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An open-label, randomized, crossover study to assess anti-inflammatory effect of Simvastatin in Rheumatoid Arthritis statin-naïve patients with associated risk factors for cardiovascular disease

By

Ayoola Oluwakayode Komolafe

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Supervisor: Prof OBW Greeff

Co-Supervisor: Prof MMTM Ally

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory condition of unknown etiology for which there is no cure. It is one of the most disabling diseases and affects about 1% of the world's population. Recent developments in the field of molecular biology have resulted in the production of new drugs used in the treatment RA. Despite these advancements, achieving optimal disease control and prevention of disease progression is still difficult in many patients, leading to a continued search for treatment methods that will improve patient outcomes. Non-biologic forms of treatment that are still being investigated include the use of statins as an adjunct therapy due to their reported anti-inflammatory effects.

Some studies have shown that the use of statins in patients with RA help in reducing disease activity and swollen joint count in addition to lowering cardiovascular risk. As evidence continue to increase on the anti-inflammatory effect of statins, researchers have started investigating possible benefits that may result from the use of statins in treatment of RA, a chronic disease marked by high levels of systemic and local inflammation.

This study investigated the anti-inflammatory effect of statins in rheumatoid arthritis patients with associated risks for cardiovascular disease, using simvastatin as the investigational product. Patients with moderately active RA despite being on maximum disease-modifying antirheumatic drugs (DMARDs) therapy and having associated risks for cardiovascular disease were screened for the study. Eligible patients were randomized into two groups, 1 and 2. Patients in group 1 received simvastatin treatment (20mg/day) for a period of 3 months in addition to their usual DMARDs after which they stopped simvastatin treatment and were followed up for a further 3 months off simvastatin treatment. Those in group 2 were allowed to continue on their usual DMARDs without simvastatin treatment for the first 3 months of the study after which they received 20mg/day simvastatin for a period of 3 months in addition to their usual DMARDs.

The anti-inflammatory effect of simvastatin was assessed by monitoring the inflammatory variables; erythrocyte sedimentation rate (ESR) and c-reactive protein (CRP) and disease activity in

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the two groups at screening, at the crossover point and at end of the study. Disease activity was significantly reduced with simvastatin treatment in the two groups. The mean change in disease activity score with simvastatin treatment was 1.30 (p = 0.01) in group 1 and 1.74 (p = 0.01) in group 2. ESR was significantly reduced with simvastatin treatment in group 1 with a mean change of 19.0 (p = 0.005) and marginally reduced in group 2 with a mean change 26.0 (p = 0.09). There was no significant change in CRP with simvastatin treatment in the two groups. The mean change in CRP with simvastatin treatment was 7.0 (p = 0.24) in group 1 and 14.7 (p =0.20) in group 2. All the patients benefited from the lipid-lowering effect of simvastatin. These findings suggest that statins may have mild anti-inflammatory properties and will be good adjuvant in RA patients with associated risks for cardiovascular disease.

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Abbreviations

ACPA	Anti-citrullinated protein antibody
ACR	American college of rheumatology
APC	Antigen-presenting cells
COX	Cyclooxygenase
CRP	C - reactive protein
DAS28	Disease activity score using 28-joint count.
DMARDs	Disease-modifying antirheumatic drugs
ESR	Erythrocyte sedimentation rate
EULAR	European League against Rheumatism
HCQ	Hydroxychloroquine
HLA	Human leukocyte antigens
HMG-CoA	Hydroxymethylglutaryl-coenzyme A inhibitors
IL	Interleukin
LDL	Low-density lipoprotein
MTX	Methotrexate
NSAIDs	Non-steroidal anti-inflammatories
NYHA	New York Heart Association
RA	Rheumatoid arthritis
SSZ	Sulfasalazine
Th	T-helper
TNF	Tumor necrosis factor

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CHAPTER 1

1.1 INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory polyarthritis that affects synovial joints and is associated with marked local and systemic inflammation as well as significant risk of cardiovascular disease (Gabriel, 2010). It is an inflammatory disorder of unknown etiology for which there is no cure. It is one of the most disabling diseases and affects about 1% of the world's population (Chopra and Abdel-Nasser, 2008).

Significant developments in the field of molecular biology over the last decade have resulted in the production of new drugs used in the treatment RA. These drugs, referred to as biologic agents include those designed to inhibit tumor necrosis factor-alpha (TNF), block T cell co-stimulation, and deplete B cells (Schur *et al.*, 2012). Despite these advancements in RA management, optimal disease control and prevention of disease progression is still difficult in many patients and the long-term poor prognosis and reduced life expectancy characteristic of RA (Wolfe *et al*., 1994) has not changed significantly. Ongoing drug therapy is required in most RA patients due to the difficulty in maintaining remission. The biologic and non-biologic disease-modifying therapies that are currently available sometimes fail to induce remission or produce only limited responses (Mclnnes and Schett, 2011). Hence, the continued search for alternative forms of treatment that will improve patients' outcomes. Non-biologic forms of treatment that are still being investigated include the use of statins as an adjunct therapy due to their reported anti-inflammatory effects.

Some recent studies have shown statins use in patients with RA help in reducing disease activity and swollen joint count in addition to lowering cardiovascular risk. These improvements in disease activity and swollen joint count are accompanied by some improvement in plasma markers of inflammation, such as CRP and ESR (Paraskevas, 2008). The increasing evidence of the anti-inflammatory effect of statins has moved researchers to keep exploring them as possible anti-inflammatory agents in management of RA, a chronic disease marked by high levels of

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inflammation. Statins have been shown to be generally safe with few side effects (Dart *et al*., 1997) and it may be reasonable to consider their use in selected cases of RA, particularly in patients with a long history of active RA in whom the risk of cardiovascular disease is high (Costenbader and Coblyn, 2005).

1.2 LITERATURE REVIEW

1.2.1 Background

RA is the most common of the systemic autoimmune diseases, affecting at least 1% of the world's population, and 2% to 3% of people over age 60 years (Chopra and Abdel-Nasser, 2008). It is an autoimmune disease that primarily affects the small joints of the hand, wrist, and feet. It is characterized by joint pain, stiffness, and swelling due to synovial inflammation and effusion. If left untreated, RA can lead to extensive destruction of the synovial joints, causing deformity, disability and premature death (Meszaros, 2006; Choi *et al*., 2002). The disabilities caused by RA can have extensive impacts on quality of life, with loss of productivity due to damaged and deformed joints inhibiting fine movements of the hand (Darmawan, 2007). Since RA is an autoimmune disease, it can affect any part of the body, especially those that rely on small vessel beds or extensive nerve systems, and can contribute to the development of many life threatening conditions (Young and Koduri, 2007). Compared with normal healthy individuals, the mortality rate is higher in RA patients and cardiovascular and other systemic complications are more frequent (Mclnnes and Schett, 2011).

1.2.2 Pathogenesis of Rheumatoid Arthritis

Although much remains uncertain, the interplay of genetic, environmental factors and autoimmunity is believed to trigger the onset and perpetuation of synovitis underlying RA (Szodoray et al., 2010). Genetic factors including class II major histocompatibility antigens/human leukocyte antigens (HLA-DR), as well as non-HLA genes have been implicated in the pathogenesis

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of RA (de Vries, 2011). The strong link between HLA–DRB1 locus and RA has been well documented especially in patients who are positive for rheumatoid factor or anti-citrullinated protein antibody (ACPA) (Gregersen et al., 1987). The link between rheumatoid arthritis and the shared epitope may be attributed to the close similarity of the shared epitope by microbial proteins, increased T-cell aging induced by shared epitope–containing HLA molecules, and a potential proinflammatory signaling function that is unrelated to the role of the shared epitope in antigen recognition (De Almeida et al., 2010). The Twin studies support the role of genetic factors in rheumatoid arthritis, reporting concordance rates of 15 to 30% among monozygotic twins and 5% among dizygotic twins (MacGregor et al., 2000).HLA and some non-HLA genes have also been linked to autoimmunity to citrullinated proteins (ACPA), as well as smoking. It has been shown that smoking and possibly other environmental and lifestyle-related factors may trigger ACPA production and the development of ACPA seropositive RA, at least in some cohorts (Lee *et al*, 2007; Besenyei et al., 2011; Scott *et al.*, 2011). The risk of developing RA increases with smoking and other bronchial stress in individuals with the susceptibility HLA–DR4 alleles (Symmons et al., 1997).

At the onset of RA, a plethora of cells enter the synovium via activated endothelial cells expressing various adhesion molecules. Several cell types, especially dendritic cells, express pattern-recognition molecules such as Toll-like receptors, which bind to various foreign and self structures as part of their innate immune response activity, become activated subsequently, and act on cells of the adaptive immune system. Antigens associated with arthritis are presented to T cells by antigen-presenting cells (APC) such as dendritic cells, macrophages, or activated B cells. This process entails binding of antigenic peptides to class II MHC molecules, and more than 80% of patients with rheumatoid arthritis carry the so-called shared epitope of the HLA-DRB1*04 cluster (Gregersen *et al*., 1987) .These alleles share a highly homologous aminoacid sequence at the third hypervariable region of the HLA-DR β chain, which confers binding of specific peptides and thus affects antigen presentation to T-cell receptors.

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T cells in the synovial membrane usually belong to the T-helper 1 subset. T-cell upregulation is associated with secretion of various lymphokines such as interleukin 2 and interferon-γ. These stimulated T-cells induce activation of macrophages, B cells, fibroblasts, and osteoclasts (Kong *et al*, 1999). B lymphocytes express various cell-surface molecules, especially their antigen receptor, immunoglobulin, and differentiation antigens, such as CD20 and CD22. They differentiate into plasma cells that secrete antibodies, including autoantibodies such as that to IgG (rheumatoid factor), to citrullainated peptides such as vimentin, fibrinogen, cyclic citrullinated peptide, or to rheumatoid arthritis antigen of 33 kDa (Steiner and Smolen, 2006). Autoantibodies in turn form immune complexes that reinforce production of proinflammatory cytokines such as TNF via complement and Fc-receptor activation. The presence of autoantibodies is associated with severe rheumatoid arthritis (Rantapaa-Dahlqvist, 2005; Nell *et al*., 2005). Activated B cells also serve as APCs leading to T-cell activation and potentially to a vicious cycle and perpetuation of the autoimmune response (Panayi, 2005)

Within the rheumatoid arthritis synovial membrane, various other cell populations accumulate via activated endothelial cells. Besides the traditional cell populations of the innate and adaptive immune system, neovascularisation takes place and there is a large increase in fibroblast-like synoviocytes, which are highly activated and produce cytokines, inflammatory mediators such as prostaglandins, and matrix metalloproteinases (Firestein, 1996). By secretion of matrix metalloproteinases into the synovial fluid, fibroblast-like synoviocytes can destroy cartilage and assist in bone destruction (Pap *et al.*, 2003).

Many cytokines are activated in the synovium by various cell populations, several of them secreted by macrophage-like cells (Zwerina *et al*, 2005). They include TNF and interleukin 1, which constitute the therapeutic targets of several compounds for rheumatoid arthritis that directly inhibit these inflammatory mediators or interfere with their receptor binding, or both. Another pro-inflammatory cytokine, interleukin-6, induces antibody production by B cells, activates T cells,

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macrophages, and osteoclasts and it is a major activator of the hepatic acute-phase response (Nishimoto *et al*, 2003).

Chemokines and adhesion molecules are other important molecules in RA synovial membrane. They bind to specific receptors and perform many functions, such as cell-cell interactions including interactions with endothelial cells, migration, and chemoattraction of and homing of cells to certain tissues, structures, or sites (Haringman et al 2005). All these molecules, on binding with their receptors, induce signal transduction pathways that lead to activation of transcription factors and, subsequently, responsive genes (Smolen and Steiner, 2003)

All these signaling pathways lead to influx, expansion, and activation of inflammatory cells in the synovium leading to the immunoinflammatory and destructive response of rheumatoid arthritis, which ultimately presents clinically as joint swelling and joint destruction.

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Pathogenesis of Rheumatoid Arthritis (Smolen *et al*., 2007)

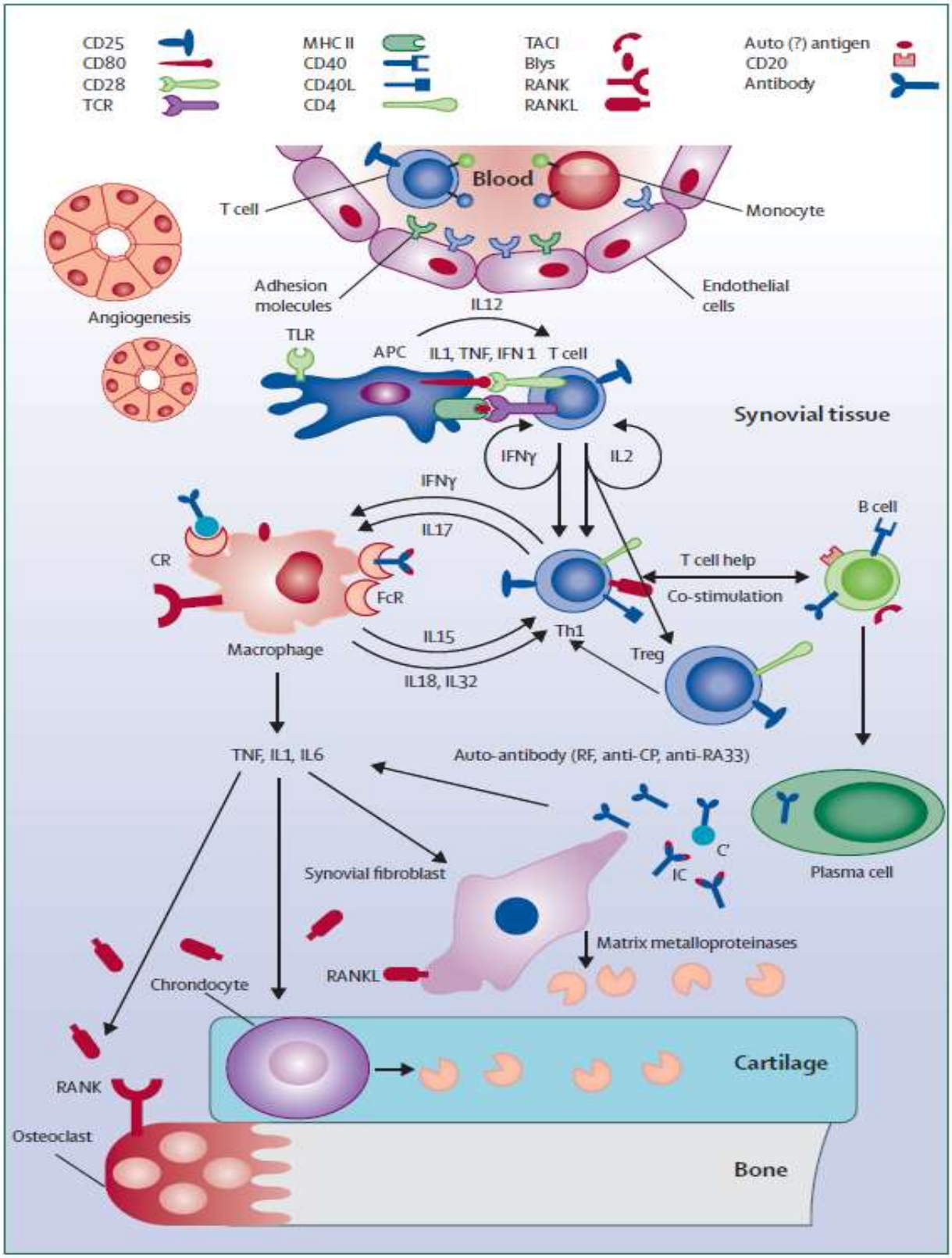

***Figure 1:* Current views on pathogenesis of rheumatoid arthritis**
Arrows show some of many interactions in rheumatoid arthritis pathogenesis. Schematic depiction of events presumably occurring in synovial membrane, as well as articular cartilage and subchondral bone, which are surrounded by aggressive rheumatoid synovitis. Blys–B lymphocyte stimulator. C'–complement. CP–citrullinated peptide. CR–complement receptor. FcR–receptor for the Fc portion of IgG. IC– immune complex. IFN–interferon. IFN1–type 1 interferons. IL–interleukin. RF–rheumatoid factor. TACI–transmembrane activator and calcium-modulator and cyclophilin ligand interactor. TCR–T-cell receptor. Th1–T-helper 1 cell. TLR–Toll-like receptor. Treg–regulatory T cell.

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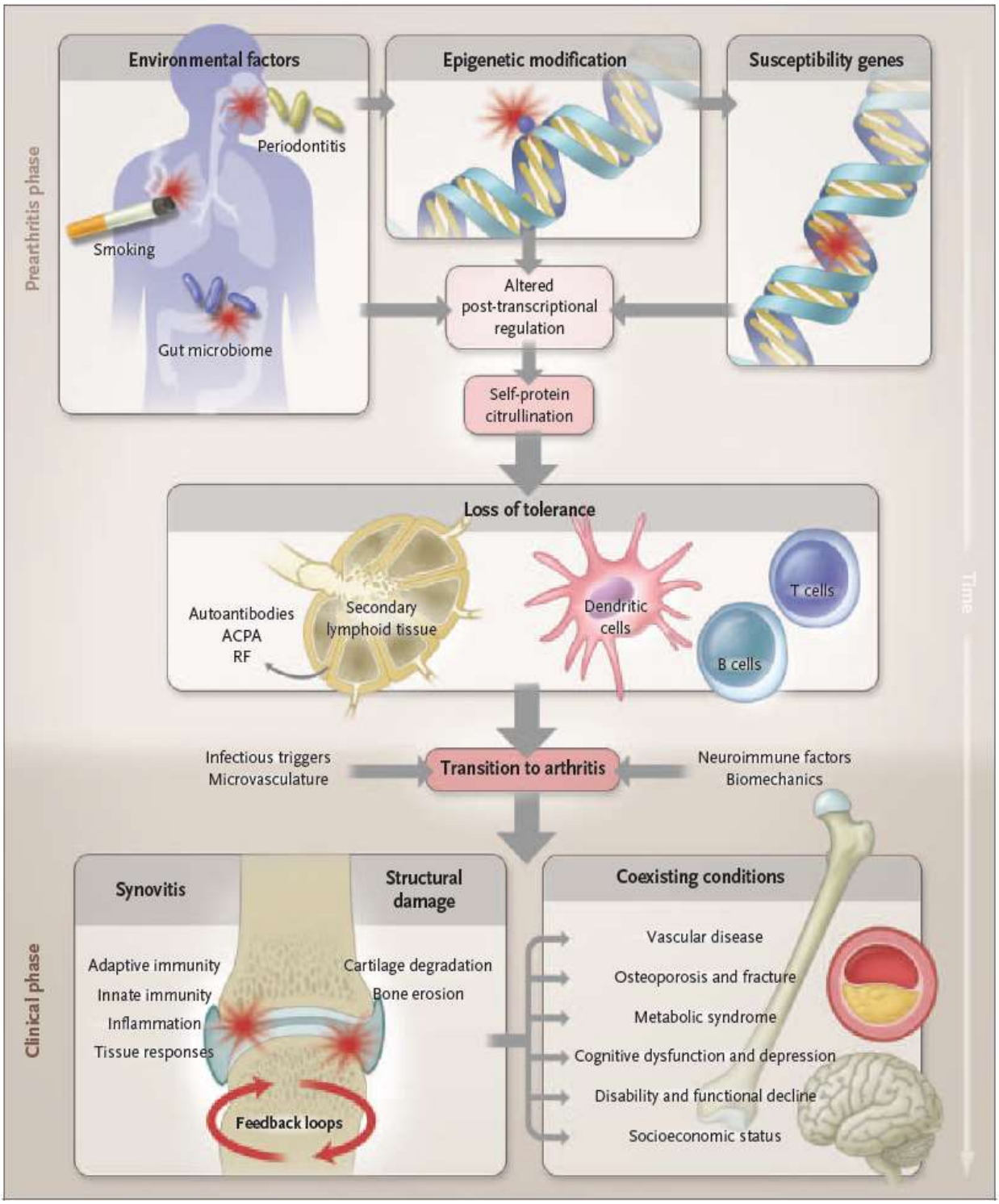

Figure 2. Multistep Progression to the Development of Rheumatoid Arthritis. (Mclnnes and Schett, 2011)
Environment–gene interactions promote loss of tolerance to self-proteins that contain a citrulline residue, which is generated by post-translational modification. This anticitrulline response can be detected in T-cell and B-cell compartments and is probably initiated in secondary lymphoid tissues or bone marrow. Thereafter, localization of the inflammatory response occurs in the joint by virtue of poorly understood mechanisms that probably involve microvascular, neurologic, biomechanical, or other tissue-specific pathways. Synovitis is initiated and perpetuated by positive feedback loops and in turn promotes systemic disorders that make up the syndrome of rheumatoid arthritis. ACPA denotes anti–citrullinated protein antibody, and RF rheumatoid factor.

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1.2.3 Rheumatoid Arthritis as an Inflammatory Disease

Rheumatoid arthritis is a chronic inflammatory disease marked by abnormal recruitment and activation of inflammatory monocyte/macrophage and lymphocytes in synovial joint, which results in persistent synovial inflammation and promotes joints destruction (Szekanecz and Koch, 2007). T-helper (Th) cells play an important role in the immune system by assisting B-cells in making antibodies, to induce macrophages to develop enhanced microbicidal activity, to recruit neutrophils, eosinophils, and basophils to sites of infection and inflammation, and, through their production of cytokines and chemokines, to orchestrate the striking array of immune responses (Gómez-Martín *et al.*, 2011). The immunologic phenomena associated with RA are consistent with dysregulation of T-helper (Th)-mediated immune responses (Miossec and van den Berg, 1997). The development and progression of RA has been shown to be due to poor regulation of levels of Th1/Th2 and Th17/Treg cells (Boissier *et al.*, 2008). Th1 and Th17 cells play a role in the inflammatory process and have been shown to be involved in many inflammatory conditions in humans and mice (Dardalhon *et al.*, 2008). Prior to the development of RA, abnormal T-cell activation usually occur with CD4+ T-cells stimulating monocytes and macrophages to produce inflammatory cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and proteolytic enzymes, initiating destruction of the synovium, cartilage, and underlying bone (Choy and Panayi, 2001). The activated T-cells in turn, induce B-cells to produce high levels of immunoglobulins, including rheumatoid factor. In addition to the local inflammation occurring in the synovial joints, the cytokines also produce a high degree of systemic inflammation which results in long term complications seen in patients with RA (Figure 3). One of such major complications which commonly co-exist with RA is cardiovascular disease.

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Mechanisms That Contribute to Clinically Observed Long-Term Complications in Patients with Rheumatoid Arthritis (McInnes and Schett, 2011)

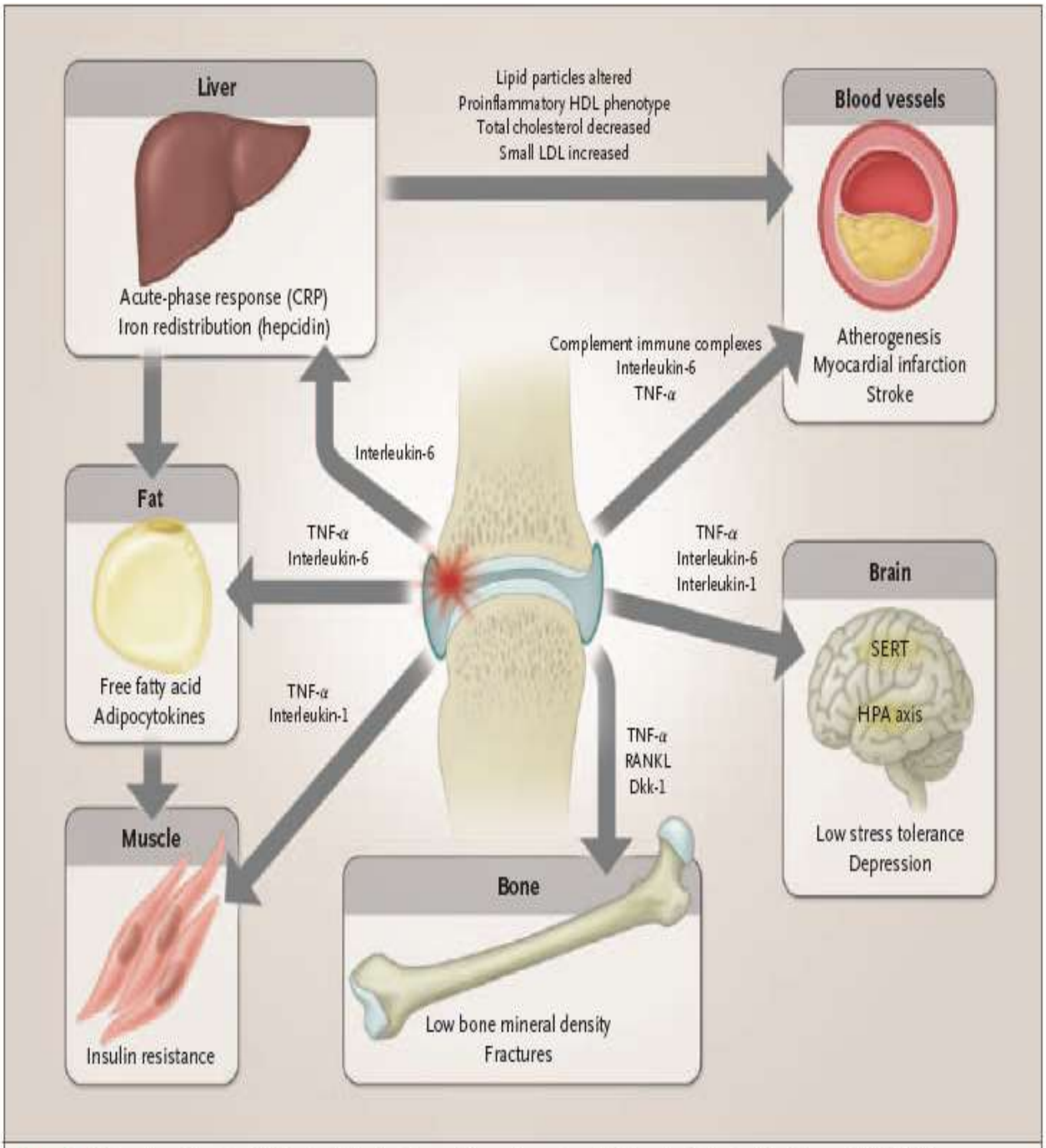

Figure 3. Mechanisms That Contribute to Clinically Observed Long-Term Complications in Patients with Rheumatoid Arthritis.
Inflammatory mediators, including cytokines, immune complexes, and altered lipid metabolism, circulate to promote several coexisting conditions in patients with rheumatoid arthritis. CRP denotes C-reactive protein, HDL high-density lipoprotein, HPA hypothalamic–pituitary–adrenal, LDL low-density lipoprotein, and SERT serotonin transporter.

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1.2.4 Cardiovascular Disease in Rheumatoid Arthritis

The risk of cardiovascular disease and death is higher in patients with RA (Turesson *et al*, 2004). Most of the premature deaths in RA are due to cardiovascular disease (Avina-Zubieta *et al*., 2008). The cardiovascular disease usually occurs at a younger age in RA patients compared to normal population and cannot be fully explained by traditional cardiovascular risk factors such as family history, smoking, and diabetes (del Rincon *et al.*, 2001). In addition to traditional cardiovascular risk factors, chronic systemic inflammation and autoimmune mechanisms have been shown to be crucial factors in atherosclerosis development and vascular damage (Fischer-Betz *et al*., 2010 and Gkaliagkousi et al.,2012).

A clearer understanding of the inflammatory immune mechanisms in atherosclerosis has provided insights into the pathogenesis of cardiovascular disease in RA. Several recent studies have revealed the association of elevated inflammatory measures and markers of autoimmunity with cardiovascular disease in RA (Rho *et al.*, 2009).Atherosclerosis and RA share many inflammatory mechanisms, including the production of cytokines, TNF-α and IL-6, activation of monocytes, macrophages, and lymphocytes, and expression of adhesion molecules (Pasceri and Yeh.,1999). CRP which is used to measure systemic inflammation and disease activity is also a strong predictor of cardiovascular events in the general population (Ridker *et al.*, 1997).

1.2.5 Pharmacological Management of Rheumatoid Arthritis

The major goal in RA management is to relieve pain, slow disease progression and bone destruction. While non-pharmacological approach such as physical, occupational and psychological approaches may contribute to therapeutic success, drugs form the mainstay of therapy in RA. Five main classes of drugs are currently used: analgesics, non-steroidal anti-inflammatories (NSAIDs), glucocorticoids, nonbiologic and biologic disease-modifying antirheumatic drugs (DMARDs). Combinations of these therapies are frequently used (Kumar and Banik, 2013). Treatment with nonbiologic DMARDs and/or biologic DMARDs is considered the standard of care for RA (Singh *et al*., 2012).

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DMARDs can be indicated for all RA patients and early use of DMARDs after diagnosis of RA is now considered an important part of RA management as most patients will show evidence of joint destruction within the first 2 years of symptom onset (Emery *et al*., 2002). Methotrexate (MTX), sulfasalazine (SSZ), leflunomide and hydroxychloroquine (HCQ) are the commonly used DMARDs. MTX is currently the anchor drug, and other conventional or biological antirheumatic drugs should be added to it for refractory patients (Kameda and Takeuchi, 2013). The combination therapy of MTX, SSZ and HCQ results in better clinical outcome than MTX alone, MTX plus SSZ, or MTX plus HCQ in patients with a poor response to MTX or another unaccompanied DMARD (Katchamart *et al*., 2009). The efficacy of SSZ plus MTX is uncertain in comparison to either drug alone. A molecular rationale for the failure of combination of SSZ and MTX to be more efficacious than either drug given alone was provided in a Dutch study which found SSZ to be a potent inhibitor of the principal cell membrane transporter for folates as well as MTX, along with inducing cellular folate depletion (Jansen *et al*., 2004).

1.2.5.1 Analgesics and NSAIDs

In RA treatment, analgesics and NSAIDs are used mainly on a temporary basis until the DMARDs take effect, as well as during disease flares. Acetaminophen (paracetamol) is the most commonly used analgesic due to its minimal side effects. NSAIDs used include ibuprofen, diclofenac, ketoprofen, indomethacin, naproxen, celecoxib, meloxicam, nabumetone, and piroxicam.

NSAIDs act majorly by inhibiting cyclooxygenase (COX) 1 and 2, thereby impairing the transformation of arachidonic acid to prostaglandins, prostacyclin, and thromboxanes. Common adverse effects of these drugs include dyspepsia, peptic ulcer disease, and bleeding. Transient elevations of liver enzymes may also occur with NSAIDs.

1.2.5.2 Glucocorticoids

Glucocorticoids are frequently included in the RA treatment regimen for a short period in order to minimize disease activity in patients with active RA while awaiting a clinical response to the given DMARD being applied. Treatment with combinations of DMARDs plus glucocorticoids provides

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greater benefit clinically (Gotzsche and Johansen, 1998) and results in less radiographic progression in comparison with DMARD monotherapy (Landewe et al., 2002). Oral glucocorticoids (prednisolone), used as a short course, or parenteral long acting glucocorticoids, such as methyl prednisolone 80–120 mg or triamcinolone 80 mg, can be given intramuscularly and when required for disease flares. Chronic use of low dose glucocorticoids in RA can cause multiple adverse events including an increased risk for osteoporosis and skeletal fractures, gastrointestinal bleeding, peptic ulcer disease, diabetes mellitus, infections, cataracts, and impaired hypothalamic-pituitary-adrenal axis response (Kumar and Banik, 2013).

1.2.5.3 Nonbiologic DMARDS

Methotrexate (MTX), sulfasalazine (SSZ), leflunomide, and hydroxychloroquine (HCQ) are the commonly used nonbiologic DMARDs in the treatment of RA. Older nonbiologic DMARDs such as gold, penicillamine, cyclosporine, and azathioprine have an adverse risk benefit ratio in RA patients and are seldom used in modern management of RA. Antibiotic DMARDs, such as doxycycline or minocycline, are also not used due to the availability of more effective drugs.

Methotrexate

MTX is the most important and most frequently prescribed substance for the treatment of RA, despite several new therapeutic options (Suarez-Almazor et al., 2000). It is a widely used first-line DMARD and can be used alone or in combination with other DMARDs. MTX has proven its efficacy as monotherapy and in combination, and the long-term safety profile is clinically acceptable .Hence, the recommendations from the European League Against Rheumatism (EULAR) state that MTX monotherapy should be the initial choice and instituted at the earliest time point if no contraindications are present (Smolen et al., 2010).

It takes 6–8 weeks for the onset of its benefit. MTX can be given orally, intramuscularly, or subcutaneously. The usual starting dose is 7.5–10 mg per week and the dose is titrated up to 20–25 mg per week on a fortnightly basis. The bioavailability of oral MTX decreases with higher doses therefore subcutaneous MTX is used in patients with inadequate response despite dose escalation. MTX primarily

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is cleared by the kidneys with most being unchanged in the urine. Thus, any decrease in glomerular filtration rate results in high serum levels of the drug that may induce bone marrow or other toxicities.

MTX is a folic acid antagonist drug. By binding to dihydrofolate reductase, MTX interferes with DNA synthesis and cell replication. For the dose used in RA, its main effect is believed to be due to the inhibition of enzymes involved in purine synthesis leading to the accumulation of adenosine and thus inhibiting the T cell activation. Common adverse effects include nausea the day after the dose is taken, mouth ulcers, reversible alopecia, rash and increased rheumatoid nodule formation. Adverse effects that rarely seen include liver cirrhosis, bone marrow suppression, and pulmonary infiltrates or allergic pneumonitis. Folic acid at the dose of 5–10 mg per week is always given 2–3 days after MTX. Taking folic acid 6 days a week reduces gastrointestinal and mucosal adverse effects and is recommended for people who develop these side effects. Blood tests monitoring must be done in patients who are taking MTX. Full blood count, liver function tests, and creatinine must be checked. The frequency of blood tests monitoring varies according to the national guidelines. A baseline chest x-ray is generally performed. MTX is usually avoided in patients with pre-existing bone marrow aplasia or cytopenias, immunodeficiency, severe hepatic disorders, or active infectious disease. Hepatotoxicity is potentially increased with the co-administration of azathioprine, SSZ, or leflunomide as part of combination therapy.

Sulfasalazine

SSZ contains an anti-inflammatory and an antibacterial agent (5-aminosalicylic acid and sulfapyridine). Its onset of action takes 6–12 weeks. Tablets are administered in evenly divided doses, taken preferably after meals at the recommended dosage range of 30–50 mg/kg/day. In clinical practice, SSZ dose is started at 500 mg/day and is increased by 500 mg weekly to 2.0–3.0 g/day.

SSZ operates by impairing folate absorption. Only 15% of the drug is absorbed as unchanged drug from small intestine. It is cleaved in the colon by bacterial enzymes to release acetylsalicylic acid and sulfapyridine. SSZ is excreted primarily by urine (as unchanged drug, conjugates, and acetylated metabolites) and in small amounts by faeces. The mechanism of action of sulfapyridine is unclear but may involve inhibition of the transcription factors which are increased in inflammation.

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Up to 30% of patients taking SSZ experience mild gastrointestinal disturbances (nausea, vomiting, loss of appetite, diarrhea), skin rash, and pruritus. Neurological symptoms of headache, dizziness, or depression also occur. In males, oligospermia with impaired motility are also observed. This, however, reverses three months after treatment is stopped. Rarer adverse effects include leucopenia, bone marrow depression, hemolytic anemia in patients with glucose-6-phosphatedehydrogenase deficiency, abnormal liver function tests, hepatitis, and abdominal pain. As SSZ inhibits absorption of folate, it can cause folate deficiency. Full blood count and liver function must be checked. The frequency of blood tests monitoring is less than what is needed for MTX and varies according to the national guidelines. SSZ should not be prescribed for patients who are hypersensitive to salicylates or sulfonamide derivatives. It is also contraindicated in patients with hematological, renal, or hepatic dysfunction. SSZ is safe to be used during pregnancy (Kumar and Banik, 2013).

Hydroxychloroquine

HCQ is primarily used in combination with other DMARDs. In patients with mildly active RA, particularly those without poor prognostic features or with findings limited to mild inflammatory arthritis and a positive antinuclear antibody test (in whom a distinction cannot be made between early RA and early systemic lupus erythematosus), HCQ is usually used rather than SSZ or MTX as the initial DMARD. It has a slow onset of action of 2–6 months. The drug is metabolized in the liver and metabolites include desethylhydroxychloroquine and desethylchloroquine. HCQ is excreted by urine as metabolites and up to 60% as unchanged drug. HCQ functions by interfering with antigen presentation and the activation of the immune response by increasing pH within macrophage phagolysosomes. Common side effects include epigastric burning, nausea, bloating, diarrhoea, skin rashes, and alopecia. Retinal toxicity with macular damage is infrequent; however it is recommended that patients wear sunglasses in strong sunlight. Corneal deposits, which are reversible if the drug is stopped, are seen in less than 0.1% of patients. However, the risk increases if the dose exceeds 6 mg/kg/day. Locally, chloroquine is available and is used at a dose of 200 mg/day. The most important of its side effects is retinopathy and requires regular ophthalmological monitoring. The frequency of ophthalmological monitoring with HCQ is controversial as it was originally developed for chloroquine which has greater ocular toxicity. Baseline

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ophthalmological review is recommended for patients with pre-existing eye disease or diabetes and then every 6 months thereafter. Patients with pre-existing maculopathy should not take HCQ. No specific laboratory monitoring is required. HCQ is considered to be safe during pregnancy (Kumar and Banik, 2013).

Leflunomide

Leflunomide is the most recent of the commonly used DMARDs given with the loading dose of 100 mg/day for three days followed by 10–20 mg/day (Smolen *et al*, 1999). In order to minimize the initial side effects, the loading dose is sometimes reduced, particularly in elderly or in patients with other co-morbid illnesses. Leflunomide is a prodrug whose active metabolite is responsible for its activity. It is metabolised by the liver to an active metabolite known as teriflunomide, which accounts for nearly all its pharmacologic activity. Further metabolism proceeds to multiple inactive metabolites which undergoes enterohepatic recirculation. The drug is excreted both in faeces and urine.

Leflunomide is an immune-modulatory agent which primarily inhibits replication of activated lymphocytes by blocking the de novo synthesis of pyrimidines and, therefore, DNA. It also has a weak anti-inflammatory action. The most common adverse effects are nausea and diarrhoea which are experienced by 20%–30% of patients, but these may settle with continued treatment. Skin rash and reversible alopecia occur in 5%–10% of patients and elevations of liver enzymes occur with sole use of leflunomide, and affect up to 60% of patients if used in combination with MTX (Emery et al., 2000). Rarer adverse effects include severe bone marrow suppression, infections, and persistent abnormal liver function tests despite dose reduction. Full blood count, creatinine, and liver function should be monitored periodically as per the national guidelines. Leflunomide is contraindicated in patients with severe immunodeficiency, impaired bone marrow function, or severe uncontrolled infections. As leflunomide inhibits cytochrome P450 2C9, it can interfere with drugs such as phenytoin and warfarin (Kumar and Banik, 2013).

1.2.5.4 Biologic DMARDs

The advancement in knowledge of the pathogenesis of RA over the last 15 years has led to the development of several biologic DMARDs and ushered a new era for the treatment of RA. Biologic

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DMARDs are used to treat moderate to severe RA that cannot be controlled by nonbiologic DMARDs. Biologic DMARDs act by blocking pro-inflammatory cytokines in a selective manner or act through B or T lymphocytes to decrease cytokine production.

Biologic therapies include the tumor necrosis factor (TNF) alpha inhibitors, anti-B cell therapy, T-cell co-stimulation blocker, anti-Interleukin 6 (IL-6), anti-Interleukin 1 (IL-1), and protein kinase inhibitors. There is a standardized nomenclature for these biologic agents: if the name ends with "cept" it is a receptor; if it ends with "mab," "zumab," "mumab," or "inib" it suggests chimeric monoclonal antibody, humanized monoclonal antibody, fully human monoclonal antibody, or small molecule kinase inhibitors, respectively (Kumar and Banik, 2013).

Anti-TNF

TNF is a cytokine involved in systemic inflammation which is abundant in the serum and synovial fluid of patients with RA and it plays a major role in the pathogenesis of RA. Infliximab, etanercept, adalimumab, certolizumab, and golimumab are anti-TNF agents and their introduction has marked the start of a revolution in the field of RA. They are very effective with 60%, 40%, and 20% of American college of rheumatology (ACR) 20, 50, and 70 responses, respectively.

Anti-TNFs common side effects include headache, abdominal pain, diarrhoea, vomiting, rash, injection site reaction, bleeding, bruising, itching, respiratory tract infection, and other infection such as cellulitis , positive anti-double-stranded DNA antibodies, positive ANA , and reactivation of latent tuberculosis (TB). Due to the risk of reactivation of *Mycobacterium tuberculosis* infection, screening for latent tuberculosis infection is recommended for every patient, regardless of the presence of risk factors of tuberculosis, prior to the initiation of a treatment with TNF inhibitors (Singh et al., 2012). In patients from high endemic regions, chest X-ray, Heaf/Mantoux test, and quantiferron gold/T-spot assay must be performed as part of screening for latent TB. Patients with latent TB must be treated first for at least one month prior to starting anti-TNF therapy. The probability of non-tuberculous mycobacterial infections are also higher with anti-TNFs and cases of TB occurring in association with TNF-alpha inhibitors have a higher likelihood of involving extrapulmonary sites and of being disseminated at presentation when compared with other TB cases.

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Anti-TNF is contraindicated in patients with the history of demyelination, active infection such as leg ulcers or long term urinary catheter, and in patients with heart failure, NYHA grade 3 or 4. It is also not currently recommended in women who are pregnant or breast feeding, though it is increasingly being used in pregnancy and thus far has been found to be safe. In patients with history of malignancy, TNFs must be used with caution. Any patient with history of malignancy must have been symptom free for at least 10 years before being placed on TNF therapy. All anti-TNFs (infliximab, etanercept, adalimumab, certolizumab, and golimumab) have been found to be more effective when used in combination with MTX (Kumar and Banik, 2013)**.**

Anti-B-cell therapy

B cells play an active role in the pathogenesis of RA. These cells are targeted by using antibodies against the pan-B-cell surface marker CD-20. Other targets such as anti-CD 19 are still under evaluation. Rituximab is currently the only licensed anti-B cell therapy in RA.

Rituximab

Rituximab is a monoclonal antibody which acts against the CD20 antigen on the surface of B-lymphocytes. CD20 controls cell cycle initiation and serves as a calcium channel. Rituximab binds to the antigen on the cell surface and B-cell cytotoxicity via the complement system, as well as to human Fc receptors, causing cell death through an antibody-dependent cellular toxicity.

1 g of Rituximab is given intravenously on days 1 and 15 in combination with MTX; subsequent courses may be administered every 24–52 weeks (based on clinical evaluation) or may be repeated earlier as necessary, but no sooner than every 16 week. B-cell recovery starts about 6 months after the completion of treatment and B-cell levels usually returns to normal by 12 months after completion of treatment. B lymphocyte depletion treatment using a combination of rituximab and MTX has been effective in randomized trials of patients resistant to MTX alone as well as those resistant to TNF inhibitors (Cohen *et al*, 2006). Despite the general long-term safety of rituximab (van Vollenhoven, 2012) an increased risk of progressive multifocal leukoencephalopathy for one case per 25,000 RA patients treated with rituximab is of concern (Molloy *et al*., 2012).

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1.2.6 Statins (Hydroxymethylglutaryl-coenzyme A inhibitors)

Statins or hydroxymethylglutaryl-coenzyme A inhibitors (HMG-CoA inhibitors) were originally identified as fungal extracts in 1976 (Endo *et al.*, 1976). They were later modified into cholesterol-lowering drugs, and have been shown reduce both cardiovascular morbidity and mortality (Shepherd *et al.*, 1995). Statins act by inhibiting the enzyme which converts HMG-CoA to mevalonic acid, thereby reducing cholesterol synthesis. It also increases low-density lipoprotein (LDL) uptake and decreases triglyceride, apolipoprotein B, and very low-density lipoprotein levels.

There have been increasing evidence and documentation suggesting that statins have therapeutic 'pleiotropic' effects which are independent of cholesterol lowering. These pleiotropic effects include anti-inflammatory and antioxidative properties, improvement of endothelial function and increased endothelial nitric oxide synthetase expression and nitric oxide bioavailability, which may explain the therapeutic benefit, observed with statin therapy. The immunomodulatory effects of statins have been shown to be independent of lipid lowering (Kwak *et al.*, 2000). Several studies have demonstrated that statins have greater mortality benefit which can not be explained by their cholesterol-lowering effects alone (Weitz-Schmidt, 2002) and these benefits occur too quickly that it can not be attributed to their effects on atherosclerotic plaque (Takemoto and Liao, 2001).

1.2.7 Pleiotropic Effects of Statins

Statins have a number of cholesterol-independent (pleiotropic) effects which are more than their well-known cholesterol-lowering properties. These effects have been well documented and include anti-inflammatory actions (Laufs, 2003). Statins affect multiple steps in the inflammatory process, including leukocyte migration and adhesion, T-cell stimulation, nitric oxide (NO) bioavailability, generation of free radicals, and angiogenesis (Palinski and Tsimikas, 2002). Statins have been shown to reduce pro-inflammatory cytokines synthesis, such as TNF-a, IL-6, and IL-1b, and also decrease serum levels of IL-2 and CRP (Ascer *et al.*, 2004; Albert et al., 2001).

Statins express most of their pleiotropic effects by interfering with the synthesis of mevalonate metabolites. The blockade of the mevalonate pathway has been shown to suppress T-cell responses

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(Kurakata *et al.*, 1996) to reduce expression of class II major histocompatibility complexes on antigen-presenting cells (Kwak *et al.*, 2000) and to inhibit chemokine synthesis in peripheral blood mononuclear cells (Romano et al., 2000).

In 1995, a decrease of rejection episodes and increase in survival of cardiac transplant recipients taking pravastatin was observed and this suggested additional anti-inflammatory and immune-modulatory effect of statins (Kobashigawa *et al.*, 1995).

1.2.8 Statins in Treating Animal Models of Inflammatory Diseases

Several studies on animal models, have shown that statin reduce inflammation and clinical symptoms of inflammatory disorders such as RA. Collagen-induced arthritis is the most widely used animal model for the evaluation of novel therapeutic strategies for rheumatoid arthritis (Williams, 2007). As in human rheumatoid arthritis, a number of both pro- and anti-inflammatory cytokines are expressed in the joints of mice with collagen-induced arthritis, including TNF-α and IL-1β, IL-6, IL-1Ra, IL-10, and transforming growth factor beta.

Intra-articular simvastatin showed anti-inflammatory properties on temporomandibular joint inflammation in growing rats (George *et al*, 2013). Simvastatin and atorvastatin showed anti-inflammatory activity in carrageenin induced paw edema in rats and formalin induced arthritis model, suggesting that simvastatin and atorvastatin may play an adjuvant role, which may be beneficial in the treatment of inflammatory disorders, especially when there is coexisting dyslipidemia (Jaiswal and Sontakke, 2012). Simvastatin prevented early and late joint inflammation in association with a decrease in articular macrophage influx and suppressed the periarticular bone destruction occurring late in the course of disease, preserving periarticular bone mineral density and preventing increases in periarticular osteoclasts and serum pyridinoline levels in arthritic animals (Funk *et al*, 2008).

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1.2.9 Statin Use in Rheumatoid Arthritis and Inflammatory Diseases

Recent studies have shown that statins may have mild anti-inflammatory effect in rheumatoid arthritis, in addition to reducing cardiovascular risk (Costenbader and Coblyn, 2005). Atorvastatin was shown to significantly upregulate the frequency and impaired function of regulatory T-cells and reduces clinical disease activity in patients with RA (Ting-Ting Tang *et al.*, 2011). A trial of atorvastatin in 116 RA patients over 6-months showed a clinically significant anti-inflammatory effect, given the fact that atorvastatin was designed primarily as a lipid-lowering drug. In this study, the RA patients were already being treated with DMARDs; however the addition of atorvastatin had a significant effect on suppressing acute-phase variables (CRP and ESR) and on reducing the swollen joint count (McCarey *et al.*, 2004). Two studies, carried out in Japan and Mexico, showed marked improvement in statin-treated RA patients. The Japanese study was a 12-week, open-label, single-arm study of 24 patients receiving 10 mg of simvastatin daily, 39% of the treatment group met the American College of Rheumatology (ACR) 50% improvement criteria (ACR50 response) (Kanda *et al.*, 2004). The study in Mexico compared the effect of simvastatin to chloroquine in 15 patients with RA who were receiving methotrexate as a single disease modifying antirheumatic drug with no satisfactory response. After eight weeks, most of the patients (9 out of 10) who received simvastatin (40mg/day) showed an ACR50 or better response , whereas such a response was not observed in any patient (0 out of 5) treated with chloroquine (Abud-Mendoza *et al.*, 2003).

1.3 Aim and Objectives

Aim:

To assess the anti-inflammatory effect of simvastatin in rheumatoid arthritis patients with associated risks for cardiovascular disease.

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Objectives:

1) To determine the effect of simvastatin therapy as an adjunct to DMARDs on inflammatory variables (ESR and CRP)

2) To determine the effect of simvastatin therapy as an adjunct to DMARDs on Disease Activity Score (DAS28).

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CHAPTER 2

METHODS AND PROCEDURES

2.1. Study Design

The study was an open-label, randomized, cross-over study to assess efficacy of simvastatin in reducing inflammatory variables and disease activity in rheumatoid arthritis. Patients with moderately active RA despite being on maximum DMARD therapy and having associated cardiovascular risks were screened at the rheumatology clinic of Steve Biko Academic Hospital. Eligible patients were randomized into two groups, 1 and 2. Patients in group 1 received simvastatin treatment (20mg/day) for a period of 3 months in addition to their usual DMARDs after which they stopped simvastatin treatment and were followed up for 3 months off simvastatin treatment. Those in group 2 were allowed to continue on their usual DMARDs without simvastatin treatment for a period of 3 months after which they received 20mg/day simvastatin for a period of 3 months in addition to their usual DMARDs. The anti-inflammatory effect of simvastatin was assessed by monitoring the inflammatory variables (ESR and CRP) and disease activity in the 2 groups over the study period.

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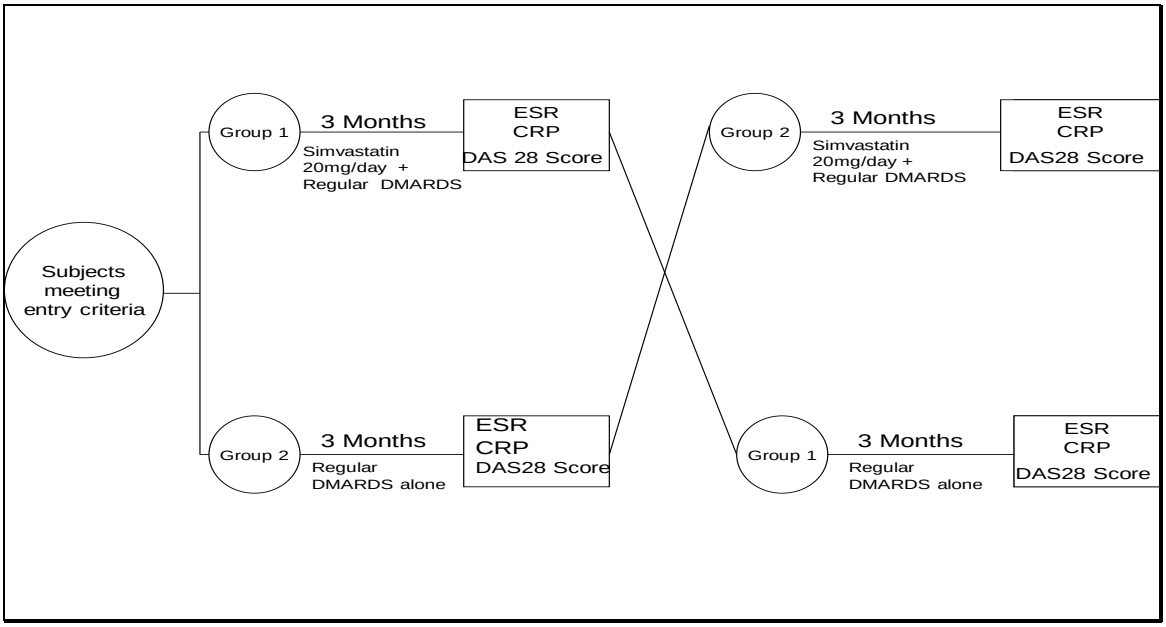
Figure 4:Study Design: An open-label, randomized crossover study



2.2. Setting

Patients were recruited from and seen at the Rheumatology Clinic of Steve Biko Academic Hospital. Participation in the study was voluntary and all participants signed a consent form prior to enrolment in the study.

2.3 Patient Selection

2.3.1 Patient population

Rheumatoid arthritis patients with moderately active disease despite being on maximum disease-modifying antirheumatic drug therapy and having associated risks for cardiovascular disease were screened at the Rheumatology Clinic of Steve Biko Academic Hospital. Eligible patients were randomized into 2 groups (statin and non-statin group).

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2.3.2 Inclusion criteria

1. Ability and willingness to provide written informed consent and comply with the requirements of the study.

2. Male and female ≥18 years of age

3. Diagnosis of RA as defined by 1987 American College of Rheumatology (ACR) criteria

4. Active disease with at least one swollen and two tender joints

5. If on corticosteroids, dose must be stable and 10 mg/day prednisone (or equivalent) or less for at least 4 weeks prior to study entry

6. DMARD dose must be stable for at least 4 weeks (methotrexate, leflunomide, etanercept, adalimumab) or at least 3 months (chloroquine or abatacept).

7. Risk for cardiovascular disease for which statin is indicated e.g hypercholesterolemia

2.3.3 Exclusion criteria

1. Currently taking a statin or have taken a statin in the past

2. History of an adverse reaction to a statin

3. Active or recent infection within 4 weeks of study entry

4. Joint replacement surgery within 60 days of study entry or plan to undergo joint replacement surgery during the course of the study

5. Parenteral cortisone injections within 4 weeks prior to study entry

6. Any change in DMARD or steroid therapy during the study

7. Chronic disorders other than RA affecting the joints, including systemic lupus erythematosus (SLE), psoriatic arthritis, gout, scleroderma, or known reactive arthritis (Reiter's syndrome)

8. History of alcohol abuse

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9. History of liver disease, current liver disease (e.g., hepatitis, cirrhosis), or abnormal liver function (AST or ALT greater than 2 times the upper limit of normal)

10. Any condition that, in the opinion of the investigator, may interfere with the study

11. Pregnancy or breastfeeding

2.4 Procedures

The study and study procedures were explained to the patients at the screening visit and willing patients signed the informed consent form approved by the University of Pretoria Research Ethics Committee.

Medical history of each patient was reviewed and full physical examination was carried out on the patients. This included an examination of the central nervous system, the cardiovascular system, the pulmonary system, the gastrointestinal system and the ear, nose and throat to rule out possible systemic diseases. Assessment of the rheumatoid arthritis disease activity was done at each visit of the study by an independent joint assessor who is a physician at the rheumatology clinic of Steve Biko Academic Hospital. Blood samples were collected at each visit and analyzed.

2.5. Measurements

All the participants had their ESR, CRP, and DAS28 score assessed at beginning of the study, at the cross over point and at the end of the study. The lipid profile was checked at the beginning of the study to identify patients with dyslipidemia requiring statin therapy and at the end of the study to assess benefit from statin therapy.

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Primary outcome was change in inflammatory variables (ESR and CRP) at the end of simvastatin treatment. Secondary outcome is change in disease activity score (DAS28) after simvastatin therapy.

2.5.1 DAS28

A simplified disease activity score using 28-joint count. It integrates measures of physical examination (swollen and tender joint counts, both 0-28), acute phase response (ESR/CRP) and patient's self-assessment of disease activity measured on a visual analogue scale (VAS) of 0-100mm. The joints included in DAS28 are (bilaterally): proximal interphalangeal joints (10 joints), metacarpophalangeal joints (10), wrists (2), elbows (2), shoulders (2) and knees (2). When looking at these joints, both the number of joints with tenderness upon touching (TEN28) and swelling (SW28) are counted. In addition, the erythrocyte sedimentation rate (ESR) is measured. Also, the patient makes a subjective assessment of disease activity during the preceding 7 days on a visual analogue scale (VAS) between 0 and 100, where 0 is "no activity" and 100 is "highest activity possible". With these parameters, DAS28 is calculated as:

$$DAS28 = 0.56 \times \sqrt{TEN28} + 0.28 \times \sqrt{SW28} + 0.70 \times \ln(ESR) + 0.014 \times SA$$

DAS28 provides absolute indication RA disease activity on a scale of 0.49 to 9.07

- DAS28 > 5.1 = High disease activity
- DAS28 3.2-5.1 = Moderate disease activity
- DAS28 2.6-3.2 = Low disease activity
- DAS28 <2.6 = Remission

By comparing a patient's DAS28 score over multiple time points, improvement or response to treatment can be assessed. The EULAR response criteria are defined as follows:

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Table 1. EULAR Response Criteria

Present DAS28	**DAS28 Improvement over time points**		
	> 1.2	**0.6 – 1.2**	**<0.6**
<3.2	Good response	Moderate response	No response
3.2 – 5.1	Moderate response	Moderate response	No response
>5.1	Moderate response	No response	No response

2.6. Data analysis

A t-test was used to test equality of mean change in ESR, CRP and disease activity score (DAS28) with statin therapy in the two groups. A change in DAS28 score ≥ 0.6 was considered clinically significant.

2.7. Ethical considerations

2.7.1 Ethics Approval

The protocol was submitted to and approved by the University of Pretoria Research Ethics Committee. The study was conducted according to ICH Good Clinical Practice Guidelines and the World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects. Patients participating in the study will be offered dietary and lifestyle advice during the period they are off simvastatin treatment as a non-pharmacologic approach to managing their risk for cardiovascular disease.

2.7.2 Consent

The patients who participated in this study made an informed decision. They were volunteers informed about the aims, methods, risks and benefits of the research study and all signed the informed consent form compiled according to international requirements and standards.

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2.7.3 Confidentiality

All personal information is kept confidential. Data that may be reported in scientific journals will not include any information which identifies the patient in this study.

2.8 Funding

The research was funded by the department of Pharmacology, University of Pretoria

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CHAPTER 3

RESULTS

27 patients with rheumatoid arthritis were screened, of whom 12 with risks for cardiovascular disease were selected and randomized into two groups. There were 3 men and 9 women with a median age of 59 years (range 40-72) enrolled in the study. The median duration after the diagnosis of RA was 5 years (range 1-27). 10 (83.3%) of the patients have positive rheumatoid factor status and 2 (16.7%) have sero-negative RA. All the patients have received at least two DMARDs, including methotrexate (*n* = 12), chloroquine (*n* = 7), sulfasalazine (*n* = 4) and leflunomide (*n* = 2).10 of the 12 patients were on a corticosteroid (prednisone).

Table 2: Change in Individual patient' Tender Joint count with and without simvastatin therapy

Patients in Group 1	Visit 1 (baseline)	Visit 2 (cross-over)	Visit 3 (Study exit)
1	6	8	10
2	2	2	2
3	2	2	4
4	5	2	4
5	7	5	7
6	3	2	2
Patients in Group 2	Visit 1 (baseline)	Visit 2 (cross-over)	Visit 3 (Study exit)
1	2	2	2
2	5	8	5
3	12	9	9
4	5	5	2
5	3	6	4
6	3	4	1

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Table 3: Change in Individual patient' Swollen Joint count with and without simvastatin therapy

Patients in Group 1	Visit 1 (baseline)	Visit 2 (cross-over)	Visit 3 (Study exit)
1	5	3	8
2	6	1	1
3	14	6	8
4	9	7	8
5	9	6	10
6	5	4	2
Patients in Group 2	Visit 1 (baseline)	Visit 2 (cross-over)	Visit 3 (Study exit)
1	7	6	3
2	5	8	5
3	2	7	8
4	9	9	6
5	4	6	5
6	4	5	3

The disease activity variables improved significantly with simvastatin treatment in the 2 groups. In particular, the swollen joint count declined by 5.83 (p = 0.003) in group 1 and 3.5 (p = 0.002) in group 2. The mean change in disease activity score with simvastatin treatment was 1.30 (CI 0.35 to 2.26; p = 0.01) in group 1 and 1.74 (CI 0.48 to 3.0; p = 0.01) in group 2.ESR was significantly reduced with simvastatin treatment in group 1 with a mean change of 19.0 (CI 8.57 to 29.43; p = 0.005) and marginally reduced in group 2 with a mean change 26.0 (CI -6.70 to 59.37; p = 0.09). There was a significant increase in ESR on stopping simvastatin treatment (p=0.008) in group 1.

No significant change in CRP with simvastatin treatment in the 2 groups. The mean change in CRP with simvastatin treatment was 7.0 (CI 6.59 to 20.59; p = 0.24) in group 1 and 14.7 (CI 11.35 to 40.75; p = 0.20) in group 2.

All of the patients benefited from the cholesterol-lowering effect of simvastatin and no adverse events were noted during simvastatin treatment.

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Table 4: Change in Individual patient' ESR, CRP and DAS28 score in response to Simvastatin therapy

	ESR			CRP			DAS 28		
Visits	**V1**	**V2**	**V3**	**V1**	**V2**	**V3**	**V1**	**V2**	**V3**
Patients in Group 1									
1	38	38	50	4.9	4.3	4.0	5.24	5.46	5.86
2	50	10	20	10.6	1.1	2.7	4.51	2.67	3.09
3	28	20	36	19.0	23.8	27.0	4.87	4.27	5.12
4	50	38	60	30.3	24.6	50.2	5.25	4.64	5.48
5	10	4	10	6.9	5.1	6.5	4.63	3.61	4.82
6	30	30	10	1.9	1.0	1.9	3.76	3.38	3.27
Patients in Group 2									
1	38	32	20	4.6	6.5	4.2	4.64	4.60	3.93
2	20	28	4	7.7	12.2	9.1	4.68	5.59	3.27
3	24	38	48	5.5	4.7	6.1	5.54	5.95	6.02
4	86	108	80	48.9	69.5	30.0	5.63	6.21	4.97
5	32	52	80	22.5	34.7	21.6	4.38	5.50	5.79
6	50	92	60	13.4	6.8	7.0	4.69	5.89	4.05

Table 5: Change in Individual patient' Total cholesterol, Triglyceride (TGD), HDL-cholesterol and LDL-cholesterol in response to Simvastatin therapy

Visits	**Visit 1 (Screening)**				**Visit 3 (Study exit)**			
Patients in Group 1	**Total Cholesterol** (mmol/L)	**TGD** (mmol/L)	**HDL** (mmol/L)	**LDL** (mmol/L)	**Total Cholesterol** (mmol/L)	**TGD** (mmol/L)	**HDL** (mmol/L)	**LDL** (mmol/L)
1	4.9	1.6	0.9	3.5	4.3	1.1	0.7	2.8
2	6.4	0.9	2.3	3.5	5.6	0.9	2.7	2.2
3	5.9	1.9	0.8	4.4	5.36	1.3	1.14	3.92
4	7.7	2.5	1.4	5.4	9.7	1.1	1.3	6.8
5	4.9	1.0	1.1	3.3	4.1	0.8	1.3	2.5
6	5.2	0.3	2.1	2.9	4.6	0.9	2.5	2.1
Patients in Group 2								
1	5.2	0.8	1.3	3.9	4.2	1.1	0.9	3.6
2	4.8	1.1	1.2	3.3	4.4	0.9	1.3	2.7
3	4.8	1.3	1.0	3.2	4.1	0.9	1.5	2.5
4	4.5	0.9	0.9	3.6	3.5	1.0	1.0	2.1
5	6.7	4.1	1.6	2.4	6.0	2.4	1.4	4.0
6	5.6	2.3	1.4	3.2	5.7	1.5	1.5	3.3

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Figure 5: Changes in ESR pattern with simvastatin therapy in group 1

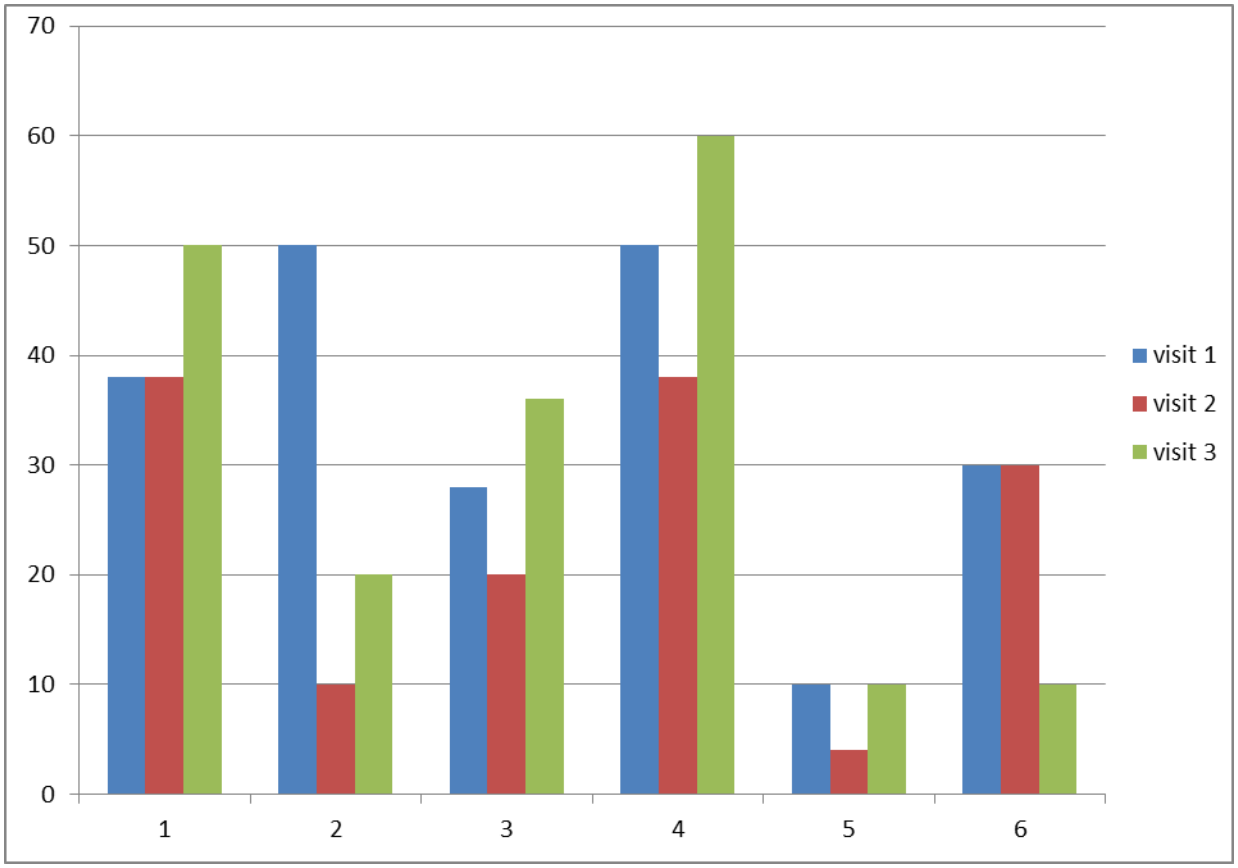

Figure 6: Changes in ESR pattern with simvastatin therapy in group 2

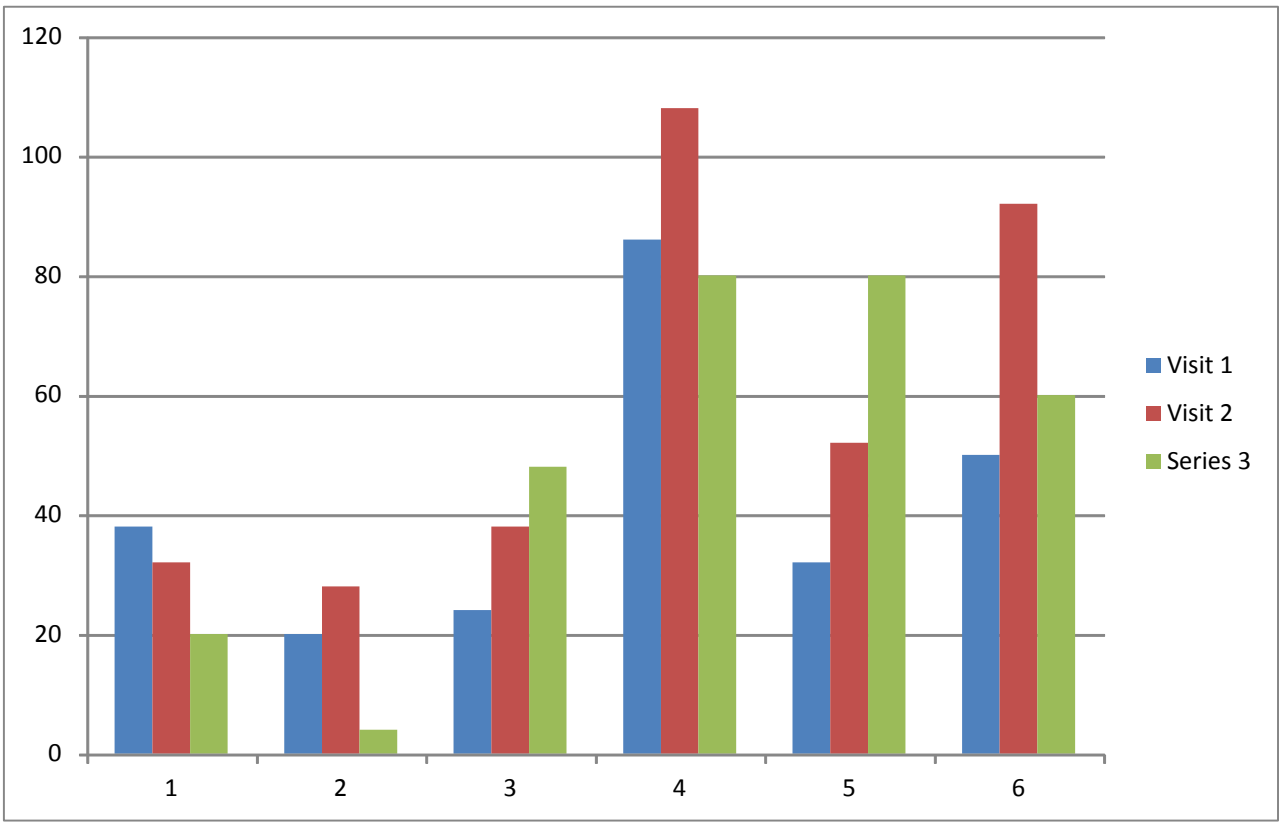

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Figure 7: Changes in DAS28 pattern with simvastatin therapy in group 1

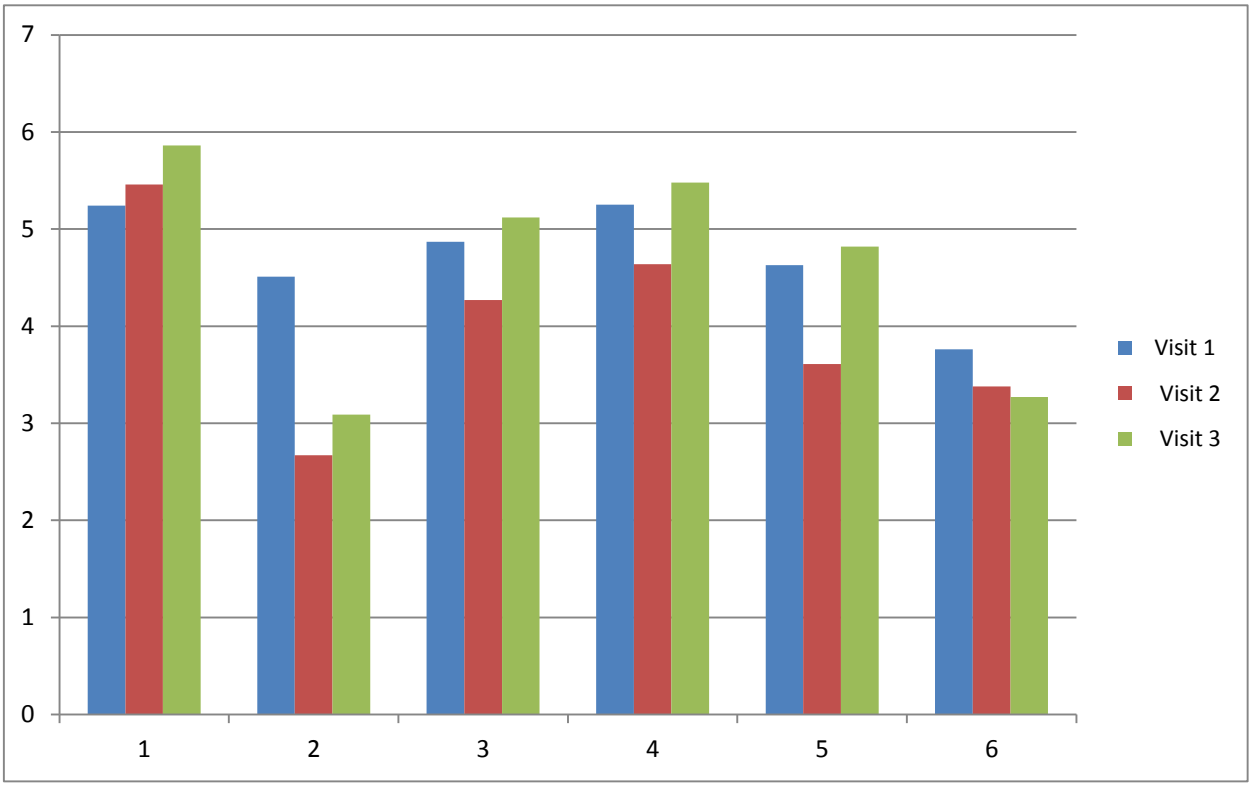
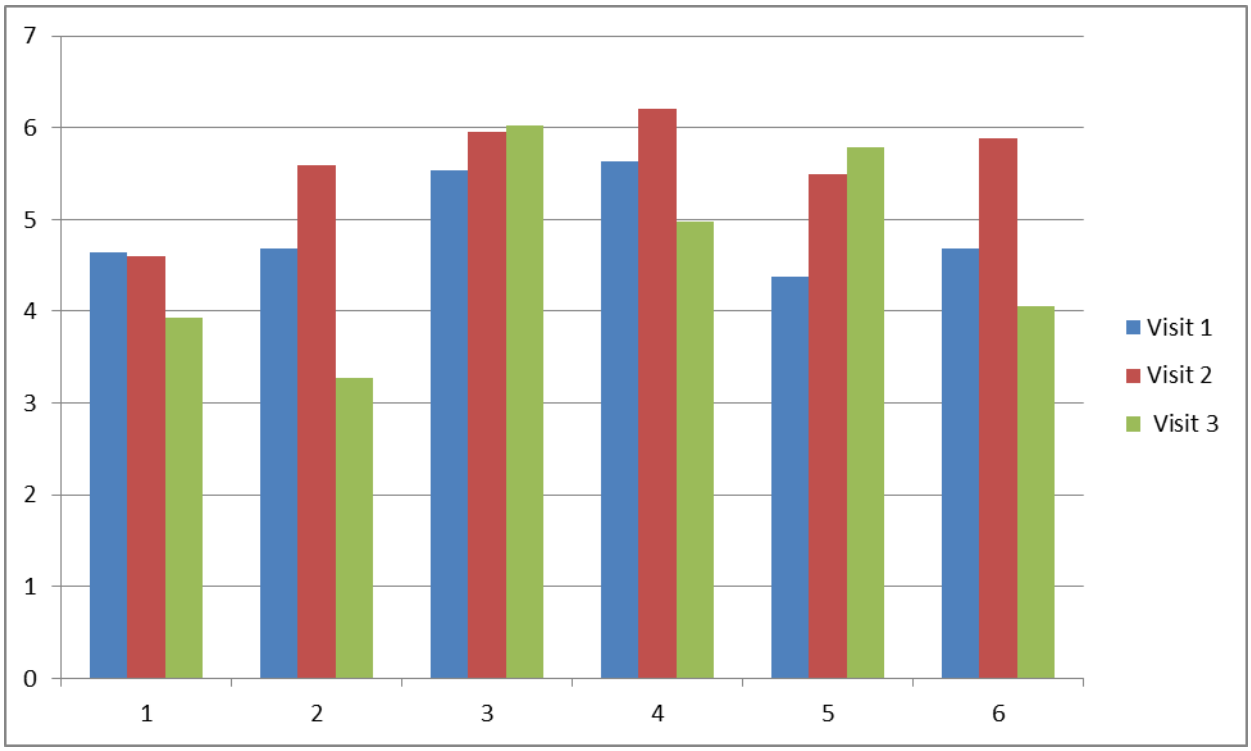


Figure 8: Changes in DAS28 pattern with simvastatin therapy in group 2



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CHAPTER 4

DISCUSSION

Despite improved control of inflammation, cardiovascular risk among people with RA remains significantly high. The increased prevalence of cardiovascular risk in RA patients was demonstrated in this study, with 44.4% of the RA patients screened for the study having associated risks for cardiovascular disease which qualified them for inclusion in the study. This prevalence is similar to that from a comprehensive meta-analysis of published mortality studies of patients with RA which showed a 50% increased risk of cardiovascular death in RA compared with the general population (Avin˜a-Zubieta *et al*., 2008). High-grade systemic inflammation and its interplay with traditional CV risk factors appear to play a major role in increased mortality observed in RA (Stevens RJ, 2005). Statins can serve a dual purpose in view of their documented anti-inflammatory effect and long-established lipid lowering effect in RA patients with associated risks for cardiovascular disease. Such dual effect has been hypothesized for statins in systemic lupus erythematosus another multisystem autoimmune disease associated with accelerated atherosclerosis (Haiyan Tu *et al*., 2012).

From this study, the effect of statin therapy in the 2 groups was generally comparable. There was marked reduction in ESR and a significant reduction in swollen joint count and clinical disease activity in patients with rheumatoid arthritis presenting with active disease despite existing DMARD therapy. Although the magnitude of change observed is mild, the significant reduction in DAS28 provides proof of concept that pathways targeted by statins offer therapeutic opportunity in inflammatory disease. This finding supports other studies that have reported that addition of low-dose simvastatin to conventional immunosuppressive therapies improved the clinical, biological, and immunological parameters in RA patients (Abud-Mendoza *et al.*, 2003, McCarey *et al.*, 2004).

ESR and CRP levels have been shown to be poorly correlated with clinical measures of disease activity in rheumatoid arthritis (Keenan *et al.*, 2008). In this study, there was significant reduction

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in level of ESR with simvastatin treatment but this was not the case with CRP. This may indicate that ESR is more sensitive than CRP to changes in RA disease activity as have been demonstrated by Ward (2004). Although the tendency in recent years has been to favor CRP as a measure of acute phase reaction, in this study, ESR was found to surpass CRP as a measure of RA activity and may be better for monitoring response to DMARD) treatment.

A limitation of the study was the few number of participants used and it would be necessary to conduct the study in a larger group of patients.

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CHAPTER 5

CONCLUSION

The influence of statins on immune mechanisms has been well-evidenced in *in vitro* studies and *in vivo* models of autoimmune disorders. Their ability to switch Th1 to Th2-type response, to support regulatory T-lymphocyte activity, and to inhibit Th17 cell functions together with concomitant inhibition of plethora of effector immune mechanisms, independent on lipid-lowering effects, justify the attempt of their potential implementation in the treatment of autoimmune diseases such as RA (Chalubinski and Broncel, 2010).

The addition of 20 mg simvastatin to conventional DMARDs not only reduced the inflammatory variable, ESR, in RA patients with associated risks for cardiovascular disease but also improved the clinical symptoms of RA as measured by the swollen joint count and disease activity score demonstrating the anti-inflammatory effect of statins. In view of the high prevalence of cardiovascular disease in RA patients, statins may be good adjuvant to DMARDs in RA patients with associated risks for cardiovascular disease.

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