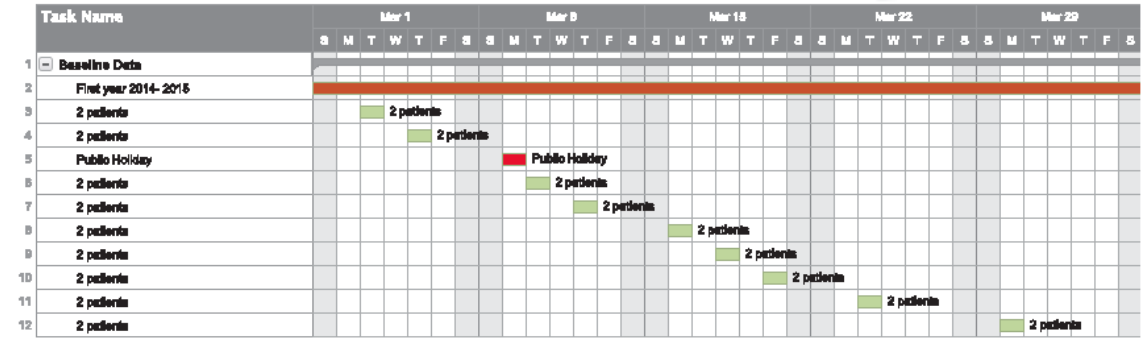
Exported on April 23, 2014 9:22:55 PM PDT

Feb



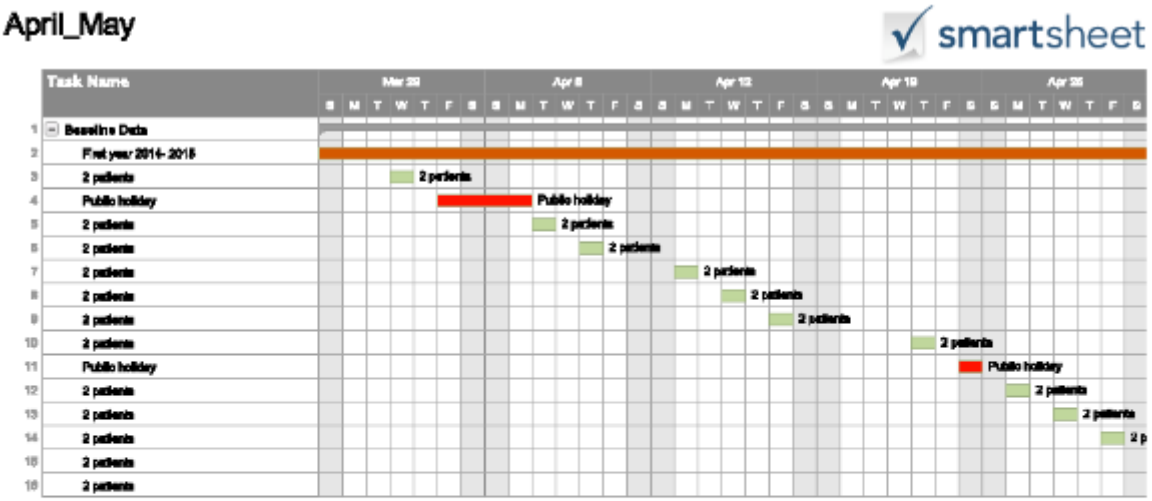
Exported on April 23, 2014 9:26:30 PM PDT

March



Exported on April 23, 2014 9:31:26 PM PDT

April_May



Exported on April 23, 2014 9:05:21 PM PDT

Appendix-5 The Stanford health assessment questionnaire (HAQ)

The STANFORD HEALTH ASSESSMENT QUESTIONNAIRE©
Stanford University School of Medicine, Division of Immunology & Rheumatology

HAQ Disability Index:
In this section we are interested in learning how your illness affects your ability to function in daily life. Please feel free to add any comments on the back of this page.

Please check the response which best describes your usual abilities OVER THE PAST WEEK:

	Without ANY difficulty[0]	With SOME difficulty[1]	With MUCH difficulty[2]	UNABLE to do[3]
DRESSING & GROOMING				
Are you able to:				
-Dress yourself, including tying shoelaces and doing buttons?	☐	☐	☐	☐
-Shampoo your hair?	☐	☐	☐	☐
ARISING				
Are you able to:				
-Stand up from a straight chair?	☐	☐	☐	☐
-Get in and out of bed?	☐	☐	☐	☐
EATING				
Are you able to:				
-Cut your meat?	☐	☐	☐	☐
-Lift a full cup or glass to your mouth?	☐	☐	☐	☐
-Open a new milk carton?	☐	☐	☐	☐
WALKING				
Are you able to:				
-Walk outdoors on flat ground?	☐	☐	☐	☐
-Climb up five steps?	☐	☐	☐	☐

Please check any AIDS OR DEVICES that you usually use for any of these activities:

- ☐ Cane
- ☐ Walker
- ☐ Crutches
- ☐ Wheelchair
- ☐ Devices used for dressing (button hook, zipper pul
  long-handled shoe horn, etc.)
- ☐ Built up or special utensils
- ☐ Special or built up chair
- ☐ Other (Specify:_____)

Please check any categories for which you usually need HELP FROM ANOTHER PERSON:

- ☐ Dressing and Grooming
- ☐ Arising
- ☐ Eating
- ☐ Walking

Please check the response which best describes your usual abilities **OVER THE PAST WEEK:**

	Without ANY difficulty [0]	With SOME difficulty [1]	With MUCH difficulty [2]	UNABLE to do [3]
HYGIENE				
Are you able to:				
-Wash and dry your body?	☐	☐	☐	☐
-Take a tub bath?	☐	☐	☐	☐
-Get on and off the toilet?	☐	☐	☐	☐
REACH				
Are you able to:				
-Reach and get down a 5-pound object (such as a bag of sugar) from just above your head?	☐	☐	☐	☐
-Bend down to pick up clothing from the floor?	☐	☐	☐	☐
GRIP				
Are you able to:				
-Open car doors?	☐	☐	☐	☐
-Open jars which have been previously opened?	☐	☐	☐	☐
-Turn faucets on and off?	☐	☐	☐	☐
ACTIVITIES				
Are you able to:				
-Run errands and shop?	☐	☐	☐	☐
-Get in and out of a car?	☐	☐	☐	☐
-Do chores such as vacuuming or yardwork	☐	☐	☐	☐

Please check any AIDS OR DEVICES that you usually use for any of these activities:

- ☐ Raised toilet seat
- ☐ Bathtub seat
- ☐ Jar opener (for jars previously opened)
- ☐ Bathtub bar
- ☐ Long-handled appliances for reach
- ☐ Long-handled appliances in bathroom
- ☐ Other (Specify:_____)

Please check any categories for which you usually need HELP FROM ANOTHER PERSON:

- ☐ Hygiene
- ☐ Reach
- ☐ Gripping and opening things
- ☐ Errands and chores

We are also interested in learning whether or not you are affected by pain because of your illness.
How much pain have you had because of your illness IN THE PAST WEEK:

PLACE A VERTICAL (|) MARK ON THE LINE TO INDICATE THE SEVERITY OF THE PAIN

No Pain — **Severe Pain**

0 — **100**

Considering all the ways that your arthritis affects you, rate how you are doing on the following scale by placing a vertical mark on the line.

Very Well — **Very Poor**

0 — **100**

Appendix-6 The Dietary Questionnaire for Epidemiological Studies Version 2 (DQES v2)

Dietary Questionnaire

QUESTIONS ABOUT WHAT YOU USUALLY EAT AND DRINK

Please fill in the date you completed this questionnaire:

DAY		MTH	YEAR
		○ JAN	○ 2004
		○ FEB	○ 2005
0	0	○ MAR	○ 2006
1	1	○ APR	○ 2007
2	2	○ MAY	○ 2008
3	3	○ JUN	○ 2009
	4	○ JUL	○ 2010
	5	○ AUG	○ 2011
	6	○ SEP	○ 2012
	7	○ OCT	○ 2013
	8	○ NOV	○ 2014
	9	○ DEC	○ 2015

INSTRUCTIONS:

This questionnaire is about your **usual** eating habits **over the past 12 months**. Where possible give only **one answer per question** for the type of food you eat **most often.**
(If you can't decide which type you have most often, answer for the types you usually eat.)

- Use a soft pencil only, preferably 2B.
- Do not use **any** biro or felt tip pen.
- Erase mistakes fully.
- Make no stray marks.

Please MARK LIKE THIS: ○■○○

1. How many pieces of fresh fruit do you usually eat per day? *(Count 1/2 cup of diced fruit, berries or grapes as one piece.)*

- ○ I didn't eat fruit
- ○ less than 1 piece of fruit per day
- ○ 1 piece of fruit per day
- ○ 2 pieces of fruit per day
- ○ 3 pieces of fruit per day
- ○ 4 or more pieces of fruit per day

2. How many different vegetables do you usually eat per day? *(Count all types, fresh, frozen or tinned.)*

- ○ less than 1 vegetable per day
- ○ 1 vegetable per day
- ○ 2 vegetables per day
- ○ 3 vegetables per day
- ○ 4 vegetables per day
- ○ 5 vegetables per day
- ○ 6 or more vegetables per day

3. What type of milk do you usually use?

- ○ none
- ○ full cream milk
- ○ reduced fat milk
- ○ skim milk
- ○ soya milk

4. How much milk do you usually use per day? *(Include flavoured milk and milk added to tea, coffee, cereal, etc.)*

- ○ none
- ○ less than 250 ml *(1 large cup or mug)*
- ○ between 250 and 500 ml *(1-2 cups)*
- ○ between 500 and 750 ml *(2-3 cups)*
- ○ 750 ml *(3 cups)* or more

5. What type of bread do you usually eat?

- ○ I don't eat bread
- ○ high fibre white bread
- ○ white bread
- ○ wholemeal bread
- ○ rye bread
- ○ multi-grain bread

6. How many slices of bread do you usually eat per day? *(Include all types, fresh or toasted and count one bread roll as 2 slices.)*

- ○ less than 1 slice per day
- ○ 1 slice per day
- ○ 2 slices per day
- ○ 3 slices per day
- ○ 4 slices per day
- ○ 5-7 slices per day
- ○ 8 or more slices per day

7. Which spread do you usually put on bread?

- ○ I don't usually use any fat spread
- ○ margarine of any kind
- ○ polyunsaturated margarine
- ○ monounsaturated margarine
- ○ butter and margarine blends
- ○ butter

8. On average, how many teaspoons of sugar do you usually use per day? *(Include sugar taken with tea and coffee and on breakfast cereal, etc.)*

- ○ none
- ○ 1 to 4 teaspoons per day
- ○ 5 to 8 teaspoons per day
- ○ 9 to 12 teaspoons per day
- ○ more than 12 teaspoons per day

9. On average, how many eggs do you usually eat per week?

- ○ I don't eat eggs
- ○ less than 1 egg per week
- ○ 1 to 2 eggs per week
- ○ 3 to 5 eggs per week
- ○ 6 or more eggs per week

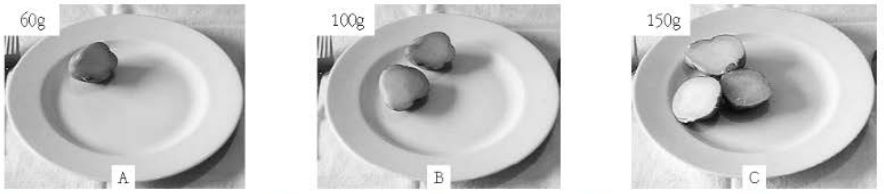
10. What types of cheese do you usually eat?

- ○ I don't eat cheese
- ○ hard cheeses, e.g. parmesan, romano
- ○ firm cheeses, e.g. cheddar, edam
- ○ soft cheeses, e.g. camembert, brie
- ○ ricotta or cottage cheese
- ○ cream cheese
- ○ low fat cheese

DO NOT WRITE IN THIS AREA.

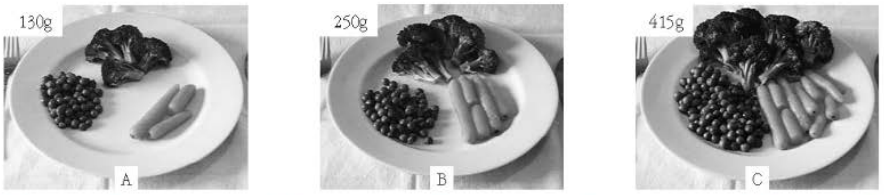
For each food shown on this page, indicate ***how much on average you would usually have eaten at main meals during the past 12 months.*** *When answering each question, think of the* ***amount*** *of that food you usually ate, even though you may rarely have eaten the food on its own.*
If you usually ate more than one helping, fill in the oval for the serving size closest to the ***total amount*** *you ate.*

11. When you ate potato, did you usually eat: ○ I never ate potato



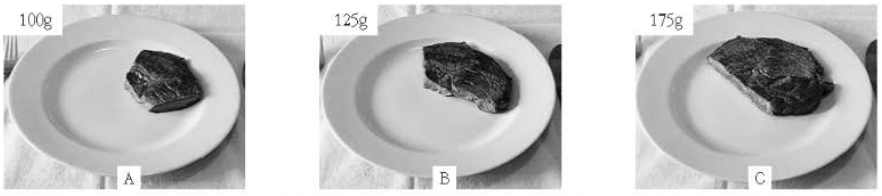
○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

12. When you ate vegetables, did you usually eat: ○ I never ate vegetables



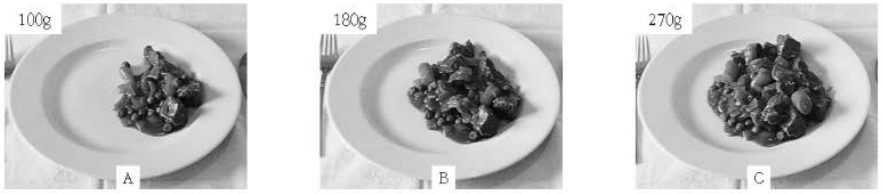
○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

13. When you ate steak, did you usually eat: ○ I never ate steak



○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

14. When you ate meat *or* vegetable casserole, did you usually eat: ○ I never ate casserole



○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

15. Over the last 12 months, on average, *how often* did you eat the following foods? *Please completely fill one oval in every line.* Please MARK LIKE THIS: ● NOT LIKE THIS: ✓ ✗

Times You Have Eaten		NEVER	less than once per month	1 to 3 times per month	1 time per week	2 times per week	3 to 4 times per week	5 to 6 times per week	1 time per day	2 times per day	3 or more times per day
CEREAL FOODS, SWEETS & SNACKS											
All Bran™	A1	○	○	○	○	○	○	○	○	○	○
Sultana Bran™, FibrePlus™, Branflakes™	A2	○	○	○	○	○	○	○	○	○	○
Weet Bix™, Vita Brits™, Weeties™	A3	○	○	○	○	○	○	○	○	○	○
Cornflakes, Nutrigrain™, Special K™	A4	○	○	○	○	○	○	○	○	○	○
Porridge	A5	○	○	○	○	○	○	○	○	○	○
Muesli	A6	○	○	○	○	○	○	○	○	○	○
Rice	A7	○	○	○	○	○	○	○	○	○	○
Pasta or noodles (include lasagne)	A8	○	○	○	○	○	○	○	○	○	○
Crackers, crispbreads, dry biscuits	A9	○	○	○	○	○	○	○	○	○	○
Sweet biscuits	A10	○	○	○	○	○	○	○	○	○	○
Cakes, sweet pies, tarts and other sweet pastries	A11	○	○	○	○	○	○	○	○	○	○
Meat pies, pasties, quiche and other savoury pastries	A12	○	○	○	○	○	○	○	○	○	○
Pizza	A13	○	○	○	○	○	○	○	○	○	○
Hamburger with a bun	A14	○	○	○	○	○	○	○	○	○	○
Chocolate	A15	○	○	○	○	○	○	○	○	○	○
Flavoured milk drink (cocoa, Milo™, etc.)	A16	○	○	○	○	○	○	○	○	○	○
Nuts	A17	○	○	○	○	○	○	○	○	○	○
Peanut butter or peanut paste	A18	○	○	○	○	○	○	○	○	○	○
Corn chips, potato crisps, Twisties™, etc.	A19	○	○	○	○	○	○	○	○	○	○
Jam, marmalade, honey or syrups	A20	○	○	○	○	○	○	○	○	○	○
Vegemite™, Marmite™ or Promite™	A21	○	○	○	○	○	○	○	○	○	○
DAIRY PRODUCTS, MEAT & FISH											
Cheese	B1	○	○	○	○	○	○	○	○	○	○
Ice-cream	B2	○	○	○	○	○	○	○	○	○	○
Yoghurt	B3	○	○	○	○	○	○	○	○	○	○
Beef	B4	○	○	○	○	○	○	○	○	○	○
Veal	B5	○	○	○	○	○	○	○	○	○	○
Chicken	B6	○	○	○	○	○	○	○	○	○	○
Lamb	B7	○	○	○	○	○	○	○	○	○	○
Pork	B8	○	○	○	○	○	○	○	○	○	○
Bacon	B9	○	○	○	○	○	○	○	○	○	○
Ham	B10	○	○	○	○	○	○	○	○	○	○
Corned beef, luncheon meats or salami	B11	○	○	○	○	○	○	○	○	○	○
Sausages or frankfurters	B12	○	○	○	○	○	○	○	○	○	○
Fish, steamed, grilled or baked	B13	○	○	○	○	○	○	○	○	○	○
Fish, fried (include take-away)	B14	○	○	○	○	○	○	○	○	○	○
Fish, tinned (salmon, tuna, sardines, etc.)	B15	○	○	○	○	○	○	○	○	○	○
FRUIT											
Tinned or frozen fruit (any kind)	C1	○	○	○	○	○	○	○	○	○	○
Fruit juice	C2	○	○	○	○	○	○	○	○	○	○
Oranges or other citrus fruit	C3	○	○	○	○	○	○	○	○	○	○
Apples	C4	○	○	○	○	○	○	○	○	○	○
Pears	C5	○	○	○	○	○	○	○	○	○	○
Bananas	C6	○	○	○	○	○	○	○	○	○	○
Watermelon, rockmelon (cantaloupe), honeydew, etc.	C7	○	○	○	○	○	○	○	○	○	○
Pineapple	C8	○	○	○	○	○	○	○	○	○	○
Strawberries	C9	○	○	○	○	○	○	○	○	○	○
Apricots	C10	○	○	○	○	○	○	○	○	○	○
Peaches or nectarines	C11	○	○	○	○	○	○	○	○	○	○
Mango or paw paw	C12	○	○	○	○	○	○	○	○	○	○
Avocado	C13	○	○	○	○	○	○	○	○	○	○

Times You Have Eaten CONTINUED

		NEVER	less than once per month	1 to 3 times per month	1 time per week	2 times per week	3 to 4 times per week	5 to 6 times per week	1 time per day	2 times per day	3 or more times per day
VEGETABLES (INCLUDING FRESH, FROZEN AND TINNED)											
Potatoes, roasted or fried (include hot chips)	D1	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Potatoes cooked without fat	D2	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tomato sauce, tomato paste or dried tomatoes	D3	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fresh or tinned tomatoes	D4	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Peppers (capsicum)	D5	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lettuce, endive, or other salad greens	D6	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cucumber	D7	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Celery	D8	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Beetroot	D9	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Carrots	D10	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cabbage or Brussels sprouts	D11	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cauliflower	D12	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Broccoli	D13	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Silverbeet or spinach	D14	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Peas	D15	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Green beans	D16	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Bean sprouts or alfalfa sprouts	D17	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Baked beans	D18	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Soy beans, soy bean curd or tofu	D19	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other beans (include chick peas, lentils, etc.)	D20	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pumpkin	D21	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Onion or leeks	D22	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Garlic (not garlic tablets)	D23	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mushrooms	D24	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Zucchini	D25	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

16. **Over the last 12 months, how often did you drink beer, wine and/or spirits?**

Times That You Drank

		NEVER	less than once a month	1-3 days per month	1 day per week	2 days per week	3 days per week	4 days per week	5 days per week	6 days per week	every day
Beer (low alcohol)	1	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Beer (full strength)	2	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Red wine	3	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
White wine (include sparkling wines)	4	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fortified wines, port, sherry, etc.	5	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Spirits, liqueurs, etc.	6	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

When answering the next two questions, please convert the amounts you drank into glasses using the examples given below. For spirits, liqueurs, and mixed drinks containing spirits, please count each nip (30 ml) as one glass.

1 can or stubby of beer = 2 glasses
1 large bottle beer (750 ml) = 4 glasses
1 bottle wine (750 ml) = 6 glasses
1 bottle of port or sherry (750 ml) = 12 glasses

17. **Over the last 12 months, on days when you were drinking, how many glasses of beer, wine and/or spirits altogether did you *usually* drink?**

TOTAL NUMBER OF GLASSES PER DAY	1	2	3	4	5	6	7	8	9	10 or more
	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

18. **Over the last 12 months, what was the *maximum* number of glasses of beer, wine and/or spirits that you drank in 24 hours?**

MAXIMUM NUMBER OF GLASSES PER 24 HOURS	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19 or more
	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Thank You for completing this questionnaire

DO NOT WRITE IN THIS AREA.

Appendix-7 Data collection questionnaire

Flinders
UNIVERSITY

Please answer the following questions. As always, your answers will be kept strictly confidential and will be aggregated with other participants' responses for the medical research.

Name:_____ **Today's date:**____/____/_____

Address:_____

_____

Telephone: Home:_____

Work: _____

A. Demographic information:

1. What is your gender?

- .Male
- .Female

2. What is your date of birth?

DD MM YYYY

| / | / |

3. What is your current work status? (Check only one answer):

- .Full-time Part-
- .time Disabled
- .Retired
- .Unemployed
-

4. What is your approximate average annual income?

- .$0-$24,999
- .$25,000-$49,999
- .$50,000-$74,999
- .$75,000-$99,999
- .$100,000-$124,999
- .$125,000-$149,999
- .$150,000-$174,999
- .$175,000-$199,999
- .$200,000 and up

5. What is your current marital status? (Check only one answer):

- .Married
- .Divorced/ Separated
- .Widowed
- .Not being married ever

Flinders
UNIVERSITY

6. What is the highest level of education you have completed? (Check only one answer):

- ○ Did not attend the school
- ○ Primary school
- ○ Secondary school
- ○ Bachelor degree
- ○ Above Bachelor degree
- ○ Vocational education
- ○ None of the above

7. Are you an Aboriginal or Torres Strait Islander?

- ○ Yes
- ○ No

8. Do you currently have health insurance, or not?

- ○ Yes
- ○ No

B. Traditional CV risk factors:

9. Have you ever been smoking?

- ○ Yes
- ○ No

If yes, please answer questions from 10-12:

10. What is your smoking status?

- ○ Current
- ○ Ex-smoker

11. How many cigarettes do you smoke in a typical day?

- ☐ 1-10 cigarettes/day
- ☐ 11-20 cigarettes/day
- ☐ 21-30 cigarettes/day
- ☐ more than 30 cigarettes/day

12. At what age did you start smoking cigarettes?

13. How important is exercise to you? (Check only one answer):

- Extremely important
- Very important
- Moderately important
- Slightly important
- Not at all important

14. Do you have a regular physical activity in a typical week?

- Yes
- No

If yes, please answer questions 15 and 16 :

15. What do you most often do for exercise? (Check only one answer):

- Lift weight
- Walk
- Run
- Hike
- Swim
- Dance
- Aerobics
- Pilates
- Play a team sport

Other (please specify)

16. How many hours per week do you spend for that activity?

Hour		Minute	AM/PM
	:		-

C. Medical history:

17. Have ever been diagnosed with one of the following medical conditions? (You can choose more than one answer)

- High blood pressure
- Diabetes mellitus
- Elevated cholesterol
- Elevated triglycerides
- Myocardial infarction (heart attack)
- Angina pectoris

Flinders UNIVERSITY

- ☐ Atrial fibrillation
- ☐ Congestive heart Failure
- ☐ Stroke (CVA)
- ☐ Transient ischemic attack (TIA)
- ☐ Deep vein thrombosis
- ☐ Chronic renal disease
- ☐ Liver disease
- ☐ Depression

Other (please specify)

D. Medication history:

18. Are you on any of the following medications? (You can choose more than one answer)

1. Anti-rheumatic medications:

○ Hydroxychloroquine (Plaquenil)

Dose	Duration

○ Leflunomide (Arabloc, Arava)

Dose	Duration

○ Methotrexate (Hospira, Methoblastin)

Dose	Duration

○ Minocycline (Minocin)

Dose	Duration

○ Sulfasalazine (Pyralin EN, Salazopyrin, Salazopyrin EN).

Dose	Duration

○ Abatacept(Orencia)

Dose	Duration

○ Rituximab (Mabthera)

Dose	Duration

Flinders UNIVERSITY

.Tocilizumab (Actemra)

Dose	Duration

.Adalimumab (Humira)

Dose	Duration

.Etanercept (Enbrel)

Dose	Duration

.Infliximab (Remicade)

Dose	Duration

.Certolizumab pegol (Cimzia)

Dose	Duration

.Golimumab (Simponi)

Dose	Duration

.Prednisolone (Panafcort, Panafcortelone, Predsone)

Dose	Duration

2. Pain Killers:

.Paracetamol (Panadol,Panadol Osteo).

Days per week	Tablets per week	Dose per tablet

.Ibuprofen (Nurofen).

Days per week	Tablets per week	Dose per tablet

Asprin (Aspro). Days per week

Days per week	Tablets per week	Dose per tablet

5

3. Cardiovascular drugs:

Antihypertensive medications.

	Name of the drug	Dose	Duration
1			
2			
3			

Lipid lowering medications including statin.

	Name of the drug	Dose	Duration
1			
2			
3			

Anti-anginal medications.

	Name of the drug	Dose	Duration
1			
2			
3			

Heart failure medications.

	Name of the drug	Dose	Duration
1			
2			
3			

Other (please specify)

4. Supplements:

Do you consume fish oil capsule?

Yes

No

Do you consume any form of vitamin D supplement?

Yes

No

Do you consume folic acid tablet?

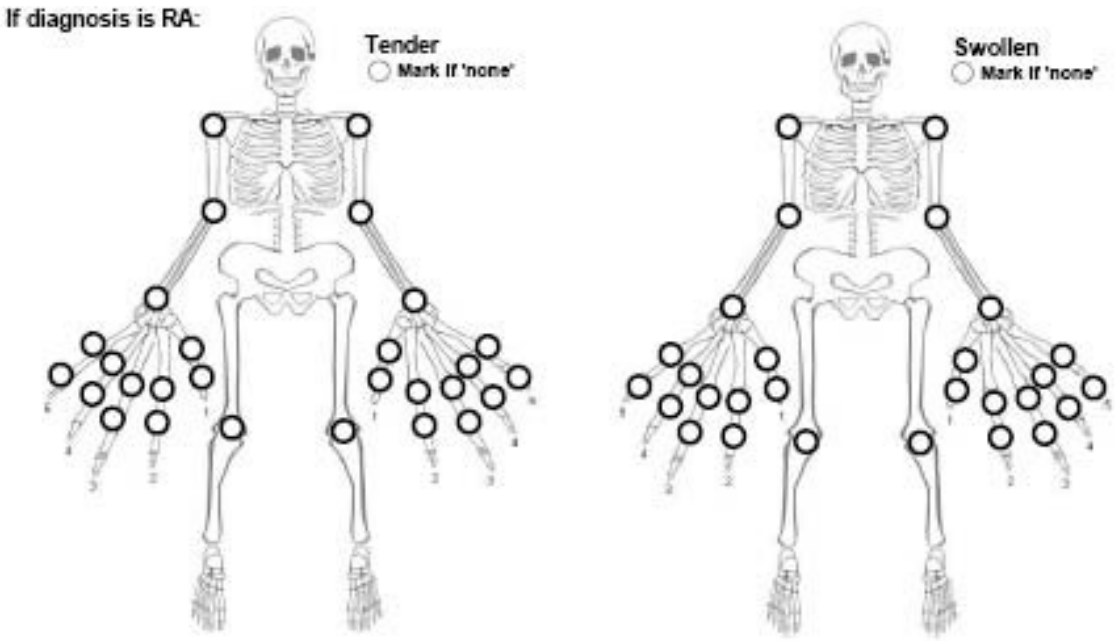
Yes

No

Appendix-8 Disease activity score on 28 joints (DAS28) form

Disease Activity Score in 28 Joints (DAS28)

Patient global assessment:



DAS28=0.56*√(28TJC) + 0.28 * √(28SJC) + 0.70*Ln(ESR/CRP) + 0.014*VAS

How to calculate a DAS28 score:

Ask the patient to make a vertical mark on a 100 mm Visual Analog Scale (VAS) corresponding to their general health or global disease activity. Using a ruler, measure from the left-hand side in mm. Note: DAS28 calculations may be performed without a VAS measurement.

Perform a swollen and tender joint examination on your patient. Add all of the swollen and tender joints and record the totals in the appropriate boxes.

Erythrocyte Sedimentation Rate (ESR) should be measured (in mm/hour). Note: C-reactive protein (CRP) levels may be used as a substitute for an ESR.

Plug the appropriate values into the formula (many online calculators are available including http://www.das-score.nl/www.das-score.nl/dasculators.html).

If using CRP instead of ESR or calculating a score from only 3 variables please see http://www.reuma-nijmegen.nl/www.das-score.nl/ for the appropriate formula.

Interpretation:

The DAS28 provides you with a number on a scale from 0 to 10 indicating current RA disease activity.

Remission: DAS28 ≤ 2.6

Low Disease activity: 2.6 < DAS28≤ 3.2

Moderate Disease Activity: 3.2 < DAS28≤ 5.1

High Disease Activity: DAS28 >5.1

Adapted from: DAS-Score.nl. Available at http://www.das-score.nl/www.das-score.nl/index.html. Accessed April 15, 2010.

Appendix-9 Patient’s data sheet

Patient name: Appointment:

ID Code:

Examination:

Blood pressure:

Note: office BP: Take three BP measurements, 3-5 min apart, and then calculate the average of the last two.

Office BP	**First**	**Second**	**Third**	**Average**
Reading				

Heart rate:

High: cm Weight: kg BMI:

Disease Activity Score with 28-joint counts:

SphygmCor:

Mobilograph:

B. Investigations:

1. Complete Blood picture:

Parameters	Hb	MCV	MCH	WBC	RBC	Platelet
Results						

2. Biochemistry:

Parameters	Sodium	Potassium	Chloride	Glucose	Creatinine	GFR
Results						

3. Liver function tests:

Parameters	GGT	ALP	AST	ALT
Results				

4. Inflammatory markers:

Parameters	CRP	ESR	RF titre	Anti CCP	ANA
Results					

5. Lipid tests

Parameters	Total Cholesterol	LDL	HDL	TG
Results				

Appendix–10 Committee for Clinical Human Research approval

Southern Adelaide Clinical

Human Research Ethics Committee

Government of South Australia
Southern Adelaide Health Service

01 June 2015

Dear Professor Mangoni

This is a formal correspondence from the Southern Adelaide Clinical Human Research Ethics Committee. Whilst this official title of the committee has changed the committee is still properly constituted under AHEC requirements with the registration number EC00188. This committee operates in accordance with the "National Statement on Ethical Conduct in Human Research (2007)." This department only uses email correspondence for all documents unless prior arrangements have been made with the manager. No hard copy correspondence will be issued.

Application Number: 76.14

Title: The relationship between methotrexate, blood pressure and arterial markers in rheumatoid arthritis

Chief Investigator: Professor Arduino Mangoni

Approved public heath sites:

Flinders Medical Centre

Repatriation General Hospital

The Issue: The Southern Adelaide Clinical Human Research Ethics Committee (SAC HREC) has approved the project amendment, and your project may now incorporate these amendments into your research. The approval extends to the following documents/changes:

Cover letter

SAC HREC Project Amendment Application form dated 05 May 2015

Low Negligible Risk Application form v6 dated 05 May 2015

Consent to Participation in Research v2 dated 01 April 2014 (tracked)

This amendment approval does not alter the current SAC HREC approval period for the study: 02 April 2016

Please read the terms and conditions of ethical approval below, as researchers have a significant responsibility to comply with reporting requirements and the other stated conditions. For example, the implications of not

providing annual reports and requesting an extension for research prior to approval expiring could lead to the suspension of the research, and has further serious consequences.

Please retain a copy of this approval for your records.

Flinders Medical Centre

The Flats G5 – Rooms 3 and 4

Flinders Drive, Bedford Park SA 5042

T: 08 8204 6453

E:Research.ethics

@health.sa.gov.au

TERMS AND CONDITIONS OF ETHICAL APPROVAL

Final ethical approval is granted subject to the researcher agreeing to meet the following terms and conditions.

As part of the Institution's responsibilities in monitoring research and complying with audit requirements, it is essential that researchers adhere to the conditions below.

Researchers have a significant responsibility to comply with the *National Statement 5.5.* in providing the SAC HREC with the required information and reporting as detailed below:

Compliance with the *National Statement on Ethical Conduct in Human Research* (2007) & the

Australian Code for the Responsible Conduct of Research (2007).

To immediately report to SAC HREC anything that may change the ethical or scientific integrity of the project.

If University personnel are involved in this project, the Principal Investigator should notify the University before commencing their research to ensure compliance with University requirements including any insurance and indemnification requirements.

It is the policy of the SAC HREC not to provide signed hardcopy or signed electronic approval letters, as our office is moving to electronic documentation. The SAC HREC office provides an unsigned electronic PDF version of the study approval letter to the Chief Investigator/Study Manager via email. These email approvals are generated via the email address research.ethics@health.sa.gov.au which can be linked back to the SAC HREC.

Report Significant Adverse events (SAE's) as per SAE requirements available at our website.

Submit an annual report on each anniversary of the date of final approval and in the correct template from the SAC HREC website.

Confidentiality of research participants MUST be maintained at all times.

A copy of the signed consent form must be given to the participant unless the project is an audit.

Any reports or publications derived from the research should be submitted to the Committee at the completion of the project.

All requests for access to medical records at any SALHN site must be accompanied by this approval email.

To regularly review the SAC HREC website and comply with all submission requirements, as they change from time to time.

The researchers agree to use electronic format for all correspondence with this department.

Researchers are reminded that all advertisements/flyers need to be approved by the committee, and that no promotion of a study can commence until final ethics and executive approval has been obtained. In addition, all media contract should be coordinated through the FMC media unit.

Yours sincerely Anna Pantelidis

Administration Officer, SAC HREC

On behalf of Professor David Gordon Chair, SAC HREC

Appendix-11 Statistical code book

Variable name	Label	Format	Codes and ranges	Missing values
mrn	Subject file number	Text	None	Not allowed
name	Subject name	Text	None	Not allowed
dob	Subject date of birth	Date (DD.MM.YYYY)	None	.(dot)
id	Subject identification number	Text	1-360	Not allowed
detailed id	Detailed Subject ID number	Text	MX001/14-	Not allowed
mtx	Methotrexate	Text	1=Yes 2=No	Not allowed
mtxduration	It is the duration from the date of commencing mx	Numeric (continuous)	Months	.
mtxdate	Date of commencing mx	Date (DD.MM.YYYY)	none	.
visitdate	Date subject visited clinic	Date (DD.MM.YYYY)	None	.
time	Time of study	00:00:00	none	Not allowed
age	Age at time of visit	Numeric	Range 18-100	.
gender	Gender	Text (nominal)	1=female 2= male	Not allowed

daynight	Blood pressure measure taken at day or night-time	Text (nominal)	1=day 2= night	.
daysbp	Averaged day systolic blood pressure taken over 24h	Numeric (continuous)	mmHg	.
nightsbp	Averaged night systolic blood pressure taken over 24h	Numeric (continuous)	mmHg	.
daydbp	Averaged day diastolic blood pressure taken over 24h	Numeric (continuous)	mmHg	.
nightdbp	Averaged night diastolic blood pressure taken over 24h	Numeric (continuous)	mmHg	.
osbp1	first office systolic blood pressure reading	Numeric (continuous)	mmHg	.
odbp1	first office diastolic blood pressure reading	Numeric (continuous)	mmHg	.
osbp2	second office systolic blood pressure reading	Numeric (continuous)	mmHg	.

odbp2	second office diastolic blood pressure reading	Numeric (continuous)	mmHg	.
osbp3	third office systolic blood pressure reading	Numeric (continuous)	mmHg	.
odbp3	third office diastolic blood pressure reading	Numeric (continuous)	mmHg	.
clinic SBP	Averaged office systolic blood pressure	Numeric (continuous)	mmHg	.
clinic DBP	Averaged office diastolic blood pressure	Numeric (continuous)	mmHg	.
24h clinical sbp	Averaged 24h systolic blood pressure	Numeric (continuous)	mmHg	.
24h clinical dbp	Averaged 24h diastolic blood pressure	Numeric (continuous)	mmHg	.
central sbp	Averaged central systolic blood pressure	Numeric (continuous)	mmHg	.
central dbp	Averaged diastolic central blood pressure	Numeric (continuous)	mmHg	.

24hacsbp	24 hours averaged central systolic blood pressure	Numeric (continuous)	mmHg	
cpp	Central pulse pressure	Numeric (continuous)	mmHg	.
ppp	Peripheral pulse pressure	Numeric (continuous)	mmHg	.
24hpwv	Averaged 24 hours Pulse wave velocity	Numeric (continuous)	m/s	.
daypwv	Averaged day pulse wave velocity	Numeric (continuous)	m/s	.
nightpwv	Averaged night pulse wave velocity	Numeric (continuous)	m/s	.
aix75	Heart rate corrected Aortic Augmentation index	Numeric (continuous)	%	.
aaix75	Averaged heart rate corrected aortic augmentation index	Numeric (continuous)	%	.
24h aaix @75	24 hours Averaged heart rate corrected aortic augmentation index	Numeric (continuous)	mmHg	.
aix	Aortic Augmentation index	Numeric (continuous)	%	.
aaix	Averaged aortic augmentation index	Numeric (continuous)	%	.

map	Mean aortic pressure	Numeric (continuous)	mmHg	.
amap	Averaged mean aortic pressure	Numeric (continuous)	%	.
ap	Aortic Augmentation pressure	Numeric (continuous)	mmHg	.
aap	Averaged aortic augmentation pressure	Numeric (continuous)	%	.
das28	Disease activity score with 28-joint counts indicating current RA disease activity	Numeric	1=”0-2.6” (remission) 2=”2.7-3.2” (low disease activity) 3=”3.3-5.1” (moderate disease activity) 4= “greater than 5.1” (high disease activity)	.
haqscore	score of Stanford Health Assessment Questionnaire to assess functional status	Numeric	range 0-3	.
vaspain	Visual analog scale for pain which is part of HAQ	Numeric (continuous)	range 0-3	.

vasglob	Visual analog scale for global health which is part of HAQ	Numeric (continuous)	range 0-3	.
wt	Weight at visit date	Numeric (continuous)	range 30-160 kg	.
ht	Height at visit date	Numeric (continuous)	range 140-220cm	.
bmi	Body mass index	Numeric (continuous)	Range 18-50 kg/m^2	
bmicat	Body mass index categories	Categorical	1= <18.5 kg/m^2 (underweight) 2= 18.5 to <25 kg/m^2 (normal weight) 3= 25 to <30 kg/m^2 (overweight) 4= ≥30 kg/m^2 (obese)	.
mtxdose	Dose of methotrexate per week	Numeric (continuous)	Range 2-30 mg/week	.
$MTXPGs_{1-5}$	Methotrexate concentration inside red blood cells (mx polyglutamate 1-5	Numeric	nmol/L packed red blood cells	.
mtxnaive	Methotrexate naïve patients	Text	1=yes 2= no	Not allowed
ADMA	Asymmetric dimethylarginine	Numeric	µmol/ l	.

	concentration in plasma	(continuous)		
SDMA	Symmetric dimethylarginine concentration in plasma	Numeric (continuous)	µmol/ l	.
Genotype	Genotype of the part of DNA sequence AC=hetrozygous. Mutant allele CC=homozygous. Wild type=normal gene	Categorical	1=AC 2=CC	.
hr	Heart rate	Numeric (continuous)	Range 50-100 beats/min	.
ahr	Averaged heart rate			
esr	Erythrocyte sedimentation rate	Numeric (continuous)	Range mm	.
crp	C-reactive protein	Numeric (continuous)	Reange mg/L	.
anticcp	Anti-citrullinated protein antibody	Numeric (continuous)	Range u/ml	.
rbs	Random blood sugar	Numeric (continuous)	Range mmol/L	.
tchol	Total cholesterol	Numeric (continuous)	Range mmol/L	.
workstat	Work status	Text (ordinal)	1=Full-time 2=Part-time	.

			3=Disabled 4=Retired 5=Unemployed	
inc$	Average annual income	Text (ordinal)	1=$0-$24,999 2=$25,000-$49,999 3=$50,000-$74,999 4=$75,000-$99,999 5=$100,000-$124,999 6=$125,000-$149,999 7=$150,000-$174,999 8=$175,000-$199,999 9=$200,000 and up	.
maristat	Marital status	Text (ordinal)	1=Married 2=Divorced/ Separated 3=Widowed 4=Not being married ever	.
edu	Completed highest level of education	Text (ordinal)	1=Did not attend the school 2=Primary school 3=Secondary	.

			school 4=Bachelor degree 5=Above Bachelor degree 6=Vocational education 7=None of the above	
aborig	Aboriginal or Torres Strait Islander	Text (nominal)	1=Yes 0=No	.
insur	Current health insurance	Text (nominal)	1=Yes 0=No	.
smok	Smoker	Text (nominal)	1=Yes 0=No	.
smokstat	Smoking status	Text	1= current 2=Ex-smoker	.
smokfreq	Cigarettes per day	Text (ordinal)	1=1-10 cigarettes/day 2=11-20 cigarettes/day 3=21-30 cigarettes/day 4=more than 30 cigarettes/day	.

smokage	Age of starting cigarettes smoking	Numeric	Range 0-100	.
exercise	Importance of exercise	Text (ordinal)	1=Extremely important 2=Very important 3=Moderately important 4=Slightly important 5=Not at all important	.
regactivity	Regular physical activity in a typical week	Text (nominal)	1=Yes 0=No	.
typeactivity	Most often type of physical activity	Text	1=Lift weight 2=Walk 3=Run 4=Hike 5=Swim 6=Dance 7=Aerobics 8=Pilates 9=Play a team sport 10=Other	.

timeactivity	minutes per week for the physical activity	Numeric	Range	.
htn	Hypertension	Text (nominal)	1=Yes 0=No	.
thtn	Treated hypertension	Text (nominal)	1=Yes 0=No	
dm	Diabetes mellitus	Text (nominal)	1=Yes 0=No	.
chol	Elevated cholesterol	Text (nominal)	1=Yes 0=No	.
tg	Elevated triglycerides	Text (nominal)	1=Yes 0=No	.
mi	Myocardial infarction Not: in the mx group it is considered present only if it is happened after at least 6 weeks of commencing the first dose of MX	Text (nominal)	1=Yes 0=No	.
angina	Angina pectoris Not: in the mx group it is considered present only if it is happened after at least 6 weeks of commencing the first dose of MX	Text (nominal)	1=Yes 0=No	.

afeb	Atrial fibrillation	Text (nominal)	1=Yes 0=No	.
chf	Congestive heart Failure Not: in the mx group it is considered present only if it is happened after at least 6 weeks of commencing the first dose of MX	Text (nominal)	1=Yes 0=No	.
cva	Stroke Not: in the mx group it is considered present only if it is happened after at least 6 weeks of commencing the first dose of MX	Text (nominal)	1=Yes 0=No	.
tia	Transient ischaemic attack	Text (nominal)	1=Yes 0=No	.
dvt	Deep vein thrombosis Not: in the mx group it is considered present only if it is happened after at least 6 weeks of commencing the first dose of MX	Text (nominal)	1=Yes 0=No	.

crd	Chronic renal disease	Text (nominal)	1=Yes 0=No	.
ld	Liver disease	Text (nominal)	1=Yes 0=No	.
raduration	RA duration	Numeric (continues)	years	.
dep	Depression	Text (nominal)	1=Yes 0=No	.
otherhist	other medical history	text	1=Yes 0=No	.
plaq	Hydroxychloroquine (Plaquenil)	Text (nominal)	1=Yes 0=No	.
plaqdose	Hydroxychloroquine dose	Numeric (continues)	mg	.
plaqdfreq	Hydroxychloroquine frequency	Numeric (continues)	Once, twice…..	.
arava	Leflunomide (Arabloc, Arava)	Text (nominal)	1=Yes 0=No	.
aravadose	arava dose	Numeric	mg	.
aravafreq	arava frequency	Numeric	Once, twice…..	.
mtx	Methotrexate (hospira, Methoblastin)	Text (nominal)	1=Yes 0=No	.
mtxdose	Methotrexate dose	Numeric	mg	.
mtxfreq	Methotrexate frequency	Numeric	Per week	.
mino	Minocycline (Minocin)	Text (nominal)	1=Yes 0=No	.

minodose	Minocycline dose	Numeric	mg	.
minofreq	Minocycline frequency	Numeric	Once, twice…..	.
sulfa	Sulfasalazine	Text (nominal)	1=Yes 0=No	.
sulfadose	Sulfasalazine dose	Numeric	mg	.
sulfafreq	Sulfasalazine frequency	Numeric	Once, twice…..	.
oren	Abatacept(Orencia)	Text (nominal)	1=Yes 0=No	.
orendose	Orencia dose	Numeric	mg	.
orenfreq	Orencia frequency	Numeric	Once, twice…..	.
mabt	Rituximab (Mabthera)	Text (nominal)	1=Yes 0=No	.
mabtdose	Mabthera dose	Numeric	mg	.
mabtfreq	Mabthera frequency	Numeric	Once, twice…..	.
actem	Tocilizumab (Actemra)	Text (nominal)	1=Yes 0=No	.
actemdose	Actemra dose	Numeric	mg	.
actemfreq	Actemra frequency	Numeric	Once, twice…..	.
hum	Adalimumab (Humira)	Text (nominal)	1=Yes 0=No	.
humdose	Humira dose	Numeric	mg	.
humfreq	Humira frequency	Numeric	Once, twice…..	.
enbr	Etanercept (Enbrel)	Text (nominal)	1=Yes 0=No	.

remi	Infliximab (Remicade)	Text (nominal)	1=Yes 0=No	.
cimz	Certolizumab pegol (Cimzia)	Text (nominal)	1=Yes 0=No	.
simpo	Golimumab (Simponi)	Text (nominal)	1=Yes 0=No	.
prednis	Prednisolone (Panafcort, Panafcortelone, Predsone)	Text (nominal)	1=Yes 0=No	.
prednisdose	Prednisolone dose	Numeric	mg	.
para	Paracetamol (Panadol,Panadol Osteo)	Text (nominal)	1=Yes 0=No	.
paradays	Paracetamol taken Days per week	Numeric	1,2,….	.
paratab	Paracetamol takenTablets per week	Numeric	1,2,…..	.
ibup	Ibuprofen (Nurofen)	Text (nominal)	1=Yes 0=No	.
ibupdays	Ibuprofen taken Days per week	Text (nominal)	1=Yes 0=No	.
ibuptab	Ibuprofen taken Tablets per week	Numeric	1,2,….	.
asa	Asprin (Aspro)	Text (nominal)	1=Yes 0=No	.
asadays	Asprin taken Days per week	Text (nominal)	1=Yes 0=No	.

asatab	Asprin takenTablets per week	Numeric	1,2,….	.
othermed	Other medications prescribed	Text	1=Yes 0=No	.
othermedlis	List of Other medications prescribed	Text	.	.
fishoil	fish oil	Text (nominal)	1=Yes 0=No	.
vitd	vitamin D	Text (nominal)	1=Yes 0=No	.
fa	Folic acid	Text (nominal)	1=Yes 0=No	.
od	Once a day	Text (nominal)	.	.
bd	Twice a day	Text (nominal)	.	.
tid	Three times a day	Text (nominal)	.	.
ow	Once per week	Text (nominal)	.	.
eow	every other week	Text (nominal)	.	.
om	Once per month	Text (nominal)	.	.
9m	Every 9 months	Text (nominal)	.	.
visit	Visit of the patient		0=first visit 1=second visit	.
mushrooms	Mushrooms including king bolete (Boletus edulis)	Text categorical	0= never eat it 1=< one/month 2=1-3 times/month	.

	oyster mushroom (Pleurotus ostreatus) portabella mushroom (Agaricus bisporus, brown strain) button mushroom (Agaricus bisporus, white strain) chanterelle (Cantharellus cibarius)		3=once/week 4=2 times/week 5=3-4 times/week 6=5-6 times/week 7=one/day 8=2times/day 9=3or more/ day	
pork fillet	pork fillet	Text categorical	0= never eat it 1=< one/month 2=1-3 times/month 3=once/week 4=2 times/week 5=3-4 times/week 6=5-6 times/week 7=one/day 8=2times/day 9=3or more/ day	.

beef steak	beef steak	Text categorical	0= never eat it 1=< one/month 2=1-3 times/month 3=once/week 4=2 times/week 5=3-4 times/week 6=5-6 times/week 7=one/day 8=2times/day 9=3or more/ day	.
lamb fillet	lamb fillet	Text categorical	0= never eat it 1=< one/month 2=1-3 times/month 3=once/week 4=2 times/week 5=3-4 times/week 6=5-6 times/week	.

			7=one/day 8=2times/day 9=3or more/ day	
chicken	chicken	Text categorical	0= never eat it 1=< one/month 2=1-3 times/month 3=once/week 4=2 times/week 5=3-4 times/week 6=5-6 times/week 7=one/day 8=2times/day 9=3or more/ day	.
ham	ham	Text categorical	0= never eat it 1=< one/month 2=1-3 times/month 3=once/week 4=2 times/week	.

			5=3-4 times/week 6=5-6 times/week 7=one/day 8=2times/day 9=3or more/ day	
salami	salami	Text categorical	0= never eat it 1=< one/month 2=1-3 times/month 3=once/week 4=2 times/week 5=3-4 times/week 6=5-6 times/week 7=one/day 8=2times/day 9=3or more/ day	.
fish	Fish, steamed, grilled or baked	Text categorical	0= never eat it 1=< one/month	.

	Fish, fried (include takeaway) Fish, tinned (salmon, tuna, sardines, etc.)		2=1-3 times/month 3=once/week 4=2 times/week 5=3-4 times/week 6=5-6 times/week 7=one/day 8=2times/day 9=3or more/ day	
rice	Rice any type	Text categorical	0= never eat it 1=< one/month 2=1-3 times/month 3=once/week 4=2 times/week 5=3-4 times/week 6=5-6 times/week 7=one/day 8=2times/day	.

			9=3or more/ day	
garlic	Garlic (not garlic tablets)	Text categorical	0= never eat it 1=< one/month 2=1-3 times/month 3=once/week 4=2 times/week 5=3-4 times/week 6=5-6 times/week 7=one/day 8=2times/day 9=3or more/ day	.
broccoli	broccoli	Text categorical	0= never eat it 1=< one/month 2=1-3 times/month 3=once/week 4=2 times/week 5=3-4 times/week	.

			6=5-6 times/week 7=one/day 8=2times/day 9=3or more/ day	
onion	onion	Text categorical	0= never eat it 1=< one/month 2=1-3 times/month 3=once/week 4=2 times/week 5=3-4 times/week 6=5-6 times/week 7=one/day 8=2times/day 9=3or more/ day	.
spinach	spinach	Text categorical	0= never eat it 1=< one/month 2=1-3 times/month	.

			3=once/week 4=2 times/week 5=3-4 times/week 6=5-6 times/week 7=one/day 8=2times/day 9=3or more/ day	
celery	celery	Text categorical	0= never eat it 1=< one/month 2=1-3 times/month 3=once/week 4=2 times/week 5=3-4 times/week 6=5-6 times/week 7=one/day 8=2times/day 9=3or more/ day	.

bran	Wheat bran	Text categorical	0= never eat it 1=< one/month 2=1-3 times/month 3=once/week 4=2 times/week 5=3-4 times/week 6=5-6 times/week 7=one/day 8=2times/day 9=3or more/ day	.
eggs	egg yolk or egg white	Text categorical	0=don't eat egg 1=<1 egg/week 2=1-2 eggs/week 3=3-5 eggs/week 4=6 or more eggs/week	.
rye bread	rye bread	Text	0= no 1=yes	.

wholemeal bread	wholemeal bread	Text	0= no 1=yes	.
beer	Low alcohol or full strength	Text categorical	0= never 1=<once a month 2=1-3 days/month 3=1 day/week 4=2 days/week 5=3 days/week 6=4 days/ week 7=5 days/week 8= 6days/week 9= every day	.
Sausages	Of any type	Text categorical	0= never 1=<once a month 2=1-3 days/month 3=1 day/week 4=2 days/week 5=3 days/week 6=4 days/ week	.

			7=5 days/week 8= 6days/week 9= every day	

Appendix-12 Tables

Table 1 Association between MTX exposure and ADMA level considering factors affecting the level of ADMA (n = 86)

ADMA	β (95% CI)	p-value
Adjusted for :		
Age (years)	-0.02 (-0.04,0.01)	0.21
DAS28 score	-0.02 (-0.05, 0.01)	0.16
eGFR (mmol/l)	-0.02 (-0.05, 0.004)	0.10
Smoking status:		
Current smoking (yes/no)	-0.02 (-0.04, 0.01)	0.25
Current and ex-smoking (yes/no)	0.001 (-0.03, 0.03)	0.97
Smoking duration (years)	0.0001 (-0.03, 0.03)	0.10
T2D (yes/no)	-0.03 (-0.05, 0.001)	0.05
Dyslipidemia (yes/no)	-0.02 (-0.05, 0.004)	0.1
Total cholesterol (mmol/L)	-0.02 (-0.05, 0.004)	0.09
Medications:		
Metformin drug (Yes/no)	-0.02 (-0.05, 0.003)	0.09
Hypertension medications (yes/no)	-0.03 (-0.06, 0.001)	0.05
Nutritional intake:		
Protein intake (g/day)	-0.02 (-0.05, 0.004)	0.10
Alcohol consumption (g/day)	-0.02 (-0.05, 0.003)	0.08

Table 2 Difference in MTX concentration between baseline and follow-up

MTXPGs (nmol/L packed RBCs)	Baseline	Follow-up	p-value
MTXPG1	31.7±19	39.1±25.5	0.09
MTXPG2	27.5±13.2	28.7±13.6	0.65
MTXPG3	38±23	36.2±21	0.69
MTXPG4	17.1±18	16.2±18.5	0.80
MTXPG5	5.8±7.7	5.03±9.5	0.68
Total MTXPGs	118.9±64.5	124.6±71.7	0.67

Measures are means± SD

REFERENCES

Actor Medical 2011, *SphygmoCor CP*, Australia, viewed 24 May 2016, <http://www.atcormedical.com>.

Agabiti-Rosei, E, Mancia, G, O'Rourke, MF, Roman, MJ, Safar, ME, Smulyan, H, Wang, JG, Wilkinson, IB, Williams, B & Vlachopoulos, C 2007, 'Central blood pressure measurements and antihypertensive therapy: a consensus document', *Hypertension*, vol. 50, no. 1, pp. 154-60. Epub 2007 Jun 11.

Aho, K, Heliovaara, M, Maatela, J, Tuomi, T & Palosuo, T 1991, 'Rheumatoid factors antedating clinical rheumatoid arthritis', *Journal of Rheumatology*, vol. 18, no. 9, pp. 1282-4.

Aho, K, Palosuo, T, Heliovaara, M, Knekt, P, Alha, P & von Essen, R 2000, 'Antifilaggrin antibodies within "normal" range predict rheumatoid arthritis in a linear fashion', *Journal of Rheumatology*, vol. 27, no. 12, pp. 2743-6.

Aho, K, von Essen, R, Kurki, P, Palosuo, T & Heliovaara, M 1993, 'Antikeratin antibody and antiperinuclear factor as markers for subclinical rheumatoid disease process', *Journal of Rheumatology*, vol. 20, no. 8, pp. 1278-81.

Ajeganova, S, Andersson, ML, Frostegard, J & Hafstrom, I 2013, 'Disease factors in early rheumatoid arthritis are associated with differential risks for cardiovascular events and mortality depending on age at onset: A 10-year observational cohort study', *Journal of Rheumatology*, vol. 15, p. 15.

Ajeganova, S, Andersson, ML & Hafström, I 2013, 'Association of obesity with worse disease severity in rheumatoid arthritis as well as with comorbidities: A long-term followup from disease onset', *Arthritis Care and Research*, vol. 65, no. 1, pp. 78-87.

Alamanos, Y, Voulgari, PV & Drosos, AA 2006, 'Incidence and prevalence of rheumatoid arthritis, based on the 1987 American College of Rheumatology criteria: a systematic review', *Seminars in Arthritis and Rheumatism*, vol. 36, no. 3, pp. 182-8. Epub 2006 Oct 11.

Aletaha, D, Neogi, T, Silman, AJ, Funovits, J, Felson, DT, Bingham, CO, 3rd, Birnbaum, NS, Burmester, GR, Bykerk, VP, Cohen, MD, Combe, B, Costenbader, KH, Dougados, M, Emery, P, Ferraccioli, G, Hazes, JM, Hobbs, K, Huizinga, TW, Kavanaugh, A, Kay, J, Kvien, TK, Laing, T, Mease, P, Menard, HA, Moreland, LW, Naden, RL, Pincus, T, Smolen, JS, Stanislawska-Biernat, E, Symmons, D, Tak, PP, Upchurch, KS, Vencovsky, J, Wolfe, F & Hawker, G 2010, '2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative', *Arthritis and Rheumatism*, vol. 62, no. 9, pp. 2569-81.

Aletaha, D & Smolen, JS 2006, 'The definition and measurement of disease modification in inflammatory rheumatic diseases', *Rheumatic Diseases Clinics of North America*, vol. 2006 Feb;32, no. 1, pp. 9-44.

Alipour, B, Homayouni-Rad, A, Vaghef-Mehrabany, E, Sharif, SK, Vaghef-Mehrabany, L, Asghari-Jafarabadi, M, Nakhjavani, MR & Mohtadi-Nia, J 2014, 'Effects of Lactobacillus casei supplementation on disease activity and inflammatory cytokines in rheumatoid arthritis patients: a randomized double-blind clinical trial', *International Journal of Rheumatic Diseases*, vol. 17, no. 5, pp. 519-27. doi: 10.1111/756-185X.12333. Epub 2014 Mar 27.

Allebeck, P 1982, 'Increased mortality in rheumatoid arthritis', *Scandinavian Journal of Rheumatology*, vol. 11, no. 2, pp. 81-6.

Allebeck, P, Ahlbom, A & Allander, E 1981, 'Increased mortality among persons with rheumatoid arthritis, but where RA does not appear on death certificate. Eleven-year follow-up of an epidemiological study', *Scandinavian Journal of Rheumatology*, vol. 10, no. 4, pp. 301-6.

Aman, S, Paimela, L, Leirisalo-Repo, M, Risteli, J, Kautiainen, H, Helve, T & Hakala, M 2000, 'Prediction of disease progression in early rheumatoid arthritis by ICTP, RF and CRP. A comparative 3-year follow-up study', *Rheumatology*, vol. 39, pp. 1009 - 13.

Amezaga Urruela, M & Suarez-Almazor, ME 2012, 'Lipid paradox in rheumatoid arthritis: changes with rheumatoid arthritis therapies', *Current Rheumatology Reports*, vol. 14, no. 5, pp. 428-37. doi: 10.1007/s11926-012-0269-z.

Amjadi, SS, Maranian, PM, Paulus, HE, Kaplan, RM, Ranganath, VK, Furst, DE, Khanna, PP & Khanna, D 2009, 'Validating and assessing the sensitivity of the Health Assessment Questionnaire-Disability Index-derived Short Form-6D in patients with early aggressive rheumatoid arthritis', *Journal of Rheumatology*, vol. 36, no. 6, pp. 1150-7. doi: 10.3899/jrheum.080959. Epub 2009 Apr 15.

An, MM, Zou, Z, Shen, H, Zhang, JD, Cao, YB & Jiang, YY 2010, 'The addition of tocilizumab to DMARD therapy for rheumatoid arthritis: a meta-analysis of randomized controlled trials', *European Journal of Clinical Pharmacology*, vol. 66, no. 1, pp. 49-59. doi: 10.1007/s00228-009-0754-0. Epub 2009 Nov 21.

Anand, SS, Yusuf, S, Vuksan, V, Devanesen, S, Teo, KK, Montague, PA, Kelemen, L, Yi, C, Lonn, E, Gerstein, H, Hegele, RA & McQueen, M 2000, 'Differences in risk factors, atherosclerosis, and cardiovascular disease between ethnic groups in Canada: the Study of Health Assessment and Risk in Ethnic groups (SHARE)', *The Lancet*, vol. 356, no. 9226, pp. 279-84.

Anderson, J, Caplan, L, Yazdany, J, Robbins, ML, Neogi, T, Michaud, K, Saag, KG, O'Dell, JR & Kazi, S 2012, 'Rheumatoid arthritis disease activity measures: American College of Rheumatology recommendations for use in clinical practice', *Arthritis Care Research (Hoboken)*, vol. 64, no. 5, pp. 640-7.

Andreozzi, F, Presta, I, Mannino, GC, Scarpelli, D, Di Silvestre, S, Di Pietro, N, Succurro, E, Sciacqua, A, Pandolfi, A, Consoli, A, Hribal, ML, Perticone, F & Sesti, G 2012, 'A functional variant of the dimethylarginine dimethylaminohydrolase-2 gene is associated with insulin sensitivity', *PLoS ONE*, vol. 7, no. 4, p. e36224. doi: 10.1371/journal.pone.0036224. Epub 2012 Apr 27.

Angel, K, Provan, SA, Gulseth, HL, Mowinckel, P, Kvien, TK & Atar, D 2010, 'Tumor necrosis factor-alpha antagonists improve aortic stiffness in patients with inflammatory arthropathies: a controlled study', *Hypertension*, vol. 55, no. 2, pp. 333-8. doi: 10.1161/HYPERTENSIONAHA.109.143982. Epub 2009 Dec 28.

Angelis-Stoforidis, P, Vajda, FJ & Christophidis, N 1999, 'Methotrexate polyglutamate levels in circulating erythrocytes and polymorphs correlate with clinical efficacy in rheumatoid arthritis', *Clinical and Experimental Rheumatology*, vol. 17, no. 3, pp. 313-20.

Anthony, S, Leiper, J & Vallance, P 2005, 'Endogenous production of nitric oxide synthase inhibitors', *Vascular Medicine*, vol. 10, no. Suppl 1, pp. S3-9.

Antman, EM, Bennett, JS, Daugherty, A, Furberg, C, Roberts, H & Taubert, KA 2007, 'Use of nonsteroidal antiinflammatory drugs: an update for clinicians: a scientific statement from the American Heart Association', *Circulation*, vol. 115, no. 12, pp. 1634-42. Epub 2007 Feb 26.

Appel, LJ, Moore, TJ, Obarzanek, E, Vollmer, WM, Svetkey, LP, Sacks, FM, Bray, GA, Vogt, TM, Cutler, JA, Windhauser, MM, Lin, PH & Karanja, N 1997, 'A clinical trial

of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group', *New England Journal of Medicine*, vol. 336, no. 16, pp. 1117-24.
Arida, A, Zampeli, E, Konstantonis, G, Fragiadaki, K, Kitas, GD, Protogerou, AD & Sfikakis, PP 2015, 'Rheumatoid arthritis is sufficient to cause atheromatosis but not arterial stiffness or hypertrophy in the absence of classical cardiovascular risk factors', *Clinical Rheumatology*, vol. 34, no. 5, pp. 853-9. doi: 10.1007/s10067-015-2914-1. Epub 2015 Mar 11.
Arnett, FC, Edworthy, SM, Bloch, DA, McShane, DJ, Fries, JF, Cooper, NS, Healey, LA, Kaplan, SR, Liang, MH, Luthra, HS & et al. 1988, 'The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 31, no. 3, pp. 315-24.
Arnold, WP, Mittal, CK, Katsuki, S & Murad, F 1977, 'Nitric oxide activates guanylate cyclase and increases guanosine 3':5'-cyclic monophosphate levels in various tissue preparations', *Proceedings of the National Academy of Sciences of the United States of America*, vol. 74, no. 8, pp. 3203-7.
Arosio, E, De Marchi, S, Rigoni, A, Prior, M, Delva, P & Lechi, A 2007, 'Forearm haemodynamics, arterial stiffness and microcirculatory reactivity in rheumatoid arthritis', *Journal of Hypertension*, vol. 25, no. 6, pp. 1273-8.
Arrigoni, FI, Vallance, P, Haworth, SG & Leiper, JM 2003, 'Metabolism of asymmetric dimethylarginines is regulated in the lung developmentally and with pulmonary hypertension induced by hypobaric hypoxia', *Circulation*, vol. 107, no. 8, pp. 1195-201.
Arts, EE, Fransen, J, den Broeder, AA, Popa, CD & van Riel, PL 2014, 'The effect of disease duration and disease activity on the risk of cardiovascular disease in rheumatoid arthritis patients', *Annals of the Rheumatic Diseases*, vol. 23, no. 10, pp. 2013-204531.
Aryaeian, N, Shahram, F, Djalali, M, Eshragian, MR, Djazayeri, A, Sarrafnejad, A, Salimzadeh, A, Naderi, N & Maryam, C 2009, 'Effect of conjugated linoleic acids, vitamin E and their combination on the clinical outcome of Iranian adults with active rheumatoid arthritis', *International Journal of Rheumatic Diseases*, vol. 12, no. 1, pp. 20-8. doi: 10.1111/j.756-185X.2009.01374.x.
Asagami, T, Abbasi, F, Stuelinger, M, Lamendola, C, McLaughlin, T, Cooke, JP, Reaven, GM & Tsao, PS 2002, 'Metformin treatment lowers asymmetric dimethylarginine concentrations in patients with type 2 diabetes', *Metabolism: Clinical and Experimental*, vol. 51, no. 7, pp. 843-6.
Ascherman, DP 2010, 'Interstitial lung disease in rheumatoid arthritis', *Current Rheumatology Reports*, vol. 12, no. 5, pp. 363-9.
Australian Bureau of Statistics 2002, *Australian Bureau of Statistics*, Australian Capital Territory in focus 2002, cat. no. 1307.8, ABS, Canberra.
Australian Bureau of Statistics 2015, *Australian Bureau of Statistics*, Australian Capital Territory in focus 2015, cat. no. 4364.0.55.001, ABS, Canberra.
Australian Institute of Health and Welfare 2009, *A picture of rheumatoid arthritis in Australia 200*9, AIHW, Canberra, viewed 11 November 2013, <http://www.aihw.gov.au>.
Australian Institute of Health and Welfare 2010, *Snapshot of arthritis in Australia 2010*, AIHW, Canberra, viewed 05 April 2016, <http://www.aihw.gov.au>.

Avalos, I, Chung, CP, Oeser, A, Gebretsadik, T, Shintani, A, Kurnik, D, Raggi, P, Sokka, T, Pincus, T & Stein, CM 2007, 'Increased augmentation index in rheumatoid arthritis and its relationship to coronary artery atherosclerosis', *Journal of Rheumatology*, vol. 34, no. 12, pp. 2388-94. Epub 007 Nov 15.

Avina-Zubieta, JA, Choi, HK, Sadatsafavi, M, Etminan, M, Esdaile, JM & Lacaille, D 2008, 'Risk of cardiovascular mortality in patients with rheumatoid arthritis: a meta-analysis of observational studies', *Arthritis and Rheumatism*, vol. 59, no. 12, pp. 1690-7.

Avina-Zubieta, JA, Thomas, J, Sadatsafavi, M, Lehman, AJ & Lacaille, D 2012, 'Risk of incident cardiovascular events in patients with rheumatoid arthritis: a meta-analysis of observational studies', *Annals of the Rheumatic Diseases*, vol. 71, no. 9, pp. 1524-9.

Avolio, A, Butlin, M & Xu, K 2012, 'Reflections on determinants of augmentation index', *Journal of Hypertension*, vol. 30, no. 2, pp. 267-8. doi: 10.1097/HJH.0b013e32834f96e0.

Baghdadi, LR, Woodman, RJ, Shanahan, EM & Mangoni, AA 2015, 'The impact of traditional cardiovascular risk factors on cardiovascular outcomes in patients with rheumatoid arthritis: a systematic review and meta-analysis', *PLoS One.*, vol. 10, no. 2, p. e0117952. doi: 10.1371/journal.pone.. eCollection 2015.

Bahadoran, Z, Mirmiran, P, Tahmasebinejad, Z & Azizi, F 2016, 'Dietary L-arginine intake and the incidence of coronary heart disease: Tehran lipid and glucose study', *Journal of Nutrition and Metabolism*, vol. 13:23., no. doi, pp. 10.1186/s12986-016-0084-z. eCollection 2016.

Bai, Y, Sun, L, Yang, T, Sun, K, Chen, J & Hui, R 2009, 'Increase in fasting vascular endothelial function after short-term oral L-arginine is effective when baseline flow-mediated dilation is low: a meta-analysis of randomized controlled trials', *American Journal of Clinical Nutrition*, vol. 89, no. 1, pp. 77-84. doi: 10.3945/ajcn.2008.26544. Epub 2008 Dec 3.

Baka, Z, Buzas, E & Nagy, G 2009, 'Rheumatoid arthritis and smoking: putting the pieces together', *Arthritis Research and Therapy*, vol. 11, no. 4, p. 238. doi: 10.1186/ar2751. Epub 009 Aug 3.

Bank, AJ & Kaiser, DR 1998, 'Smooth muscle relaxation: effects on arterial compliance, distensibility, elastic modulus, and pulse wave velocity', *Hypertension*, vol. 32, no. 2, pp. 356-9.

Barrera, P, Laan, RF, van Riel, PL, Dekhuijzen, PN, Boerbooms, AM & van de Putte, LB 1994, 'Methotrexate-related pulmonary complications in rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 53, no. 7, pp. 434-9.

Barrera, P, Van Ede, A, Laan, RF, Van Riel, PL, Boerbooms, AM & Van De Putte, LB 1994, 'Methotrexate-related pulmonary complications in patients with rheumatoid arthritis: cluster of five cases in a period of three months', *Annals of the Rheumatic Diseases*, vol. 53, no. 7, pp. 479-80.

Barzilai, O, Sherer, Y, Ram, M, Izhaky, D, Anaya, JM & Shoenfeld, Y 2007, 'Epstein-Barr virus and cytomegalovirus in autoimmune diseases: are they truly notorious? A preliminary report', *Annals of the New York Academy of Sciences*, vol. 1108, pp. 567-77.

Barzilay, JI, Abraham, L, Heckbert, SR, Cushman, M, Kuller, LH, Resnick, HE & Tracy, RP 2001, 'The relation of markers of inflammation to the development of glucose disorders in the elderly: the Cardiovascular Health Study', *Diabetes*, vol. 50, no. 10, pp. 2384-9.

Baslund, B, Gregers, J & Nielsen, CH 2008, 'Reduced folate carrier polymorphism determines methotrexate uptake by B cells and CD4+ T cells', *Rheumatology*

(Oxford), vol. 47, no. 4, pp. 451-3. doi: 10.1093/rheumatology/ken073. Epub 2008 Mar 3.

Bathon, JM, Martin, RW, Fleischmann, RM, Tesser, JR, Schiff, MH, Keystone, EC, Genovese, MC, Wasko, MC, Moreland, LW, Weaver, AL, Markenson, J & Finck, BK 2000, 'A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis', *New England Journal of Medicine*, vol. 343, no. 22, pp. 1586-93.

Bautista, LE, Lopez-Jaramillo, P, Vera, LM, Casas, JP, Otero, AP & Guaracao, AI 2001, 'Is C-reactive protein an independent risk factor for essential hypertension?', *Journal of Hypertension*, vol. 19, no. 5, pp. 857-61.

Behrendt, D & Ganz, P 2002, 'Endothelial function: From vascular biology to clinical applications', *The American Journal of Cardiology*, vol. 90, no. 10, Supplement 3, pp. L40-L8.

Belanger, CF, Hennekens, CH, Rosner, B & Speizer, FE 1978, 'The nurses' health study', *American Journal of Nursing*, vol. 78, no. 6, pp. 1039-40.

Belhassen, L, Pelle, G, Dubois-Rande, JL & Adnot, S 2003, 'Improved endothelial function by the thromboxane A2 receptor antagonist S 18886 in patients with coronary artery disease treated with aspirin', *Journal of the American College of Cardiology*, vol. 41, no. 7, pp. 1198-204.

Benatti, FB & Pedersen, BK 2015, 'Exercise as an anti-inflammatory therapy for rheumatic diseases—myokine regulation', *Nature Reviews. Rheumatology*, vol. 11, no. 2, pp. 86-97.

Benedek, TG 2006, 'The history of bacteriologic concepts of rheumatic fever and rheumatoid arthritis', *Seminars in Arthritis and Rheumatism*, vol. 36, no. 2, pp. 109-23.

Berglin, E, Padyukov, L, Sundin, U, Hallmans, G, Stenlund, H, Van Venrooij, WJ, Klareskog, L & Dahlqvist, SR 2004, 'A combination of autoantibodies to cyclic citrullinated peptide (CCP) and HLA-DRB1 locus antigens is strongly associated with future onset of rheumatoid arthritis', *Arthritis Research and Therapy*, vol. 6, no. 4, pp. R303-8. Epub 2004 May 11.

Bernatsky, S, Hudson, M & Suissa, S 2007, 'Anti-rheumatic drug use and risk of serious infections in rheumatoid arthritis', *Rheumatology (Oxford)*, vol. 46, no. 7, pp. 1157-60. Epub 2007 May 3.

Beyersmann, J, Gastmeier, P, Wolkewitz, M & Schumacher, M 2008, 'An easy mathematical proof showed that time-dependent bias inevitably leads to biased effect estimation', *Journal of Clinical Epidemiology*, vol. 61, no. 12, pp. 1216-21. doi: 10.016/j.jclinepi.2008.02.008. Epub Jul 10.

Bhole, VM, Choi, HK, Burns, LC, Vera Kellet, C, Lacaille, DV, Gladman, DD & Dutz, JP 2012, 'Differences in body mass index among individuals with PsA, psoriasis, RA and the general population', *Rheumatology (Oxford)*, vol. 51, no. 3, pp. 552-6. doi: 10.1093/rheumatology/ker349. Epub 2011 Nov 25.

Bilecik, NA, Tuna, S, Samanci, N, Balci, N & Akbas, H 2014, 'Prevalence of metabolic syndrome in women with rheumatoid arthritis and effective factors', *International Journal of Clinical and Experimental Medicine*, vol. 7, no. 8, pp. 2258-65. eCollection 014.

Bjelakovic, G, Nikolova, D, Gluud, LL, Simonetti, RG & Gluud, C 2007, 'Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis', *Journal of the American Medical Association*, vol. 297, no. 8, pp. 842-57.

Blacher, J, Asmar, R, Djane, S, London, GM & Safar, ME 1999, 'Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients', *Hypertension*, vol. 33, no. 5, pp. 1111-7.

Blackburn, WD, Jr. 1994, 'Validity of acute phase proteins as markers of disease activity', *Journal of Rheumatology*, vol. 42, pp. 9-13.

Blanch, N, Clifton, PM & Keogh, JB 2015, 'A systematic review of vascular and endothelial function: Effects of fruit, vegetable and potassium intake', *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 25, no. 3, pp. 253-66.

Boers, GH 2000, 'Mild hyperhomocysteinemia is an independent risk factor of arterial vascular disease', *Seminars in Thrombosis and Hemostasis*, vol. 26, no. 3, pp. 291-5.

Boers, M & Tugwell, P 1993, 'The validity of pooled outcome measures (indices) in rheumatoid arthritis clinical trials', *J ournal of Rheumatology*, vol. 20, no. 3, pp. 568-74.

Boger, RH 2003, 'Association of asymmetric dimethylarginine and endothelial dysfunction', *Clinical Chemistry and Laboratory Medicine*, vol. 41, no. 11, pp. 1467-72.

---- 2004, 'Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, explains the "L-arginine paradox" and acts as a novel cardiovascular risk factor', *Journal of Nutrition*, vol. 134, no. 10 Suppl, pp. 2842S-7S; discussion 53S.

Böger, RH 2003, 'The emerging role of asymmetric dimethylarginine as a novel cardiovascular risk factor', *Cardiovascular Research*, vol. 59, no. 4, pp. 824-33.

Boger, RH, Bode-Böger, SM, Kienke, S, Stan, AC, Nafe, R & Frolich, JC 1998, 'Dietary L-arginine decreases myointimal cell proliferation and vascular monocyte accumulation in cholesterol-fed rabbits', *Atherosclerosis*, vol. 136, no. 1, pp. 67-77.

Böger, RH, Bode-Böger, SM, Sydow, K, Heistad, DD & Lentz, SR 2000, 'Plasma concentration of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, is elevated in monkeys with hyperhomocyst(e)inemia or hypercholesterolemia', *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 20, no. 6, pp. 1557-64.

Boger, RH, Bode-Boger, SM, Szuba, A, Tsao, PS, Chan, JR, Tangphao, O, Blaschke, TF & Cooke, JP 1998, 'Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia', *Circulation*, vol. 98, no. 18, pp. 1842-7.

Boger, RH, Bode-Boger, SM, Tsao, PS, Lin, PS, Chan, JR & Cooke, JP 2000, 'An endogenous inhibitor of nitric oxide synthase regulates endothelial adhesiveness for monocytes', *Journal of the American College of Cardiology*, vol. 36, no. 7, pp. 2287-95.

Boger, RH, Sydow, K, Borlak, J, Thum, T, Lenzen, H, Schubert, B, Tsikas, D & Bode-Boger, SM 2000, 'LDL cholesterol upregulates synthesis of asymmetrical dimethylarginine in human endothelial cells: involvement of S-adenosylmethionine-dependent methyltransferases', *Circulation Research*, vol. 87, no. 2, pp. 99-105.

Boggia, J, Li, Y, Thijs, L, Hansen, TW, Kikuya, M, Bjorklund-Bodegard, K, Richart, T, Ohkubo, T, Kuznetsova, T, Torp-Pedersen, C, Lind, L, Ibsen, H, Imai, Y, Wang, J, Sandoya, E, O'Brien, E & Staessen, JA 2007, 'Prognostic accuracy of day versus night ambulatory blood pressure: a cohort study', *The Lancet*, vol. 370, no. 9594, pp. 1219-29.

Bohanec Grabar, P, Leandro-Garcia, LJ, Inglada-Perez, L, Logar, D, Rodriguez-Antona, C & Dolzan, V 2012, 'Genetic variation in the SLC19A1 gene and methotrexate toxicity in rheumatoid arthritis patients', *Pharmacogenomics*, vol. 13, no. 14, pp. 1583-94. doi: 10.2217/pgs.12.150.

Bohanec Grabar, P, Logar, D, Lestan, B & Dolzan, V 2008, 'Genetic determinants of methotrexate toxicity in rheumatoid arthritis patients: a study of polymorphisms affecting methotrexate transport and folate metabolism', *European Journal of Clinical Pharmacology*, vol. 64, no. 11, pp. 1057-68. doi: 10.07/s00228-008-0521-7. Epub 2008 Jul 8.

Borst, P & Elferink, RO 2002, 'Mammalian ABC transporters in health and disease', *Annual Review of Biochemistry*, vol. 71, pp. 537-92. Epub 2001 Nov 9.

Bouma, MG, van den Wildenberg, FA & Buurman, WA 1996, 'Adenosine inhibits cytokine release and expression of adhesion molecules by activated human endothelial cells', *American Journal of Physiology*, vol. 270, no. 2 Pt 1, pp. C522-9.

Bournia, VK, Dania, V, Lachanis, S, Konsta, M & Iliopoulos, A 2012, 'B12 deficiency myelopathy in a patient with long-standing rheumatoid arthritis', *Journal of Clinical Rheumatology*, vol. 18, no. 7, pp. 372-4. doi: 10.1097/RHU.0b013e318273312f.

Bowes, J & Barton, A 2008, 'Recent advances in the genetics of RA susceptibility', *Rheumatology (Oxford)*, vol. 47, no. 4, pp. 399-402. doi: 10.1093/rheumatology/ken005. Epub 2008 Feb 7.

Boyer, JF, Gourraud, PA, Cantagrel, A, Davignon, JL & Constantin, A 2011, 'Traditional cardiovascular risk factors in rheumatoid arthritis: a meta-analysis', *Joint, Bone, Spine*, vol. 78, no. 2, pp. 179-83.

Brady, SR, de Courten, B, Reid, CM, Cicuttini, FM, de Courten, MP & Liew, D 2009, 'The role of traditional cardiovascular risk factors among patients with rheumatoid arthritis', *Journal of Rheumatology*, vol. 36, no. 1, pp. 34-40.

Bramwell, JC & Hill, AV 1922, 'The velocity of the pulse wave in man', *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character*, vol. 93, no. 652, pp. 298-306.

Brandt, C & Pedersen, BK 2010, 'The role of exercise-induced myokines in muscle homeostasis and the defense against chronic diseases', *Journal of Biomedicine and Biotechnology*, vol. 2010:520258., no. doi, p. 10.1155/2010/520258. Epub 2010 Mar 9.

Braun, J 2010, 'Optimal administration and dosage of methotrexate', *Clinical and Experimental Rheumatology*, vol. 28, no. 5 Suppl 61, pp. S46-51. Epub 2010 Oct 28.

Braun, J 2011, 'Methotrexate: optimizing the efficacy in rheumatoid arthritis', *Therapeutic Advances in Musculoskeletal Disease*, vol. 3, no. 3, pp. 151-8. doi: 10.1177/1759720X11408635.

Breuninger, LM, Paul, S, Gaughan, K, Miki, T, Chan, A, Aaronson, SA & Kruh, GD 1995, 'Expression of multidrug resistance-associated protein in NIH/3T3 cells confers multidrug resistance associated with increased drug efflux and altered intracellular drug distribution', *Cancer Research*, vol. 55, no. 22, pp. 5342-7.

Bridges, SL 2004, 'Update on autoantibodies in rheumatoid arthritis', *Current Rheumatology Reports*, vol. 6, no. 5, pp. 343-50.

Briganti, EM, Shaw, JE, Chadban, SJ, Zimmet, PZ, Welborn, TA, McNeil, JJ & Atkins, RC 2003, 'Untreated hypertension among Australian adults: the 1999-2000 Australian Diabetes, Obesity and Lifestyle Study (AusDiab)', *Medical Journal of Australia*, vol. 179, no. 3, pp. 135-9.

Brink, M, Verheul, MK, Ronnelid, J, Berglin, E, Holmdahl, R, Toes, RE, Klareskog, L, Trouw, LA & Rantapaa-Dahlqvist, S 2015, 'Anti-carbamylated protein antibodies in the pre-symptomatic phase of rheumatoid arthritis, their relationship with multiple anti-citrulline peptide antibodies and association with radiological damage', *Arthritis*

Research & Therapy, vol. 17, no. 1, pp. 536. doi: 10.1186/s13075-015-0536-2. Epub 2015 Feb 7.

Brinker, RR & Ranganathan, P 2010, 'Methotrexate pharmacogenetics in rheumatoid arthritis', *Clinical and Experimental Rheumatology*, vol. 28, no. 5 Suppl 61, pp. S33-9. Epub 2010 Oct 28.

Britt H, Miller GC, Charles J, Henderson J, Bayram C, Valenti L, Pan Y, Harrison C, O'Halloran J, Fahridin S & T, C 2010, *General practice activity in Australia 2000–01 to 2009–10: 10-year data tables*, Cat. no. GEP 28, AIHW, Canberra.

Brooks, BA, Molyneaux, LM & Yue, DK 2001, 'Augmentation of central arterial pressure in Type 2 diabetes', *Diabetic Medicine*, vol. 18, no. 5, pp. 374-80.

Bruce, B & Fries, JF 2003, 'The Stanford Health Assessment Questionnaire: dimensions and practical applications', *Health and Quality of Life Outcomes*, vol. 1, p. 20.

Bryan, PT & Marshall, JM 1999, 'Cellular mechanisms by which adenosine induces vasodilatation in rat skeletal muscle: significance for systemic hypoxia', *Journal of Physiology*, vol. 514, no. Pt 1, pp. 163-75.

Bucci, M, Gratton, JP, Rudic, RD, Acevedo, L, Roviezzo, F, Cirino, G & Sessa, WC 2000, 'In vivo delivery of the caveolin-1 scaffolding domain inhibits nitric oxide synthesis and reduces inflammation', *Nature Medicine*, vol. 6, no. 12, pp. 1362-7.

Bunting, S, Moncada, S & Vane, JR 1983, 'The prostacyclin--thromboxane A2 balance: pathophysiological and therapeutic implications', *British Medical Bulletin*, vol. 39, no. 3, pp. 271-6.

Busso, N, So, A, Chobaz-Peclat, V, Morard, C, Martinez-Soria, E, Talabot-Ayer, D & Gabay, C 2002, 'Leptin signaling deficiency impairs humoral and cellular immune responses and attenuates experimental arthritis', *Journal of Immunology*, vol. 168, no. 2, pp. 875-82.

Caballero, B 2007, 'The global epidemic of obesity: an overview', *Epidemiologic Reviews*, vol. 29, no. 1, pp. 1-5.

Calder, PC 2006, 'n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases', *American Journal of Clinical Nutrition*, vol. 83, no. 6 Suppl, pp. 1505S-19S.

Caliz, R, del Amo, J, Balsa, A, Blanco, F, Silva, L, Sanmarti, R, Martinez, FG, Collado, MD, Ramirez Mdel, C, Tejedor, D, Artieda, M, Pascual-Salcedo, D, Oreiro, N, Andreu, JL, Graell, E, Simon, L, Martinez, A & Mulero, J 2012, 'The C677T polymorphism in the MTHFR gene is associated with the toxicity of methotrexate in a Spanish rheumatoid arthritis population', *Scandinavian Journal of Rheumatology*, vol. 41, no. 1, pp. 10-4. doi:.3109/03009742.2011.617312. Epub 2011 Nov 1.

Callahan, LF, Cordray, DS, Wells, G & Pincus, T 1996, 'Formal education and five-year mortality in rheumatoid arthritis: mediation by helplessness scale score', *Arthritis Care and Research*, vol. 9, no. 6, pp. 463-72.

Campbell, L, Chen, C, Bhagat, SS, Parker, RA & Östör, AJK 2011, 'Risk of adverse events including serious infections in rheumatoid arthritis patients treated with tocilizumab: a systematic literature review and meta-analysis of randomized controlled trials', *Rheumatology*, vol. 50, no. 3, pp. 552-62.

Cannella, AC & O'Dell, JR 2012, 'Traditional DMARDs: Methotrexate, leflunomide, sulfasalazine, hydroxychloroquine, and combination therapies ', in *Firestein: Kelley's Textbook of Rheumatology* 9 edn, W.B. Saunders, St. Louis, MO pp. 917-40.

Cannon, GW, Holden, WL, Juhaeri, J, Dai, W, Scarazzini, L & Stang, P 2004, 'Adverse events with disease modifying antirheumatic drugs (DMARD): a cohort study of leflunomide compared with other DMARD', *Journal of Rheumatology*, vol. 31, no. 10, pp. 1906-11.

Capell, HA, Madhok, R, Hunter, JA, Porter, D, Morrison, E, Larkin, J, Thomson, EA, Hampson, R & Poon, FW 2004, 'Lack of radiological and clinical benefit over two years of low dose prednisolone for rheumatoid arthritis: results of a randomised controlled trial', *Annals of the Rheumatic Diseases*, vol. 63, no. 7, pp. 797-803.

Caplin, B, Nitsch, D, Gill, H, Hoefield, R, Blackwell, S, MacKenzie, D, Cooper, JA, Middleton, RJ, Talmud, PJ, Veitch, P, Norman, J, Wheeler, DC & Leiper, JM 2010, 'Circulating methylarginine levels and the decline in renal function in patients with chronic kidney disease are modulated by DDAH1 polymorphisms', *Kidney International*, vol. 77, no. 5, pp. 459-67. doi: 10.1038/ki.2009.463. Epub Dec 9.

Carson, CW, Cannon, GW, Egger, MJ, Ward, JR & Clegg, DO 1987, 'Pulmonary disease during the treatment of rheumatoid arthritis with low dose pulse methotrexate', *Seminars in Arthritis and Rheumatism*, vol. 16, no. 3, pp. 186-95.

Carter, JD, Lodhi, AB, Nagda, SR, Ricca, L, Ward, C, Traina, E, Thompson, ZJ, Huang, Y, Valeriano, J & Vasey, FB 2007, 'Determining rheumatologists' accuracy at assessing functional disability in rheumatoid arthritis patients using the Health Assessment Questionnaire-Disability Index', *Journal of Rheumatology*, vol. 34, no. 5, pp. 958-63. Epub 2007 Apr 1.

Caruso, I, Santandrea, S, Sarzi Puttini, P, Boccassini, L, Montrone, F, Cazzola, M, Azzolini, V & Segre, D 1990, 'Clinical, laboratory and radiographic features in early rheumatoid arthritis', *Journal of Rheumatology*, vol. 17, pp. 1263-7.

Cathcart, ES & Spodick, DH 1962, 'Rheumatoid heart disease. A study of the incidence and nature of cardiac lesions in rheumatoid arthritis', *New England Journal of Medicine*, vol. 266, pp. 959-64.

Cavelaars, M, Tulen, JH, van Bemmel, JH & van den Meiracker, AH 2004, 'Physical activity, dipping and haemodynamics', *Journal of Hypertension*, vol. 22, no. 12, pp. 2303-9.

Cerhan, JR, Saag, KG, Criswell, LA, Merlino, LA & Mikuls, TR 2002, 'Blood transfusion, alcohol use, and anthropometric risk factors for rheumatoid arthritis in older women', *Journal of Rheumatology*, vol. 29, no. 2, pp. 246-54.

Cerutti, H, Muzzi, C, Leoncini, R, Scapellato, C, Cortelazzo, A, Furlani, E & Guerranti, R 2011, 'Erythrocyte sedimentation rate measurement by VES Matic Cube 80 in relation to inflammation plasma proteins', *Journal of Clinical Laboratory Analysis*, vol. 25, no. 3, pp. 198-202. doi: 10.1002/jcla.20457.

Chae, CU, Lee, RT, Rifai, N & Ridker, PM 2001, 'Blood pressure and inflammation in apparently healthy men', *Hypertension*, vol. 38, no. 3, pp. 399-403.

Chaer, F, Lucena, J, Reis, R, Brasil, F, Melo, M & Callegari, A 2012, 'Evaluation of metabolic syndrome in 97 patients with rheumatoid arthritis', *Arthritis Rheumatology*, vol. 64, no. 10, p. 1266.

Chalon, S, Moreno, H, Jr., Benowitz, NL, Hoffman, BB & Blaschke, TF 2000, 'Nicotine impairs endothelium-dependent dilatation in human veins in vivo', *Clinical Pharmacology & Therapeutics*, vol. 67, no. 4, pp. 391-7.

Chambers, JC, McGregor, A, Jean-Marie, J & Kooner, JS 1998, 'Acute hyperhomocysteinaemia and endothelial dysfunction', *The Lancet.*, vol. 351, no. 9095, pp. 36-7.

Chan, ES & Cronstein, BN 2002, 'Molecular action of methotrexate in inflammatory diseases', *Arthritis Research*, vol. 4, no. 4, pp. 266-73. Epub 2002 Mar 19.

---- 2013, 'Mechanisms of action of methotrexate', *Bulletin of the NYU Hospital for Joint Diseases (2013)*, vol. 71, no. Suppl 1, pp. S5-8.

Chango, A, Emery-Fillon, N, de Courcy, GP, Lambert, D, Pfister, M, Rosenblatt, DS & Nicolas, JP 2000, 'A polymorphism (80G->A) in the reduced folate carrier gene and its associations with folate status and homocysteinemia', *Molecular Genetics and Metabolism*, vol. 70, no. 4, pp. 310-5.

Chatzikyriakidou, A, Georgiou, I, Voulgari, PV, Papadopoulos, CG, Tzavaras, T & Drosos, AA 2007, 'Transcription regulatory polymorphism -43T>C in the 5'-flanking region of SLC19A1 gene could affect rheumatoid arthritis patient response to methotrexate therapy', *Rheumatology International*, vol. 27, no. 11, pp. 1057-61. Epub 2007 Apr 3.

Chave, KJ, Ryan, TJ, Chmura, SE & Galivan, J 2003, 'Identification of single nucleotide polymorphisms in the human gamma-glutamyl hydrolase gene and characterization of promoter polymorphisms', *Gene*, vol. 319, pp. 167-75.

Cheah, SY, Clark, C, Goldberg, L, Li Wan Po, A & Phillips, R 1996, 'Outcome measures, pooled index and quality of life instruments in rheumatoid arthritis', *Journal of Clinical Pharmacy and Therapeutics*, vol. 21, no. 5, pp. 297-316.

Chemla, D, Antony, I, Lecarpentier, Y & Nitenberg, A 2003, 'Contribution of systemic vascular resistance and total arterial compliance to effective arterial elastance in humans', *American Journal of Physiology*, vol. 285, no. 2, pp. H614-20. Epub 2003 Apr 10.

Chen, MY, Chung, SW, Tran, JC, Tong, KS, Ho, YY, Chan, CH & Xiao, Y 2011, 'Dietary exposure of Hong Kong adults to nitrate and nitrite from vegetable intake', *Food Additives & Contaminants*, vol. 4, no. 2, pp. 94-8. doi: 10.1080/19393210.2011.574158. Epub 2011 May 23.

Chenevard, R, Hurlimann, D, Bechir, M, Enseleit, F, Spieker, L, Hermann, M, Riesen, W, Gay, S, Gay, RE, Neidhart, M, Michel, B, Luscher, TF, Noll, G & Ruschitzka, F 2003, 'Selective COX-2 inhibition improves endothelial function in coronary artery disease', *Circulation*, vol. 107, no. 3, pp. 405-9.

Chimenti, MS, Triggianese, P, Conigliaro, P, Candi, E, Melino, G & Perricone, R 2015, 'The interplay between inflammation and metabolism in rheumatoid arthritis', *Cell Death and Disease*, vol. 6:e1887., no. doi, p. 10.1038/cddis.2015.246.

Chobanian, AV, Bakris, GL, Black, HR, Cushman, WC, Green, LA, Izzo, JL, Jr., Jones, DW, Materson, BJ, Oparil, S, Wright, JT, Jr. & Roccella, EJ 2003, 'The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report', *Journal of the American Medical Association*, vol. 289, no. 19, pp. 2560-72. Epub 003 May 14.

Choi, CB, Sung, YK, Cho, SK, Yoo, DH, Lee, SS, Lee, J, Kim, J, Lee, HS, Kim, TH, Yoon, BY, Yoo, WH, Choe, JY, Lee, SH, Shim, SC, Chung, WT, Hong, SJ, Lee, CK, Koh, E, Jun, JB, Bang, SY, Kim, SK, Cha, HS, Shim, J & Bae, SC 2013, 'Methotrexate dose and outcomes in patients with rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 72, no. Suppl 3, p. A836.

Choi, HK 2005, 'Dietary risk factors for rheumatic diseases', *Current Opinion in Rheumatology*, vol. 17, no. 2, pp. 141-6.

Choi, HK, Hernan, MA, Seeger, JD, Robins, JM & Wolfe, F 2002, 'Methotrexate and mortality in patients with rheumatoid arthritis: a prospective study', *The Lancet*, vol. 359, no. 9313, pp. 1173-7.

Choi, HK, Nguyen, US, Niu, J, Danaei, G & Zhang, Y 2014, 'Selection bias in rheumatic disease research', *Nature Reviews Rheumatology*, vol. 10, no. 7, pp. 403-12. doi: 10.1038/nrrheum.2014.36. Epub Apr 1.

Choi, HK, Rho, YH, Zhu, Y, Cea-Soriano, L, Avina-Zubieta, JA & Zhang, Y 2013, 'The risk of pulmonary embolism and deep vein thrombosis in rheumatoid arthritis: a UK

population-based outpatient cohort study', *Annals of the Rheumatic Diseases*, vol. 72, no. 7, pp. 1182-7. doi: 10.36/annrheumdis-2012-201669. Epub 2012 Aug 28.
Choy, E & Panayi, G 2001, 'Cytokine pathways and joint inflammation in rheumatoid arthritis', *New England Journal of Medicine*, vol. 344, no. 12, pp. 907-16.
Choy, E & Sattar, N 2009, 'Interpreting lipid levels in the context of high-grade inflammatory states with a focus on rheumatoid arthritis: a challenge to conventional cardiovascular risk actions', *Annals of the Rheumatic Diseases*, vol. 68, no. 4, pp. 460-9. doi: 10.1136/ard.2008.101964.
Choy, E, Smith, C, Dore, C & Scott, D 2005, 'A meta-analysis of the efficacy and toxicity of combining disease-modifying anti-rheumatic drugs in rheumatoid arthritis based on patient withdrawal', *Rheumatology (Oxford)*, vol. 44, no. 11, pp. 1414-21. Epub 2005 Jul 19.
Chung, CP, Giles, JT, Petri, M, Szklo, M, Post, W, Blumenthal, RS, Gelber, AC, Ouyang, P, Jenny, NS & Bathon, JM 2012, 'Prevalence of traditional modifiable cardiovascular risk factors in patients with rheumatoid arthritis: comparison with control subjects from the multi-ethnic study of atherosclerosis', *Seminars in Arthritis and Rheumatism*, vol. 41, no. 4, pp. 535-44.
Chung, CP, Oeser, A, Avalos, I, Gebretsadik, T, Shintani, A, Raggi, P, Sokka, T, Pincus, T & Stein, CM 2006, 'Utility of the Framingham risk score to predict the presence of coronary atherosclerosis in patients with rheumatoid arthritis', *Arthritis Research and Therapy*, vol. 8, no. 6, p. R186.
Chung, CP, Oeser, A, Raggi, P, Gebretsadik, T, Shintani, AK, Sokka, T, Pincus, T, Avalos, I & Stein, CM 2005, 'Increased coronary-artery atherosclerosis in rheumatoid arthritis: relationship to disease duration and cardiovascular risk factors', *Arthritis and Rheumatism*, vol. 52, no. 10, pp. 3045-53.
Clarke, S 1993, 'Protein methylation', *Curr Opin Cell Biol.*, vol. 5, no. 6, pp. 977-83.
Cleland, LG, Caughey, GE, James, MJ & Proudman, SM 2006, 'Reduction of cardiovascular risk factors with longterm fish oil treatment in early rheumatoid arthritis', *Journal of Rheumatology*, vol. 33, no. 10, pp. 1973-9. Epub 2006 Aug 1.
Cleland, LG, French, JK, Betts, WH, Murphy, GA & Elliott, MJ 1988, 'Clinical and biochemical effects of dietary fish oil supplements in rheumatoid arthritis', *Journal of Rheumatology*, vol. 15, no. 10, pp. 1471-5.
Cleland, LG, James, MJ & Proudman, SM 2003, 'The role of fish oils in the treatment of rheumatoid arthritis', *Drugs*, vol. 63, no. 9, pp. 845-53.
Cobb, S, Anderson, F & Bauer, W 1953, 'Length of life and cause of death in rheumatoid arthritis', *New England Journal of Medicine*, vol. 249, no. 14, pp. 553-6.
Colebatch, AN, Marks, JL, van der Heijde, DM & Edwards, CJ 2012, 'Safety of nonsteroidal antiinflammatory drugs and/or paracetamol in people receiving methotrexate for inflammatory arthritis: a Cochrane systematic review', *Journal of Rheumatology*, vol. 90:62-73., no. doi, p. 10.3899/jrheum.120345.
Collins, CE, Boggess, MM, Watson, JF, Guest, M, Duncanson, K, Pezdirc, K, Rollo, M, Hutchesson, MJ & Burrows, TL 2014, 'Reproducibility and comparative validity of a food frequency questionnaire for Australian adults', *Clinical Nutrition*, vol. 33, no. 5, pp. 906-14.
Collis, MG 1989, 'The vasodilator role of adenosine', *Pharmacology and Therapeutics*, vol. 41, no. 1-2, pp. 143-62.
Conn, DL 2001, 'Resolved: Low-dose prednisone is indicated as a standard treatment in patients with rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 45, no. 5, pp. 462-7.

Cooke, JP 2000, 'Does ADMA cause endothelial dysfunction?', *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 20, no. 9, pp. 2032-7.
Cooke, JP 2005, 'ADMA: its role in vascular disease', *Vascular Medicine*, vol. 10, no. Suppl 1, pp. S11-7.
Corretti, MC, Anderson, TJ, Benjamin, EJ, Celermajer, D, Charbonneau, F, Creager, MA, Deanfield, J, Drexler, H, Gerhard-Herman, M, Herrington, D, Vallance, P, Vita, J & Vogel, R 2002, 'Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force', *Journal of the American College of Cardiology*, vol. 39, no. 2, pp. 257-65.
Costenbader, KH & Karlson, EW 2006, 'Cigarette smoking and autoimmune disease: what can we learn from epidemiology?', *Lupus*, vol. 15, no. 11, pp. 737-45.
Crilly, MA, Kumar, V, Clark, HJ, Scott, NW, Macdonald, AG & Williams, DJ 2009, 'Arterial stiffness and cumulative inflammatory burden in rheumatoid arthritis: a dose-response relationship independent of established cardiovascular risk factors', *Rheumatology (Oxford)*, vol. 48, no. 12, pp. 1606-12. doi: 10.093/rheumatology/kep305. Epub 2009 Oct 25.
Crilly, MA & McNeill, G 2012, 'Arterial dysfunction in patients with rheumatoid arthritis and the consumption of daily fruits and daily vegetables', *European Journal of Clinical Nutrition*, vol. 66, no. 3, pp. 345-52.
Cross, M, Smith, E, Hoy, D, Carmona, L, Wolfe, F, Vos, T, Williams, B, Gabriel, S, Lassere, M, Johns, N, Buchbinder, R, Woolf, A & March, L 2014, 'The global burden of rheumatoid arthritis: estimates from the Global Burden of Disease 2010 study', *Annals of the Rheumatic Diseases*.
Crowson, CS, Matteson, EL, Roger, VL, Therneau, TM & Gabriel, SE 2012, 'Usefulness of risk scores to estimate the risk of cardiovascular disease in patients with rheumatoid arthritis', *American Journal of Cardiology*, vol. 110, no. 3, pp. 420-4. doi: 10.1016/j.amjcard.2012.03.044. Epub Apr 20.
Cuchacovich, R & Espinoza, LR 2009, 'Does TNF-alpha blockade play any role in cardiovascular risk among rheumatoid arthritis (RA) patients?', *Clinical Rheumatology*, vol. 28, no. 10, pp. 1217-20. doi: 10.007/s10067-009-1208-x. Epub 2009 Jun 11.
Cutolo, M, Sulli, A, Pizzorni, C, Seriolo, B & Straub, RH 2001, 'Anti-inflammatory mechanisms of methotrexate in rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 60, no. 8, pp. 729-35.
Cypiene, A, Laucevicius, A, Venalis, A, Ryliskyte, L, Dadoniene, J, Petrulioniene, Z, Kovaite, M, Laskova, V & Gintautas, J 2007, 'Non-invasive assessment of arterial stiffness indices by applanation tonometry and pulse wave analysis in patients with rheumatoid arthritis treated with TNF-alpha blocker remicade (infliximab)', *Proceedings of the Western Pharmacology Society*, vol. 50, pp. 119-22.
Dalrymple, JM, Stamp, LK, O'Donnell, JL, Chapman, PT, Zhang, M & Barclay, ML 2008, 'Pharmacokinetics of oral methotrexate in patients with rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 58, no. 11, pp. 3299-308. doi: 10.1002/art.24034.
Danialou, G, Vicaut, E, Sambe, A, Aubier, M & Boczkowski, J 1997, 'Predominant role of A1 adenosine receptors in mediating adenosine induced vasodilatation of rat diaphragmatic arterioles: involvement of nitric oxide and the ATP-dependent K+ channels', *British Journal of Pharmacology*, vol. 121, no. 7, pp. 1355-63.

Daniels, JW, Mole, PA, Shaffrath, JD & Stebbins, CL 1998, 'Effects of caffeine on blood pressure, heart rate, and forearm blood flow during dynamic leg exercise', *Journal of Applied Physiology (1985).* vol. 85, no. 1, pp. 154-9.

Das, UN 2001, 'Is obesity an inflammatory condition?', *Nutrition*, vol. 17, no. 11-12, pp. 953-66.

Davies, JE, Baksi, J, Francis, DP, Hadjiloizou, N, Whinnett, ZI, Manisty, CH, Aguado-Sierra, J, Foale, RA, Malik, IS, Tyberg, JV, Parker, KH, Mayet, J & Hughes, AD 2010, 'The arterial reservoir pressure increases with aging and is the major determinant of the aortic augmentation index', *The American Journal of Physiology-Heart and Circulatory Physiology*, vol. 298, no. 2, pp. H580-6. doi: 10.1152/ajpheart.00875.2009. Epub 2009 Dec 11.

Davies, PF 2009, 'Hemodynamic shear stress and the endothelium in cardiovascular pathophysiology', *Nature Clinical Practice Cardiovascular Medicine*, vol. 6, no. 1, pp. 16-26. doi: 10.1038/ncpcardio397. Epub 2008 Nov 25.

Davis, LA, Polk, B, Mann, A, Wolff, RK, Kerr, GS, Reimold, AM, Cannon, GW, Mikuls, TR & Caplan, L 2014, 'Folic acid pathway single nucleotide polymorphisms associated with methotrexate significant adverse events in United States veterans with rheumatoid arthritis', *Clinical and Experimental Rheumatology*, vol. 32, no. 3, pp. 324-32. Epub 2014 Jan 20.

Dayal, S & Lentz, SR 2005, 'ADMA and hyperhomocysteinemia', *Vascular Medicine*, vol. 10, no. Suppl 1, pp. S27-33.

Dayer, JM & Demczuk, S 1984, 'Cytokines and other mediators in rheumatoid arthritis', *Springer Seminars in Immunopathology*, vol. 7, no. 4, pp. 387-413.

de Jong, Z, Munneke, M, Zwinderman, AH, Kroon, HM, Ronday, KH, Lems, WF, Dijkmans, BA, Breedveld, FC, Vliet Vlieland, TP, Hazes, JM & Huizinga, TW 2004, 'Long term high intensity exercise and damage of small joints in rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 63, no. 11, pp. 1399-405.

de Rotte, MC, Bulatovic, M, Heijstek, MW, Jansen, G, Heil, SG, van Schaik, RH, Wulffraat, NM & de Jonge, R 2012, 'ABCB1 and ABCC3 gene polymorphisms are associated with first-year response to methotrexate in juvenile idiopathic arthritis', *Journal of Rheumatology*, vol. 39, no. 10, pp. 2032-40. doi: 10.3899/jrheum.111593. Epub 2012 Aug 1.

de Vries, R 2011, 'Genetics of rheumatoid arthritis: time for a change!', *Current Opinion in Rheumatology*, vol. 23, no. 3, pp. 227-32. doi: 10.1097/BOR.0b013e3283457524.

Deighton, CM, Walker, DJ, Griffiths, ID & Roberts, DF 1989, 'The contribution of HLA to rheumatoid arthritis', *Clinical Genetics*, vol. 36, no. 3, pp. 178-82.

del Rincon, I, Freeman, GL, Haas, RW, O'Leary, DH & Escalante, A 2005, 'Relative contribution of cardiovascular risk factors and rheumatoid arthritis clinical manifestations to atherosclerosis', *Arthritis and Rheumatism*, vol. 52, no. 11, pp. 3413-23.

del Rincon, I, Haas, RW, Pogosian, S & Escalante, A 2005, 'Lower limb arterial incompressibility and obstruction in rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 64, no. 3, pp. 425-32. Epub 2004 Jul 22.

del Rincon, I, O'Leary, DH, Haas, RW & Escalante, A 2004, 'Effect of glucocorticoids on the arteries in rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 50, no. 12, pp. 3813-22.

del Rincon, I, Williams, K, Stern, MP, Freeman, GL, O'Leary, DH & Escalante, A 2003, 'Association between carotid atherosclerosis and markers of inflammation in

rheumatoid arthritis patients and healthy subjects', *Arthritis and Rheumatism*, vol. 48, no. 7, pp. 1833-40.

del Rincon, ID, Williams, K, Stern, MP, Freeman, GL & Escalante, A 2001, 'High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors', *Arthritis and Rheumatism*, vol. 44, no. 12, pp. 2737-45.

Dervieux, T, Furst, D, Lein, DO, Capps, R, Smith, K, Caldwell, J & Kremer, J 2005, 'Pharmacogenetic and metabolite measurements are associated with clinical status in patients with rheumatoid arthritis treated with methotrexate: results of a multicentred cross sectional observational study', *Annals of the Rheumatic Diseases*, vol. 64, no. 8, pp. 1180-5. Epub 2005 Jan 27.

Dervieux, T, Furst, D, Lein, DO, Capps, R, Smith, K, Walsh, M & Kremer, J 2004, 'Polyglutamation of methotrexate with common polymorphisms in reduced folate carrier, aminoimidazole carboxamide ribonucleotide transformylase, and thymidylate synthase are associated with methotrexate effects in rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 50, no. 9, pp. 2766-74.

Dervieux, T, Greenstein, N & Kremer, J 2006, 'Pharmacogenomic and metabolic biomarkers in the folate pathway and their association with methotrexate effects during dosage escalation in rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 54, no. 10, pp. 3095-103.

Dervieux, T, Kremer, J, Lein, DO, Capps, R, Barham, R, Meyer, G, Smith, K, Caldwell, J & Furst, DE 2004, 'Contribution of common polymorphisms in reduced folate carrier and gamma-glutamylhydrolase to methotrexate polyglutamate levels in patients with rheumatoid arthritis', *Pharmacogenetics*, vol. 14, no. 11, pp. 733-9.

Dervieux, T, Wessels, JA, van der Straaten, T, Penrod, N, Moore, JH, Guchelaar, HJ & Kremer, JM 2009, 'Gene-gene interactions in folate and adenosine biosynthesis pathways affect methotrexate efficacy and tolerability in rheumatoid arthritis', *Pharmacogenetics and Genomics*, vol. 19, no. 12, pp. 935-44. doi: 10.1097/FPC.0b013e32833315d1.

Di Franco, M, Spinelli, FR, Metere, A, Gerardi, MC, Conti, V, Boccalini, F, Iannuccelli, C, Ciciarello, F, Agati, L & Valesini, G 2012, 'Serum levels of asymmetric dimethylarginine and apelin as potential markers of vascular endothelial dysfunction in early rheumatoid arthritis', *Mediators of Inflammation*, vol. 2012:347268., no. doi, p. 10.1155/2012/347268. Epub 2012 Aug 7.

Diaz-Borjon, A, Richaud-Patin, Y, Alvarado de la Barrera, C, Jakez-Ocampo, J, Ruiz-Arguelles, A & Llorente, L 2000, 'Multidrug resistance-1 (MDR-1) in rheumatic autoimmune disorders. Part II: Increased P-glycoprotein activity in lymphocytes from systemic lupus erythematosus patients might affect steroid requirements for disease control', *Joint Bone Spine*, vol. 67, no. 1, pp. 40-8.

Dimitroulas, T, Hodson, J, Sandoo, A, Smith, JP, Douglas, KM & Kitas, GD 2015, 'Symmetric Dimethylarginine Is Not Associated with Cumulative Inflammatory Load or Classical Cardiovascular Risk Factors in Rheumatoid Arthritis: A 6-Year Follow-Up Study', *Mediators of Inflammation*, vol. 2015:796562., no. doi, p. 10.1155/2015/796562. Epub 2015 Oct 12.

Dimitroulas, T, Sandoo, A, Hodson, J, Smith, J, Douglas, KM & Kitas, GD 2016, 'Associations between asymmetric dimethylarginine, homocysteine, and the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism (rs1801133) in rheumatoid arthritis', *Scandinavian Journal of Rheumatology*, vol. 45, no. 4, pp. 267-73. doi: 10.3109/03009742.2015.1086433. Epub 2015 Nov 24.

Dimitroulas, T, Sandoo, A, Hodson, J, Smith, J, Panoulas, VF & Kitas, GD 2014, 'Relationship between dimethylarginine dimethylaminohydrolase gene variants and asymmetric dimethylarginine in patients with rheumatoid arthritis', *Atherosclerosis*, vol. 237, no. 1, pp. 38-44. doi: 10.1016/j.atherosclerosis.2014.07.033. Epub Aug 27.

Dimitroulas, T, Sandoo, A & Kitas, GD 2012, 'Asymmetric dimethylarginine as a surrogate marker of endothelial dysfunction and cardiovascular risk in patients with systemic rheumatic diseases', *International Journal of Molecular Sciences*, vol. 13, no. 10, pp. 12315-35. doi: 10.3390/ijms131012315.

Dimitroulas, T, Sandoo, A, Veldhuijzen van Zanten, JJ, Smith, JP, Hodson, J, Metsios, GS, Stavropoulos-Kalinoglou, A & Kitas, GD 2013, 'Predictors of asymmetric dimethylarginine levels in patients with rheumatoid arthritis: the role of insulin resistance', *Scandinavian Journal of Rheumatology*, vol. 42, no. 3, pp. 176-81. doi: 10.3109/03009742.2012.747627. Epub 2013 Jan 14.

Ding, FH, Fan, WX, Zhang, RY, Zhang, Q, Li, Y & Wang, JG 2011, 'Validation of the noninvasive assessment of central blood pressure by the SphygmoCor and Omron devices against the invasive catheter measurement', *American Journal of Hypertension*, vol. 24, no. 12, pp. 1306-11. doi: 10.038/ajh.2011.145. Epub Oct 6.

Ding, FH, Li, Y, Zhang, RY, Zhang, Q & Wang, JG 2013, 'Comparison of the SphygmoCor and Omron devices in the estimation of pressure amplification against the invasive catheter measurement', *Journal of Hypertension*, vol. 31, no. 1, pp. 86-93. doi: 10.1097/HJH.0b013e32835a8eca.

Diouf, ML, Diallo, S, Mbengue, M & Moreira-Diop, T 2001, '[Methotrexate, liver and rheumatoid arthritis in tropical areas]', *Sante.*, vol. 11, no. 3, pp. 195-200.

Dixey, J, Solymossy, C, Young, A & Early, R 2004, '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)', *Journal of Rheumatology*, vol. 69, pp. 48-54.

Dixon, WG, Watson, KD, Lunt, M, Hyrich, KL, Silman, AJ & Symmons, DP 2007, 'Reduction in the incidence of myocardial infarction in patients with rheumatoid arthritis who respond to anti-tumor necrosis factor alpha therapy: results from the British Society for Rheumatology Biologics Register', *Arthritis and Rheumatism*, vol. 56, no. 9, pp. 2905-12.

Doll, R, Peto, R, Boreham, J & Sutherland, I 2004, 'Mortality in relation to smoking: 50 years' observations on male British doctors', *British Medical Journal*, vol. 328, no. 7455, p. 1519. Epub 2004 Jun 22.

Doll, R, Peto, R, Wheatley, K, Gray, R & Sutherland, I 1994, 'Mortality in relation to smoking: 40 years' observations on male British doctors', *British Medical Journal*, vol. 309, no. 6959, pp. 901-11.

Donahue, KE, Gartlehner, G, Jonas, DE, Lux, LJ, Thieda, P, Jonas, BL, Hansen, RA, Morgan, LC & Lohr, KN 2008, 'Systematic review: comparative effectiveness and harms of disease-modifying medications for rheumatoid arthritis', *Annals of Internal Medicine*, vol. 148, no. 2, pp. 124-34. Epub 2007 Nov 19.

Dong, JY, Qin, LQ, Zhang, Z, Zhao, Y, Wang, J, Arigoni, F & Zhang, W 2011, 'Effect of oral L-arginine supplementation on blood pressure: a meta-analysis of randomized, double-blind, placebo-controlled trials', *American Heart Journal*, vol. 162, no. 6, pp. 959-65. doi: 10.1016/j.ahj.2011.09.012. Epub Nov 8.

Drozdzik, M, Rudas, T, Pawlik, A, Gornik, W, Kurzawski, M & Herczynska, M 2007, 'Reduced folate carrier-1 80G>A polymorphism affects methotrexate treatment

outcome in rheumatoid arthritis', *Pharmacogenomics Journal*, vol. 7, no. 6, pp. 404-7. Epub 2007 Feb 27.
Drury, AN & Szent-Gyorgyi, A 1929, 'The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart', *Journal of Physiology*, vol. 68, no. 3, pp. 213-37.
Duthie, JJ, Brown, PE, Truelove, LH, Baragar, FD & Lawrie, AJ 1964, 'Course and prognosis in rheumatoid arthritis. A further report', *Annals of the Rheumatic Diseases*, vol. 23, pp. 193-204.
Eikelboom, JW, Lonn, E, Genest, J, Jr., Hankey, G & Yusuf, S 1999, 'Homocyst(e)ine and cardiovascular disease: a critical review of the epidemiologic evidence', *Annals of Internal Medicine*, vol. 131, no. 5, pp. 363-75.
El-Barbary, AM, Hussein, MS, Rageh, EM, Hamouda, HE, Wagih, AA & Ismail, RG 2011, 'Effect of atorvastatin on inflammation and modification of vascular risk factors in rheumatoid arthritis', *Journal of Rheumatology*, vol. 38, no. 2, pp. 229-35. doi: 10.3899/jrheum.100582. Epub 2010 Nov 1.
Emery, P, Breedveld, FC, Dougados, M, Kalden, JR, Schiff, MH & Smolen, JS 2002, 'Early referral recommendation for newly diagnosed rheumatoid arthritis: evidence based development of a clinical guide', *Annals of the Rheumatic Diseases*, vol. 61, no. 4, pp. 290-7.
Emery, P, Gabay, C, Kraan, M & Gomez-Reino, J 2007, 'Evidence-based review of biologic markers as indicators of disease progression and remission in rheumatoid arthritis', *Rheumatology International*, vol. 27, no. 9, pp. 793-806. Epub 2007 May 16.
Erne, P, Bolli, P, Burgisser, E & Buhler, FR 1984, 'Correlation of platelet calcium with blood pressure. Effect of antihypertensive therapy', *New England Journal of Medicine*, vol. 310, no. 17, pp. 1084-8.
Ernst, E & Resch, KL 1993, 'Fibrinogen as a cardiovascular risk factor: a meta-analysis and review of the literature', *Annals of Internal Medicine*, vol. 118, no. 12, pp. 956-63.
Essouma, M & Noubiap, JJ 2015, 'Therapeutic potential of folic acid supplementation for cardiovascular disease prevention through homocysteine lowering and blockade in rheumatoid arthritis patients', *Biomarker Research*, vol. 3:24., no. doi, pp. 10.1186/s40364-015-0049-9. eCollection 2015.
Ezzati, M, Hoorn, SV, Lopez, AD, Danaei, G, Rodgers, A, Mathers, CD & Murray, CJL 2006, 'Comparative quantification of mortality and burden of disease attributable to selected risk factors', in *Global Burden of Disease and Risk Factors*, The International Bank for Reconstruction and Development /The World Bank, Washington (DC)., pp. 241-268.
Fagard, R, Brguljan, J, Thijs, L & Staessen, J 1996, 'Prediction of the actual awake and asleep blood pressures by various methods of 24 h pressure analysis', *Journal of Hypertension*, vol. 14, no. 5, pp. 557-63.
Fantuzzi, G 2005, 'Adipose tissue, adipokines, and inflammation', *Journal of Allergy and Clinical Immunology*, vol. 115, no. 5, pp. 911-9.
Fard, A, Tuck, CH, Donis, JA, Sciacca, R, Di Tullio, MR, Wu, HD, Bryant, TA, Chen, NT, Torres-Tamayo, M, Ramasamy, R, Berglund, L, Ginsberg, HN, Homma, S & Cannon, PJ 2000, 'Acute elevations of plasma asymmetric dimethylarginine and impaired endothelial function in response to a high-fat meal in patients with type 2 diabetes', *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 20, no. 9, pp. 2039-44.
Fardet, L, Petersen, I & Nazareth, I 2011, 'Prevalence of long-term oral glucocorticoid prescriptions in the UK over the past 20 years', *Rheumatology*, vol. 50, no. 11, pp. 1982-90.

Fehlauer, CS, Carson, CW, Cannon, GW, Ward, JR, Samuelson, CO, Williams, HJ & Clegg, DO 1989, 'Methotrexate therapy in rheumatoid arthritis: 2-year retrospective followup study', *Journal of Rheumatology*, vol. 16, no. 3, pp. 307-12.

Felson, DT, Anderson, JJ, Boers, M, Bombardier, C, Chernoff, M, Fried, B, Furst, D, Goldsmith, C, Kieszak, S, Lightfoot, R & et al. 1993, 'The American College of Rheumatology preliminary core set of disease activity measures for rheumatoid arthritis clinical trials. The Committee on Outcome Measures in Rheumatoid Arthritis Clinical Trials', *Arthritis and Rheumatism*, vol. 36, no. 6, pp. 729-40.

Felson, DT, Anderson, JJ, Boers, M, Bombardier, C, Furst, D, Goldsmith, C, Katz, LM, Lightfoot, R, Jr., Paulus, H, Strand, V & et al. 1995, 'American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 38, no. 6, pp. 727-35.

Feng, J, Chen, Q, Yu, F, Wang, Z, Chen, S, Jin, Z, Cai, Q, Liu, Y & He, J 2016, 'Body mass index and risk of rheumatoid arthritis: a meta-analysis of observational studies', *Medicine (Baltimore)*, vol. 95, no. 8, p. e2859. doi: 10.1097/MD.0000000000002859.

Feoktistov, I 2002, 'Differential expression of adenosine receptors in human endothelial cells: role of a2b receptors in angiogenic factor regulation', *Circulation Research*, vol. 90, no. 5, pp. 531-8.

Ferrier, KE, Muhlmann, MH, Baguet, JP, Cameron, JD, Jennings, GL, Dart, AM & Kingwell, BA 2002, 'Intensive cholesterol reduction lowers blood pressure and large artery stiffness in isolated systolic hypertension', *Journal of the American College of Cardiology*, vol. 39, no. 6, pp. 1020-5.

Festa, A, D'Agostino, R, Jr., Howard, G, Mykkanen, L, Tracy, RP & Haffner, SM 2000, 'Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS)', *Circulation*, vol. 102, no. 1, pp. 42-7.

Fichtlscherer, S, Rossig, L, Breuer, S, Vasa, M, Dimmeler, S & Zeiher, AM 2001, 'Tumor necrosis factor antagonism with etanercept improves systemic endothelial vasoreactivity in patients with advanced heart failure', *Circulation*, vol. 104, no. 25, pp. 3023-5.

Finckh, A & Turesson, C 2014, 'The impact of obesity on the development and progression of rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 73, no. 11, pp. 1911-3.

Firestein, GS 2003, 'Evolving concepts of rheumatoid arthritis', *Nature*, vol. 423, no. 6937, pp. 356-61.

Fisslthaler, B & Fleming, I 2009, 'Activation and signaling by the AMP-activated protein kinase in endothelial cells', *Circulation Research*, vol. 105, no. 2, pp. 114-27. doi: 10.1161/CIRCRESAHA.109.201590.

Forstermann, U & Munzel, T 2006, 'Endothelial nitric oxide synthase in vascular disease: from marvel to menace', *Circulation*, vol. 113, no. 13, pp. 1708-14.

Fortin, PR, Lew, RA, Liang, MH, Wright, EA, Beckett, LA, Chalmers, TC & Sperling, RI 1995, 'Validation of a meta-analysis: the effects of fish oil in rheumatoid arthritis', *Journal of Clinical Epidemiology*, vol. 48, no. 11, pp. 1379-90.

Fox, KR & Hillsdon, M 2007, 'Physical activity and obesity', *Obesity Reviews*, vol. 8, no. Suppl 1, pp. 115-21.

Fox, RI, Herrmann, ML, Frangou, CG, Wahl, GM, Morris, RE, Strand, V & Kirschbaum, BJ 1999, 'Mechanism of action for leflunomide in rheumatoid arthritis', *Clinical Immunology*, vol. 93, no. 3, pp. 198-208.

Franklin, SS 2005, 'Arterial stiffness and hypertension: a two-way street?', *Hypertension*, vol. 45, no. 3, pp. 349-51. Epub 2005 Feb 14.
Franssen, PM & Imholz, BP 2010, 'Evaluation of the Mobil-O-Graph new generation ABPM device using the ESH criteria', *Blood Pressure Monitoring*, vol. 15, no. 4, pp. 229-31.
Frisell, T, Hellgren, K, Alfredsson, L, Raychaudhuri, S, Klareskog, L & Askling, J 2016, 'Familial aggregation of arthritis-related diseases in seropositive and seronegative rheumatoid arthritis: a register-based case-control study in Sweden', *Annals of the Rheumatic Diseases*, vol. 75, no. 1, pp. 183-9. doi: 10.1136/annrheumdis-2014-206133. Epub 2014 Dec 12.
Fromm, MF, Kauffmann, HM, Fritz, P, Burk, O, Kroemer, HK, Warzok, RW, Eichelbaum, M, Siegmund, W & Schrenk, D 2000, 'The effect of rifampin treatment on intestinal expression of human MRP transporters', *American Journal of Pathology*, vol. 157, no. 5, pp. 1575-80.
Frosst, P, Blom, HJ, Milos, R, Goyette, P, Sheppard, CA, Matthews, RG, Boers, GJ, den Heijer, M, Kluijtmans, LA, van den Heuvel, LP & et al. 1995, 'A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase', *Nature Genetics*, vol. 10, no. 1, pp. 111-3.
Fujimaki, C, Hayashi, H, Tsuboi, S, Matsuyama, T, Kosuge, K, Yamada, H, Inoue, K & Itoh, K 2009, 'Plasma total homocysteine level and methylenetetrahydrofolate reductase 677C>T genetic polymorphism in Japanese patients with rheumatoid arthritis', *Biomarkers*, vol. 14, no. 1, pp. 49-54. doi: 10.1080/13547500902730664.
Furst, DE 1997, 'The rational use of methotrexate in rheumatoid arthritis and other rheumatic diseases', *British Journal of Rheumatology*, vol. 36, no. 11, pp. 1196-204.
Gabb, GM, Mangoni, AA, Anderson, CS, Cowley, D, Dowden, JS, Golledge, J, Hankey, GJ, Howes, FS, Leckie, L, Perkovic, V, Schlaich, M, Zwar, NA, Medley, TL & Arnolda, L 2016, 'Guideline for the diagnosis and management of hypertension in adults - 2016', *Medical Journal of Australia*, vol. 205, no. 2, pp. 85-9.
Gabriel, SE, Crowson, CS, Kremers, HM, Doran, MF, Turesson, C, O'Fallon, WM & Matteson, EL 2003, 'Survival in rheumatoid arthritis: a population-based analysis of trends over 40 years', *Arthritis and Rheumatism*, vol. 48, no. 1, pp. 54-8.
Gabriel, SE, Crowson, CS & O'Fallon, WM 1999a, 'Comorbidity in arthritis', *Journal of Rheumatology*, vol. 26, no. 11, pp. 2475-9.
Gabriel, SE, Crowson, CS & O'Fallon, WM 1999b, 'Mortality in rheumatoid arthritis: have we made an impact in 4 decades?', *Journal of Rheumatology*, vol. 26, no. 12, pp. 2529-33.
Galarraga, B, Khan, F, Kumar, P, Pullar, T & Belch, JJ 2009, 'Etanercept improves inflammation-associated arterial stiffness in rheumatoid arthritis', *Rheumatology (Oxford)*, vol. 48, no. 11, pp. 1418-23. doi: 10.093/rheumatology/kep251. Epub 2009 Sep 4.
Geleijnse, JM, Giltay, EJ, Grobbee, DE, Donders, AR & Kok, FJ 2002, 'Blood pressure response to fish oil supplementation: metaregression analysis of randomized trials', *Journal of Hypertension*, vol. 20, no. 8, pp. 1493-9.
Giles, JT, Fernandes, V, Lima, JA & Bathon, JM 2005, 'Myocardial dysfunction in rheumatoid arthritis: epidemiology and pathogenesis', *Arthritis Research and Therapy*, vol. 7, no. 5, pp. 195-207. Epub 2005 Aug 24.
Giles, JT, Post, WS, Blumenthal, RS, Polak, J, Petri, M, Gelber, AC, Szklo, M & Bathon, JM 2011, 'Longitudinal predictors of progression of carotid atherosclerosis in rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 63, no. 11, pp. 3216-25. doi: 10.1002/art.30542.

Giovannelli, J, Dallongeville, J, Wagner, A, Bongard, V, Laillet, B, Marecaux, N, Ruidavets, JB, Haas, B, Ferrieres, J, Arveiler, D, Simon, C & Dauchet, L 2014, 'Validation of a short, qualitative food frequency questionnaire in French adults participating in the MONA LISA-NUT study 2005-2007', *Journal of the Academy of Nutrition and Dietetics*, vol. 114, no. 4, pp. 552-61. doi: 10.1016/j.jand.2013.07.002. Epub Sep 29.

Girod, JP & Brotman, DJ 2004, 'Does altered glucocorticoid homeostasis increase cardiovascular risk?', *Cardiovascular Research*, vol. 64, no. 2, pp. 217-26.

Glasser, SP, Arnett, DK, McVeigh, GE, Finkelstein, SM, Bank, AJ, Morgan, DJ & Cohn, JN 1997, 'Vascular compliance and cardiovascular disease: a risk factor or a marker?', *American Journal of Hypertension*, vol. 10, no. 10 Pt 1, pp. 1175-89.

Goldberg, RJ & Katz, J 2007, 'A meta-analysis of the analgesic effects of omega-3 polyunsaturated fatty acid supplementation for inflammatory joint pain', *Pain*, vol. 129, no. 1-2, pp. 210-23. Epub 2007 Mar 1.

Goldsmith, CH, Smythe, HA & Helewa, A 1993, 'Interpretation and power of a pooled index', *Journal of Rheumatology*, vol. 20, no. 3, pp. 575-8.

Goligorsky, MS 2005, 'Endothelial cell dysfunction: can't live with it, how to live without it', *American Journal of Physiology*, vol. 288, no. 5, pp. F871-80.

Gonzalez-Juanatey, C, Llorca, J, Testa, A, Revuelta, J, Garcia-Porrua, C & Gonzalez-Gay, MA 2003, 'Increased prevalence of severe subclinical atherosclerotic findings in long-term treated rheumatoid arthritis patients without clinically evident atherosclerotic disease', *Medicine (Baltimore)*, vol. 82, no. 6, pp. 407-13.

Gonzalez, A, Maradit Kremers, H, Crowson, CS, Ballman, KV, Roger, VL, Jacobsen, SJ, O'Fallon, WM & Gabriel, SE 2008, 'Do cardiovascular risk factors confer the same risk for cardiovascular outcomes in rheumatoid arthritis patients as in non-rheumatoid arthritis patients?', *Annals of the Rheumatic Diseases*, vol. 67, no. 1, pp. 64-9.

Gonzalez, A, Maradit Kremers, H, Crowson, CS, Nicola, PJ, Davis, JM, 3rd, Therneau, TM, Roger, VL & Gabriel, SE 2007, 'The widening mortality gap between rheumatoid arthritis patients and the general population', *Arthritis and Rheumatism*, vol. 56, no. 11, pp. 3583-7.

Goodman, SM, Cronstein, BN & Bykerk, VP 2015, 'Outcomes related to methotrexate dose and route of administration in patients with rheumatoid arthritis: a systematic literature review', *Clinical and Experimental Rheumatology*, vol. 33, no. 2, pp. 272-8. Epub 2014 Dec 23.

Goodson, NJ, Wiles, NJ, Lunt, M, Barrett, EM, Silman, AJ & Symmons, DP 2002, 'Mortality in early inflammatory polyarthritis: cardiovascular mortality is increased in seropositive patients', *Arthritis and Rheumatism*, vol. 46, no. 8, pp. 2010-9.

Goodwin, JE & Geller, DS 2012, 'Glucocorticoid-induced hypertension', *Pediatric Nephrology*, vol. 27, no. 7, pp. 1059-66. doi: 10.07/s00467-011-1928-4. Epub 2011 Jul 9.

Goonasekera, CD, Rees, DD, Woolard, P, Frend, A, Shah, V & Dillon, MJ 1997, 'Nitric oxide synthase inhibitors and hypertension in children and adolescents', *Journal of Hypertension*, vol. 15, no. 8, pp. 901-9.

Goralczyk, T, Tisonczyk, J, Fijorek, K & Undas, A 2012, 'High tea and vegetable consumption is associated with low ADMA generation in older healthy subjects', *Metabolism: Clinical and Experimental*, vol. 61, no. 8, pp. 1171-6.

Greenberg, JD, Kremer, JM, Curtis, JR, Hochberg, MC, Reed, G, Tsao, P, Farkouh, ME, Nasir, A, Setoguchi, S & Solomon, DH 2011, 'Tumour necrosis factor antagonist use and associated risk reduction of cardiovascular events among patients with

rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 70, no. 4, pp. 576-82. doi: 10.1136/ard.2010.129916. Epub 2010 Nov 24.
Greenberg, JD, Reed, G, Kremer, JM, Tindall, E, Kavanaugh, A, Zheng, C, Bishai, W & Hochberg, MC 2010, 'Association of methotrexate and tumour necrosis factor antagonists with risk of infectious outcomes including opportunistic infections in the CORRONA registry', *Annals of the Rheumatic Diseases*, vol. 69, no. 2, pp. 380-6. doi: 10.1136/ard.2008.089276. Epub 2009 Apr 8.
Gregersen, PK, Silver, J & Winchester, RJ 1987, 'The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 30, no. 11, pp. 1205-13.
Gremese, E & Ferraccioli, G 2011, 'The metabolic syndrome: the crossroads between rheumatoid arthritis and cardiovascular risk', *Autoimmunity Reviews*, vol. 10, no. 10, pp. 582-9. doi: 10.1016/j.autrev.2011.04.018. Epub Apr 22.
Griendling, KK, Sorescu, D & Ushio-Fukai, M 2000, 'NAD(P)H oxidase: role in cardiovascular biology and disease', *Circulation Research*, vol. 86, no. 5, pp. 494-501.
Grimes, DA & Schulz, KF 2002, 'Bias and causal associations in observational research', *The Lancet*, vol. 359, no. 9302, pp. 248-52.
Grosser, T, Fries, S & FitzGerald, GA 2006, 'Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities', *Journal of Clinical Investigation*, vol. 116, no. 1, pp. 4-15.
Grundy, SM, Brewer, HB, Jr., Cleeman, JI, Smith, SC, Jr. & Lenfant, C 2004, 'Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition', *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 24, no. 2, pp. e13-8.
Gubner, R, August, S & Ginsberg, V 1951, 'Therapeutic suppression of tissue reactivity. II. Effect of aminopterin in rheumatoid arthritis and psoriasis', *American Journal of the Medical Sciences*, vol. 221, no. 2, pp. 176-82.
Hackam, DG, Quinn, RR, Ravani, P, Rabi, DM, Dasgupta, K, Daskalopoulou, SS, Khan, NA, Herman, RJ, Bacon, SL, Cloutier, L, Dawes, M, Rabkin, SW, Gilbert, RE, Ruzicka, M, McKay, DW, Campbell, TS, Grover, S, Honos, G, Schiffrin, EL, Bolli, P, Wilson, TW, Feldman, RD, Lindsay, P, Hill, MD, Gelfer, M, Burns, KD, Vallee, M, Prasad, GV, Lebel, M, McLean, D, Arnold, JM, Moe, GW, Howlett, JG, Boulanger, JM, Larochelle, P, Leiter, LA, Jones, C, Ogilvie, RI, Woo, V, Kaczorowski, J, Trudeau, L, Petrella, RJ, Milot, A, Stone, JA, Drouin, D, Lavoie, KL, Lamarre-Cliche, M, Godwin, M, Tremblay, G, Hamet, P, Fodor, G, Carruthers, SG, Pylypchuk, GB, Burgess, E, Lewanczuk, R, Dresser, GK, Penner, SB, Hegele, RA, McFarlane, PA, Sharma, M, Reid, DJ, Tobe, SW, Poirier, L & Padwal, RS 2013, 'The 2013 Canadian Hypertension Education Program recommendations for blood pressure measurement, diagnosis, assessment of risk, prevention, and treatment of hypertension', *Canadian Journal of Cardiology*, vol. 29, no. 5, pp. 528-42. doi: 10.1016/j.cjca.2013.01.005. Epub Mar 29.
Hakoda, M, Oiwa, H, Kasagi, F, Masunari, N, Yamada, M, Suzuki, G & Fujiwara, S 2005, 'Mortality of rheumatoid arthritis in Japan: a longitudinal cohort study', *Annals of the Rheumatic Diseases*, vol. 64, no. 10, pp. 1451-5. Epub 2005 May 5.
Hametner, B, Wassertheurer, S, Kropf, J, Mayer, C, Eber, B & Weber, T 2013, 'Oscillometric estimation of aortic pulse wave velocity: comparison with intra-aortic catheter

measurements', *Blood Pressure Monitoring*, vol. 18, no. 3, pp. 173-6. doi: 10.1097/MBP.0b013e3283614168.
Hamilton, CA, Brosnan, MJ, Al-Benna, S, Berg, G & Dominiczak, AF 2002, 'NAD(P)H oxidase inhibition improves endothelial function in rat and human blood vessels', *Hypertension*, vol. 40, no. 5, pp. 755-62.
Han, C, Robinson, DW, Jr., Hackett, MV, Paramore, LC, Fraeman, KH & Bala, MV 2006, 'Cardiovascular disease and risk factors in patients with rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis', *Journal of Rheumatology*, vol. 33, no. 11, pp. 2167-72. Epub 006 Sep 1.
Han, C, Robinson Jr, DW, Hackett, MV, Paramore, LC, Fraeman, KH & Bala, MV 2006, 'Cardiovascular disease and risk factors in patients with rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis', *Journal of Rheumatology*, vol. 33, no. 11, pp. 2167-72.
Hansel, S, Lassig, G, Pistrosch, F & Passauer, J 2003, 'Endothelial dysfunction in young patients with long-term rheumatoid arthritis and low disease activity', *Atherosclerosis*, vol. 170, no. 1, pp. 177-80.
Hansson, GK 2005, 'Inflammation, atherosclerosis, and coronary artery disease', *New England Journal of Medicine*, vol. 352, no. 16, pp. 1685-95.
Hansson, GK & Libby, P 2006, 'The immune response in atherosclerosis: a double-edged sword', *Nature Reviews Immunology*, vol. 6, no. 7, pp. 508-19. Epub 2006 Jun 16.
Harrington, JT 2009, 'The uses of disease activity scoring and the physician global assessment of disease activity for managing rheumatoid arthritis in rheumatology practice', *Journal of Rheumatology*, vol. 36, no. 5, pp. 925-9. doi: 10.3899/jrheum.081046. Epub 2009 Apr 15.
Hartley, L, May, MD, Loveman, E, Colquitt, JL & Rees, K 2016, 'Dietary fibre for the primary prevention of cardiovascular disease', *Cochrane Database of Systematic Reviews*, no. 1, p. CD011472. doi: 10.1002/14651858.CD011472.pub2.
Haruna, Y, Morita, Y, Komai, N, Yada, T, Sakuta, T, Tomita, N, Fox, DA & Kashihara, N 2006, 'Endothelial dysfunction in rat adjuvant-induced arthritis: vascular superoxide production by NAD(P)H oxidase and uncoupled endothelial nitric oxide synthase', *Arthritis and Rheumatism*, vol. 54, no. 6, pp. 1847-55.
Hasko, G & Cronstein, BN 2004, 'Adenosine: an endogenous regulator of innate immunity', *Trends in Immunology*, vol. 25, no. 1, pp. 33-9.
Haydar, AA, Hujairi, N, Kirkham, B, Hangartner, R & Goldsmith, DJ 2004, 'Chronic overdose of leflunomide inducing interstitial nephritis', *Nephrology, Dialysis, Transplantation*, vol. 19, no. 5, pp. 1334-5.
Hazes, JM 1991, 'Pregnancy and its effect on the risk of developing rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 50, no. 2, pp. 71-2.
He, FJ, Nowson, CA, Lucas, M & MacGregor, GA 2007, 'Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: meta-analysis of cohort studies', *Journal of Human Hypertension*, vol. 21, no. 9, pp. 717-28. Epub 2007 Apr 19.
He, FJ, Nowson, CA & MacGregor, GA 2006, 'Fruit and vegetable consumption and stroke: meta-analysis of cohort studies', *The Lancet*, vol. 367, no. 9507, pp. 320-6.
He, K, Song, Y, Daviglus, ML, Liu, K, Van Horn, L, Dyer, AR, Goldbourt, U & Greenland, P 2004a, 'Fish consumption and incidence of stroke: a meta-analysis of cohort studies', *Stroke*, vol. 35, no. 7, pp. 1538-42. Epub 2004 May 20.

He, K, Song, Y, Daviglus, ML, Liu, K, Van Horn, L, Dyer, AR & Greenland, P 2004b, 'Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies', *Circulation*, vol. 109, no. 22, pp. 2705-11.

Head, GA, McGrath, BP, Mihailidou, AS, Nelson, MR, Schlaich, MP, Stowasser, M, Mangoni, AA, Cowley, D, Brown, MA, Ruta, LA & Wilson, A 2012, 'Ambulatory blood pressure monitoring in Australia: 2011 consensus position statement', *Journal of Hypertension*, vol. 30, no. 2, pp. 253-66. doi: 10.1097/HJH.0b013e32834de621.

Head, GA, Mihailidou, AS, Duggan, KA, Beilin, LJ, Berry, N, Brown, MA, Bune, AJ, Cowley, D, Chalmers, JP, Howe, PR, Hodgson, J, Ludbrook, J, Mangoni, AA, McGrath, BP, Nelson, MR, Sharman, JE & Stowasser, M 2010, 'Definition of ambulatory blood pressure targets for diagnosis and treatment of hypertension in relation to clinic blood pressure: prospective cohort study', *British Medical Journal*, vol. 340:c1104., no. doi, p. 10.1136/bmj.c04.

Heil, SG, Van der Put, NM, Waas, ET, den Heijer, M, Trijbels, FJ & Blom, HJ 2001, 'Is mutated serine hydroxymethyltransferase (SHMT) involved in the etiology of neural tube defects?', *Molecular Genetics and Metabolism*, vol. 73, no. 2, pp. 164-72.

Henke, PK, Sukheepod, P, Proctor, MC, Upchurch Jr, GR & Stanley, JC 2003, 'Clinical relevance of peripheral vascular occlusive disease in patients with rheumatoid arthritis and systemic lupus erythematosus 1', *Journal of Vascular Surgery*, vol. 38, no. 1, pp. 111-5.

Hensor, EMA, Emery, P, Bingham, SJ, Conaghan, PG & Consortium, tY 2010, 'Discrepancies in categorizing rheumatoid arthritis patients by DAS-28(ESR) and DAS-28(CRP): can they be reduced?', *Rheumatology*, vol. 49, no. 8, pp. 1521-9.

Herman, S, Zurgil, N & Deutsch, M 2005, 'Low dose methotrexate induces apoptosis with reactive oxygen species involvement in T lymphocytic cell lines to a greater extent than in monocytic lines', *Inflammation Research*, vol. 54, no. 7, pp. 273-80.

Hermida, RC, Ayala, DE, Mojon, A, Smolensky, MH, Portaluppi, F & Fernandez, JR 2014, 'Sleep-time ambulatory blood pressure as a novel therapeutic target for cardiovascular risk reduction', *Journal of Human Hypertension*, vol. 28, no. 10, pp. 567-74. doi: 10.1038/jhh.2014.1. Epub Feb 6.

Hernandez-Baldizon, S 2012, '[How to effectively use methotrexate in rheumatoid arthritis?]', *Reumatología Clínica*, vol. 8, no. 1, pp. 42-5. doi: 10.1016/j.reuma.2011.01.010. Epub Sep 25.

Hernandez-Diaz, S, Varas-Lorenzo, C & Garcia Rodriguez, LA 2006, 'Non-steroidal antiinflammatory drugs and the risk of acute myocardial infarction', *Basic & Clinical Pharmacology & Toxicology*, vol. 98, no. 3, pp. 266-74.

Hetland, ML, Stengaard-Pedersen, K, Junker, P, Lottenburger, T, Ellingsen, T, Andersen, LS, Hansen, I, Skjodt, H, Pedersen, JK, Lauridsen, UB, Svendsen, A, Tarp, U, Podenphant, J, Hansen, G, Lindegaard, H, de Carvalho, A, Ostergaard, M & Horslev-Petersen, K 2006, 'Combination treatment with methotrexate, cyclosporine, and intraarticular betamethasone compared with methotrexate and intraarticular betamethasone in early active rheumatoid arthritis: an investigator-initiated, multicenter, randomized, double-blind, parallel-group, placebo-controlled study', *Arthritis and Rheumatism*, vol. 54, no. 5, pp. 1401-9.

Hider, SL, Bruce, IN & Thomson, W 2007, 'The pharmacogenetics of methotrexate', *Rheumatology*, vol. 46, no. 10, pp. 1520-4.

Hider, SL, Thomson, W, Mack, LF, Armstrong, DJ, Shadforth, M & Bruce, IN 2008, 'Polymorphisms within the adenosine receptor 2a gene are associated with adverse

events in RA patients treated with MTX', *Rheumatology (Oxford)*, vol. 47, no. 8, pp. 1156-9. doi: 10.093/rheumatology/ken182. Epub 2008 Jun 6.
Higashi, Y, Noma, K, Yoshizumi, M & Kihara, Y 2009, 'Endothelial function and oxidative stress in cardiovascular diseases', *Circulation Journal*, vol. 73, no. 3, pp. 411-8. Epub 2009 Feb 4.
Hippisley-Cox, J & Coupland, C 2005, 'Risk of myocardial infarction in patients taking cyclo-oxygenase-2 inhibitors or conventional non-steroidal anti-inflammatory drugs: population based nested case-control analysis', *British Medical Journal*, vol. 330, no. 7504, p. 1366.
Hippisley-Cox, J, Coupland, C, Vinogradova, Y, Robson, J, Minhas, R, Sheikh, A & Brindle, P 2008, 'Predicting cardiovascular risk in England and Wales: prospective derivation and validation of QRISK2', *British Medical Journal*, vol. 336, no. 7659, pp. 1475-82. doi: 10.136/bmj.39609.449676.25. Epub 2008 Jun 23.
Hodge, A, Patterson, AJ, Brown, WJ, Ireland, P & Giles, G 2000, 'The Anti Cancer Council of Victoria FFQ: relative validity of nutrient intakes compared with weighed food records in young to middle-aged women in a study of iron supplementation', *Australian and New Zealand Journal of Public Health*, vol. 24, no. 6, pp. 576-83.
Hodge, AM, Simpson, JA, Fridman, M, Rowley, K, English, DR, Giles, GG, Su, Q & O'Dea, K 2009, 'Evaluation of an FFQ for assessment of antioxidant intake using plasma biomarkers in an ethnically diverse population', *Public Health Nutrition*, vol. 12, no. 12, pp. 2438-47. doi: 10.1017/S1368980009005539. Epub 2009 Apr 16.
Hoekstra, M, Haagsma, C, Neef, C, Proost, J, Knuif, A & van de Laar, M 2004, 'Bioavailability of higher dose methotrexate comparing oral and subcutaneous administration in patients with rheumatoid arthritis', *Journal of Rheumatology*, vol. 31, no. 4, pp. 645-8.
Hoekstra, M, van Ede, AE, Haagsma, CJ, van de Laar, MA, Huizinga, TW, Kruijsen, MW & Laan, RF 2003, 'Factors associated with toxicity, final dose, and efficacy of methotrexate in patients with rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 62, no. 5, pp. 423-6.
Hogg, N, Kalyanaraman, B, Joseph, J, Struck, A & Parthasarathy, S 1993, 'Inhibition of low-density lipoprotein oxidation by nitric oxide. Potential role in atherogenesis', *FEBS Letters*, vol. 334, no. 2, pp. 170-4.
Holden, DP, Cartwright, JE, Nussey, SS & Whitley, GS 2003, 'Estrogen stimulates dimethylarginine dimethylaminohydrolase activity and the metabolism of asymmetric dimethylarginine', *Circulation*, vol. 108, no. 13, pp. 1575-80. Epub 2003 Sep 8.
Hooijberg, JH, Broxterman, HJ, Kool, M, Assaraf, YG, Peters, GJ, Noordhuis, P, Scheper, RJ, Borst, P, Pinedo, HM & Jansen, G 1999, 'Antifolate resistance mediated by the multidrug resistance proteins MRP1 and MRP2', *Cancer Research*, vol. 59, no. 11, pp. 2532-5.
Horton, MR 2004, 'Rheumatoid arthritis associated interstitial lung disease', *Critical Reviews in Computed Tomography*, vol. 45, no. 5-6, pp. 429-40.
Howes, F, Hansen, E, Williams, D & Nelson, M 2010, 'Barriers to diagnosing and managing hypertension - a qualitative study in Australian general practice', *Australian Family Physician*, vol. 39, no. 7, pp. 511-6.
Hu, Y, Costenbader, KH, Gao, X, Al-Daabil, M, Sparks, JA, Solomon, DH, Hu, FB, Karlson, EW & Lu, B 2014, 'Sugar-sweetened soda consumption and risk of developing rheumatoid arthritis in women', *American Journal of Clinical Nutrition*, vol. 100, no. 3, pp. 959-67. doi: 10.3945/ajcn.114.086918. Epub 2014 Jul 16.

Huizinga, TW & Landewe, RB 2005, 'Early aggressive therapy in rheumatoid arthritis: a 'window of opportunity'?', *Nature Clinical Practice Rheumatology*, vol. 1, no. 1, pp. 2-3.

Hulot, JS, Villard, E, Maguy, A, Morel, V, Mir, L, Tostivint, I, William-Faltaos, D, Fernandez, C, Hatem, S, Deray, G, Komajda, M, Leblond, V & Lechat, P 2005, 'A mutation in the drug transporter gene ABCC2 associated with impaired methotrexate elimination', *Pharmacogenetics and Genomics*, vol. 15, no. 5, pp. 277-85.

Hurlimann, D, Forster, A, Noll, G, Enseleit, F, Chenevard, R, Distler, O, Bechir, M, Spieker, LE, Neidhart, M, Michel, BA, Gay, RE, Luscher, TF, Gay, S & Ruschitzka, F 2002, 'Anti-tumor necrosis factor-alpha treatment improves endothelial function in patients with rheumatoid arthritis', *Circulation*, vol. 106, no. 17, pp. 2184-7.

Hyndman, ME, Verma, S, Rosenfeld, RJ, Anderson, TJ & Parsons, HG 2002, 'Interaction of 5-methyltetrahydrofolate and tetrahydrobiopterin on endothelial function', *American Journal of Physiology-Heart and Circulatory Physiology*, vol. 282, no. 6, pp. H2167-72.

Ibiebele, TI, Parekh, S, Mallitt, KA, Hughes, MC, O'Rourke, PK & Webb, PM 2009, 'Reproducibility of food and nutrient intake estimates using a semi-quantitative FFQ in Australian adults', *Public Health Nutrition*, vol. 12, no. 12, pp. 2359-65. doi: 10.1017/S1368980009005023. Epub 2009 Mar 4.

Ignarro, LJ 2002, 'Nitric oxide as a unique signaling molecule in the vascular system: a historical overview', *Journal of Physiology and Pharmacology*, vol. 53, no. 4 Pt 1, pp. 503-14.

Ignarro, LJ, Balestrieri, ML & Napoli, C 2007, 'Nutrition, physical activity, and cardiovascular disease: an update', *Cardiovascular Research*, vol. 73, no. 2, pp. 326-40. Epub 2006 Jul 21.

Ignarro, LJ, Harbison, RG, Wood, KS & Kadowitz, PJ 1986, 'Activation of purified soluble guanylate cyclase by endothelium-derived relaxing factor from intrapulmonary artery and vein: stimulation by acetylcholine, bradykinin and arachidonic acid', *Journal of Pharmacology and Experimental Therapeutics*, vol. 237, no. 3, pp. 893-900.

Ignarro, LJ & Napoli, C 2004, 'Novel features of nitric oxide, endothelial nitric oxide synthase, and atherosclerosis', *Current Atherosclerosis Reports*, vol. 6, no. 4, pp. 281-7.

Ikdahl, E, Rollefstad, S, Hisdal, J, Olsen, IC, Pedersen, TR, Kvien, TK & Semb, AG 2016, 'Sustained improvement of arterial stiffness and blood pressure after long-term rosuvastatin treatment in patients with inflammatory joint diseases: results from the RORA-AS Study', *PLoS One.*, vol. 11, no. 4, p. e0153440. doi: 10.1371/journal.pone.. eCollection 2016.

Ikeda, K, Hirayama, T, Iwamoto, K, Takazawa, T, Kawase, Y, Yoshii, Y, Kano, O, Kawabe, K, Tamura, M & Iwasaki, Y 2011, 'Pulse wave velocity study in middle-aged migraineurs at low cardiovascular disease risk', *Headache*, vol. 51, no. 8, pp. 1239-44. doi: 10.111/j.526-4610.2011.01960.x. Epub 2011 Jul 28.

Innala, L, Moller, B, Ljung, L, Magnusson, S, Smedby, T, Sodergren, A, Ohman, ML, Rantapaa-Dahlqvist, S & Wallberg-Jonsson, S 2011, 'Cardiovascular events in early RA are a result of inflammatory burden and traditional risk factors: a five year prospective study', *Arthritis Research and Therapy*, vol. 13, no. 4, p. R131.

Inoue, K & Yuasa, H 2014, 'Molecular basis for pharmacokinetics and pharmacodynamics of methotrexate in rheumatoid arthritis therapy', *Drug Metabolism and Pharmacokinetics*, vol. 29, no. 1, pp. 12-9. Epub 2013 Nov 26.

Iqbal, R, Anand, S, Ounpuu, S, Islam, S, Zhang, X, Rangarajan, S, Chifamba, J, Al-Hinai, A, Keltai, M & Yusuf, S 2008, 'Dietary patterns and the risk of acute myocardial infarction in 52 countries: results of the INTERHEART study', *Circulation*, vol. 118, no. 19, pp. 1929-37. doi: 10.161/CIRCULATIONAHA.107.738716. Epub 2008 Oct 20.

Irace, C, Mancuso, G, Fiaschi, E, Madia, A, Sesti, G & Gnasso, A 2004, 'Effect of anti TNFalpha therapy on arterial diameter and wall shear stress and HDL cholesterol', *Atherosclerosis*, vol. 177, no. 1, pp. 113-8.

Ireland, P, Jolley, D, Giles, G, O'Dea, K, Powles, J, Rutishauser, I, Wahlqvist, ML & Williams, J 1994, 'Development of the Melbourne FFQ: a food frequency questionnaire for use in an Australian prospective study involving an ethnically diverse cohort', *Asia Pacific Journal of Clinical Nutrition*, vol. 3, no. 1, pp. 19-31.

Iso, H 2011, 'Lifestyle and cardiovascular disease in Japan', *Journal of Atherosclerosis and Thrombosis*, vol. 18, no. 2, pp. 83-8. Epub 2011 Feb 5.

Isomaki, HA, Mutru, O & Koota, K 1975, 'Death rate and causes of death in patients with rheumatoid arthritis', *Scandinavian Journal of Rheumatology*, vol. 4, no. 4, pp. 205-8.

Izzo, JL, Jr. 2004, 'Arterial stiffness and the systolic hypertension syndrome', *Current Opinion in Cardiology*, vol. 19, no. 4, pp. 341-52.

Jacobsson, LT, Knowler, WC, Pillemer, S, Hanson, RL, Pettitt, DJ, Nelson, RG, del Puente, A, McCance, DR, Charles, MA & Bennett, PH 1993, 'Rheumatoid arthritis and mortality. A longitudinal study in Pima Indians', *Arthritis and Rheumatism*, vol. 36, no. 8, pp. 1045-53.

Jain, S, Khera, R, Corrales-Medina, VF, Townsend, RR & Chirinos, JA 2014, '"Inflammation and arterial stiffness in humans"', *Atherosclerosis*, vol. 237, no. 2, pp. 381-90. doi: 10.1016/j.atherosclerosis.2014.09.011. Epub Sep 28.

Jalili, M, Kolahi, S, Aref-Hosseini, SR, Mamegani, ME & Hekmatdoost, A 2014, 'Beneficial role of antioxidants on clinical outcomes and erythrocyte antioxidant parameters in rheumatoid arthritis patients', *International Journal of Preventive Medicine*, vol. 5, no. 7, pp. 835-40.

Jansen, A, van der Horst-Bruinsma, I, van Schaardenburg, D, van de Stadt, R, de Koning, M & Dijkmans, B 2002, 'Rheumatoid factor and antibodies to cyclic citrullinated Peptide differentiate rheumatoid arthritis from undifferentiated polyarthritis in patients with early arthritis', *Journal of Rheumatology*, vol. 29, no. 10, pp. 2074-6.

Jansen, L, van der Horst-Bruinsma, I, van Schaardenburg, D, Bezemer, P & Dijkmans, B 2001, 'Predictors of radiographic joint damage in patients with early rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 60, no. 10, pp. 924-7.

Jansen, L, van Schaardenburg, D, van Der Horst-Bruinsma, I, Bezemer, P & Dijkmans, B 2000, 'Predictors of functional status in patients with early rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 59, pp. 223 - 6.

Jekic, B, Lukovic, L, Bunjevacki, V, Milic, V, Novakovic, I, Damnjanovic, T, Milasin, J, Popovic, B, Maksimovic, N, Damjanov, N, Radunovic, G, Kovacevic, L & Krajinovic, M 2013, 'Association of the TYMS 3G/3G genotype with poor response and GGH 354GG genotype with the bone marrow toxicity of the methotrexate in RA patients', *European Journal of Clinical Pharmacology*, vol. 69, no. 3, pp. 377-83. doi: 10.1007/s00228-012-1341-3. Epub 2012 Jul 5.

Jensen, MP, Chen, C & Brugger, AM 2003, 'Interpretation of visual analog scale ratings and change scores: a reanalysis of two clinical trials of postoperative pain', *Journal of Pain*, vol. 4, no. 7, pp. 407-14.

Jiang, X, Frisell, T, Askling, J, Karlson, EW, Klareskog, L, Alfredsson, L & Källberg, H 2015, 'To what extent is the familial risk of rheumatoid arthritis explained by established rheumatoid arthritis risk factors?', *Arthritis & Rheumatology*, vol. 67, no. 2, pp. 352-62.

Johnson, C, Giles, JT, Bathon, J, Lederer, D, Hoffman, EA, Barr, RG & Danoff, SK 2016, 'Smoking and subclinical ILD in RA versus the multi-ethnic study of atherosclerosis', *PLoS One*, vol. 11, no. 4, p. e0153024. doi: 10.1371/journal.pone.. eCollection 2016.

Jones, CR, Taylor, K, Chowienczyk, P, Poston, L & Shennan, AH 2000, 'A validation of the Mobil O Graph (version 12) ambulatory blood pressure monitor', *Blood Pressure Monitoring*, vol. 5, no. 4, pp. 233-8.

Jones, G, Sebba, A, Gu, J, Lowenstein, MB, Calvo, A, Gomez-Reino, JJ, Siri, DA, Tomsic, M, Alecock, E, Woodworth, T & Genovese, MC 2010, 'Comparison of tocilizumab monotherapy versus methotrexate monotherapy in patients with moderate to severe rheumatoid arthritis: the AMBITION study', *Annals of the Rheumatic Diseases*, vol. 69, no. 1, pp. 88-96. doi: 10.1136/ard.2008.105197. Epub.

Jorgensen, KT, Wiik, A, Pedersen, M, Hedegaard, CJ, Vestergaard, BF, Gislefoss, RE, Kvien, TK, Wohlfahrt, J, Bendtzen, K & Frisch, M 2008, 'Cytokines, autoantibodies and viral antibodies in premorbid and postdiagnostic sera from patients with rheumatoid arthritis: case-control study nested in a cohort of Norwegian blood donors', *Annals of the Rheumatic Diseases*, vol. 67, no. 6, pp. 860-6. Epub 2007 Jul 20.

Kapil, V, Webb, AJ & Ahluwalia, A 2010, 'Inorganic nitrate and the cardiovascular system', *Heart*, vol. 96, no. 21, pp. 1703-9. doi: 10.136/hrt.2009.180372. Epub 2010 Aug 23.

Karvounaris, SA, Sidiropoulos, PI, Papadakis, JA, Spanakis, EK, Bertsias, GK, Kritikos, HD, Ganotakis, ES & Boumpas, DT 2007, 'Metabolic syndrome is common among middle-to-older aged Mediterranean patients with rheumatoid arthritis and correlates with disease activity: a retrospective, cross-sectional, controlled, study', *Annals of the Rheumatic Diseases*, vol. 66, no. 1, pp. 28-33. Epub 2006 Jun 22.

Kato, T, Hamada, A, Mori, S & Saito, H 2012, 'Genetic polymorphisms in metabolic and cellular transport pathway of methotrexate impact clinical outcome of methotrexate monotherapy in Japanese patients with rheumatoid arthritis', *Drug Metabolism and Pharmacokinetics*, vol. 27, no. 2, pp. 192-9. Epub 2011 Nov 22.

Kay, J, Morgacheva, O, Messing, S, Kremer, J, Greenberg, J, Reed, G, Gravallese, E & Furst, D 2014, 'Clinical disease activity and acute phase reactant levels are discordant among patients with active rheumatoid arthritis: acute phase reactant levels contribute separately to predicting outcome at one year', *Arthritis Research and Therapy*, vol. 16, no. 1, p. R40.

Kayacelebi, AA, Pham, VV, Willers, J, Hahn, A, Stichtenoth, DO, Jordan, J & Tsikas, D 2014, 'Plasma homoarginine (hArg) and asymmetric dimethylarginine (ADMA) in patients with rheumatoid arthritis: is homoarginine a cardiovascular corrective in rheumatoid arthritis, an anti-ADMA?', *International Journal of Cardiology*, vol. 176, no. 3, pp. 1129-31. doi: 10.016/j.ijcard.2014.07.296. Epub Aug 13.

Kearney, PM, Baigent, C, Godwin, J, Halls, H, Emberson, JR & Patrono, C 2006, 'Do selective cyclo-oxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials', *British Medical Journal*, vol. 332, no. 7553, pp. 1302-8.

Kearney, PM, Whelton, M, Reynolds, K, Muntner, P, Whelton, PK & He, J 2005, 'Global burden of hypertension: analysis of worldwide data', *The Lancet*, vol. 365, no. 9455, pp. 217-23.

Kellgren, JH, Lawrence, JS & Aitken-Swan, J 1953, 'Rheumatic complaints in an urban population', *Annals of the Rheumatic Diseases*, vol. 12, no. 1, pp. 5-15.
Kerekes, G, Soltesz, P, Szucs, G, Szamosi, S, Der, H, Szabo, Z, Csathy, L, Vancsa, A, Szodoray, P, Szegedi, G & Szekanecz, Z 2011, 'Effects of adalimumab treatment on vascular disease associated with early rheumatoid arthritis', *Israel Medicine Association Journal*, vol. 13, no. 3, pp. 147-52.
Kielstein, JT, Donnerstag, F, Gasper, S, Menne, J, Kielstein, A, Martens-Lobenhoffer, J, Scalera, F, Cooke, JP, Fliser, D & Bode-Böger, SM 2006, 'ADMA increases arterial stiffness and decreases cerebral blood flow in humans', *Stroke*, vol. 37, no. 8, pp. 2024-9. Epub 06 Jun 29.
Kim, HJ, Kim, MJ, Lee, CK & Hong, YH 2015, 'Effects of methotrexate on carotid intima-media thickness in patients with rheumatoid arthritis', *Journal of Korean Med Science*, vol. 30, no. 11, pp. 1589-96. doi: 10.3346/jkms.2015.30.11.1589. Epub 2015 Oct 16.
Kim, RB 2002, 'Drugs as P-glycoprotein substrates, inhibitors, and inducers', *Drug Metabolism Reviews*, vol. 34, no. 1-2, pp. 47-54.
Kim, YS, Sung, YK, Choi, CB, Uhm, WS, Kim, TH, Shin, JH & Jun, JB 2012, 'The major determinants of arterial stiffness in Korean patients with rheumatoid arthritis are age and systolic blood pressure, not disease-related factors', *Rheumatology International*, vol. 32, no. 11, pp. 3455-61. doi: 10.1007/s00296-011-2198-y. Epub 2011 Nov 6.
Kimoto, M, Whitley, GS, Tsuji, H & Ogawa, T 1995, 'Detection of NG,NG-dimethylarginine dimethylaminohydrolase in human tissues using a monoclonal antibody', *Journal of Biochemistry*, vol. 117, no. 2, pp. 237-8.
King, DE & DeLegge, M 2009, 'The impact of fiber supplementation on ADMA levels', *Nutrition in Clinical Practice*, vol. 24, no. 1, pp. 80-3. doi: 10.1177/0884533608329229.
King, DE, Egan, BM & Geesey, ME 2003, 'Relation of dietary fat and fiber to elevation of C-reactive protein', *American Journal of Cardiology*, vol. 92, no. 11, pp. 1335-9.
King, DE, Mainous, AG, 3rd, Egan, BM, Woolson, RF & Geesey, ME 2008, 'Effect of psyllium fiber supplementation on C-reactive protein: the trial to reduce inflammatory markers (TRIM)', *Annals of Family Medicine*, vol. 6, no. 2, pp. 100-6. doi: 10.1370/afm.819.
Kirwan, JR & Reeback, JS 1986, 'Stanford Health Assessment Questionnaire modified to assess disability in British patients with rheumatoid arthritis', *British Journal of Rheumatology*, vol. 25, no. 2, pp. 206-9.
Kisiel, B, Kruszewski, R, Juszkiewicz, A, Raczkiewicz, A, Bachta, A, Tlustochowicz, M, Staniszewska-Varga, J, Klos, K, Duda, K, Boguslawska-Walecka, R, Ploski, R & Tlustochowicz, W 2015, 'Methotrexate, Cyclosporine A, and Biologics Protect against Atherosclerosis in Rheumatoid Arthritis', *Journal of Immunology Research*, vol. 2015:759610., no. doi, p. 10.1155/2015/759610. Epub 2015 May 18.
Kjeldsen-Kragh, J, Lund, JA, Riise, T, Finnanger, B, Haaland, K, Finstad, R, Mikkelsen, K & Forre, O 1992, 'Dietary omega-3 fatty acid supplementation and naproxen treatment in patients with rheumatoid arthritis', *Journal of Rheumatology*, vol. 19, no. 10, pp. 1531-6.
Klareskog, L, Padyukov, L, Lorentzen, J & Alfredsson, L 2006a, 'Mechanisms of disease: Genetic susceptibility and environmental triggers in the development of rheumatoid arthritis', *Nature Clinical Practice Rheumatology*, vol. 2, no. 8, pp. 425-33.
Klareskog, L, Stolt, P, Lundberg, K, Källberg, H, Bengtsson, C, Grunewald, J, Ronnelid, J, Harris, HE, Ulfgren, AK, Rantapaa-Dahlqvist, S, Eklund, A, Padyukov, L &

Alfredsson, L 2006b, 'A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination', *Arthritis and Rheumatism*, vol. 54, no. 1, pp. 38-46.
Klocke, R, Cockcroft, JR, Taylor, GJ, Hall, IR & Blake, DR 2003, 'Arterial stiffness and central blood pressure, as determined by pulse wave analysis, in rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 62, no. 5, pp. 414-8.
Kocabay, G, Hasdemir, H & Yildiz, M 2012, 'Evaluation of pulse wave velocity in systemic lupus erythematosus, rheumatoid arthritis and Behçet's disease', *Journal of Cardiology*, vol. 59, no. 1, pp. 72-7.
Koivuniemi, R, Paimela, L, Suomalainen, R & Leirisalo-Repo, M 2013, 'Cardiovascular diseases in patients with rheumatoid arthritis', *Scandinavian Journal of Rheumatology*, vol. 42, no. 2, pp. 131-5.
Komai, N, Morita, Y, Sakuta, T, Kuwabara, A & Kashihara, N 2007, 'Anti-tumor necrosis factor therapy increases serum adiponectin levels with the improvement of endothelial dysfunction in patients with rheumatoid arthritis', *Modern Rheumatology*, vol. 17, no. 5, pp. 385-90. Epub 2007 Oct 19.
Koupenova, M, Johnston-Cox, H & Ravid, K 2012, 'Regulation of atherosclerosis and associated risk factors by adenosine and adenosine receptors', *Current Atherosclerosis Reports*, vol. 14, no. 5, pp. 460-8. doi: 10.1007/s11883-012-0263-y.
Koutroumbas, G, Georgianos, PI, Sarafidis, PA, Protogerou, A, Karpetas, A, Vakianis, P, Raptis, V, Liakopoulos, V, Panagoutsos, S, Syrganis, C & Passadakis, P 2015, 'Ambulatory aortic blood pressure, wave reflections and pulse wave velocity are elevated during the third in comparison to the second interdialytic day of the long interval in chronic haemodialysis patients', *Nephrology Dialysis Transplantation*, vol. 30, no. 12, pp. 2046-53. doi: 10.1093/ndt/gfv090. Epub 2015 Apr 28.
Krajinovic, M, Costea, I, Primeau, M, Dulucq, S & Moghrabi, A 2005, 'Combining several polymorphisms of thymidylate synthase gene for pharmacogenetic analysis', *Pharmacogenomics Journal*, vol. 5, no. 6, pp. 374-80.
Krajinovic, M, Lemieux-Blanchard, E, Chiasson, S, Primeau, M, Costea, I & Moghrabi, A 2004, 'Role of polymorphisms in MTHFR and MTHFD1 genes in the outcome of childhood acute lymphoblastic leukemia', *Pharmacogenomics Journal*, vol. 4, no. 1, pp. 66-72.
Kremer, J & Lee, J 1986, 'The safety and efficacy of the use of methotrexate in long-term therapy for rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 29, pp. 822 - 31.
Kremer, JM 2004, 'Toward a better understanding of methotrexate', *Arthritis and Rheumatism*, vol. 50, no. 5, pp. 1370-82.
Kremer, JM, Jubiz, W, Michalek, A, Rynes, RI, Bartholomew, LE, Bigaouette, J, Timchalk, M, Beeler, D & Lininger, L 1987, 'Fish-oil fatty acid supplementation in active rheumatoid arthritis. A double-blinded, controlled, crossover study', *Annals of Internal Medicine*, vol. 106, no. 4, pp. 497-503.
Kremer, JM, Lawrence, DA, Jubiz, W, DiGiacomo, R, Rynes, R, Bartholomew, LE & Sherman, M 1990, 'Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis. Clinical and immunologic effects', *Arthritis and Rheumatism*, vol. 33, no. 6, pp. 810-20.
Kremers, HM, Crowson, CS, Therneau, TM, Roger, VL & Gabriel, SE 2008, 'High ten-year risk of cardiovascular disease in newly diagnosed rheumatoid arthritis patients: a population-based cohort study', *Arthritis and Rheumatism*, vol. 58, no. 8, pp. 2268-74.
Kris-Etherton, PM, Harris, WS & Appel, LJ 2002, 'Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease', *Circulation*, vol. 106, no. 21, pp. 2747-57.

Kroeker, EJ & Wood, EH 1955, 'Comparison of simultaneously recorded central and peripheral arterial pressure pulses during rest, exercise and tilted position in man', *Circulation Research*, vol. 3, no. 6, pp. 623-32.

Kromann, N & Green, A 1980, 'Epidemiological studies in the Upernavik district, Greenland. Incidence of some chronic diseases 1950-1974', *Acta Medica Scandinavica*, vol. 208, no. 5, pp. 401-6.

Kroot, EJ, de Jong, BA, van Leeuwen, MA, Swinkels, H, van den Hoogen, FH, van't Hof, M, van de Putte, LB, van Rijswijk, MH, van Venrooij, WJ & van Riel, PL 2000, 'The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 43, no. 8, pp. 1831-5.

Kroot, EJ, van Leeuwen, MA, van Rijswijk, MH, Prevoo, ML, Van 't Hof, MA, van De Putte, LB & van Riel, PL 2000, 'No increased mortality in patients with rheumatoid arthritis: up to 10 years of follow up from disease onset', *Annals of the Rheumatic Diseases*, vol. 59, no. 12, pp. 954-8.

Kumagai, K, Hiyama, K, Oyama, T, Maeda, H & Kohno, N 2003, 'Polymorphisms in the thymidylate synthase and methylenetetrahydrofolate reductase genes and sensitivity to the low-dose methotrexate therapy in patients with rheumatoid arthritis', *International Journal of Molecular Medicine*, vol. 11, no. 5, pp. 593-600.

Kume, K, Amano, K, Yamada, S, Hatta, K, Ohta, H & Kuwaba, N 2011, 'Tocilizumab monotherapy reduces arterial stiffness as effectively as etanercept or adalimumab monotherapy in rheumatoid arthritis: an open-label randomized controlled trial', *Journal of Rheumatology*, vol. 38, no. 10, pp. 2169-71. doi: 10.3899/jrheum.110340. Epub 2011 Aug 1.

Kumeda, Y, Inaba, M, Goto, H, Nagata, M, Henmi, Y, Furumitsu, Y, Ishimura, E, Inui, K, Yutani, Y, Miki, T, Shoji, T & Nishizawa, Y 2002, 'Increased thickness of the arterial intima-media detected by ultrasonography in patients with rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 46, no. 6, pp. 1489-97.

Kurko, J, Besenyei, T, Laki, J, Glant, TT, Mikecz, K & Szekanecz, Z 2013, 'Genetics of rheumatoid arthritis - a comprehensive review', *Clinical Reviews in Allergy and Immunology*, vol. 45, no. 2, pp. 170-9.

Kvalvik, AG, Jones, MA & Symmons, DP 2000, 'Mortality in a cohort of Norwegian patients with rheumatoid arthritis followed from 1977 to 1992', *Scandinavian Journal of Rheumatology*, vol. 29, no. 1, pp. 29-37.

Lafforgue, P, Monjanel-Mouterde, S, Durand, A, Catalin, J & Acquaviva, PC 1995, 'Lack of correlation between pharmacokinetics and efficacy of low dose methotrexate in patients with rheumatoid arthritis', *Journal of Rheumatology*, vol. 22, no. 5, pp. 844-9.

Lai, L & Ghebremariam, YT 2016, 'Modulating DDAH/NOS pathway to discover vasoprotective insulin sensitizers', *Journal of Diabetes Research*, vol. 2016:1982096., no. doi, p. 10.1155/2016/1982096. Epub 2015 Dec 6.

Lakatua, DJ, Haus, E, Halberg, F, Halberg, E, Wendt, HW, Sackett-Lundeen, LL, Berg, HG, Kawasaki, T, Ueno, M, Uezono, K & et al. 1986, 'Circadian characteristics of urinary epinephrine and norepinephrine from healthy young women in Japan and U.S.A', *Chronobiology International*, vol. 3, no. 3, pp. 189-95.

Lambert, TL, Kent, RS & Whorton, AR 1986, 'Bradykinin stimulation of inositol polyphosphate production in porcine aortic endothelial cells', *Journal of Biological Chemistry*, vol. 261, no. 32, pp. 15288-93.

Landim, MB, Casella Filho, A & Chagas, AC 2009, 'Asymmetric dimethylarginine (ADMA) and endothelial dysfunction: implications for atherogenesis', *Clinics (Sao Paulo)*, vol. 64, no. 5, pp. 471-8.

Lantelme, P, Mestre, C, Lievre, M, Gressard, A & Milon, H 2002, 'Heart rate: an important confounder of pulse wave velocity assessment', *Hypertension*, vol. 39, no. 6, pp. 1083-7.

Lash, TL & Fink, AK 2003, 'Semi-automated sensitivity analysis to assess systematic errors in observational data', *Epidemiology*, vol. 14, no. 4, pp. 451-8.

Lassegue, B & Clempus, RE 2003, 'Vascular NAD(P)H oxidases: specific features, expression, and regulation', *American Journal of Physiology, Regulatory, Integrative and Comparative Physiology*, vol. 285, no. 2, pp. R277-97.

Lassere, MN, Rappo, J, Portek, IJ, Sturgess, A & Edmonds, JP 2013, 'How many life years are lost in patients with rheumatoid arthritis? Secular cause-specific and all-cause mortality in rheumatoid arthritis, and their predictors in a long-term Australian cohort study', *Internal Medicine Journal*, vol. 43, no. 1, pp. 66-72. doi: 10.1111/j.445-5994.2012.02727.x.

Lawrence, JS 1970, 'Heberden Oration, 1969. Rheumatoid arthritis--nature or nurture?', *Annals of the Rheumatic Diseases*, vol. 29, no. 4, pp. 357-79.

Lee, H-Y & Oh, B-H 2010, 'Aging and arterial stiffness', *Circulation Journal*, vol. 74, no. 11, pp. 2257-62.

Lee, SH, Lee, MS, Lee, JH, Kim, SW, Kang, RH, Choi, MJ, Park, SJ, Kim, SJ, Lee, JM, Cole, SP & Lee, MG 2010, 'MRP1 polymorphisms associated with citalopram response in patients with major depression', *Journal of Clinical Psychopharmacology*, vol. 30, no. 2, pp. 116-25. doi: 10.1097/JCP.0b013e3181d2ef42.

Lee, Y-H, Bae, S-C & Song, G-G 2012, 'Omega-3 polyunsaturated fatty acids and the treatment of rheumatoid arthritis: a meta-analysis', *Archives of Medical Research*, vol. 43, no. 5, pp. 356-62.

Leiper, J, Nandi, M, Torondel, B, Murray-Rust, J, Malaki, M, O'Hara, B, Rossiter, S, Anthony, S, Madhani, M, Selwood, D, Smith, C, Wojciak-Stothard, B, Rudiger, A, Stidwill, R, McDonald, NQ & Vallance, P 2007, 'Disruption of methylarginine metabolism impairs vascular homeostasis', *Nature Medicine*, vol. 13, no. 2, pp. 198-203. Epub 2007 Feb 4.

Leiper, JM, Santa Maria, J, Chubb, A, MacAllister, RJ, Charles, IG, Whitley, GS & Vallance, P 1999, 'Identification of two human dimethylarginine dimethylaminohydrolases with distinct tissue distributions and homology with microbial arginine deiminases', *Biochemical Journal*, vol. 343, no. Pt 1, pp. 209-14.

Lemmey, AB, Marcora, SM, Chester, K, Wilson, S, Casanova, F & Maddison, PJ 2009, 'Effects of high-intensity resistance training in patients with rheumatoid arthritis: a randomized controlled trial', *Arthritis and Rheumatism*, vol. 61, no. 12, pp. 1726-34. doi: 10.002/art.24891.

Lerman, A, Burnett, JC, Jr., Higano, ST, McKinley, LJ & Holmes, DR, Jr. 1998, 'Long-term L-arginine supplementation improves small-vessel coronary endothelial function in humans', *Circulation*, vol. 97, no. 21, pp. 2123-8.

Lerman, A & Zeiher, AM 2005, 'Endothelial function: cardiac events', *Circulation*, vol. 111, no. 3, pp. 363-8.

Lewington, S, Clarke, R, Qizilbash, N, Peto, R & Collins, R 2002, 'Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies', *The Lancet*, vol. 360, no. 9349, pp. 1903-13.

Lewis, P, Hazleman, BL, Hanka, R & Roberts, S 1980, 'Cause of death in patients with rheumatoid arthritis with particular reference to azathioprine', *Annals of the Rheumatic Diseases*, vol. 39, no. 5, pp. 457-61.

Li, J, Fenton, RA, Wheeler, HB, Powell, CC, Peyton, BD, Cutler, BS & Dobson, JG, Jr. 1998, 'Adenosine A2a receptors increase arterial endothelial cell nitric oxide', *Journal of Surgical Research*, vol. 80, no. 2, pp. 357-64.

Li, P, Han, CX, Ma, CL, Guo, JL, Liu, B, Du, J & Bi, LQ 2013, 'Determinants of brachial-ankle pulse wave velocity in Chinese patients with rheumatoid arthritis', *Clinical and Developmental Immunology*, vol. 2013:342869., no. doi, p. 10.1155/2013/342869. Epub 2013 Jul 31.

Li, R, Zhao, J, Su, Y, He, J, Chen, L, Gu, F, Zhao, C, Deng, X, Zhou, W, Hao, Y, Xue, Y, Liu, H, Zhao, Y, Zou, Q, Liu, X, Zhu, P, Sun, L, Zhang, Z, Zou, H, Li, X, Liu, Y, Fang, Y, Keystone, E, McInnes, I & Li, Z 2016, 'High remission and low relapse with prolonged intensive DMARD therapy in rheumatoid arthritis (PRINT): A multicenter randomized clinical trial', *Medicine (Baltimore)*, vol. 95, no. 28, p. e3968. doi: 10.1097/MD.0000000000003968.

Liakos, CI, Grassos, CA & Babalis, DK 2015, '2013 ESH/ESC guidelines for the management of arterial hypertension: what has changed in daily clinical practice?', *High Blood Pressure & Cardiovascular Prevention*, vol. 22, no. 1, pp. 43-53. doi: 10.1007/s40292-014-0071-2. Epub 2014 Sep 25.

Libby, P, Ridker, PM & Hansson, GK 2009, 'Inflammation in atherosclerosis: from pathophysiology to practice', *Journal of the American College of Cardiology*, vol. 54, no. 23, pp. 2129-38. doi: 10.1016/j.jacc.2009.09.009.

Libby, P, Ridker, PM & Maseri, A 2002, 'Inflammation and atherosclerosis', *Circulation*, vol. 105, no. 9, pp. 1135-43.

Lima, A, Seabra, V, Martins, S, Coelho, A, Araujo, A & Medeiros, R 2014, 'Thymidylate synthase polymorphisms are associated to therapeutic outcome of advanced non-small cell lung cancer patients treated with platinum-based chemotherapy', *Molecular Biology Reports*, vol. 41, no. 5, pp. 3349-57. doi: 10.1007/s11033-014-3197-3. Epub 2014 Feb 20.

Lima, A, Sousa, H, Monteiro, J, Azevedo, R, Medeiros, R & Seabra, V 2014, 'Genetic polymorphisms in low-dose methotrexate transporters: current relevance as methotrexate therapeutic outcome biomarkers', *Pharmacogenomics*, vol. 15, no. 12, pp. 1611-35. doi: 10.2217/pgs.14.116.

Lin, KY, Ito, A, Asagami, T, Tsao, PS, Adimoolam, S, Kimoto, M, Tsuji, H, Reaven, GM & Cooke, JP 2002, 'Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase', *Circulation*, vol. 106, no. 8, pp. 987-92.

Lind, L, Ingelsson, E, Kumar, J, Syvanen, AC, Axelsson, T & Teerlink, T 2013, 'Genetic variation in the dimethylarginine dimethylaminohydrolase 1 gene (DDAH1) is related to asymmetric dimethylarginine (ADMA) levels, but not to endothelium-dependent vasodilation', *Vascular Medicine*, vol. 18, no. 4, pp. 192-9. doi: 10.1177/1358863X13496488. Epub 2013 Jul 26.

Listing, J, Strangfeld, A, Kekow, J, Schneider, M, Kapelle, A, Wassenberg, S & Zink, A 2008, 'Does tumor necrosis factor alpha inhibition promote or prevent heart failure in patients with rheumatoid arthritis?', *Arthritis and Rheumatism*, vol. 58, no. 3, pp. 667-77. doi: 10.1002/art.23281.

Littlejohn, G, Roberts, L, Bird, P, de Jager, J, Griffiths, H, Nicholls, D, Young, J, Zochling, J & Tymms, KE 2015, 'Patients with rheumatoid arthritis in the Australian OPAL cohort show significant improvement in disease activity over 5 years: a multicenter observational study', *Journal of Rheumatology*, vol. 1, p. 141575.

Lu, B, Hiraki, LT, Sparks, JA, Malspeis, S, Chen, C-Y, Awosogba, JA, Arkema, EV, Costenbader, KH & Karlson, EW 2014, 'Being overweight or obese and risk of developing rheumatoid arthritis among women: a prospective cohort study', *Annals of the Rheumatic Diseases*, vol. 73, no. 11, pp. 1914-22.

Lundman, P, Eriksson, MJ, Stuhlinger, M, Cooke, JP, Hamsten, A & Tornvall, P 2001, 'Mild-to-moderate hypertriglyceridemia in young men is associated with endothelial dysfunction and increased plasma concentrations of asymmetric dimethylarginine', *Journal of the American College of Cardiology*, vol. 38, no. 1, pp. 111-6.

Luzardo, L, Lujambio, I, Sottolano, M, da Rosa, A, Thijs, L, Noboa, O, Staessen, JA & Boggia, J 2012, '24-h ambulatory recording of aortic pulse wave velocity and central systolic augmentation: a feasibility study', *Hypertension Research*, vol. 35, no. 10, pp. 980-7. doi: 10.1038/hr.2012.78. Epub May 24.

Ma, MH, Scott, IC, Kingsley, GH & Scott, DL 2010, 'Remission in early rheumatoid arthritis', *Journal of Rheumatology*, vol. 37, no. 7, pp. 1444-53. doi: 10.3899/jrheum.091131. Epub 2010 Jun 1.

MacAllister, RJ, Rambausek, MH, Vallance, P, Williams, D, Hoffmann, KH & Ritz, E 1996, 'Concentration of dimethyl-L-arginine in the plasma of patients with end-stage renal failure', *Nephrology Dialysis Transplantation*, vol. 11, no. 12, pp. 2449-52.

MacGregor, AJ, Snieder, H, Rigby, AS, Koskenvuo, M, Kaprio, J, Aho, K & Silman, AJ 2000, 'Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins', *Arthritis and Rheumatism*, vol. 43, no. 1, pp. 30-7.

MacLean, CH, Mojica, WA, Morton, SC, Pencharz, J, Hasenfeld Garland, R, Tu, W, Newberry, SJ, Jungvig, LK, Grossman, J, Khanna, P, Rhodes, S & Shekelle, P 2004, 'Effects of omega-3 fatty acids on lipids and glycemic control in type II diabetes and the metabolic syndrome and on inflammatory bowel disease, rheumatoid arthritis, renal disease, systemic lupus erythematosus, and osteoporosis', *Evidence Report/Technology Assessment (Summary).* no. 89, pp. 1-4.

Maehre, HK, Jensen, IJ, Elvevoll, EO & Eilertsen, KE 2015, 'Omega-3 fatty acids and cardiovascular diseases: effects, mechanisms and dietary relevance', *International Journal of Molecular Sciences*, vol. 16, no. 9, pp. 22636-61. doi: 10.3390/ijms160922636.

Mahmud, A & Feely, J 2001, 'Acute effect of caffeine on arterial stiffness and aortic pressure waveform', *Hypertension*, vol. 38, no. 2, pp. 227-31.

Mahmud, A & Feely, J 2003, 'Effect of smoking on arterial stiffness and pulse pressure amplification', *Hypertension*, vol. 41, no. 1, pp. 183-7.

Mahomed, FA 1872, 'The physiology and clinical use of the sphygmograph', *Medical Times and Gazette*, vol. 1, no. 62.

Mailloux, A, Grenet, K, Bruneel, A, Bénéteau-Burnat, B, Vaubourdolle, M & Baudin, B 2001, 'Anticancer drugs induce necrosis of human endothelial cells involving both oncosis and apoptosis', *European Journal of Cell Biology*, vol. 80, no. 6, pp. 442-9.

Maini, RN 1998, 'Rheumatoid arthritis. A paradigm of inflammatory disease of the musculoskeletal system', *Acta Orthopaedica Scandinavica*, vol. 281, pp. 6-13.

Maini, RN, Breedveld, FC, Kalden, JR, Smolen, JS, Davis, D, Macfarlane, JD, Antoni, C, Leeb, B, Elliott, MJ, Woody, JN, Schaible, TF & Feldmann, M 1998, 'Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 41, no. 9, pp. 1552-63.

Maki-Petaja, KM, Elkhawad, M, Cheriyan, J, Joshi, FR, Ostor, AJ, Hall, FC, Rudd, JH & Wilkinson, IB 2012, 'Anti-tumor necrosis factor-alpha therapy reduces aortic

inflammation and stiffness in patients with rheumatoid arthritis', *Circulation*, vol. 126, no. 21, pp. 2473-80. doi: 10.1161/CIRCULATIONAHA.112.120410. Epub 2012 Oct 24.

Maki-Petaja, KM, Hall, FC, Booth, AD, Wallace, SM, Yasmin, Bearcroft, PW, Harish, S, Furlong, A, McEniery, CM, Brown, J & Wilkinson, IB 2006, 'Rheumatoid arthritis is associated with increased aortic pulse-wave velocity, which is reduced by anti-tumor necrosis factor-alpha therapy', *Circulation*, vol. 114, no. 11, pp. 1185-92. Epub 2006 Sep 4.

Malone, AF & Reddan, DN 2010, 'Pulse pressure. Why is it important?', *Peritoneal Dialysis International*, vol. 30, no. 3, pp. 265-8. doi: 10.3747/pdi.2010.00002.

Manavathongchai, S, Bian, A, Rho, YH, Oeser, A, Solus, JF, Gebretsadik, T, Shintani, A & Stein, CM 2013, 'Inflammation and hypertension in rheumatoid arthritis', *Journal of Rheumatology*, vol. 40, no. 11, pp. 1806-11. doi: 10.3899/jrheum.130394. Epub 2013 Sep 1.

Mancia, G, De Backer, G, Dominiczak, A, Cifkova, R, Fagard, R, Germano, G, Grassi, G, Heagerty, AM, Kjeldsen, SE, Laurent, S, Narkiewicz, K, Ruilope, L, Rynkiewicz, A, Schmieder, RE, Boudier, HA, Zanchetti, A, Vahanian, A, Camm, J, De Caterina, R, Dean, V, Dickstein, K, Filippatos, G, Funck-Brentano, C, Hellemans, I, Kristensen, SD, McGregor, K, Sechtem, U, Silber, S, Tendera, M, Widimsky, P, Zamorano, JL, Erdine, S, Kiowski, W, Agabiti-Rosei, E, Ambrosioni, E, Lindholm, LH, Viigimaa, M, Adamopoulos, S, Agabiti-Rosei, E, Ambrosioni, E, Bertomeu, V, Clement, D, Erdine, S, Farsang, C, Gaita, D, Lip, G, Mallion, JM, Manolis, AJ, Nilsson, PM, O'Brien, E, Ponikowski, P, Redon, J, Ruschitzka, F, Tamargo, J, van Zwieten, P, Waeber, B & Williams, B 2007, '2007 Guidelines for the management of arterial hypertension: the task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC)', *Journal of Hypertension*, vol. 25, no. 6, pp. 1105-87.

Mancia, G, Fagard, R, Narkiewicz, K, Redon, J, Zanchetti, A, Bohm, M, Christiaens, T, Cifkova, R, De Backer, G, Dominiczak, A, Galderisi, M, Grobbee, DE, Jaarsma, T, Kirchhof, P, Kjeldsen, SE, Laurent, S, Manolis, AJ, Nilsson, PM, Ruilope, LM, Schmieder, RE, Sirnes, PA, Sleight, P, Viigimaa, M, Waeber, B & Zannad, F 2013, '2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC)', *Journal of Hypertension*, vol. 31, no. 7, pp. 1281-357. doi: 10.097/01.hjh.0000431740.32696.cc.

Mandola, MV, Stoehlmacher, J, Muller-Weeks, S, Cesarone, G, Yu, MC, Lenz, HJ & Ladner, RD 2003, 'A novel single nucleotide polymorphism within the 5' tandem repeat polymorphism of the thymidylate synthase gene abolishes USF-1 binding and alters transcriptional activity', *Cancer Research*, vol. 63, no. 11, pp. 2898-904.

Manfredsdottir, VF, Vikingsdottir, T, Jonsson, T, Geirsson, AJ, Kjartansson, O, Heimisdottir, M, Sigurdardottir, SL, Valdimarsson, H & Vikingsson, A 2006, 'The effects of tobacco smoking and rheumatoid factor seropositivity on disease activity and joint damage in early rheumatoid arthritis', *Rheumatology (Oxford)*, vol. 45, no. 6, pp. 734-40. Epub 2006 Jan 10.

Mangoni, AA 2009, 'The emerging role of symmetric dimethylarginine in vascular disease', *Advances in Clinical Chemistry*, vol. 48, pp. 73-94.

Mangoni, AA, Sherwood, RA, Asonganyi, B, Swift, CG, Thomas, S & Jackson, SH 2005, 'Short-term oral folic acid supplementation enhances endothelial function in patients

with type 2 diabetes', *American Journal of Hypertension*, vol. 18, no. 2 Pt 1, pp. 220-6.
Mangoni, AA, Sherwood, RA, Swift, CG & Jackson, SHD 2002, 'Folic acid enhances endothelial function and reduces blood pressure in smokers: a randomized controlled trial', *Journal of Internal Medicine*, vol. 252, no. 6, pp. 497-503.
Mann, CJ 2003, 'Observational research methods. Research design II: cohort, cross sectional, and case-control studies', *Emergency Medicine Journal*, vol. 20, no. 1, pp. 54-60.
Maradit-Kremers, H, Crowson, CS, Nicola, PJ, Ballman, KV, Roger, VL, Jacobsen, SJ & Gabriel, SE 2005a, 'Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a population-based cohort study', *Arthritis and Rheumatism*, vol. 52, no. 2, pp. 402-11.
Maradit-Kremers, H, Crowson, CS, Nicola, PJ, Ballman, KV, Roger, VL, Jacobsen, SJ & Gabriel, SE 2005b, 'Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a population-based cohort study', *Arthritis and Rheumatism*, vol. 52, no. 2, pp. 402-11.
Maradit-Kremers, H, Nicola, P, Crowson, C, Ballman, K & Gabriel, S 2005, 'Cardiovascular death in rheumatoid arthritis: a population-based study', *Arthritis and Rheumatism*, vol. 52, pp. 722 - 32.
Marey, EJ 1876, 'Pression et vitesse du sang', *Physiologie Experimentale, Paris: Pratique des hautes etudes de M Marey*.
Marsh, S, McKay, JA, Cassidy, J & McLeod, HL 2001, 'Polymorphism in the thymidylate synthase promoter enhancer region in colorectal cancer', *International Journal of Oncology*, vol. 19, no. 2, pp. 383-6.
Mason, JC & Libby, P 2015, 'Cardiovascular disease in patients with chronic inflammation: mechanisms underlying premature cardiovascular events in rheumatologic conditions', *European Heart Journal*, vol. 36, no. 8, pp. 482-9.
Masuda, S, Ibaramoto, K, Takeuchi, A, Saito, H, Hashimoto, Y & Inui, KI 1999, 'Cloning and functional characterization of a new multispecific organic anion transporter, OAT-K2, in rat kidney', *Molecular Pharmacology*, vol. 55, no. 4, pp. 743-52.
Matherly, LH & Goldman, DI 2003, 'Membrane transport of folates', *Vitamins and Hormones*, vol. 66, pp. 403-56.
Mathew, AV, Zeng, L, Byun, J & Pennathur, S 2015, 'Metabolomic profiling of arginine metabolome links altered methylation to chronic kidney disease accelerated atherosclerosis', *Journal of Proteomics & Bioinformatics*, vol. Suppl, no. 14. pii, p. 001. Epub 2015 May 18.
Matsui, T, Kuga, Y, Kaneko, A, Nishino, J, Eto, Y, Chiba, N, Yasuda, M, Saisho, K, Shimada, K & Tohma, S 2007, 'Disease Activity Score 28 (DAS28) using C-reactive protein underestimates disease activity and overestimates EULAR response criteria compared with DAS28 using erythrocyte sedimentation rate in a large observational cohort of rheumatoid arthritis patients in Japan', *Annals of the Rheumatic Diseases*, vol. 66, no. 9, pp. 1221-6.
Mauck, GW, Smith, CR, Geddes, LA & Bourland, JD 1980, 'The meaning of the point of maximum oscillations in cuff pressure in the indirect measurement of blood pressure--part ii', *Journal of Biomechanical Engineering*, vol. 102, no. 1, pp. 28-33.
Mayhan, WG, Arrick, DM, Sharpe, GM & Sun, H 2009, 'Nitric oxide synthase-dependent responses of the basilar artery during acute infusion of nicotine', *Nicotine & Tobacco Research*, vol. 11, no. 3, pp. 270-7. doi: 10.1093/ntr/ntn025. Epub 2009 Feb 23.
McBride, AE & Silver, PA 2001, 'State of the Arg: protein methylation at arginine comes of age', *Cell*, vol. 106, no. 1, pp. 5-8.

McCarey, DW, McInnes, IB, Madhok, R, Hampson, R, Scherbakov, O, Ford, I, Capell, HA & Sattar, N 2004, 'Trial of Atorvastatin in Rheumatoid Arthritis (TARA): double-blind, randomised placebo-controlled trial', *The Lancet*, vol. 363, no. 9426, pp. 2015-21.

McDermott, JR 1976, 'Studies on the catabolism of Ng-methylarginine, Ng, Ng-dimethylarginine and Ng, Ng-dimethylarginine in the rabbit', *Biochemical Journal*, vol. 154, no. 1, pp. 179-84.

McDonald, D 1960, 'Blood flow in arteries', *London: Edward Arnold*, vol. 119, pp. 286-288.

McEniery, CM, Cockcroft, JR, Roman, MJ, Franklin, SS & Wilkinson, IB 2014, 'Central blood pressure: current evidence and clinical importance', *European Heart Journal*, vol. 35, no. 26, pp. 1719-25. doi: 10.093/eurheartj/eht565. Epub 2014 Jan 23.

McEntegart, A, Capell, HA, Creran, D, Rumley, A, Woodward, M & Lowe, GDO 2001, 'Cardiovascular risk factors, including thrombotic variables, in a population with rheumatoid arthritis', *Rheumatology*, vol. 40, no. 6, pp. 640-4.

McInnes, IB & O'Dell, JR 2010, 'State-of-the-art: rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 69, no. 11, pp. 1898-906. doi: 10.136/ard.2010.134684.

McKellar, G, Morrison, E, McEntegart, A, Hampson, R, Tierney, A, Mackle, G, Scoular, J, Scott, JA & Capell, HA 2007, 'A pilot study of a Mediterranean-type diet intervention in female patients with rheumatoid arthritis living in areas of social deprivation in Glasgow', *Annals of the Rheumatic Diseases*, vol. 66, no. 9, pp. 1239-43. Epub 2007 Jul 5.

McLean-Tooke, A, Aldridge, C, Waugh, S, Spickett, GP & Kay, L 2009, 'Methotrexate, rheumatoid arthritis and infection risk: what is the evidence?', *Rheumatology (Oxford)*, vol. 48, no. 8, pp. 867-71. doi: 10.1093/rheumatology/kep101. Epub 2009 May 15.

McLennan, P, Howe, P, Abeywardena, M, Muggli, R, Raederstorff, D, Mano, M, Rayner, T & Head, R 1996, 'The cardiovascular protective role of docosahexaenoic acid', *European Journal of Pharmacology*, vol. 300, no. 1-2, pp. 83-9.

McVeigh, GE, Burns, DE, Finkelstein, SM, McDonald, KM, Mock, JE, Feske, W, Carlyle, PF, Flack, J, Grimm, R & Cohn, JN 1991, 'Reduced vascular compliance as a marker for essential hypertension', *American Journal of Hypertension*, vol. 4, no. 3 Pt 1, pp. 245-51.

Mena, JP, Salazar-Paramo, M, Gonzalez-Lopez, L, Gamez-Nava, JI, Sandoval-Ramirez, L, Sanchez, JD, Figuera, LE, Munoz-Valle, FJ, Vazquez del Mercado, M & Davalos, IP 2011, 'Polymorphisms C677T and A1298C in the MTHFR gene in Mexican patients with rheumatoid arthritis treated with methotrexate: implication with elevation of transaminases', *Pharmacogenomics Journal*, vol. 11, no. 4, pp. 287-91. doi: 10.1038/tpj.2010.32. Epub Jun 1.

Menard, C, Heraud, F, Volatier, JL & Leblanc, JC 2008, 'Assessment of dietary exposure of nitrate and nitrite in France', *Food Additives and Contaminants. Part A, Chemistry, Analysis, Control, Exposure and Risk Assessment*, vol. 25, no. 8, pp. 971-88. doi: 10.1080/02652030801946561.

Mente, A, de Koning, L, Shannon, HS & Anand, SS 2009, 'A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease', *Archives of Internal Medicine*, vol. 169, no. 7, pp. 659-69. doi: 10.1001/archinternmed.2009.38.

Merkel, LA, Lappe, RW, Rivera, LM, Cox, BF & Perrone, MH 1992, 'Demonstration of vasorelaxant activity with an A1-selective adenosine agonist in porcine coronary

artery: involvement of potassium channels', *Journal of Pharmacology and Experimental Therapeutics*, vol. 260, no. 2, pp. 437-43.
Metsios, GS, Stavropoulos-Kalinoglou, A, Panoulas, VF, Wilson, M, Nevill, AM, Koutedakis, Y & Kitas, GD 2009, 'Association of physical inactivity with increased cardiovascular risk in patients with rheumatoid arthritis', *European Journal of Cardiovascular Prevention and Rehabilitation*, vol. 16, no. 2, pp. 188-94.
Metsios, GS, Stavropoulos-Kalinoglou, A, Veldhuijzen van Zanten, JJ, Treharne, GJ, Panoulas, VF, Douglas, KM, Koutedakis, Y & Kitas, GD 2008, 'Rheumatoid arthritis, cardiovascular disease and physical exercise: a systematic review', *Rheumatology (Oxford)*, vol. 47, no. 3, pp. 239-48. Epub 2007 Nov 28.
Mezzano, SA, Aros, CA, Droguett, A, Burgos, ME, Ardiles, LG, Flores, CA, Carpio, D, Vio, CP, Ruiz-Ortega, M & Egido, J 2003, 'Renal angiotensin II up-regulation and myofibroblast activation in human membranous nephropathy', *Kidney International Supplements*, no. 86, pp. S39-45.
Mikkelsen, TS, Thorn, CF, Yang, JJ, Ulrich, CM, French, D, Zaza, G, Dunnenberger, HM, Marsh, S, McLeod, HL, Giacomini, K, Becker, ML, Gaedigk, R, Leeder, JS, Kager, L, Relling, MV, Evans, W, Klein, TE & Altman, RB 2011, 'PharmGKB summary: methotrexate pathway', *Pharmacogenetics and Genomics*, vol. 21, no. 10, pp. 679-86. doi: 10.1097/FPC.0b013e328343dd93.
Miller, PE, Van Elswyk, M & Alexander, DD 2014, 'Long-chain omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid and blood pressure: a meta-analysis of randomized controlled trials', *American Journal of Hypertension*, vol. 27, no. 7, pp. 885-96. doi: 10.1093/ajh/hpu024. Epub 2014 Mar 6.
Minaur, N, Sawyers, S, Parker, J & Darmawan, J 2004, 'Rheumatic disease in an Australian Aboriginal community in North Queensland, Australia. A WHO-ILAR COPCORD survey', *Journal of Rheumatology*, vol. 31, no. 5, pp. 965-72.
Mirfeizi, Z, Noubakht, Z, Rezaie, AE, Jokar, MH & Sarabi, ZS 2014, 'Plasma levels of leptin and visfatin in rheumatoid arthritis patients; is there any relationship with joint damage?', *Iranian Journal of Basic Medical Sciences*, vol. 17, no. 9, pp. 662-6.
Mitchell, DM, Spitz, PW, Young, DY, Bloch, DA, McShane, DJ & Fries, JF 1986, 'Survival, prognosis, and causes of death in rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 29, no. 6, pp. 706-14.
Mitchell, GF, Lacourciere, Y, Ouellet, JP, Izzo, JL, Jr., Neutel, J, Kerwin, LJ, Block, AJ & Pfeffer, MA 2003, 'Determinants of elevated pulse pressure in middle-aged and older subjects with uncomplicated systolic hypertension: the role of proximal aortic diameter and the aortic pressure-flow relationship', *Circulation*, vol. 108, no. 13, pp. 1592-8. Epub 2003 Sep 15.
Miyazaki, H, Matsuoka, H, Cooke, JP, Usui, M, Ueda, S, Okuda, S & Imaizumi, T 1999, 'Endogenous nitric oxide synthase inhibitor: a novel marker of atherosclerosis', *Circulation*, vol. 99, no. 9, pp. 1141-6.
Mohammad, A, Hartery, K, Bond, U & Phelan, M 2010, 'Increased occurrence of cardiovascular events and comorbidities in a general rheumatology cohort', *Irish Journal of Medical Science*, vol. 179, no. 2, pp. 273-6.
Mohamed, HJ, Sorich, MJ, Kowalski, SM, McKinnon, R, Proudman, SM, Cleland, L & Wiese, MD 2015, 'The role and utility of measuring red blood cell methotrexate polyglutamate concentrations in inflammatory arthropathies—a systematic review', *European Journal of Clinical Pharmacology*, vol. 71, no. 4, pp. 411-23.

Monk, HL, Muller, S, Mallen, CD & Hider, SL 2013, 'Cardiovascular screening in rheumatoid arthritis: a cross-sectional primary care database study', *BMC Family Practice*, vol. 14, no. 1, p. 150.

Monson, RR & Hall, AP 1976, 'Mortality among arthritics', *Journal of Chronic Diseases*, vol. 29, no. 7, pp. 459-67.

Morisaki, T, Gross, M, Morisaki, H, Pongratz, D, Zollner, N & Holmes, EW 1992, 'Molecular basis of AMP deaminase deficiency in skeletal muscle', *Proceedings of the National Academy of Sciences USA*, vol. 89, no. 14, pp. 6457-61.

Morrow, VA, Foufelle, F, Connell, JM, Petrie, JR, Gould, GW & Salt, IP 2003, 'Direct activation of AMP-activated protein kinase stimulates nitric-oxide synthesis in human aortic endothelial cells', *Journal of Biological Chemistry*, vol. 278, no. 34, pp. 31629-39. Epub 2003 Jun 4.

Mottonen, T 1988, 'Prediction of erosiveness and rate of development of new erosions in early rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 47, pp. 648 - 53.

Moya, P, Salazar, J, Arranz, MJ, Diaz-Torne, C, del Rio, E, Casademont, J, Corominas, H & Baiget, M 2016, 'Methotrexate pharmacokinetic genetic variants are associated with outcome in rheumatoid arthritis patients', *Pharmacogenomics*, vol. 17, no. 1, pp. 25-9. doi: 10.2217/pgs.15.150. Epub 015 Dec 14.

Mulders, TA, van den Bogaard, B, Bakker, A, Trip, MD, Stroes, ES, van den Born, BJ & Pinto-Sietsma, SJ 2012, 'Arterial stiffness is increased in families with premature coronary artery disease', *Heart*, vol. 98, no. 6, pp. 490-4. doi: 10.1136/heartjnl-2011-300999. Epub 2011 Dec 22.

Mutru, O, Laakso, M, Isomaki, H & Koota, K 1985, 'Ten year mortality and causes of death in patients with rheumatoid arthritis', *British Medical Journal*, vol. 290, no. 6484, pp. 1797-9.

Mutru, O, Laakso, M, Isomaki, H & Koota, K 1989, 'Cardiovascular mortality in patients with rheumatoid arthritis', *Cardiology*, vol. 76, no. 1, pp. 71-7.

Myasoedova, E, Crowson, CS, Kremers, HM, Fitz-Gibbon, PD, Therneau, TM & Gabriel, SE 2010, 'Total cholesterol and LDL levels decrease before rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 69, no. 7, pp. 1310-4. doi: 10.136/ard.2009.122374. Epub 2009 Oct 23.

Myasoedova, E, Crowson, CS, Nicola, PJ, Maradit-Kremers, H, Davis, JM, 3rd, Roger, VL, Therneau, TM & Gabriel, SE 2011, 'The influence of rheumatoid arthritis disease characteristics on heart failure', *Journal of Rheumatology*, vol. 38, no. 8, pp. 1601-6. doi: 10.3899/jrheum.100979. Epub 2011 May 15.

Myllykangas-Luosujarvi, RA, Aho, K & Isomaki, HA 1995, 'Mortality in rheumatoid arthritis', *Seminars in Arthritis and Rheumatism*, vol. 25, no. 3, pp. 193-202.

Nadareishvili, Z, Michaud, K, Hallenbeck, JM & Wolfe, F 2008, 'Cardiovascular, rheumatologic, and pharmacologic predictors of stroke in patients with rheumatoid arthritis: a nested, case-control study', *Arthritis and Rheumatism*, vol. 59, no. 8, pp. 1090-6.

Nakajima, A, Inoue, E, Tanaka, E, Singh, G, Sato, E, Hoshi, D, Shidara, K, Hara, M, Momohara, S, Taniguchi, A, Kamatani, N & Yamanaka, H 2010, 'Mortality and cause of death in Japanese patients with rheumatoid arthritis based on a large observational cohort, IORRA', *Scandinavian Journal of Rheumatology*, vol. 39, no. 5, pp. 360-7.

Naranjo, A, Sokka, T, Descalzo, MA, Calvo-Alen, J, Horslev-Petersen, K, Luukkainen, RK, Combe, B, Burmester, GR, Devlin, J, Ferraccioli, G, Morelli, A, Hoekstra, M, Majdan, M, Sadkiewicz, S, Belmonte, M, Holmqvist, AC, Choy, E, Tunc, R, Dimic, A, Bergman, M, Toloza, S, Pincus, T & Group, Q-R 2008, 'Cardiovascular disease in

patients with rheumatoid arthritis: results from the QUEST-RA study', *Arthritis Research and Therapy*, vol. 10, no. 2, p. R30.
Nassonov, EL, Samsonov, MY, Chichasova, NV, Nikiphorova, EL, Tilz, GP, Demel, U, Widner, B & Fuchs, D 2000, 'Soluble adhesion molecules in rheumatoid arthritis', *Rheumatology (Oxford)*, vol. 39, no. 7, pp. 808-10.
Natali, A, Toschi, E, Baldeweg, S, Ciociaro, D, Favilla, S, Sacca, L & Ferrannini, E 2006, 'Clustering of insulin resistance with vascular dysfunction and low-grade inflammation in type 2 diabetes', *Diabetes*, vol. 55, no. 4, pp. 1133-40.
Newby, AC 1988, 'Metabolic vasodilatation: the role of adenosine', *Biochemical Society Transactions*, vol. 16, no. 4, pp. 479-80.
Nguyen, B, Bauman, A, Gale, J, Banks, E, Kritharides, L & Ding, D 2016, 'Fruit and vegetable consumption and all-cause mortality: evidence from a large Australian cohort study', *International Journal of Behavioral Nutrition and Physical Activity*, vol. 13, no. 1, pp. 9. doi: 10.1186/s12966-016-0334-5.
Nguyen, HN, Fujiyoshi, A, Abbott, RD & Miura, K 2013, 'Epidemiology of cardiovascular risk factors in Asian countries', *Circulation Journal*, vol. 77, no. 12, pp. 2851-9. Epub 013 Nov 15.
Nicholas, NS & Panayi, GS 1988, 'Rheumatoid arthritis and pregnancy', *Clinical and Experimental Rheumatology*, vol. 6, no. 2, pp. 179-82.
Nichols, W & O'Rourke, M 1998, *McDonald's Blood Flow in Arteries: Theoretical, practical and clinical principles*, London: Arnold.
Nichols, WW 2005, 'Clinical measurement of arterial stiffness obtained from noninvasive pressure waveforms', *American Journal of Hypertension*, vol. 18, no. 1 Pt 2, pp. 3S-10S.
Nicola, PJ, Maradit-Kremers, H, Roger, VL, Jacobsen, SJ, Crowson, CS, Ballman, KV & Gabriel, SE 2005, 'The risk of congestive heart failure in rheumatoid arthritis: a population-based study over 46 years', *Arthritis & Rheumatology*, vol. 52, no. 2, pp. 412-20.
Nielen, MM, van Schaardenburg, D, Reesink, HW, van de Stadt, RJ, van der Horst-Bruinsma, IE, de Koning, MH, Habibuw, MR, Vandenbroucke, JP & Dijkmans, BA 2004, 'Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors', *Arthritis and Rheumatism*, vol. 50, no. 2, pp. 380-6.
Nielsen, GL, Faarvang, KL, Thomsen, BS, Teglbjaerg, KL, Jensen, LT, Hansen, TM, Lervang, HH, Schmidt, EB, Dyerberg, J & Ernst, E 1992, 'The effects of dietary supplementation with n-3 polyunsaturated fatty acids in patients with rheumatoid arthritis: a randomized, double blind trial', *European Journal of Clinical Investigation*, vol. 22, no. 10, pp. 687-91.
Nielung, L, Christensen, R, Danneskiold-Samsøe, B, Bliddal, H, Holm, CC, Ellegaard, K, Slott Jensen, H & Bartels, EM 2015, 'Validity and agreement between the 28-joint disease activity score based on c-reactive protein and erythrocyte sedimentation rate in patients with rheumatoid arthritis', *Arthritis*, vol. 2015, p. 401690.
Nishimura, K, Sugiyama, D, Kogata, Y, Tsuji, G, Nakazawa, T, Kawano, S, Saigo, K, Morinobu, A, Koshiba, M, Kuntz, KM, Kamae, I & Kumagai, S 2007, 'Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis', *Annals of Internal Medicine*, vol. 146, no. 11, pp. 797-808.
Niskanen, L, Laaksonen, DE, Nyyssonen, K, Punnonen, K, Valkonen, VP, Fuentes, R, Tuomainen, TP, Salonen, R & Salonen, JT 2004, 'Inflammation, abdominal obesity,

and smoking as predictors of hypertension', *Hypertension*, vol. 44, no. 6, pp. 859-65. Epub 2004 Oct 18.
Noguchi, K, Hamadate, N, Matsuzaki, T, Sakanashi, M, Nakasone, J, Uchida, T, Arakaki, K, Kubota, H, Ishiuchi, S, Masuzaki, H, Sugahara, K, Ohya, Y, Sakanashi, M & Tsutsui, M 2011, 'Increasing dihydrobiopterin causes dysfunction of endothelial nitric oxide synthase in rats in vivo', *American Journal of Physiology-Heart and Circulatory Physiology*, vol. 301, no. 3, pp. H721-9. doi: 10.1152/ajpheart.01089.2010. Epub 2011 May 27.
Nordemar, R, Ekblom, B, Zachrisson, L & Lundqvist, K 1981, 'Physical training in rheumatoid arthritis: a controlled long-term study. I', *Scandinavian Journal of Rheumatology*, vol. 10, no. 1, pp. 17-23.
O'Brien, E, Asmar, R, Beilin, L, Imai, Y, Mallion, JM, Mancia, G, Mengden, T, Myers, M, Padfield, P, Palatini, P, Parati, G, Pickering, T, Redon, J, Staessen, J, Stergiou, G & Verdecchia, P 2003, 'European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement', *Journal of Hypertension*, vol. 21, no. 5, pp. 821-48.
O'Keefe, JH & Bell, DS 2007, 'Postprandial hyperglycemia/hyperlipidemia (postprandial dysmetabolism) is a cardiovascular risk factor', *American Journal of Cardiology*, vol. 100, no. 5, pp. 899-904. Epub 2007 Jun 26.
O'Keefe, JH, Jr. & Harris, WS 2000, 'From Inuit to implementation: omega-3 fatty acids come of age', *Mayo Clinic Proceedings*, vol. 75, no. 6, pp. 607-14.
O'Kelly, S, Andersen, K, Capewell, S & Ryden, L 2011, 'Bringing prevention to the population: an important role for cardiologists in policy-making', *European Heart Journal*, vol. 32, no. 16, pp. 1964-7. doi: 10.093/eurheartj/ehr128. Epub 2011 May 26.
O'Rielly, DD & Rahman, P 2010, 'Pharmacogenetics of rheumatoid arthritis: Potential targets from susceptibility genes and present therapies', *Journal of Pharmacogenomics and Personalized Medicine*, vol. 3, pp. 15-31. Epub 2010 Mar 30.
O'Rourke, M 1990, 'Arterial stiffness, systolic blood pressure, and logical treatment of arterial hypertension', *Hypertension*, vol. 15, no. 4, pp. 339-47.
O'Rourke, M 1995, 'Mechanical principles in arterial disease', *Hypertension*, vol. 26, no. 1, pp. 2-9.
O'Rourke, MF & Mancia, G 1999, 'Arterial stiffness', *Journal of Hypertension*, vol. 17, no. 1, pp. 1-4.
O'Rourke, MF, Pauca, A & Jiang, X 2001, 'Pulse wave analysis', *British Journal of Clinical Pharmacology*, vol. 51, pp. 507–22.
O'Rourke, MF, Staessen, JA, Vlachopoulos, C, Duprez, D & Plante, GE 2002, 'Clinical applications of arterial stiffness; definitions and reference values', *American Journal of Hypertension*, vol. 15, no. 5, pp. 426-44.
Ogawa, T, Kimoto, M, Watanabe, H & Sasaoka, K 1987, 'Metabolism of NG,NG-and NG,N'G-dimethylarginine in rats', *Archives of Biochemistry and Biophysics*, vol. 252, no. 2, pp. 526-37.
Ogdie, A, Yu, Y, Haynes, K, Love, TJ, Maliha, S, Jiang, Y, Troxel, AB, Hennessy, S, Kimmel, SE, Margolis, DJ, Choi, H, Mehta, NN & Gelfand, JM 2015, 'Risk of major cardiovascular events in patients with psoriatic arthritis, psoriasis and rheumatoid arthritis: a population-based cohort study', *Annals of the Rheumatic Diseases*, vol. 74, no. 2, pp. 326-32.
Ohkubo, T, Hozawa, A, Yamaguchi, J, Kikuya, M, Ohmori, K, Michimata, M, Matsubara, M, Hashimoto, J, Hoshi, H, Araki, T, Tsuji, I, Satoh, H, Hisamichi, S & Imai, Y 2002,

'Prognostic significance of the nocturnal decline in blood pressure in individuals with and without high 24-h blood pressure: the Ohasama study', *Journal of Hypertension*, vol. 20, no. 11, pp. 2183-9.

Ohte, N, Saeki, T, Miyabe, H, Sakata, S, Mukai, S, Hayano, J, Niki, K, Sugawara, M & Kimura, G 2007, 'Relationship between blood pressure obtained from the upper arm with a cuff-type sphygmomanometer and central blood pressure measured with a catheter-tipped micromanometer', *Heart and Vessels*, vol. 22, no. 6, pp. 410-5. Epub 2007 Nov 26.

Ong, KL, Wu, BJ, Cheung, BM, Barter, PJ & Rye, KA 2013, 'Arthritis: its prevalence, risk factors, and association with cardiovascular diseases in the United States, 1999 to 2008', *Annals of Epidemiology*, vol. 23, no. 2, pp. 80-6. doi: 10.1016/j.annepidem.2012.11.008. Epub Dec 5.

Osanai, T, Saitoh, M, Sasaki, S, Tomita, H, Matsunaga, T & Okumura, K 2003, 'Effect of shear stress on asymmetric dimethylarginine release from vascular endothelial cells', *Hypertension*, vol. 42, no. 5, pp. 985-90. Epub 2003 Oct 13.

Ospelt, C & Gay, S 2008, 'The role of resident synovial cells in destructive arthritis', *Best Practice & Research: Clinical Rheumatology*, vol. 22, no. 2, pp. 239-52. doi: 10.1016/j.berh.2008.01.004.

Ostensen, M & Villiger, PM 2007, 'The remission of rheumatoid arthritis during pregnancy', *Seminars in Immunopathology*, vol. 29, no. 2, pp. 185-91.

Østergaard, M, Ejbjerg, B & Szkudlarek, M 2005, 'Imaging in early rheumatoid arthritis: roles of magnetic resonance imaging, ultrasonography, conventional radiography and computed tomography', *Best Practice & Research: Clinical Rheumatology*, vol. 19, no. 1, pp. 91-116.

Østergaard, M, Pedersen, SJ & Døhn, UM 2008, 'Imaging in rheumatoid arthritis – status and recent advances for magnetic resonance imaging, ultrasonography, computed tomography and conventional radiography', *Best Practice & Research Clinical Rheumatology*, vol. 22, no. 6, pp. 1019-44.

Ouchi, N, Kihara, S, Funahashi, T, Matsuzawa, Y & Walsh, K 2003, 'Obesity, adiponectin and vascular inflammatory disease', *Current Opinion in Lipidology*, vol. 14, no. 6, pp. 561-6.

Owen, SA, Hider, SL, Martin, P, Bruce, IN, Barton, A & Thomson, W 2013, 'Genetic polymorphisms in key methotrexate pathway genes are associated with response to treatment in rheumatoid arthritis patients', *Pharmacogenomics Journal*, vol. 13, no. 3, pp. 227-34. doi: 10.1038/tpj.2012.7. Epub Mar 27.

Owen, SA, Lunt, M, Bowes, J, Hider, SL, Bruce, IN, Thomson, W & Barton, A 2013, 'MTHFR gene polymorphisms and outcome of methotrexate treatment in patients with rheumatoid arthritis: analysis of key polymorphisms and meta-analysis of C677T and A1298C polymorphisms', *Pharmacogenomics Journal*, vol. 13, no. 2, pp. 137-47. doi: 10.1038/tpj.2011.42. Epub Sep 20.

Owlia, MB 2006, ' Clinical spectrum of connective tissue disorders', *Journal, Indian Academy of Clinical Medicine*, vol. 7, no. 3, pp. 217–24.

Padyukov, L, Silva, C, Stolt, P, Alfredsson, L & Klareskog, L 2004, 'A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 50, no. 10, pp. 3085-92.

Paiva, H, Lehtimaki, T, Laakso, J, Ruokonen, I, Tervonen, R, Metso, S, Nikkila, M, Wuolijoki, E & Laaksonen, R 2004, 'Dietary composition as a determinant of plasma

asymmetric dimethylarginine in subjects with mild hypercholesterolemia', *Metabolism: Clinical and Experimental*, vol. 53, no. 8, pp. 1072-5.
Palatini, P, Frigo, G, Bertolo, O, Roman, E, Da Corta, R & Winnicki, M 1998, 'Validation of the A&D TM-2430 device for ambulatory blood pressure monitoring and evaluation of performance according to subjects' characteristics', *Blood Pressure Monitoring*, vol. 3, no. 4, pp. 255-60.
Palm, F, Onozato, ML, Luo, Z & Wilcox, CS 2007, 'Dimethylarginine dimethylaminohydrolase (DDAH): expression, regulation, and function in the cardiovascular and renal systems', *American Journal of Physiology-Heart and Circulatory Physiology*, vol. 293, no. 6, pp. H3227-45. Epub 2007 Oct 12.
Palmer, RM, Ashton, DS & Moncada, S 1988, 'Vascular endothelial cells synthesize nitric oxide from L-arginine', *Nature*, vol. 333, no. 6174, pp. 664-6.
Pandey, VG, Jain, S, Rana, A, Puri, N, Arudra, SK, Mopidevi, B, Kaw, M, Nasjletti, A & Kumar, A 2015, 'Dexamethasone promotes hypertension by allele-specific regulation of the human angiotensinogen gene', *Journal of Biological Chemistry*, vol. 290, no. 9, pp. 5749-58. doi: 10.1074/jbc.M114.601922. Epub 2015 Jan 7.
Panoulas, VF, Douglas, KM, Milionis, HJ, Stavropoulos-Kalinglou, A, Nightingale, P, Kita, MD, Tselios, AL, Metsios, GS, Elisaf, MS & Kitas, GD 2007, 'Prevalence and associations of hypertension and its control in patients with rheumatoid arthritis', *Rheumatology (Oxford)*, vol. 46, no. 9, pp. 1477-82.
Panoulas, VF, Douglas, KM, Stavropoulos-Kalinoglou, A, Metsios, GS, Nightingale, P, Kita, MD, Elisaf, MS & Kitas, GD 2008, 'Long-term exposure to medium-dose glucocorticoid therapy associates with hypertension in patients with rheumatoid arthritis', *Rheumatology (Oxford)*, vol. 47, no. 1, pp. 72-5.
Panoulas, VF, Metsios, GS, Pace, AV, John, H, Treharne, GJ, Banks, MJ & Kitas, GD 2008, 'Hypertension in rheumatoid arthritis', *Rheumatology*, vol. 47, no. 9, pp. 1286-98.
Parati, G, Stergiou, GS, Asmar, R, Bilo, G, de Leeuw, P, Imai, Y, Kario, K, Lurbe, E, Manolis, A, Mengden, T, O'Brien, E, Ohkubo, T, Padfield, P, Palatini, P, Pickering, TG, Redon, J, Revera, M, Ruilope, LM, Shennan, A, Staessen, JA, Tisler, A, Waeber, B, Zanchetti, A & Mancia, G 2010, 'European Society of Hypertension practice guidelines for home blood pressure monitoring', *Journal of Human Hypertension*, vol. 24, no. 12, pp. 779-85. doi: 10.1038/jhh.2010.54. Epub Jun 3.
Park, YB, Ahn, CW, Choi, HK, Lee, SH, In, BH, Lee, HC, Nam, CM & Lee, SK 2002, 'Atherosclerosis in rheumatoid arthritis: morphologic evidence obtained by carotid ultrasound', *Arthritis and Rheumatism*, vol. 46, no. 7, pp. 1714-9.
Pasceri, V & Yeh, ET 1999, 'A tale of two diseases: atherosclerosis and rheumatoid arthritis', *Circulation*, vol. 100, no. 21, pp. 2124-6.
Paul, B, Whiting, MJ, De Pasquale, CG & Mangoni, AA 2010, 'Acute effects of 5-methyltetrahydrofolate on endothelial function and asymmetric dimethylarginine in patients with chronic heart failure', *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 20, no. 5, pp. 341-9. doi: 10.1016/j.numecd.2009.04.008. Epub Sep 12.
Pawlik, A, Wrzesniewska, J, Fiedorowicz-Fabrycy, I & Gawronska-Szklarz, B 2004, 'The MDR1 3435 polymorphism in patients with rheumatoid arthritis', *International Journal of Clinical Pharmacology and Therapeutics*, vol. 42, no. 9, pp. 496-503.
Pepine, CJ 2009, 'The impact of nitric oxide in cardiovascular medicine: untapped potential utility', *Am J Med.*, vol. 122, no. 5 Suppl, pp. S10-5. doi:.1016/j.amjmed.2009.03.003.
Pepys, MB & Hirschfield, GM 2003, 'C-reactive protein: a critical update', *Journal of Clinical Investigation*, vol. 111, no. 12, pp. 1805-12.

Petros, A, Lamb, G, Leone, A, Moncada, S, Bennett, D & Vallance, P 1994, 'Effects of a nitric oxide synthase inhibitor in humans with septic shock', *Cardiovascular Research*, vol. 28, no. 1, pp. 34-9.

Philippe, F, Chemaly, E, Blacher, J, Mourad, J-J, Dibie, A, Larrazet, F, Laborde, F & E. Safar, M 2002, 'Aortic pulse pressure and extent of coronary artery disease in percutaneous transluminal coronary angioplasty candidates', *American Journal of Hypertension*, vol. 15, no. 8, pp. 672-7.

Phivthong-ngam, L, Bode-Böger, SM, Böger, RH, Bohme, M, Brandes, RP, Mugge, A & Frolich, JC 1998, 'Dietary L-arginine normalizes endothelin-induced vascular contractions in cholesterol-fed rabbits', *Journal of Cardiovascular Pharmacology*, vol. 32, no. 2, pp. 300-7.

Pieringer, H, Brummaier, T, Schmid, M, Pichler, M, Hayat-Khayyati, A, Ebner, S, Biesenbach, G & Pohanka, E 2012, 'Rheumatoid arthritis is an independent risk factor for an increased augmentation index regardless of the coexistence of traditional cardiovascular risk factors', *Seminars in Arthritis and Rheumatism*, vol. 42, no. 1, pp. 17-22. doi: 10.1016/j.semarthrit.2012.02.003. Epub Apr 3.

Pieringer, H & Pichler, M 2011, 'Cardiovascular morbidity and mortality in patients with rheumatoid arthritis: vascular alterations and possible clinical implications', *Quarterly Journal of Medicine*, vol. 104, no. 1, pp. 13-26. doi: 10.1093/qjmed/hcq203. Epub 2010 Nov 10.

Pieringer, H, Schumacher, S, Stuby, U & Biesenbach, G 2009, 'Augmentation index and large-artery remodeling in patients with longstanding rheumatoid arthritis compared with healthy controls', *Seminars in Arthritis and Rheumatism*, vol. 39, no. 3, pp. 163-9. doi: 10.1016/j.semarthrit.2008.08.005. Epub Sep 27.

Pikula, A, Böger, RH, Beiser, AS, Maas, R, DeCarli, C, Schwedhelm, E, Himali, JJ, Schulze, F, Au, R, Kelly-Hayes, M, Kase, CS, Vasan, RS, Wolf, PA & Seshadri, S 2009, 'Association of plasma ADMA levels with MRI markers of vascular brain injury: Framingham offspring study', *Stroke*, vol. 40, no. 9, pp. 2959-64. doi: 10.1161/STROKEAHA.109.557116. Epub 2009 Jul 30.

Pincus, T, Callahan, LF, Sale, WG, Brooks, AL, Payne, LE & Vaughn, WK 1984, 'Severe functional declines, work disability, and increased mortality in seventy-five rheumatoid arthritis patients studied over nine years', *Arthritis and Rheumatism*, vol. 27, no. 8, pp. 864-72.

Pincus, T, Cronstein, B & Braun, J 2010, 'Methotrexate--the anchor drug--an introduction', *Clinical and Experimental Rheumatology*, vol. 28, no. 5 Suppl 61, pp. S1-2. Epub 2010 Oct 28.

Pincus, T & Sokka, T 2004, 'Quantitative measures and indices to assess rheumatoid arthritis in clinical trials and clinical care', *Rheumatic Diseases Clinics of North America*, vol. 2004 Nov;30, no. 4, pp. 725-51.

Pittas, AG, Joseph, NA & Greenberg, AS 2004, 'Adipocytokines and insulin resistance', *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 2, pp. 447-52.

Plant, M, Jones, P, Saklatvala, J, Ollier, W & Dawes, P 1998, 'Patterns of radiological progression in early rheumatoid arthritis: results of an 8 year prospective study', *Journal of Rheumatology*, vol. 25, no. 3, pp. 417-26.

Plant, M, Williams, A, O'Sullivan, M, Lewis, P, Coles, E & Jessop, J 2000, 'Relationship between time-integrated C-reactive protein levels and radiologic progression in patients with rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 43, pp. 1473-7.

Plaza-Plaza, JC, Aguilera, M, Canadas-Garre, M, Chemello, C, Gonzalez-Utrilla, A, Faus Dader, MJ & Calleja, MA 2012, 'Pharmacogenetic polymorphisms contributing to

toxicity induced by methotrexate in the southern Spanish population with rheumatoid arthritis', *OMICS:A Journal of Integrative Biology*, vol. 16, no. 11, pp. 589-95. doi: 10.1089/omi.2011.0142. Epub 2012 Oct 24.

Prasad, M, Hermann, J, Gabriel, SE, Weyand, CM, Mulvagh, S, Mankad, R, Oh, JK, Matteson, EL & Lerman, A 2015, 'Cardiorheumatology: cardiac involvement in systemic rheumatic disease', *Nature Reviews Cardiology*, vol. 12, no. 3, pp. 168-76. doi: 10.1038/nrcardio.2014.206. Epub Dec 23.

Prevoo, ML, van 't Hof, MA, Kuper, HH, van Leeuwen, MA, van de Putte, LB & van Riel, PL 1995, 'Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 38, no. 1, pp. 44-8.

Prior, P, Symmons, DP, Scott, DL, Brown, R & Hawkins, CF 1984, 'Cause of death in rheumatoid arthritis', *British Journal of Rheumatology*, vol. 23, no. 2, pp. 92-9.

Protogerou, AD, Argyris, A, Nasothimiou, E, Vrachatis, D, Papaioannou, TG, Tzamouranis, D, Blacher, J, Safar, ME, Sfikakis, P & Stergiou, GS 2012, 'Feasibility and reproducibility of noninvasive 24-h ambulatory aortic blood pressure monitoring with a brachial cuff-based oscillometric device', *American Journal of Hypertension*, vol. 25, no. 8, pp. 876-82.

Protogerou, AD, Zampeli, E, Fragiadaki, K, Stamatelopoulos, K, Papamichael, C & Sfikakis, PP 2011, 'A pilot study of endothelial dysfunction and aortic stiffness after interleukin-6 receptor inhibition in rheumatoid arthritis', *Atherosclerosis*, vol. 219, no. 2, pp. 734-6. doi: 10.1016/j.atherosclerosis.2011.09.015. Epub Sep 16.

Provan, SA, Angel, K, Semb, AG, Mowinckel, P, Agewall, S, Atar, D & Kvien, TK 2011, 'Early prediction of increased arterial stiffness in patients with chronic inflammation: a 15-year followup study of 108 patients with rheumatoid arthritis', *Journal of Rheumatology*, vol. 38, no. 4, pp. 606-12. doi: 10.3899/jrheum.100689. Epub 2011 Jan 15.

Provan, SA, Berg, IJ, Hammer, HB, Mathiessen, A, Kvien, TK & Semb, AG 2015, 'The impact of newer biological disease modifying anti-rheumatic drugs on cardiovascular risk factors: a 12-month longitudinal study in rheumatoid arthritis patients treated with rituximab, abatacept and tociliziumab', *PLoS One*, vol. 10, no. 6, p. e0130709. doi: 10.1371/journal.pone.. eCollection 2015.

Psaty, BM, Koepsell, TD, Lin, D, Weiss, NS, Siscovick, DS, Rosendaal, FR, Pahor, M & Furberg, CD 1999, 'Assessment and control for confounding by indication in observational studies', *Journal of the American Geriatrics Society*, vol. 47, no. 6, pp. 749-54.

Pullmann, R, Jr., Abdelmohsen, K, Lal, A, Martindale, JL, Ladner, RD & Gorospe, M 2006, 'Differential stability of thymidylate synthase 3'-untranslated region polymorphic variants regulated by AUF1', *Journal of Biological Chemistry*, vol. 281, no. 33, pp. 23456-63. Epub 2006 Jun 20.

Putney, JW, Jr. 1986, 'A model for receptor-regulated calcium entry', *Cell Calcium*, vol. 7, no. 1, pp. 1-12.

Qi, S, Xin, R, Guo, W & Liu, Y 2014, 'Meta-analysis of oral contraceptives and rheumatoid arthritis risk in women', *Therapeutics and Clinical Risk Management*, vol. 10:915-23., no. doi, p. 10.2147/TCRM.S70867. eCollection 2014.

Qin, B, Yang, M, Fu, H, Ma, N, Wei, T, Tang, Q, Hu, Z, Liang, Y, Yang, Z & Zhong, R 2015, 'Body mass index and the risk of rheumatoid arthritis: a systematic review and dose response meta-analysis', *Arthritis Research and Therapy*, vol. 17, no. 1.

Radhakutty, A, Mangelsdorf, BL, Drake, SM, Samocha-Bonet, D, Jenkins, AB, Heilbronn, LK, Smith, MD, Thompson, CH & Burt, MG 2016, 'Effect of acute and chronic glucocorticoid therapy on insulin sensitivity and postprandial vascular function', *Clinical Endocrinology*, vol. 84, no. 4, pp. 501-8. doi: 10.1111/cen.12966. Epub 2015 Nov 23.

Ranganathan, P 2008, 'An update on methotrexate pharmacogenetics in rheumatoid arthritis', *Pharmacogenomics*, vol. 9, no. 4, pp. 439-51. doi: 10.2217/14622416.9.4.439.

Ranganathan, P, Culverhouse, R, Marsh, S, Mody, A, Scott-Horton, TJ, Brasington, R, Joseph, A, Reddy, V, Eisen, S & McLeod, HL 2008, 'Methotrexate (MTX) pathway gene polymorphisms and their effects on MTX toxicity in Caucasian and African American patients with rheumatoid arthritis', *Journal of Rheumatology*, vol. 35, no. 4, pp. 572-9. Epub 2008 Mar 15.

Ranganathan, P & McLeod, HL 2006, 'Methotrexate pharmacogenetics: the first step toward individualized therapy in rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 54, no. 5, pp. 1366-77.

Rantapaa-Dahlqvist, S, de Jong, BA, Berglin, E, Hallmans, G, Wadell, G, Stenlund, H, Sundin, U & van Venrooij, WJ 2003, 'Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 48, no. 10, pp. 2741-9.

Rau, T, Erney, B, Gores, R, Eschenhagen, T, Beck, J & Langer, T 2006, 'High-dose methotrexate in pediatric acute lymphoblastic leukemia: impact of ABCC2 polymorphisms on plasma concentrations', *Clinical Pharmacology & Therapeutics*, vol. 80, no. 5, pp. 468-76.

Ray, CJ, Abbas, MR, Coney, AM & Marshall, JM 2002, 'Interactions of adenosine, prostaglandins and nitric oxide in hypoxia-induced vasodilatation: in vivo and in vitro studies', *The Journal of Physiology*, vol. 544, no. Pt 1, pp. 195-209.

Raynauld, JP 1997, 'Cardiovascular mortality in rheumatoid arthritis: how harmful are corticosteroids?', *Journal of Rheumatology*, vol. 24, no. 3, pp. 415-6.

Refai, TM, Al-Salem, IH, Nkansa-Dwamena, D & Al-Salem, MH 2002, 'Hyperhomocysteinaemia and risk of thrombosis in systemic lupus erythematosus patients', *Clinical Rheumatology*, vol. 21, no. 6, pp. 457-61.

Reid, DM, Kennedy, NS, Smith, MA, Tothill, P & Nuki, G 1982, 'Total body calcium in rheumatoid arthritis: effects of disease activity and corticosteroid treatment', *British Medical Journal*, vol. 285, no. 6338, pp. 330-2.

Reilly, PA, Cosh, JA, Maddison, PJ, Rasker, JJ & Silman, AJ 1990, 'Mortality and survival in rheumatoid arthritis: a 25 year prospective study of 100 patients', *Annals of the Rheumatic Diseases*, vol. 49, no. 6, pp. 363-9.

Reimann, M, Dierkes, J, Carlsohn, A, Talbot, D, Ferrari, M, Hallund, J, Hall, WL, Vafeiadou, K, Huebner, U, Branca, F, Bugel, S, Williams, CM, Zunft, HJ & Koebnick, C 2006, 'Consumption of soy isoflavones does not affect plasma total homocysteine or asymmetric dimethylarginine concentrations in healthy postmenopausal women', *Journal of Nutrition*, vol. 136, no. 1, pp. 100-5.

Reiss, AB, Carsons, SE, Anwar, K, Rao, S, Edelman, SD, Zhang, H, Fernandez, P, Cronstein, BN & Chan, ES 2008, 'Atheroprotective effects of methotrexate on reverse cholesterol transport proteins and foam cell transformation in human THP-1 monocyte/macrophages', *Arthritis and Rheumatism*, vol. 58, no. 12, pp. 3675-83. doi: 10.1002/art.24040.

Rhen, T & Cidlowski, JA 2005, 'Antiinflammatory action of glucocorticoids--new mechanisms for old drugs', *New England Journal of Medicine*, vol. 353, no. 16, pp. 1711-23.
Rho, YH, Chung, CP, Oeser, A, Solus, J, Asanuma, Y, Sokka, T, Pincus, T, Raggi, P, Gebretsadik, T, Shintani, A & Stein, CM 2009, 'Inflammatory mediators and premature coronary atherosclerosis in rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 61, no. 11, pp. 1580-5.
Rho, YH, Oeser, A, Chung, CP, Milne, GL & Stein, CM 2009, 'Drugs used in the treatment of rheumatoid arthritis: relationship between current use and cardiovascular risk factors', *Archives of Drug Information*, vol. 2, no. 2, pp. 34-40.
Ridker, PM, Buring, JE, Rifai, N & Cook, NR 2007, 'Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds Risk Score', *Journal of the American Medical Association*, vol. 297, no. 6, pp. 611-9.
Ridker, PM, Buring, JE, Shih, J, Matias, M & Hennekens, CH 1998, 'Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women', *Circulation*, vol. 98, no. 8, pp. 731-3.
Ridker, PM, Danielson, E, Fonseca, FA, Genest, J, Gotto, AM, Jr., Kastelein, JJ, Koenig, W, Libby, P, Lorenzatti, AJ, MacFadyen, JG, Nordestgaard, BG, Shepherd, J, Willerson, JT & Glynn, RJ 2008, 'Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein', *New England Journal of Medicine*, vol. 359, no. 21, pp. 2195-207. doi: 10.1056/NEJMoa0807646. Epub 2008 Nov 9.
Ridker, PM, Hennekens, CH, Buring, JE & Rifai, N 2000, 'C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women', *New England Journal of Medicine*, vol. 342, no. 12, pp. 836-43.
Ridker, PM, Paynter, NP, Rifai, N, Gaziano, JM & Cook, NR 2008, 'C-reactive protein and parental history improve global cardiovascular risk prediction: the Reynolds Risk Score for men', *Circulation*, vol. 2008 Nov 25;118, no. 22, pp. 2243-51.
Ridker, PM, Stampfer, MJ & Rifai, N 2001, 'Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease', *Journal of the American Medical Association*, vol. 285, no. 19, pp. 2481-5.
Rihacek, I, Frana, P, Soucek, M, Plachy, M & Kianicka, B 2009, '[The diurnal variability of blood pressure in patients with hypertension and rheumatoid arthritis]', *Vnitrni Lekarstvi*, vol. 55, no. 2, pp. 111-6.
Riise, T, Jacobsen, BK, Gran, JT, Haga, HJ & Arnesen, E 2001, 'Total mortality is increased in rheumatoid arthritis. A 17-year prospective study', *Clinical Rheumatology*, vol. 20, no. 2, pp. 123-7.
Rindfleisch, JA & Muller, D 2005, 'Diagnosis and management of rheumatoid arthritis', *American Family Physician*, vol. 72, no. 6, pp. 1037-47.
Robb, AO, Mills, NL, Din, JN, Smith, IBJ, Paterson, F, Newby, DE & Denison, FC 2009, 'Influence of the menstrual cycle, pregnancy, and preeclampsia on arterial stiffness', *Hypertension*, vol. 53, no. 6, pp. 952-8.
Roberts-Thomson, RA & Roberts-Thomson, PJ 1999, 'Rheumatic disease and the Australian aborigine', *Annals of the Rheumatic Diseases*, vol. 58, no. 5, pp. 266-70.
Roberts, RS 1993, 'Pooled outcome measures in arthritis: the pros and cons', *Journal of Rheumatology*, vol. 20, no. 3, pp. 566-7.
Rodriguez, LA, Tolosa, LB, Ruigomez, A, Johansson, S & Wallander, MA 2009, 'Rheumatoid arthritis in UK primary care: incidence and prior morbidity',

Scandinavian Journal of Rheumatology, vol. 38, no. 3, pp. 173-7. doi: 10.1080/03009740802448825.

Rogoza, AN, Pavlova, TS & Sergeeva, MV 2000, 'Validation of A&D UA-767 device for the self-measurement of blood pressure', *Blood Pressure Monitoring*, vol. 5, no. 4, pp. 227-31.

Rolland, PH, Friggi, A, Barlatier, A, Piquet, P, Latrille, V, Faye, MM, Guillou, J, Charpiot, P, Bodard, H, Ghiringhelli, O & et al. 1995, 'Hyperhomocysteinemia-induced vascular damage in the minipig. Captopril-hydrochlorothiazide combination prevents elastic alterations', *Circulation*, vol. 91, no. 4, pp. 1161-74.

Roman, MJ, Devereux, RB, Kizer, JR, Lee, ET, Galloway, JM, Ali, T, Umans, JG & Howard, BV 2007, 'Central pressure more strongly relates to vascular disease and outcome than does brachial pressure: the Strong Heart Study', *Hypertension*, vol. 50, no. 1, pp. 197-203. Epub 2007 May 7.

Romao, VC, Lima, A, Bernardes, M, Canhao, H & Fonseca, JE 2014, 'Three decades of low-dose methotrexate in rheumatoid arthritis: can we predict toxicity?', *Immunologic Research*, vol. 60, no. 2-3, pp. 289-310. doi: 10.1007/s12026-014-8564-6.

Ropes, MW, Bennett, GA, Cobb, S, Jacox, R & Jessar, RA 1957, 'Proposed diagnostic criteria for rheumatoid arthritis; report of a study conducted by a committee of the American Rheumatism Association', *Journal of Chronic Diseases*, vol. 5, no. 6, pp. 630-5.

Ropes, MW, Bennett, GA, Cobb, S, Jacox, R & Jessar, RA 1958, '1958 Revision of diagnostic criteria for rheumatoid arthritis', *Bulletin on the Rheumatic Diseases*, vol. 9, no. 4, pp. 175-6.

Ross, R 1999, 'Atherosclerosis--an inflammatory disease', *New England Journal of Medicine*, vol. 340, no. 2, pp. 115-26.

Roubenoff, R, Roubenoff, RA, Cannon, JG, Kehayias, JJ, Zhuang, H, Dawson-Hughes, B, Dinarello, CA & Rosenberg, IH 1994, 'Rheumatoid cachexia: cytokine-driven hypermetabolism accompanying reduced body cell mass in chronic inflammation', *Journal of Clinical Investigation*, vol. 93, no. 6, pp. 2379-86.

Roubille, C, Richer, V, Starnino, T, McCourt, C, McFarlane, A, Fleming, P, Siu, S, Kraft, J, Lynde, C, Pope, J, Gulliver, W, Keeling, S, Dutz, J, Bessette, L, Bissonnette, R & Haraoui, B 2015, 'The effects of tumour necrosis factor inhibitors, methotrexate, non-steroidal anti-inflammatory drugs and corticosteroids on cardiovascular events in rheumatoid arthritis, psoriasis and psoriatic arthritis: a systematic review and meta-analysis', *Annals of the Rheumatic Diseases*, vol. 74, no. 3, pp. 480-9. doi: 10.1136/annrheumdis-2014-206624. Epub 2015 Jan 5.

Roux, N, Flipo, RM, Cortet, B, Lafitte, JJ, Tonnel, AB, Duquesnoy, B & Delcambre, B 1996, 'Pneumocystis carinii pneumonia in rheumatoid arthritis patients treated with methotrexate. A report of two cases', *Review Rheumatism, English Edition*, vol. 63, no. 6, pp. 453-6.

Rozman, B, Praprotnik, S, Logar, D, Tomšič, M, Hojnik, M, Kos-Golja, M, Accetto, R & Dolenc, P 2002, 'Leflunomide and hypertension', *Annals of the Rheumatic Diseases*, vol. 61, no. 6, pp. 567-9.

Ruderman, EM 2013, 'The role of concomitant methotrexate in biologic therapy for rheumatoid arthritis', *Bulletin of the Hospital for Joint Diseases (2013)*, vol. 71, no. Suppl 1, pp. S29-32.

Ruiz-Esquide, V & Sanmarti, R 2012, 'Tobacco and other environmental risk factors in rheumatoid arthritis', *Reumatología Clínica*, vol. 8, no. 6, pp. 342-50. doi: 10.1016/j.reuma.2012.02.011. Epub May 17.

Ryu, J, Cha, RH, Kim, DK, Lee, JH, Yoon, SA, Ryu, DR, Oh, J, Kim, S, Han, SY, Lee, EY & Kim, YS 2015, 'Time points for obtaining representative values of 24-hour blood pressure in chronic kidney disease', *Korean Journal of Internal Medicine*, vol. 30, no. 5, pp. 665-74. doi: 10.3904/kjim.2015.30.5.665. Epub Aug 27.

Saag, KG 2001, 'Resolved: Low-dose glucocorticoids are neither safe nor effective for the long-term treatment of rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 45, no. 5, pp. 468-71.

Saag, KG, Koehnke, R, Caldwell, JR, Brasington, R, Burmeister, LF, Zimmerman, B, Kohler, JA & Furst, DE 1994, 'Low dose long-term corticosteroid therapy in rheumatoid arthritis: An analysis of serious adverse events', *The American Journal of Medicine*, vol. 96, no. 2, pp. 115-23.

Saag, KG, Teng, GG, Patkar, NM, Anuntiyo, J, Finney, C, Curtis, JR, Paulus, HE, Mudano, A, Pisu, M, Elkins-Melton, M, Outman, R, Allison, JJ, Suarez Almazor, M, Bridges, SL, Jr., Chatham, WW, Hochberg, M, MacLean, C, Mikuls, T, Moreland, LW, O'Dell, J, Turkiewicz, AM & Furst, DE 2008, 'American College of Rheumatology 2008 recommendations for the use of nonbiologic and biologic disease-modifying antirheumatic drugs in rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 59, no. 6, pp. 762-84. doi: 10.1002/art.23721.

Sabio, JM, Vargas-Hitos, J, Zamora-Pasadas, M, Mediavilla, JD, Navarrete, N, Ramirez, A, Hidalgo-Tenorio, C, Jaimez, L, Martin, J & Jimenez-Alonso, J 2009, 'Metabolic syndrome is associated with increased arterial stiffness and biomarkers of subclinical atherosclerosis in patients with systemic lupus erythematosus', *Journal of Rheumatology*, vol. 36, no. 10, pp. 2204-11. doi: 10.3899/jrheum.081253. Epub 2009 Sep 1.

Safar, ME, London, GM, Asmar, RG, Hugues, CJ & Laurent, SA 1986, 'An indirect approach for the study of the elastic modulus of the brachial artery in patients with essential hypertension', *Cardiovascular Research*, vol. 20, no. 8, pp. 563-7.

Saito, H, Masuda, S & Inui, K 1996, 'Cloning and functional characterization of a novel rat organic anion transporter mediating basolateral uptake of methotrexate in the kidney', *Journal of Biological Chemistry*, vol. 271, no. 34, pp. 20719-25.

Sakai, R, Hirano, F, Kihara, M, Yokoyama, W, Yamazaki, H, Harada, S, Nanki, T, Koike, R, Miyasaka, N & Harigai, M 2015, 'High prevalence of cardiovascular comorbidities in patients with rheumatoid arthritis from a population-based cross-sectional study of a Japanese health insurance database', *Modern Rheumatology*, vol. 14, pp. 1-7.

Sakurai, M, Yamakado, T, Kurachi, H, Kato, T, Kuroda, K, Ishisu, R, Okamoto, S, Isaka, N, Nakano, T & Ito, M 2007, 'The relationship between aortic augmentation index and pulse wave velocity: an invasive study', *Journal of Hypertension*, vol. 25, no. 2, pp. 391-7.

Sandoo, A, Carroll, D, Metsios, GS, Kitas, GD & Veldhuijzen van Zanten, JJ 2011, 'The association between microvascular and macrovascular endothelial function in patients with rheumatoid arthritis: a cross-sectional study', *Arthritis Research & Therapy*, vol. 13, no. 3, p. R99. doi: 10.1186/ar3374.

Sandoo, A, Dimitroulas, T, Hodson, J, Smith, JP, Douglas, KM & Kitas, GD 2015, 'Cumulative inflammation associates with asymmetric dimethylarginine in rheumatoid arthritis: a 6 year follow-up study', *Rheumatology (Oxford)*, vol. 54, no. 7, pp. 1145-52. doi: 10.093/rheumatology/keu349. Epub 2014 Sep 3.

Sandoo, A, Dimitroulas, T, Toms, TE, Hodson, J, Veldhuijzen van Zanten, JJ, Smith, JP & Kitas, GD 2012, 'Clinical remission following treatment with tumour necrosis factor-alpha antagonists is not accompanied by changes in asymmetric dimethylarginine in

patients with rheumatoid arthritis', *Clinical Biochemistry*, vol. 45, no. 16-17, pp. 1399-403. doi: 10.016/j.clinbiochem.2012.07.092. Epub Jul 20.
Sandoo, A, Dimitroulas, T, Veldhuijzen van Zanten, JJ, Smith, JP, Metsios, GS, Nightingale, P, Stavropoulos-Kalinoglou, A & Kitas, GD 2012, 'Lack of association between asymmetric dimethylarginine and in vivo microvascular and macrovascular endothelial function in patients with rheumatoid arthritis', *Clinical and Experimental Rheumatology*, vol. 30, no. 3, pp. 388-96. Epub 2012 Jun 26.
Sandoo, A, van Zanten, JJ, Metsios, GS, Carroll, D & Kitas, GD 2010, 'The endothelium and its role in regulating vascular tone', *Open Cardiovascular Medicine Journal*, vol. 4:302-12., no. doi, p. 10.2174/1874192401004010302.
Saravanan, V & Kelly, CA 2004, 'Reducing the risk of methotrexate pneumonitis in rheumatoid arthritis', *Rheumatology*, vol. 43, no. 2, pp. 143-7.
Schaub, TP, Kartenbeck, J, Konig, J, Vogel, O, Witzgall, R, Kriz, W & Keppler, D 1997, 'Expression of the conjugate export pump encoded by the mrp2 gene in the apical membrane of kidney proximal tubules', *Journal of the American Society of Nephrology*, vol. 8, no. 8, pp. 1213-21.
Schellekens, GA, de Jong, BA, van den Hoogen, FH, van de Putte, LB & van Venrooij, WJ 1998, 'Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies', *Journal of Clinical Investigation*, vol. 101, no. 1, pp. 273-81.
Schiff, MH & Whelton, A 2000, 'Renal toxicity associated with disease-modifying antirheumatic drugs used for the treatment of rheumatoid arthritis', *Seminars in Arthritis and Rheumatism*, vol. 30, no. 3, pp. 196-208.
Schilling, WP, Cabello, OA & Rajan, L 1992, 'Depletion of the inositol 1,4,5-trisphosphate-sensitive intracellular Ca2+ store in vascular endothelial cells activates the agonist-sensitive Ca(2+)-influx pathway', *Biochemical Journal*, vol. 284, no. Pt 2, pp. 521-30.
Schilling, WP & Elliott, SJ 1992, 'Ca2+ signaling mechanisms of vascular endothelial cells and their role in oxidant-induced endothelial cell dysfunction', *American Journal of Physiology*, vol. 262, no. 6 Pt 2, pp. H1617-30.
Schmidt, MI, Duncan, BB, Sharrett, AR, Lindberg, G, Savage, PJ, Offenbacher, S, Azambuja, MI, Tracy, RP & Heiss, G 1999, 'Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study', *The Lancet*, vol. 353, no. 9165, pp. 1649-52.
Schnabel, A & Gross, WL 1994, 'Low-dose methotrexate in rheumatic diseases—Efficacy, side effects, and risk factors for side effects', *Seminars in Arthritis and Rheumatism*, vol. 23, no. 5, pp. 310-27.
Schnabel, A, Reinhold-Keller, E, Willmann, V & Gross, WL 1994, 'Tolerability of methotrexate starting with 15 or 25 mg/week for rheumatoid arthritis', *Rheumatology International*, vol. 14, no. 1, pp. 33-8.
Schoels, M, Aletaha, D, Smolen, JS, Bijlsma, JW, Burmester, G, Breedveld, FC, Cutolo, M, Combe, B, Dougados, M, Emery, P, Kalden, J, Keystone, EC, Kvien, TK, Martin-Mola, E, Montecucco, C & De Wit, M 2010, 'Follow-up standards and treatment targets in rheumatoid arthritis: results of a questionnaire at the EULAR 2008', *Annals of the Rheumatic Diseases*, vol. 69, no. 3, pp. 575-8. doi: 10.1136/ard.2009.108472. Epub 2009 Apr 22.
Schroder, H, Fogh, K & Herlin, T 1988, 'In vivo decline of methotrexate and methotrexate polyglutamates in age-fractionated erythrocytes', *Cancer Chemotherapy and Pharmacology*, vol. 21, no. 2, pp. 150-5.

Scott, D, Grindulis, K, Struthers, G, Coulton, B, Popert, A & Bacon, P 1984, 'Progression of radiological changes in rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 43, pp. 8 - 17.

Scott, D, Pugner, K, Kaarela, K, Doyle, D, Woolf, A, Holmes, J & Hieke, K 2000, 'The links between joint damage and disability in rheumatoid arthritis', *Rheumatology (Oxford)*, vol. 39, no. 2, pp. 122-32.

Scott, DL, Symmons, DP, Coulton, BL & Popert, AJ 1987, 'Long-term outcome of treating rheumatoid arthritis: results after 20 years', *The Lancet*, vol. 1, no. 8542, pp. 1108-11.

Segers, P, Stergiopulos, N & Westerhof, N 2000, 'Quantification of the contribution of cardiac and arterial remodeling to hypertension', *Hypertension*, vol. 36, no. 5, pp. 760-5.

Sekine, T, Cha, SH, Tsuda, M, Apiwattanakul, N, Nakajima, N, Kanai, Y & Endou, H 1998, 'Identification of multispecific organic anion transporter 2 expressed predominantly in the liver', *Federation of European Biochemical Societies Letters*, vol. 429, no. 2, pp. 179-82.

Sekine, T, Watanabe, N, Hosoyamada, M, Kanai, Y & Endou, H 1997, 'Expression cloning and characterization of a novel multispecific organic anion transporter', *Journal of Biological Chemistry*, vol. 272, no. 30, pp. 18526-9.

Sen, U, Mishra, PK, Tyagi, N & Tyagi, SC 2010, 'Homocysteine to hydrogen sulfide or hypertension', *Cell Biochemistry and Biophysics*, vol. 57, no. 2-3, pp. 49-58. doi: 10.1007/s12013-010-9079-y.

Serelis, J, Panagiotakos, DB, Mavrommati, M & Skopouli, FN 2011, 'Cardiovascular disease is related to hypertension in patients with rheumatoid arthritis: a Greek cohort study', *Journal of Rheumatology*, vol. 38, no. 2, pp. 236-41.

Sesso, HD, Buring, JE, Rifai, N, Blake, GJ, Gaziano, JM & Ridker, PM 2003, 'C-reactive protein and the risk of developing hypertension', *Journal of the American Medical Association*, vol. 290, no. 22, pp. 2945-51.

Sesti, G, Mannino, GC, De Lorenzo, C, Greco, A, Sciacqua, A, Marini, MA, Andreozzi, F & Perticone, F 2013, 'A functional variant of the dimethylarginine dimethylaminohydrolase-2 gene is associated with chronic kidney disease', *Atherosclerosis*, vol. 231, no. 1, pp. 141-4. doi: 10.1016/j.atherosclerosis.2013.08.041. Epub Sep 14.

Shapiro, Y, Boaz, M, Matas, Z, Fux, A & Shargorodsky, M 2008, 'The association between the renin-angiotensin-aldosterone system and arterial stiffness in young healthy subjects', *Clinical Endocrinology*, vol. 68, no. 4, pp. 510-2.

Sharman, JE, Howes, FS, Head, GA, McGrath, BP, Stowasser, M, Schlaich, M, Glasziou, P & Nelson, MR 2015, 'Home blood pressure monitoring: Australian Expert Consensus Statement', *Journal of Hypertension*, vol. 33, no. 9, pp. 1721-8. doi: 10.097/HJH.0000000000000673.

Shen, TC, Lin, CL, Chen, CH, Tu, CY, Hsia, TC, Shih, CM, Hsu, WH & Sung, FC 2014, 'Increased risk of chronic obstructive pulmonary disease in patients with rheumatoid arthritis: a population-based cohort study', *QJM: An International Journal of Medicine*, vol. 107, no. 7, pp. 537-43. doi: 10.1093/qjmed/hcu027. Epub 2014 Feb 3.

Sherrer, YS, Bloch, DA, Mitchell, DM, Young, DY & Fries, JF 1986, 'The development of disability in rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 29, no. 4, pp. 494-500.

Shige, H, Dart, A & Nestel, P 2001, 'Simvastatin improves arterial compliance in the lower limb but not in the aorta', *Atherosclerosis*, vol. 155, no. 1, pp. 245-50.

Shiina, T, Hosomichi, K, Inoko, H & Kulski, JK 2009, 'The HLA genomic loci map: expression, interaction, diversity and disease', *Journal of Human Genetics*, vol. 54, no. 1, pp. 15-39. doi: 10.1038/jhg.2008.5. Epub 9 Jan 9.

Siddiqi, N 2011, 'Publication bias in epidemiological studies', *Central European Journal of Public Health*, vol. 19, no. 2, pp. 118-20.

Silman, AJ, MacGregor, AJ, Thomson, W, Holligan, S, Carthy, D, Farhan, A & Ollier, WE 1993, 'Twin concordance rates for rheumatoid arthritis: results from a nationwide study', *British Journal of Rheumatology*, vol. 32, no. 10, pp. 903-7.

Silman, AJ, Newman, J & MacGregor, AJ 1996, 'Cigarette smoking increases the risk of rheumatoid arthritis. Results from a nationwide study of disease-discordant twins', *Arthritis and Rheumatism*, vol. 39, no. 5, pp. 732-5.

Silverstein, FE, Faich, G, Goldstein, JL, Simon, LS, Pincus, T, Whelton, A, Makuch, R, Eisen, G, Agrawal, NM, Stenson, WF, Burr, AM, Zhao, WW, Kent, JD, Lefkowith, JB, Verburg, KM & Geis, GS 2000, 'Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: A randomized controlled trial. Celecoxib Long-term Arthritis Safety Study', *Journal of the American Medical Association*, vol. 284, no. 10, pp. 1247-55.

Singal, VK, Chaturvedi, VP & Brar, KS 2005, 'Efficacy and toxicity profile of methotrexate chloroquine combination in treatment of active rheumatoid arthritis', *Medical Journal Armed Forces India*, vol. 61, no. 1, pp. 29-32. doi: 10.1016/S0377-1237(05)80114-5. Epub 2011 Jul 21.

Singh, JA, Furst, DE, Bharat, A, Curtis, JR, Kavanaugh, AF, Kremer, JM, Moreland, LW, O'Dell, J, Winthrop, KL, Beukelman, T, Bridges, SL, Jr., Chatham, WW, Paulus, HE, Suarez-Almazor, M, Bombardier, C, Dougados, M, Khanna, D, King, CM, Leong, AL, Matteson, EL, Schousboe, JT, Moynihan, E, Kolba, KS, Jain, A, Volkmann, ER, Agrawal, H, Bae, S, Mudano, AS, Patkar, NM & Saag, KG 2012, '2012 update of the 2008 American College of Rheumatology recommendations for the use of disease-modifying antirheumatic drugs and biologic agents in the treatment of rheumatoid arthritis', *Arthritis Care Res (Hoboken)*, vol. 64, no. 5, pp. 625-39. doi: 10.1002/acr.21641.

Singh, JA, Saag, KG, Bridges, SL, Jr., Akl, EA, Bannuru, RR, Sullivan, MC, Vaysbrot, E, McNaughton, C, Osani, M, Shmerling, RH, Curtis, JR, Furst, DE, Parks, D, Kavanaugh, A, O'Dell, J, King, C, Leong, A, Matteson, EL, Schousboe, JT, Drevlow, B, Ginsberg, S, Grober, J, St Clair, EW, Tindall, E, Miller, AS & McAlindon, T 2016, '2015 American College of Rheumatology guideline for the treatment of rheumatoid arthritis', *Arthritis & Rheumatology*, vol. 68, no. 1, pp. 1-26. doi: 10.1002/art.39480. Epub 2015 Nov 6.

Singwe-Ngandeu, M, Essouma, M, Ama Moor, VJ, Musa, AJ, Menanga, AP, Ngoufack, C, Lontsi, SSa, Ntandzi, T, Ayi Efoua, YV & Melong Pianta, MC 2016, 'Cardiovascular risk burden in Sub-Saharan Africans with rheumatoid arthritis: a hospital-based study in Yaounde, Cameroon', *Open Journal of Rheumatology and Autoimmune Diseases*, vol. 06, no. 01, pp. 1-9.

Sirenko, O, Kuryata, O & Lysunets, T 2015, 'AB0377 Endothelial dysfunction in patients with rheumatoid arthritis combined with hypertension and its correction with l-arginine aspartate', *Annals of the Rheumatic Diseases*, vol. 74, no. Suppl 2, p. 1019.

Skafar, DF, Xu, R, Morales, J, Ram, J & Sowers, JR 1997, 'Clinical review 91: Female sex hormones and cardiovascular disease in women', *Journal of Clinical Endocrinology & Metabolism*, vol. 82, no. 12, pp. 3913-8.

Skinner, MR & Marshall, JM 1996, 'Studies on the roles of ATP, adenosine and nitric oxide in mediating muscle vasodilatation induced in the rat by acute systemic hypoxia', *Journal of Physiology*, vol. 495, no. Pt 2, pp. 553-60.

Skoldstam, L, Borjesson, O, Kjallman, A, Seiving, B & Akesson, B 1992, 'Effect of six months of fish oil supplementation in stable rheumatoid arthritis. A double-blind, controlled study', *Scandinavian Journal of Rheumatology*, vol. 21, no. 4, pp. 178-85.

Smith, CJ, Zhang, Y, Koboldt, CM, Muhammad, J, Zweifel, BS, Shaffer, A, Talley, JJ, Masferrer, JL, Seibert, K & Isakson, PC 1998, 'Pharmacological analysis of cyclooxygenase-1 in inflammation', *Proceedings of the National Academy of Sciences USA*, vol. 95, no. 22, pp. 13313-8.

Smits, P, Boekema, P, De Abreu, R, Thien, T & van 't Laar, A 1987, 'Evidence for an antagonism between caffeine and adenosine in the human cardiovascular system', *Journal of Cardiovascular Pharmacology and Therapeutics*, vol. 10, no. 2, pp. 136-43.

Smits, P, Williams, SB, Lipson, DE, Banitt, P, Rongen, GA & Creager, MA 1995, 'Endothelial release of nitric oxide contributes to the vasodilator effect of adenosine in humans', *Circulation*, vol. 92, no. 8, pp. 2135-41.

Smolen, JS, Aletaha, D, Koeller, M, Weisman, MH & Emery, P 2007, 'New therapies for treatment of rheumatoid arthritis', *The Lancet*, vol. 370, no. 9602, pp. 1861-74.

Smolen, JS, Breedveld, FC, Eberl, G, Jones, I, Leeming, M, Wylie, GL & Kirkpatrick, J 1995, 'Validity and reliability of the twenty-eight-joint count for the assessment of rheumatoid arthritis activity', *Arthritis and Rheumatism*, vol. 38, no. 1, pp. 38-43.

Smolen, JS, Landewe, R, Breedveld, FC, Dougados, M, Emery, P, Gaujoux-Viala, C, Gorter, S, Knevel, R, Nam, J, Schoels, M, Aletaha, D, Buch, M, Gossec, L, Huizinga, T, Bijlsma, JW, Burmester, G, Combe, B, Cutolo, M, Gabay, C, Gomez-Reino, J, Kouloumas, M, Kvien, TK, Martin-Mola, E, McInnes, I, Pavelka, K, van Riel, P, Scholte, M, Scott, DL, Sokka, T, Valesini, G, van Vollenhoven, R, Winthrop, KL, Wong, J, Zink, A & van der Heijde, D 2010, 'EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs', *Annals of the Rheumatic Diseases*, vol. 69, no. 6, pp. 964-75. doi: 10.1136/ard.2009.126532. Epub 2010 May 5.

Smyrnova, VM 2015, 'Hematological abnormalities in Ukrainian patients with rheumatoid arthritis', *Journal of Arthritis*, vol. 04, no. 01.

Sokka, T, Mottonen, T & Hannonen, P 1999, 'Mortality in early "sawtooth" treated rheumatoid arthritis patients during the first 8-14 years', *Scandinavian Journal of Rheumatology*, vol. 28, no. 5, pp. 282-7.

Solomon, A, Tsang, L, Woodiwiss, AJ, Millen, AM, Norton, GR & Dessein, PH 2014, 'Cardiovascular disease risk amongst African black patients with rheumatoid arthritis: the need for population specific stratification', *BioMed Research International*, vol. 2014:826095., no. doi, p. 10.1155/2014/826095. Epub 2014 Jul 23.

Solomon, DH, Curtis, JR, Saag, KG, Lii, J, Chen, L, Harrold, LR, Herrinton, LJ, Graham, DJ, Kowal, MK, Kuriya, B, Liu, L, Griffin, MR, Lewis, JD & Rassen, JA 2013, 'Cardiovascular risk in rheumatoid arthritis: comparing TNF-alpha blockade with nonbiologic DMARDs', *American Journal of Medicine*, vol. 126, no. 8, pp. 730.e9-.e17. doi: 10.1016/j.amjmed.2013.02.016.

Solomon, DH, Goodson, NJ, Katz, JN, Weinblatt, ME, Avorn, J, Setoguchi, S, Canning, C & Schneeweiss, S 2006, 'Patterns of cardiovascular risk in rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 65, no. 12, pp. 1608-12. Epub 2006 Jun 22.

Solomon, DH, Karlson, EW, Rimm, EB, Cannuscio, CC, Mandl, LA, Manson, JE, Stampfer, MJ & Curhan, GC 2003, 'Cardiovascular morbidity and mortality in women diagnosed with rheumatoid arthritis', *Circulation*, vol. 107, no. 9, pp. 1303-7.

Solomon, DH, Love, TJ, Canning, C & Schneeweiss, S 2010, 'Risk of diabetes among patients with rheumatoid arthritis, psoriatic arthritis and psoriasis', *Annals of the Rheumatic Diseases*, vol. 69, no. 12, pp. 2114-7. doi: 10.1136/ard.2009.125476. Epub 2010 Jun 28.

Sonnenberg, GE, Krakower, GR & Kissebah, AH 2004, 'A novel pathway to the manifestations of metabolic syndrome', *Obesity Research*, vol. 12, no. 2, pp. 180-6.

Sopori, ML & Kozak, W 1998, 'Immunomodulatory effects of cigarette smoke', *Journal of Neuroimmunology*, vol. 83, no. 1-2, pp. 148-56.

Soubrier, M & Dougados, M 2005, 'How to assess early rheumatoid arthritis in daily clinical practice', *Best Practice & Research: Clinical Rheumatology*, vol. 19, no. 1, pp. 73-89.

Souverein, PC, Berard, A, Van Staa, TP, Cooper, C, Egberts, AC, Leufkens, HG & Walker, BR 2004, 'Use of oral glucocorticoids and risk of cardiovascular and cerebrovascular disease in a population based case-control study', *Heart*, vol. 90, no. 8, pp. 859-65.

Spasovski, D, Latifi, A, Osmani, B, Krstevska-Balkanov, S, Kafedizska, I, Slaninka-Micevska, M, Dejanova, B, Alabakovska, S & Balkanov, T 2013, 'Determination of the diagnostic values of asymmetric dimethylarginine as an indicator for evaluation of the endothelial dysfunction in patients with rheumatoid arthritis', *Arthritis*, vol. 2013:818037., no. doi, p. 10.1155/2013/818037. Epub 2013 May 15.

Spinelli, FR, Di Franco, M, Metere, A, Conti, F, Iannuccelli, C, Agati, L & Valesini, G 2014, 'Decrease of asymmetric dimethyl arginine after anti-TNF therapy in patients with rheumatoid arthritis', *Drug Development and Research*, vol. 75, no. Suppl 1, pp. S67-9. doi: 10.1002/ddr.21200.

Stamatelopoulos, KS, Kitas, GD, Papamichael, CM, Chryssohoou, E, Kyrkou, K, Georgiopoulos, G, Protogerou, A, Panoulas, VF, Sandoo, A, Tentolouris, N, Mavrikakis, M & Sfikakis, PP 2009, 'Atherosclerosis in rheumatoid arthritis versus diabetes: a comparative study', *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 29, no. 10, pp. 1702-8. doi: 10.161/ATVBAHA.109.190108. Epub 2009 Jul 16.

Stamatelopoulos, KS, Kitas, GD, Papamichael, CM, Kyrkou, K, Zampeli, E, Fragiadaki, K, Panoulas, VF, Mavrikakis, M & Sfikakis, PP 2010, 'Subclinical peripheral arterial disease in rheumatoid arthritis', *Atherosclerosis*, vol. 212, no. 1, pp. 305-9. doi: 10.1016/j.atherosclerosis.2010.05.007. Epub May 12.

Stamatelopoulos, KS, Lekakis, JP, Poulakaki, NA, Papamichael, CM, Venetsanou, K, Aznaouridis, K, Protogerou, AD, Papaioannou, TG, Kumar, S & Stamatelopoulos, SF 2004, 'Tamoxifen improves endothelial function and reduces carotid intima-media thickness in postmenopausal women', *American Heart Journal*, vol. 147, no. 6, pp. 1093-9.

Stamp, LK, Chapman, PT, O'Donnell, JL, Zhang, M, James, J, Frampton, C, Barclay, ML, Kennedy, MA & Roberts, RL 2010, 'Polymorphisms within the folate pathway predict folate concentrations but are not associated with disease activity in rheumatoid arthritis patients on methotrexate', *Pharmacogenets Genomics*, vol. 20, no. 6, pp. 367-76. doi: 10.1097/FPC.0b013e3283398a71.

Stamp, LK, O'Donnell, JL, Chapman, PT, Zhang, M, Frampton, C, James, J & Barclay, ML 2009, 'Determinants of red blood cell methotrexate polyglutamate concentrations in rheumatoid arthritis patients receiving long-term methotrexate treatment', *Arthritis and Rheumatism*, vol. 60, no. 8, pp. 2248-56. doi: 10.1002/art.24653.

Stamp, LK, O'Donnell, JL, Chapman, PT, Zhang, M, James, J, Frampton, C & Barclay, ML 2010, 'Methotrexate polyglutamate concentrations are not associated with disease control in rheumatoid arthritis patients receiving long-term methotrexate therapy', *Arthritis and Rheumatism*, vol. 62, no. 2, pp. 359-68. doi: 10.1002/art.27201.

Stamp, LK & Roberts, RL 2011, 'Effect of genetic polymorphisms in the folate pathway on methotrexate therapy in rheumatic diseases', *Pharmacogenomics*, vol. 12, no. 10, pp. 1449-63. doi: 10.2217/pgs.11.86.

Stenehjem, AE & Os, I 2004, 'Reproducibility of blood pressure variability, white-coat effect and dipping pattern in untreated, uncomplicated and newly diagnosed essential hypertension', *Blood Pressure*, vol. 13, no. 4, pp. 214-24.

Stenstrom, CH & Minor, MA 2003, 'Evidence for the benefit of aerobic and strengthening exercise in rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 49, no. 3, pp. 428-34.

Steunebrink, LM, Vonkeman, HE, ten Klooster, PM, Hoekstra, M, van Riel, PL & van de Laar, MA 2016, 'Recently diagnosed rheumatoid arthritis patients benefit from a treat-to-target strategy: results from the DREAM registry', *Clinical Rheumatology*, vol. 35, no. 3, pp. 609-15. doi: 10.1007/s10067-016-3191-3. Epub 2016 Feb 6.

Stolt, P, Bengtsson, C, Nordmark, B, Lindblad, S, Lundberg, I, Klareskog, L & Alfredsson, L 2003, 'Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases', *Annals of the Rheumatic Diseases*, vol. 62, no. 9, pp. 835-41.

Stoner, L, Young, JM & Fryer, S 2012, 'Assessments of arterial stiffness and endothelial function using pulse wave analysis', *International Journal of Vascular Medicine*, vol. 2012:903107., no. doi, p. 10.1155/2012/903107. Epub 2012 May 14.

Strand, V, Cohen, S, Schiff, M, Weaver, A, Fleischmann, R, Cannon, G, Fox, R, Moreland, L, Olsen, N, Furst, D, Caldwell, J, Kaine, J, Sharp, J, Hurley, F & Loew-Friedrich, I 1999, 'Treatment of active rheumatoid arthritis with leflunomide compared with placebo and methotrexate. Leflunomide Rheumatoid Arthritis Investigators Group', *Archives of Internal Medicine*, vol. 159, no. 21, pp. 2542-50.

Stuhlinger, MC, Abbasi, F, Chu, JW, Lamendola, C, McLaughlin, TL, Cooke, JP, Reaven, GM & Tsao, PS 2002, 'Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor', *Journal of American Medical Association*, vol. 287, no. 11, pp. 1420-6.

Stuhlinger, MC, Tsao, PS, Her, JH, Kimoto, M, Balint, RF & Cooke, JP 2001, 'Homocysteine impairs the nitric oxide synthase pathway: role of asymmetric dimethylarginine', *Circulation*, vol. 104, no. 21, pp. 2569-75.

Subar, AF, Thompson, FE, Kipnis, V, Midthune, D, Hurwitz, P, McNutt, S, McIntosh, A & Rosenfeld, S 2001, 'Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires: the Eating at America's Table Study', *American Journal of Epidemiology*, vol. 154, no. 12, pp. 1089-99.

Sugiyama, D, Nishimura, K, Tamaki, K, Tsuji, G, Nakazawa, T, Morinobu, A & Kumagai, S 2010, 'Impact of smoking as a risk factor for developing rheumatoid arthritis: a meta-analysis of observational studies', *Annals of the Rheumatic Diseases*, vol. 69, no. 1, pp. 70-81. doi: 10.1136/ard.2008.096487. Epub.

Sung, JY, Hong, JH, Kang, HS, Choi, I, Lim, SD, Lee, JK, Seok, JH, Lee, JH & Hur, GM 2000, 'Methotrexate suppresses the interleukin-6 induced generation of reactive oxygen species in the synoviocytes of rheumatoid arthritis', *Immunopharmacology*, vol. 47, no. 1, pp. 35-44.

Sutton-Tyrrell, K, Bostom, A, Selhub, J & Zeigler-Johnson, C 1997, 'High homocysteine levels are independently related to isolated systolic hypertension in older adults', *Circulation*, vol. 96, no. 6, pp. 1745-9.

Svensson, B, Boonen, A, Albertsson, K, van der Heijde, D, Keller, C & Hafström, I 2005, 'Low-dose prednisolone in addition to the initial disease-modifying antirheumatic drug in patients with early active rheumatoid arthritis reduces joint destruction and increases the remission rate: a two-year randomized trial', *Arthritis and Rheumatism*, vol. 52, no. 11, pp. 3360-70.

Svetkey, LP, Sacks, FM, Obarzanek, E, Vollmer, WM, Appel, LJ, Lin, PH, Karanja, NM, Harsha, DW, Bray, GA, Aickin, M, Proschan, MA, Windhauser, MM, Swain, JF, McCarron, PB, Rhodes, DG & Laws, RL 1999, 'The DASH diet, sodium intake and blood pressure trial (DASH-sodium): rationale and design. DASH-Sodium Collaborative Research Group', *Journal of the American Dietetic Association*, vol. 99, no. 8 Suppl, pp. S96-104.

Swierkot, J & Szechinski, J 2006, 'Methotrexate in rheumatoid arthritis', *Pharmacological Reports*, vol. 58, no. 4, pp. 473-92.

Sydow, K, Mondon, CE & Cooke, JP 2005, 'Insulin resistance: potential role of the endogenous nitric oxide synthase inhibitor ADMA', *Vascular Medicine*, vol. 10, no. Suppl 1, pp. S35-43.

Symmons, DP, Jones, MA, Scott, DL & Prior, P 1998, 'Longterm mortality outcome in patients with rheumatoid arthritis: early presenters continue to do well', *Journal of Rheumatology*, vol. 25, no. 6, pp. 1072-7.

Takano, M, Yumoto, R & Murakami, T 2006, 'Expression and function of efflux drug transporters in the intestine', *Pharmacology and Therapeutics*, vol. 109, no. 1–2, pp. 137-61.

Takatori, R, Takahashi, KA, Tokunaga, D, Hojo, T, Fujioka, M, Asano, T, Hirata, T, Kawahito, Y, Satomi, Y, Nishino, H, Tanaka, T, Hirota, Y & Kubo, T 2006, 'ABCB1 C3435T polymorphism influences methotrexate sensitivity in rheumatoid arthritis patients', *Clinical and Experimental Rheumatology*, vol. 24, no. 5, pp. 546-54.

Taler, SJ, Textor, SC, Canzanello, VJ & Schwartz, L 1999, 'Cyclosporin-induced hypertension: incidence, pathogenesis and management', *Drug Safety*, vol. 20, no. 5, pp. 437-49.

Teede, HJ 2007, 'Sex hormones and the cardiovascular system: effects on arterial function in women', *Clinical and Experimental Pharmacology and Physiology*, vol. 34, no. 7, pp. 672-6.

Teplan, V, Schuck, O, Racek, J, Mareckova, O, Stollova, M, Hanzal, V & Maly, J 2008, 'Reduction of plasma asymmetric dimethylarginine in obese patients with chronic kidney disease after three years of a low-protein diet supplemented with keto-amino acids: a randomized controlled trial', *Wiener Klinische Wochenschrift*, vol. 120, no. 15-16, pp. 478-85. doi: 10.1007/s00508-008-0987-4.

Textor, SC, Canzanello, VJ, Taler, SJ, Wilson, DJ, Schwartz, LL, Augustine, JE, Raymer, JM, Romero, JC, Wiesner, RH, Krom, RA & et al. 1994, 'Cyclosporine-induced hypertension after transplantation', *Mayo Clin Proc.*, vol. 69, no. 12, pp. 1182-93.

The Heart Foundation & The National Stroke Foundation 2016, *Innovative proposals to tackle our most costly disease group: HEART DISEASE AND STROKE*, The Heart Foundation, viewed 16 October 2016, http://www.heartfoundation.org.au.

Thomas, JA & Aithal, GP 2005, 'Monitoring liver function during methotrexate therapy for psoriasis: are routine biopsies really necessary?', *American Journal of Clinical Dermatology*, vol. 6, no. 6, pp. 357-63.

Thornton, CC, Al-Rashed, F, Calay, D, Birdsey, GM, Bauer, A, Mylroie, H, Morley, BJ, Randi, AM, Haskard, DO, Boyle, JJ & Mason, JC 2016, 'Methotrexate-mediated activation of an AMPK-CREB-dependent pathway: a novel mechanism for vascular protection in chronic systemic inflammation', *Annals of the Rheumatic Diseases*, vol. 75, no. 2, pp. 439-48. doi: 10.1136/annrheumdis-2014-206305. Epub 2015 Jan 9.

Tian, H & Cronstein, BN 2007, 'Understanding the mechanisms of action of methotrexate: implications for the treatment of rheumatoid arthritis', *Bulletin of the NYU Hospital for Joint Diseases*, vol. 65, no. 3, pp. 168-73.

Tian, L & Chesler, NC 2012, 'In vivo and in vitro measurements of pulmonary arterial stiffness: A brief review', *Pulmonary Circulation*, vol. 2, no. 4, pp. 505-17. doi: 10.4103/2045-8932.105040.

Tikiz, H, Arslan, O, Pirildar, T, Tikiz, C & Bayindir, P 2010, 'The effect of anti-tumor necrosis factor (TNF)-alpha therapy with etanercept on endothelial functions in patients with rheumatoid arthritis', *Anatolian Journal of Cardiology*, vol. 10, no. 2, pp. 98-103. doi: 10.5152/akd.2010.031.

Tomiyama, H & Yamashina, A 2010, 'Non-invasive vascular function tests: their pathophysiological background and clinical application', *Circulation Journal*, vol. 74, no. 1, pp. 24-33. Epub 2009 Nov 17.

Tommasi, S, Zanato, C, Lewis, BC, Nair, PC, Dall'Angelo, S, Zanda, M & Mangoni, AA 2015, 'Arginine analogues incorporating carboxylate bioisosteric functions are micromolar inhibitors of human recombinant DDAH-1', *Organic & Biomolecular Chemistry*, vol. 13, no. 46, pp. 11315-30. doi: 10.1039/c5ob01843a. Epub 2015 Sep 30.

Toms, TE, Panoulas, VF, Douglas, KM, Nightingale, P, Smith, JP, Griffiths, H, Sattar, N, Symmons, DP & Kitas, GD 2011, 'Are lipid ratios less susceptible to change with systemic inflammation than individual lipid components in patients with rheumatoid arthritis?', *Angiology*, vol. 62, no. 2, pp. 167-75. doi: 10.1177/0003319710373749. Epub 2010 Aug 3.

Toms, TE, Symmons, DP & Kitas, GD 2010, 'Dyslipidaemia in rheumatoid arthritis: the role of inflammation, drugs, lifestyle and genetic factors', *Current Vascular Pharmacology*, vol. 8, no. 3, pp. 301-26.

Totoson, P, Maguin-Gate, K, Nappey, M, Wendling, D & Demougeot, C 2016, 'Endothelial Dysfunction in Rheumatoid Arthritis: Mechanistic Insights and Correlation with Circulating Markers of Systemic Inflammation', *PLoS One*, vol. 11, no. 1, p. e0146744. doi: 10.1371/journal.pone.. eCollection 2016.

Touroutoglou, N & Pazdur, R 1996, 'Thymidylate synthase inhibitors', *Clinical Cancer Research*, vol. 2, no. 2, pp. 227-43.

Toutouzas, K, Riga, M, Stefanadi, E & Stefanadis, C 2008, 'Asymmetric dimethylarginine (ADMA) and other endogenous nitric oxide synthase (NOS) inhibitors as an important cause of vascular insulin resistance', *Hormone and Metabolic Research*, vol. 40, no. 9, pp. 655-9. doi: 10.1055/s-0028-1083814. Epub 2008 Sep 15.

Trayhurn, P & Wood, IS 2005, 'Signalling role of adipose tissue: adipokines and inflammation in obesity', *Biochemical Society Transactions*, vol. 33, no. Pt 5, pp. 1078-81.

Treharne, GJ, Lyons, AC, Hale, ED, Douglas, KM & Kitas, GD 2005, 'Predictors of medication adherence in people with rheumatoid arthritis: studies are necessary but non-validated measures of medication adherence are of concern', *Rheumatology (Oxford)*, vol. 44, no. 10, p. 1330; author reply 1. Epub 2005 Jun 21.

Turesson, C, Jarenros, A & Jacobsson, L 2004, 'Increased incidence of cardiovascular disease in patients with rheumatoid arthritis: results from a community based study', *Annals of the Rheumatic Diseases*, vol. 63, no. 8, pp. 952-5. Epub 2004 Mar 29.

Turesson, C, O'Fallon, WM, Crowson, CS, Gabriel, SE & Matteson, EL 2002, 'Occurrence of extraarticular disease manifestations is associated with excess mortality in a community based cohort of patients with rheumatoid arthritis', *Journal of Rheumatology*, vol. 29, no. 1, pp. 62-7.

Turiel, M, Tomasoni, L, Sitia, S, Cicala, S, Gianturco, L, Ricci, C, Atzeni, F, De Gennaro Colonna, V, Longhi, M & Sarzi-Puttini, P 2010, 'Effects of long-term disease-modifying antirheumatic drugs on endothelial function in patients with early rheumatoid arthritis', *Cardiovascular Therapeutics*, vol. 28, no. 5, pp. e53-64. doi: 10.1111/j.755-5922.2009.00119.x.

Uddin, J, Kraus, AS & Kelly, HG 1970, 'Survivorship and death in rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 13, no. 2, pp. 125-30.

Uhlig, T, Hagen, KB & Kvien, TK 1999, 'Current tobacco smoking, formal education, and the risk of rheumatoid arthritis', *Journal of Rheumatology*, vol. 26, no. 1, pp. 47-54.

Ulu, A, Harris, TR, Morisseau, C, Miyabe, C, Inoue, H, Schuster, G, Dong, H, Iosif, AM, Liu, JY, Weiss, RH, Chiamvimonvat, N, Imig, JD & Hammock, BD 2013, 'Anti-inflammatory effects of omega-3 polyunsaturated fatty acids and soluble epoxide hydrolase inhibitors in angiotensin-II-dependent hypertension', *Journal of Cardiovascular Pharmacology*, vol. 62, no. 3, pp. 285-97. doi: 10.1097/FJC.0b013e318298e460.

Ulu, A, Stephen Lee, KS, Miyabe, C, Yang, J, Hammock, BG, Dong, H & Hammock, BD 2014, 'An omega-3 epoxide of docosahexaenoic acid lowers blood pressure in angiotensin-II-dependent hypertension', *Journal of Cardiovascular Pharmacology*, vol. 64, no. 1, pp. 87-99. doi: 10.1097/FJC.0000000000000094.

Vaghef-Mehrabany, E, Alipour, B, Homayouni-Rad, A, Sharif, SK, Asghari-Jafarabadi, M & Zavvari, S 2014, 'Probiotic supplementation improves inflammatory status in patients with rheumatoid arthritis', *Nutrition*, vol. 30, no. 4, pp. 430-5. doi: 10.1016/j.nut.2013.09.007. Epub Dec 17.

Vaidya, D, Golden, SH, Haq, N, Heckbert, SR, Liu, K & Ouyang, P 2015, 'Association of sex hormones with carotid artery distensibility in men and postmenopausal women: multi-ethnic study of atherosclerosis', *Hypertension*, vol. 65, no. 5, pp. 1020-5. doi: 10.161/HYPERTENSIONAHA.114.04826. Epub 2015 Mar 9.

Valkonen, VP, Paiva, H, Salonen, JT, Lakka, TA, Lehtimaki, T, Laakso, J & Laaksonen, R 2001, 'Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine', *The Lancet*, vol. 358, no. 9299, pp. 2127-8.

Vallance, P & Leiper, J 2004, 'Cardiovascular biology of the asymmetric dimethylarginine:dimethylarginine dimethylaminohydrolase pathway', *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 24, no. 6, pp. 1023-30. Epub 2004 Apr 22.

Vallance, P, Leone, A, Calver, A, Collier, J & Moncada, S 1992, 'Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure', *The Lancet*, vol. 339, no. 8793, pp. 572-5.

van Aubel, RA, Smeets, PH, Peters, JG, Bindels, RJ & Russel, FG 2002, 'The MRP4/ABCC4 gene encodes a novel apical organic anion transporter in human kidney proximal tubules: putative efflux pump for urinary cAMP and cGMP', *Journal of the American Society of Nephrology*, vol. 13, no. 3, pp. 595-603.

van der Heijde, D, Klareskog, L, Rodriguez-Valverde, V, Codreanu, C, Bolosiu, H, Melo-Gomes, J, Tornero-Molina, J, Wajdula, J, Pedersen, R & Fatenejad, S 2006, 'Comparison of etanercept and methotrexate, alone and combined, in the treatment of rheumatoid arthritis: two-year clinical and radiographic results from the TEMPO study, a double-blind, randomized trial', *Arthritis and Rheumatism*, vol. 54, no. 4, pp. 1063-74.

van der Heijde, DM, van 't Hof, MA, van Riel, PL, Theunisse, LA, Lubberts, EW, van Leeuwen, MA, van Rijswijk, MH & van de Putte, LB 1990, 'Judging disease activity in clinical practice in rheumatoid arthritis: first step in the development of a disease activity score', *Annals of the Rheumatic Diseases*, vol. 49, no. 11, pp. 916-20.

van der Heijden, JW, Oerlemans, R, Tak, PP, Assaraf, YG, Kraan, MC, Scheffer, GL, van der Laken, CJ, Lems, WF, Scheper, RJ, Dijkmans, BA & Jansen, G 2009, 'Involvement of breast cancer resistance protein expression on rheumatoid arthritis synovial tissue macrophages in resistance to methotrexate and leflunomide', *Arthritis and Rheumatism*, vol. 60, no. 3, pp. 669-77. doi: 10.1002/art.24354.

van der Put, NM, Gabreels, F, Stevens, EM, Smeitink, JA, Trijbels, FJ, Eskes, TK, van den Heuvel, LP & Blom, HJ 1998, 'A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects?', *American Journal of Human Genetics*, vol. 62, no. 5, pp. 1044-51.

van der Straaten, RJ, Wessels, JA, de Vries-Bouwstra, JK, Goekoop-Ruiterman, YP, Allaart, CF, Bogaartz, J, Tiller, M, Huizinga, TW & Guchelaar, HJ 2007, 'Exploratory analysis of four polymorphisms in human GGH and FPGS genes and their effect in methotrexate-treated rheumatoid arthritis patients', *Pharmacogenomics*, vol. 8, no. 2, pp. 141-50.

van der Tempel, H, Tulleken, JE, Limburg, PC, Muskiet, FA & van Rijswijk, MH 1990, 'Effects of fish oil supplementation in rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 49, no. 2, pp. 76-80.

van der Woude, D, Houwing-Duistermaat, JJ, Toes, RE, Huizinga, TW, Thomson, W, Worthington, J, van der Helm-van Mil, AH & de Vries, RR 2009, 'Quantitative heritability of anti-citrullinated protein antibody-positive and anti-citrullinated protein antibody-negative rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 60, no. 4, pp. 916-23. doi: 10.1002/art.24385.

Van Doornum, S, Brand, C, King, B & Sundararajan, V 2006, 'Increased case fatality rates following a first acute cardiovascular event in patients with rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 54, no. 7, pp. 2061-8.

Van Doornum, S, McColl, G & Wicks, IP 2004, 'Atorvastatin reduces arterial stiffness in patients with rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 63, no. 12, pp. 1571-5.

Van Doornum, S, McColl, G & Wicks, IP 2005, 'Tumour necrosis factor antagonists improve disease activity but not arterial stiffness in rheumatoid arthritis', *Rheumatology (Oxford)*, vol. 44, no. 11, pp. 1428-32. Epub 2005 Aug 2.

van Ede, AE, Laan, RF, Blom, HJ, De Abreu, RA & van de Putte, LB 1998, 'Methotrexate in rheumatoid arthritis: an update with focus on mechanisms involved in toxicity', *Seminars in Arthritis and Rheumatism*, vol. 27, no. 5, pp. 277-92.

van Ede, AE, Laan, RF, Blom, HJ, Huizinga, TW, Haagsma, CJ, Giesendorf, BA, de Boo, TM & van de Putte, LB 2001, 'The C677T mutation in the methylenetetrahydrofolate reductase gene: a genetic risk factor for methotrexate-related elevation of liver enzymes in rheumatoid arthritis patients', *Arthritis and Rheumatism*, vol. 44, no. 11, pp. 2525-30.

van Ede, AE, Laan, RFJM, Blom, HJ, Boers, GHJ, Haagsma, CJ, Thomas, CMG, de Boo, TM & van de Putte, LBA 2002, 'Homocysteine and folate status in methotrexate-treated patients with rheumatoid arthritis', *Rheumatology*, vol. 41, no. 6, pp. 658-65.

van Gestel, AM, Prevoo, ML, van 't Hof, MA, van Rijswijk, MH, van de Putte, LB & van Riel, PL 1996, 'Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria', *Arthritis and Rheumatism*, vol. 39, no. 1, pp. 34-40.

van Haandel, L, Becker, ML, Williams, TD, Leeder, JS & Stobaugh, JF 2011, 'Measurement of methotrexate polyglutamates in human erythrocytes by ion-pair UPLC-MS/MS', *Bioanalysis*, vol. 3, no. 24, pp. 2783-96. doi: 10.4155/bio.11.288.

van Haandel, L, Becker, ML, Williams, TD, Stobaugh, JF & Leeder, JS 2012, 'Comprehensive quantitative measurement of folate polyglutamates in human erythrocytes by ion pairing ultra-performance liquid chromatography/tandem mass spectrometry', *Rapid Communications in Mass Spectrometry*, vol. 26, no. 14, pp. 1617-30. doi: 10.002/rcm.6268.

van Halm, VP, Nurmohamed, MT, Twisk, JW, Dijkmans, BA & Voskuyl, AE 2006, 'Disease-modifying antirheumatic drugs are associated with a reduced risk for cardiovascular disease in patients with rheumatoid arthritis: a case control study', *Arthritis Research and Therapy*, vol. 8, no. 5, pp. 1-6.

van Hoorn, EC, van Middelaar-Voskuilen, MC, van Limpt, CJP, Lamb, KJ, Bouritius, H, Vriesema, AJM, van Leeuwen, PAM & van Norren, K 2005, 'Preoperative supplementation with a carbohydrate mixture decreases organ dysfunction-associated risk factors', *Clinical Nutrition*, vol. 24, no. 1, pp. 114-23.

van Leeuwen, M, van Rijswijk, M, van der Heijde, D, Te Meerman, G, van Riel, P, Houtman, P, van De Putte, L & Limburg, P 1993, 'The acute-phase response in relation to radiographic progression in early rheumatoid arthritis: a prospective study during the first three years of the disease', *British Journal of Rheumatology*, vol. 32, pp. 9 - 13.

van Riel, PL & Schumacher, HR, Jr. 2001, 'How does one assess early rheumatoid arthritis in daily clinical practice?', *Best Practice & Research: Clinical Rheumatology*, vol. 15, no. 1, pp. 67-76.

van Roon, EN & van de Laar, MA 2010, 'Methotrexate bioavailability', *Clinical and Experimental Rheumatology*, vol. 28, no. 5 Suppl 61, pp. S27-32. Epub 2010 Oct 28.

van Varik, BJ, Rennenberg, RJ, Reutelingsperger, CP, Kroon, AA, de Leeuw, PW & Schurgers, LJ 2012, 'Mechanisms of arterial remodeling: lessons from genetic diseases', *Frontiers in Genetics*, vol. 3:290., no. doi, p. 10.3389/fgene.2012.00290. eCollection 2012.

Vandenbroucke, JP, Hazevoet, HM & Cats, A 1984, 'Survival and cause of death in rheumatoid arthritis: a 25-year prospective followup', *Journal of Rheumatology*, vol. 11, no. 2, pp. 158-61.

Vane, JR, Änggård, EE & Botting, RM 1990, 'Regulatory Functions of the Vascular Endothelium', *New England Journal of Medicine*, vol. 323, no. 1, pp. 27-36.

Varani, K, Padovan, M, Govoni, M, Vincenzi, F, Trotta, F & Borea, PA 2010, 'The role of adenosine receptors in rheumatoid arthritis', *Autoimmunity Reviews*, vol. 10, no. 2, pp. 61-4. doi: 10.1016/j.autrev.2010.07.019. Epub Aug 5.

Vasan, RS, Larson, MG, Leip, EP, Kannel, WB & Levy, D 2001, 'Assessment of frequency of progression to hypertension in non-hypertensive participants in the Framingham Heart Study: a cohort study', *The Lancet*, vol. 358, no. 9294, pp. 1682-6.

Vasdev, S & Gill, V 2008, 'The antihypertensive effect of arginine', *International Journal of Angiology*, vol. 17, no. 1, pp. 7-22.

Verboom, CN 2006, 'Highly purified omega-3 polyunsaturated fatty acids are effective as adjunct therapy for secondary prevention of myocardial infarction', *Herz*, vol. 31, no. Suppl 3, pp. 49-59.

Verdecchia, P, Porcellati, C, Schillaci, G, Borgioni, C, Ciucci, A, Battistelli, M, Guerrieri, M, Gatteschi, C, Zampi, I, Santucci, A, Santucci, C, Reboldi, G & et al. 1994, 'Ambulatory blood pressure. An independent predictor of prognosis in essential hypertension', *Hypertension*, vol. 24, no. 6, pp. 793-801.

Verdecchia, P, Schillaci, G & Porcellati, C 1991, 'Dippers versus non-dippers', *Journal of Hypertension. Supplement.*, vol. 9, no. 8, pp. S42-4.

Verma, S, Li, SH, Badiwala, MV, Weisel, RD, Fedak, PW, Li, RK, Dhillon, B & Mickle, DA 2002, 'Endothelin antagonism and interleukin-6 inhibition attenuate the proatherogenic effects of C-reactive protein', *Circulation*, vol. 105, no. 16, pp. 1890-6.

Versini, M, Jeandel, PY, Rosenthal, E & Shoenfeld, Y 2014, 'Obesity in autoimmune diseases: not a passive bystander', *Autoimmunity Reviews*, vol. 13, no. 9, pp. 981-1000. doi: 10.16/j.autrev.2014.07.001. Epub Aug 2.

Verstappen, SM, Jacobs, JW, van der Veen, MJ, Heurkens, AH, Schenk, Y, ter Borg, EJ, Blaauw, AA & Bijlsma, JW 2007, 'Intensive treatment with methotrexate in early rheumatoid arthritis: aiming for remission. Computer Assisted Management in Early Rheumatoid Arthritis (CAMERA, an open-label strategy trial)', *Annals of the Rheumatic Diseases*, vol. 66, no. 11, pp. 1443-9. Epub 2007 May 22.

Vessey, MP, Villard-Mackintosh, L & Yeates, D 1987, 'Oral contraceptives, cigarette smoking and other factors in relation to arthritis', *Contraception*, vol. 35, no. 5, pp. 457-64.

Vials, A & Burnstock, G 1993, 'A2-purinoceptor-mediated relaxation in the guinea-pig coronary vasculature: a role for nitric oxide', *British Journal of Pharmacology*, vol. 109, no. 2, pp. 424-9.

Vlachopoulos, CV, Terentes-Printzios, DG, Ioakeimidis, NK, Aznaouridis, KA & Stefanadis, CI 2013, 'Prediction of cardiovascular events and all-cause mortality with erectile dysfunction: a systematic review and meta-analysis of cohort studies', *Circulation: Cardiovascular Quality and Outcomes*, vol. 6, no. 1, pp. 99-109. doi: 10.1161/CIRCOUTCOMES.112.966903. Epub 2013 Jan 8.

Vlaming, ML, Pala, Z, van Esch, A, Wagenaar, E, de Waart, DR, van de Wetering, K, van der Kruijssen, CM, Oude Elferink, RP, van Tellingen, O & Schinkel, AH 2009, 'Functionally overlapping roles of Abcg2 (Bcrp1) and Abcc2 (Mrp2) in the elimination of methotrexate and its main toxic metabolite 7-hydroxymethotrexate in vivo', *Clinical Cancer Research*, vol. 15, no. 9, pp. 3084-93. doi: 10.1158/078-0432.CCR-08-2940. Epub 009 Apr 21.

Vlaming, ML, Pala, Z, van Esch, A, Wagenaar, E, van Tellingen, O, de Waart, DR, Oude Elferink, RP, van de Wetering, K & Schinkel, AH 2008, 'Impact of Abcc2 (Mrp2) and Abcc3 (Mrp3) on the in vivo elimination of methotrexate and its main toxic metabolite 7-hydroxymethotrexate', *Clinical Cancer Research*, vol. 14, no. 24, pp. 8152-60. doi: 10.1158/078-0432.CCR-08-1609.

Vlaming, ML, van Esch, A, Pala, Z, Wagenaar, E, van de Wetering, K, van Tellingen, O & Schinkel, AH 2009, 'Abcc2 (Mrp2), Abcc3 (Mrp3), and Abcg2 (Bcrp1) are the main determinants for rapid elimination of methotrexate and its toxic metabolite 7-hydroxymethotrexate in vivo', *Molecular Cancer Therapeutics*, vol. 8, no. 12, pp. 3350-9. doi: 10.1158/535-7163.MCT-09-0668. Epub.

Vokonas, PS, Kannel, WB & Cupples, LA 1988, 'Epidemiology and risk of hypertension in the elderly: the Framingham Study', *Journal of Hypertension*, vol. 6, no. 1, pp. S3-9.

Volk, EL & Schneider, E 2003, 'Wild-type breast cancer resistance protein (BCRP/ABCG2) is a methotrexate polyglutamate transporter', *Cancer Research*, vol. 63, no. 17, pp. 5538-43.

Vyssoulis, G, Karpanou, E, Kyvelou, SM, Adamopoulos, D, Gialernios, T, Gymnopoulou, E, Cokkinos, D & Stefanadis, C 2010, 'Associations between plasma homocysteine levels, aortic stiffness and wave reflection in patients with arterial hypertension, isolated office hypertension and normotensive controls', *Journal of Human Hypertension*, vol. 24, no. 3, pp. 183-9. doi: 10.1038/jhh.2009.50. Epub Jun 11.

Wadham, C & Mangoni, AA 2009, 'Dimethylarginine dimethylaminohydrolase regulation: a novel therapeutic target in cardiovascular disease', *Expert Opinion on Drug Metabolism & Toxicology*, vol. 5, no. 3, pp. 303-19. doi: 10.1517/17425250902785172.

Wallberg-Jonsson, S, Caidahl, K, Klintland, N, Nyberg, G & Rantapaa-Dahlqvist, S 2008, 'Increased arterial stiffness and indication of endothelial dysfunction in long-standing rheumatoid arthritis', *Scandinavian Journal of Rheumatology*, vol. 37, no. 1, pp. 1-5. doi: 10.1080/03009740701633238.

Wallberg-Jonsson, S, Ohman, ML & Dahlqvist, SR 1997, 'Cardiovascular morbidity and mortality in patients with seropositive rheumatoid arthritis in Northern Sweden', *Journal of Rheumatology*, vol. 24, no. 3, pp. 445-51.

Wang, CH, Li, SH, Weisel, RD, Fedak, PW, Dumont, AS, Szmitko, P, Li, RK, Mickle, DA & Verma, S 2003, 'C-reactive protein upregulates angiotensin type 1 receptors in vascular smooth muscle', *Circulation*, vol. 107, no. 13, pp. 1783-90. Epub 2003 Mar 17.

Wang, D, Johnson, AD, Papp, AC, Kroetz, DL & Sadee, W 2005, 'Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435C>T affects mRNA stability', *Pharmacogenets Genomics*, vol. 15, no. 10, pp. 693-704.

Wang, GS & Cooper, TA 2007, 'Splicing in disease: disruption of the splicing code and the decoding machinery', *Nature Reviews Genetics*, vol. 8, no. 10, pp. 749-61. Epub 2007 Aug 29.

Wang, KL, Cheng, HM, Sung, SH, Chuang, SY, Li, CH, Spurgeon, HA, Ting, CT, Najjar, SS, Lakatta, EG, Yin, FC, Chou, P & Chen, CH 2010, 'Wave reflection and arterial stiffness in the prediction of 15-year all-cause and cardiovascular mortalities: a community-based study', *Hypertension*, vol. 55, no. 3, pp. 799-805. doi: 10.1161/HYPERTENSIONAHA.109.139964. Epub 2010 Jan 11.

Wang, X, Qin, X, Demirtas, H, Li, J, Mao, G, Huo, Y, Sun, N, Liu, L & Xu, X 2007, 'Efficacy of folic acid supplementation in stroke prevention: a meta-analysis', *The Lancet*, vol. 369, no. 9576, pp. 1876-82.

Warren, RB, Smith, RL, Campalani, E, Eyre, S, Smith, CH, Barker, JN, Worthington, J & Griffiths, CE 2008, 'Genetic variation in efflux transporters influences outcome to methotrexate therapy in patients with psoriasis', *Journal of Investigative Dermatology*, vol. 128, no. 8, pp. 1925-9. doi: 10.038/jid.2008.16. Epub Feb 7.

Wascher, TC, Graier, WF, Dittrich, P, Hussain, MA, Bahadori, B, Wallner, S & Toplak, H 1997, 'Effects of low-dose L-arginine on insulin-mediated vasodilatation and insulin sensitivity', *European Journal of Clinical Investigation*, vol. 27, no. 8, pp. 690-5.

Wassertheurer, S, Kropf, J, Weber, T, van der Giet, M, Baulmann, J, Ammer, M, Hametner, B, Mayer, CC, Eber, B & Magometschnigg, D 2010, 'A new oscillometric method for pulse wave analysis: comparison with a common tonometric method', *Journal of Human Hypertension*, vol. 24, no. 8, pp. 498-504. doi: 10.1038/jhh.2010.27. Epub Mar 18.

Wassertheurer, S, Mayer, C & Breitenecker, F 2008, 'Modeling arterial and left ventricular coupling for non-invasive measurements', *Simulation Modelling Practice and Theory*, vol. 16, no. 8, pp. 988-97.

Watson, DJ, Rhodes, T & Guess, HA 2003, 'All-cause mortality and vascular events among patients with rheumatoid arthritis, osteoarthritis, or no arthritis in the UK General Practice Research Database', *Journal of Rheumatology*, vol. 30, no. 6, pp. 1196-202.

Weber, T, Ammer, M, Rammer, M, Adji, A, O'Rourke, MF, Wassertheurer, S, Rosenkranz, S & Eber, B 2009, 'Noninvasive determination of carotid-femoral pulse wave velocity depends critically on assessment of travel distance: a comparison with invasive measurement', *Journal of Hypertension*, vol. 27, no. 8, pp. 1624-30. doi: 10.097/HJH.0b013e32832cb04e.

Weber, T, Wassertheurer, S, Rammer, M, Maurer, E, Hametner, B, Mayer, CC, Kropf, J & Eber, B 2011, 'Validation of a brachial cuff-based method for estimating central systolic blood pressure', *Hypertension*, vol. 58, no. 5, pp. 825-32. doi: 10.1161/HYPERTENSIONAHA.111.176313. Epub 2011 Sep 12.

Wei, L, MacDonald, TM & Walker, BR 2004, 'Taking glucocorticoids by prescription is associated with subsequent cardiovascular disease', *Annals of Internal Medicine*, vol. 141, no. 10, pp. 764-70.

Wei, W, Tolle, M, Zidek, W & van der Giet, M 2010, 'Validation of the mobil-O-Graph: 24 h-blood pressure measurement device', *Blood Pressure Monitoring*, vol. 15, no. 4, pp. 225-8. doi: 10.1097/MBP.0b013e328338892f.

Weinblatt, ME, Coblyn, JS, Fox, DA, Fraser, PA, Holdsworth, DE, Glass, DN & Trentham, DE 1985, 'Efficacy of low-dose methotrexate in rheumatoid arthritis', *The New England Journal of Medicine*, vol. 312, no. 13, pp. 818-22.

Weinblatt, ME, Trentham, DE, Fraser, PA, Holdsworth, DE, Falchuk, KR, Weissman, BN & Coblyn, JS 1988, 'Long-term prospective trial of low-dose methotrexate in rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 31, no. 2, pp. 167-75.

Weisberg, SP, McCann, D, Desai, M, Rosenbaum, M, Leibel, RL & Ferrante, AW, Jr. 2003, 'Obesity is associated with macrophage accumulation in adipose tissue', *Journal of Clinical Investigation*, vol. 112, no. 12, pp. 1796-808.

Weisman, MH, Furst, DE, Park, GS, Kremer, JM, Smith, KM, Wallace, DJ, Caldwell, JR & Dervieux, T 2006, 'Risk genotypes in folate-dependent enzymes and their association with methotrexate-related side effects in rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 54, no. 2, pp. 607-12.

Weiss, W, Gohlisch, C, Harsch-Gladisch, C, Tolle, M, Zidek, W & van der Giet, M 2012, 'Oscillometric estimation of central blood pressure: validation of the Mobil-O-Graph in comparison with the SphygmoCor device', *Blood Pressure Monitoring*, vol. 17, no. 3, pp. 128-31. doi: 10.1097/MBP.0b013e328353ff63.

Welch, GN & Loscalzo, J 1998, 'Homocysteine and Atherothrombosis', *New England Journal of Medicine*, vol. 338, no. 15, pp. 1042-50.

Wells, JC 2012, 'The evolution of human adiposity and obesity: where did it all go wrong?', *Disease Models & Mechanisms*, vol. 5, no. 5, pp. 595-607. doi: 10.1242/dmm.009613.

Wesley, A, Bengtsson, C, Elkan, AC, Klareskog, L, Alfredsson, L & Wedren, S 2013, 'Association between body mass index and anti-citrullinated protein antibody-positive and anti-citrullinated protein antibody-negative rheumatoid arthritis: results from a population-based case-control study', *Arthritis Care & Research (Hoboken)*, vol. 65, no. 1, pp. 107-12. doi: 10.1002/acr.21749.

Wessels, JA, de Vries-Bouwstra, JK, Heijmans, BT, Slagboom, PE, Goekoop-Ruiterman, YP, Allaart, CF, Kerstens, PJ, van Zeben, D, Breedveld, FC, Dijkmans, BA, Huizinga, TW & Guchelaar, HJ 2006, 'Efficacy and toxicity of methotrexate in early rheumatoid arthritis are associated with single-nucleotide polymorphisms in genes coding for folate pathway enzymes', *Arthritis and Rheumatism*, vol. 54, no. 4, pp. 1087-95.

Wessels, JA, Huizinga, TW & Guchelaar, HJ 2008, 'Recent insights in the pharmacological actions of methotrexate in the treatment of rheumatoid arthritis', *Rheumatology (Oxford)*, vol. 47, no. 3, pp. 249-55. Epub 2007 Nov 28.

Wessels, JAM, Kooloos, WM, Jonge, RD, De Vries-Bouwstra, JK, Allaart, CF, Linssen, A, Collee, G, Sonnaville, PD, Lindemans, J, Huizinga, TWJ & Guchelaar, H-J 2006, 'Relationship between genetic variants in the adenosine pathway and outcome of methotrexate treatment in patients with recent-onset rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 54, no. 9, pp. 2830-9.

Westenberg, JJ, van Poelgeest, EP, Steendijk, P, Grotenhuis, HB, Jukema, JW & de Roos, A 2012, 'Bramwell-Hill modeling for local aortic pulse wave velocity estimation: a validation study with velocity-encoded cardiovascular magnetic resonance and invasive pressure assessment', *Journal of Cardiovascular Magnetic Resonance*, vol. 14:2., no. doi, pp. 10.1186/532-429X-14-2.

Westhoff, TH, Straub-Hohenbleicher, H, Schmidt, S, Tolle, M, Zidek, W & van der Giet, M 2005, 'Convenience of ambulatory blood pressure monitoring: comparison of different devices', *Blood Pressure Monitoring*, vol. 10, no. 5, pp. 239-42.

Wettergren, Y, Odin, E, Carlsson, G & Gustavsson, B 2010, 'MTHFR, MTR, and MTRR polymorphisms in relation to p16INK4A hypermethylation in mucosa of patients with colorectal cancer', *Molecular Medicine*, vol. 16, no. 9-10, pp. 425-32. doi: 10.2119/molmed.009.00156. Epub 2010 Jun 11.

White, WB 2001, 'The systolic blood pressure versus pulse pressure controversy', *American Journal of Cardiology*, vol. 87, no. 11, pp. 1278-81.

Whiting-O'Keefe, QE, Fye, KH & Sack, KD 1991, 'Methotrexate and histologic hepatic abnormalities: A meta-analysis', *The American Journal of Medicine*, vol. 90, no. 1, pp. 711-6.

Wilkinson, IB, Franklin, SS & Cockcroft, JR 2004, 'Nitric oxide and the regulation of large artery stiffness: from physiology to pharmacology', *Hypertension*, vol. 44, no. 2, pp. 112-6. Epub 2004 Jul 19.

Wilkinson, IB, Fuchs, SA, Jansen, IM, Spratt, JC, Murray, GD, Cockcroft, JR & Webb, DJ 1998, 'Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis', *Journal of Hypertension*, vol. 16, no. 12 Pt 2, pp. 2079-84.

Wilkinson, IB, Hall, IR, MacCallum, H, Mackenzie, IS, McEniery, CM, van der Arend, BJ, Shu, YE, MacKay, LS, Webb, DJ & Cockcroft, JR 2002, 'Pulse-wave analysis: clinical evaluation of a noninvasive, widely applicable method for assessing

endothelial function', *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 22, no. 1, pp. 147-52.
Wilkinson, IB, Prasad, K, Hall, IR, Thomas, A, MacCallum, H, Webb, DJ, Frenneaux, MP & Cockcroft, JR 2002, 'Increased central pulse pressure and augmentation index in subjects with hypercholesterolemia', *Journal of the American College of Cardiology*, vol. 39, no. 6, pp. 1005-11.
Willeit, P, Freitag, DF, Laukkanen, JA, Chowdhury, S, Gobin, R, Mayr, M, Di Angelantonio, E & Chowdhury, R 2015, 'Asymmetric dimethylarginine and cardiovascular risk: systematic review and meta-analysis of 22 prospective studies', *Journal of the American Heart Association*, vol. 4, no. 6, p. e001833. doi: 10.1161/JAHA.115..
Willers, J 2011, 'Combined Add-on Supplementation of Omega-3 Fatty Acids, Vitamin E, Vitamin A, Copper, and Selenium in Rheumatoid Arthritis', *Food and Nutrition Sciences*, vol. 02, no. 07, pp. 714-23.
Williams, B, Lacy, PS, Thom, SM, Cruickshank, K, Stanton, A, Collier, D, Hughes, AD, Thurston, H & O'Rourke, M 2006, 'Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study', *Circulation*, vol. 113, no. 9, pp. 1213-25. Epub 2006 Feb 13.
Williams, HJ, Willkens, RF, Samuelson, CO, Jr., Alarcon, GS, Guttadauria, M, Yarboro, C, Polisson, RP, Weiner, SR, Luggen, ME, Billingsley, LM & et al. 1985, 'Comparison of low-dose oral pulse methotrexate and placebo in the treatment of rheumatoid arthritis. A controlled clinical trial', *Arthritis and Rheumatism*, vol. 28, no. 7, pp. 721-30.
Williams, W & Huang, K 1982, 'Renal tubular transport of folic acid and methotrexate in the monkey', *American Journal of Physiology*, vol. 242, no. 5, pp. F484-90.
Wilson, AM, Ryan, MC & Boyle, AJ 2006, 'The novel role of C-reactive protein in cardiovascular disease: risk marker or pathogen', *International Journal of Cardiology*, vol. 106, no. 3, pp. 291-7.
Wilson, AM, Swan, JD, Ding, H, Zhang, Y, Whitbourn, RJ, Gurry, J, Yii, M, Wilson, AC, Hill, M, Triggle, C, Best, JD & Jenkins, AJ 2007, 'Widespread vascular production of C-reactive protein (CRP) and a relationship between serum CRP, plaque CRP and intimal hypertrophy', *Atherosclerosis*, vol. 191, no. 1, pp. 175-81. Epub 2006 Jun 9.
Wislowska, M, Rok, M, Jaszczyk, B, Stepien, K & Cicha, M 2007, 'Serum leptin in rheumatoid arthritis', *Rheumatology International*, vol. 27, no. 10, pp. 947-54. Epub 2007 Apr 19.
Wolfe, F, Caplan, L & Michaud, K 2006, 'Treatment for rheumatoid arthritis and the risk of hospitalization for pneumonia: associations with prednisone, disease-modifying antirheumatic drugs, and anti-tumor necrosis factor therapy', *Arthritis and Rheumatism*, vol. 54, no. 2, pp. 628-34.
Wolfe, F, Freundlich, B & Straus, WL 2003, 'Increase in cardiovascular and cerebrovascular disease prevalence in rheumatoid arthritis', *Journal of Rheumatology*, vol. 30, no. 1, pp. 36-40.
Wolfe, F & Hawley, D 1998, 'The longterm outcomes of rheumatoid arthritis: Work disability: a prospective 18 year study of 823 patients', *Journal of Rheumatology*, vol. 25, pp. 2108-17.
Wolfe, F, Hawley, DJ & Cathey, MA 1990, 'Termination of slow acting antirheumatic therapy in rheumatoid arthritis: a 14-year prospective evaluation of 1017 consecutive starts', *Journal of Rheumatology*, vol. 17, no. 8, pp. 994-1002.

Wolfe, F & Michaud, K 2004a, 'Heart failure in rheumatoid arthritis: rates, predictors, and the effect of anti-tumor necrosis factor therapy', *American Journal of Medicine*, vol. 116, no. 5, pp. 305-11.
Wolfe, F & Michaud, K 2004b, 'Lymphoma in rheumatoid arthritis: the effect of methotrexate and anti-tumor necrosis factor therapy in 18,572 patients', *Arthritis and Rheumatism*, vol. 50, no. 6, pp. 1740-51.
Wolfe, F, Mitchell, DM, Sibley, JT, Fries, JF, Bloch, DA, Williams, CA, Spitz, PW, Haga, M, Kleinheksel, SM & Cathey, MA 1994, 'The mortality of rheumatoid arthritis', *Arthritis and Rheumatism*, vol. 37, no. 4, pp. 481-94.
Wong, M, Chowienczyk, P & Kirkham, B 2005, 'Cardiovascular issues of COX-2 inhibitors and NSAIDs', A*ustralian Family Physician*, vol. 34, no. 11, pp. 945-8.
Wong, M, Oakley, SP, Young, L, Jiang, BY, Wierzbicki, A, Panayi, G, Chowienczyk, P & Kirkham, B 2009, 'Infliximab improves vascular stiffness in patients with rheumatoid arthritis', *Annals of the Rheumatic Diseases*, vol. 68, no. 8, pp. 1277-84.
Wong, M, Toh, L, Wilson, A, Rowley, K, Karschimkus, C, Prior, D, Romas, E, Clemens, L, Dragicevic, G, Harianto, H, Wicks, I, McColl, G, Best, J & Jenkins, A 2003, 'Reduced arterial elasticity in rheumatoid arthritis and the relationship to vascular disease risk factors and inflammation', *Arthritis and Rheumatism*, vol. 48, no. 1, pp. 81-9.
Woo, KS, Chook, P, Lolin, YI, Sanderson, JE, Metreweli, C & Celermajer, DS 1999, 'Folic acid improves arterial endothelial function in adults with hyperhomocystinemia', *Journal of the American College of Cardiology*, vol. 34, no. 7, pp. 2002-6.
Woods, RK, Stoney, RM, Ireland, PD, Bailey, MJ, Raven, JM, Thien, FC, Walters, EH & Abramson, MJ 2002, 'A valid food frequency questionnaire for measuring dietary fish intake', *Asia Pacific Journal of Clinical Nutrition*, vol. 11, no. 1, pp. 56-61.
World Health Organization 2003, *Prevention of recurrent heart attacks and strokes in low and middle income populations. Evidence-based recommendations for policy makers and health professionals*, Geneva, viewed 02 May 2016, <http://www.who.int/bookorders >.
Worthington, J, Ollier, WE, Leach, MK, Smith, I, Hay, EM, Thomson, W, Pepper, L, Carthy, D, Farhan, A, Martin, S & et al. 1994, 'The Arthritis and Rheumatism Council's National Repository of Family Material: pedigrees from the first 100 rheumatoid arthritis families containing affected sibling pairs', *British Journal of Rheumatology*, vol. 33, no. 10, pp. 970-6.
Wright, GE, Crowley, DC, Charpie, JR, Ohye, RG, Bove, EL & Kulik, TJ 2004, 'High systemic vascular resistance and sudden cardiovascular collapse in recovering Norwood patients', *Annals of Thoracic Surgery*, vol. 77, no. 1, pp. 48-52.
Wucherpfennig, KW 2001, 'Structural Basis of Molecular Mimicry', *Journal of Autoimmunity*, vol. 16, no. 3, pp. 293-302.
Xiao, W, Bai, Y, Ye, P, Luo, L, Liu, D, Wu, H & Bai, J 2014, 'Plasma homocysteine is associated with aortic arterial stiffness but not wave reflection in Chinese hypertensive subjects', *PLoS One*, vol. 9, no. 1, p. e85938. doi: 10.1371/journal.pone.0085938. eCollection 2014.
Xiong, Y, Fu, YF, Fu, SH & Zhou, HH 2003, 'Elevated levels of the serum endogenous inhibitor of nitric oxide synthase and metabolic control in rats with streptozotocin-induced diabetes', *Journal of Cardiovascular Pharmacology*, vol. 42, no. 2, pp. 191-6.
Xun, P, Qin, B, Song, Y, Nakamura, Y, Kurth, T, Yaemsiri, S, Djousse, L & He, K 2012, 'Fish consumption and risk of stroke and its subtypes: accumulative evidence from a

meta-analysis of prospective cohort studies', *European Journal of Clinical Nutrition*, vol. 66, no. 11, pp. 1199-207. doi: 10.038/ejcn.2012.133. Epub Oct 3.

Yang, HT, Lee, M, Hong, KS, Ovbiagele, B & Saver, JL 2012, 'Efficacy of folic acid supplementation in cardiovascular disease prevention: an updated meta-analysis of randomized controlled trials', *European Journal of Internal Medicine*, vol. 23, no. 8, pp. 745-54. doi: 10.1016/j.ejim.2012.07.004. Epub Aug 11.

Yasmin, McEniery, CM, Wallace, S, Mackenzie, IS, Cockcroft, JR & Wilkinson, IB 2004, 'C-reactive protein is associated with arterial stiffness in apparently healthy individuals', *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 24, no. 5, pp. 969-74. Epub 2004 Mar 4.

Yildiz, M, Soy, M, Kurum, T & Ozbay, G 2004, 'Increased pulse wave velocity and shortened pulse wave propagation time in young patients with rheumatoid arthritis', *Canadian Journal of Cardiology*, vol. 20, no. 11, pp. 1097-100.

Ysart, G, Miller, P, Barrett, G, Farrington, D, Lawrance, P & Harrison, N 1999, 'Dietary exposures to nitrate in the UK', *Food Additives & Contaminants*, vol. 16, no. 12, pp. 521-32.

Zachariae, H 2000, 'Liver biopsies and methotrexate: a time for reconsideration?', *Journal of the American Academy of Dermatology*, vol. 42, no. 3, pp. 531-4.

Zachariae, H, Sogaard, H & Heickendorff, L 1996, 'Methotrexate-induced liver cirrhosis. Clinical, histological and serological studies--a further 10-year follow-up', *Dermatology*, vol. 192, no. 4, pp. 343-6.

Zentilin, P, Seriolo, B, Dulbecco, P, Caratto, E, Iiritano, E, Fasciolo, D, Bilardi, C, Mansi, C, Testa, E & Savarino, V 2002, 'Eradication of Helicobacter pylori may reduce disease severity in rheumatoid arthritis', *Alimentary Pharmacology & Therapeutics*, vol. 16, no. 7, pp. 1291-9.

Zhang, GG, Bai, YP, Chen, MF, Shi, RZ, Jiang, DJ, Fu, QM, Tan, GS & Li, YJ 2008, 'Asymmetric dimethylarginine induces TNF-alpha production via ROS/NF-kappaB dependent pathway in human monocytic cells and the inhibitory effect of reinioside C', *Vascular Pharmacology*, vol. 48, no. 2-3, pp. 115-21.

Zhang, L, Spencer, KL, Voruganti, VS, Jorgensen, NW, Fornage, M, Best, LG, Brown-Gentry, KD, Cole, SA, Crawford, DC, Deelman, E, Franceschini, N, Gaffo, AL, Glenn, KR, Heiss, G, Jenny, NS, Kottgen, A, Li, Q, Liu, K, Matise, TC, North, KE, Umans, JG & Kao, WH 2013, 'Association of functional polymorphism rs2231142 (Q141K) in the ABCG2 gene with serum uric acid and gout in 4 US populations: the PAGE Study', *American Journal of Epidemiology*, vol. 177, no. 9, pp. 923-32. doi: 10.1093/aje/kws330. Epub 2013 Apr 3.

Zhang, Y, Kollias, G, Argyris, AA, Papaioannou, TG, Tountas, C, Konstantonis, GD, Achimastos, A, Blacher, J, Safar, ME, Sfikakis, PP & Protogerou, AD 2015, 'Association of left ventricular diastolic dysfunction with 24-h aortic ambulatory blood pressure: the SAFAR study', *Journal of Human Hypertension*, vol. 29, no. 7, pp. 442-8. doi: 10.1038/jhh.2014.101. Epub Nov 13.

Zhang, Z, Shi, Q, Sturgis, EM, Spitz, MR, Hong, WK & Wei, Q 2004, 'Thymidylate synthase 5'- and 3'-untranslated region polymorphisms associated with risk and progression of squamous cell carcinoma of the head and neck', *Clinical Cancer Research*, vol. 10, no. 23, pp. 7903-10.

Zhang, Z, Zhao, P, Li, A, Lv, X, Gao, Y, Sun, H, Ding, Y & Liu, J 2009, 'Effects of methotrexate on plasma cytokines and cardiac remodeling and function in postmyocarditis rats', *Mediators of Inflammation*, vol. 2009:389720., no. doi, p. 10.1155/2009/389720. Epub 2009 Oct 26.

Zoller, B, Li, X, Sundquist, J & Sundquist, K 2012, 'Risk of pulmonary embolism in patients with autoimmune disorders: a nationwide follow-up study from Sweden', *The Lancet*, vol. 379, no. 9812, pp. 244-9. doi: 10.1016/S0140-6736(11)61306-8. Epub 2011 Nov 25.