THE PREVALENCE OF SIDE EFFECTS ON METHOTREXATE AND ITS ASSOCIATED RISK FACTORS IN SOUTH AFRICAN PATIENTS WITH RHEUMATOID ARTHRITIS

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DECLARATION

I, Dr Nabeela Kajee, declare that this research report is my own, unaided work. It is being submitted for the Degree of Master of Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

_____ (Signature of candidate)

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of_____20_____in_____

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Abstract

Background

Methotrexate (MTX) remains one of the anchor disease-modifying antirheumatic drugs (DMARDS) used to treat rheumatoid arthritis (RA). Methotrexate usage can be associated with a variety of side effects which range from mild to severe. This can lead to premature discontinuation or dose adjustments of a drug which is very efficacious in the treatment of RA.

Objectives

To document the prevalence and nature of MTX toxicities in patients with RA, in a South African population. We also tried to identify potential risk factors for developing these toxicities and determined whether MTX doses of more than 30mg a week as based on a clinician's judgement are associated with an increased frequency of toxic events.

Methods

A retrospective chart review of 200 consecutive RA patients was carried out in the Department of Rheumatology at Charlotte Maxeke Johannesburg Academic Hospital. Information was captured from the files and patients were not clinically assessed. Patient characteristics were reviewed and recorded at three time intervals i.e. entry to the clinic, at the time toxicity developed and at the time of data collection. Data was expressed as mean, median or percentages as appropriate. Age and gender adjusted associations of recorded variables other than MTX with toxicity characteristics were determined in multivariable regression models. Those factors that were associated in age and sex adjusted models with toxicity were entered together with MTX variables in a single model with consideration of collinearity, interactions and cause-effect relations.

Results

Overall toxicity was recorded as 25% (60 toxic events in 50 patients). There were 9 patients who developed more than 1 adverse event. Leucopenia was most prevalent (9.5%), followed by a transaminitis (8.5%). Stomatitis (4.5%), nausea (3%) and anaemia (1.5%) accounted for the other toxic events. Leucopenia was associated

with age at entry (OR 0.96, 95%CI: 0.91-0.99), concomitant sulphasalazine usage at entry or exit (OR 3.76, 95%CI: 1.08-13.09 and OR 2.8, 95%CI: 1.07-7.34 respectively) and the development of anaemia (OR 21.18, 95%CI: 1.83-245.7; p=0.01). Transaminitis was borderline associated with age at entry (OR 0.96, 95%CI: 0.91-1.0; p=0.06) and with the simultaneous development of nausea and stomatitis (OR 5.97, 95%CI: 1.01-35.29; p=0.04 and OR 6.32, 95%CI: 1.43-28.2; p=0.01 respectively). Alcohol usage was present in 4 patients who developed a transaminitis (OR 2.25, 95%CI: 0.66-7.69; p=0.2). Doses higher than 30mg/week were prescribed in 45 patients of which only 12 developed toxic events, 6 leucopenia (OR 1.68, 95%CI: 0.6-4.7; p=0.3), 5 transaminitis (OR 1.49, 95%CI: 0.5-4.43; p=0.5) and 1 nausea.

Conclusions

This study delineates the prevalence of toxicities on MTX in a predominantly Black, South African population. The most frequently occurring toxicity in our population was leucopenia. Most other studies in RA patients looking at MTX toxicity described transaminitis as the most prevalent toxic event. Doses of MTX higher than 30mg a week were not associated with an increased prevalence of toxicity. In patients with refractory disease, high doses of MTX comprise a potential alternative preceding the use of a biologic agent.

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Abbreviations

ABC: adenosine triphosphate-binding cassettes

ACE: angiotensin-converting enzyme

ACR: American College of Rheumatology

ACPA: anti-cyclic citrullinated protein antibodies

AICAR: 5-amino-imidazole-4-carboxamide ribonucleotide

AST: aspartate aminotransferase

ALT: alanine transaminase

BMI: body mass index

CKD: chronic kidney disease

CMJAH: Charlotte Maxeke Johannesburg Academic Hospital

CXR: chest x-ray

DARC: Duffy antigen receptor for chemokines

DHFR: dihydrofolate reductase

DMARDs: disease modifying anti-rheumatic drugs

DNA: deoxyribonucleic acid

eGFR: estimated glomerular filtration rate

EULAR: European League Against Rheumatism

FBC: full blood count

GIT: gastrointestinal

GM-CSF: granulocyte macrophage- colony stimulating factors

HIV: human immunodeficiency virus

IQR: interquartile range

IL: interleukin

IFN: interferon

LFT: liver function test

MTX: methotrexate

MTHFR: methylenetetrahydrofolate reductase

NSAIDs: non-steroidal anti-inflammatory drugs

RA: rheumatoid arthritis

RNA: ribonucleic acid

RFC: reduced folate carrier

SARAA: South African Rheumatism and Arthritis Association

SD: standard deviation

SLC: solute carrier

SNP: single nucleotide polymorphisms

SZP: sulphasalazine

TNFα: tumour necrosis factor alpha

Chapter 1: Introduction and literature review

1.1 Background

Rheumatoid arthritis (RA) is an autoimmune disease that results in a chronic, systemic inflammatory process, which may affect many tissues and organs. The disease principally attacks synovial joints. Rheumatoid arthritis is a disabling and painful condition, which leads to substantial loss of function and mobility if not adequately treated. The prevalence of the disease is between 0.9-1% in urban South Africans (1). This is similar to the worldwide prevalence in industrialized countries which is reported at between 0.5- 1.4% (2). A more recent study by Usenbo et al (65) reported a higher prevalence of up to 2.5%. Rural South African communities display a lower prevalence of 0.07% (65).

Genetic and environmental factors are implicated in the pathogenesis of RA. Human leukocyte antigen (especially HLA-DRB1) and the major histocompatibility complex are important and well-studied genetic factors in RA (3). Minor genes such as cytokine promoters and T cell-signalling genes also contribute to susceptibility and severity of RA and are thus targets for newer biological therapies (4). A key element in RA pathogenesis is increased citrullination of proteins i.e. vimentin, fibrinogen, type 2 collagen and alfaenolase, which increase the propensity for immune reactivity to the neo-epitopes created by this process. This results in the production of anti-cyclic citrullinated protein antibodies (ACPA) (5). Smoking or tobacco exposure has been shown to correlate strongly with citrullination of tissue proteins in the lung and is considered to be one of the main mechanisms involved in inducing or worsening auto-immune disease (6). A striking gene-environment interaction between HLA-DR 4 and smoking is described in patients who are ACPA positive (7). Nesse et al, describe additional citrullinated proteins which are formed in periodontitis and are similar to those formed in RA-affected tissue (8). This suggests that periodontitis induced citrullination may contribute to the aetiology of RA. Anti-cyclic citrullinated protein antibodies can actually pre-date the onset of clinical symptoms by up to ten years (5), thus creating a window of opportunity to treat early RA and prevent the formation of debilitating joint destruction. Other environmental factors implicated include infectious agents such as Epstein-Barr virus, mycobacterium tuberculosis, escherichia coli, proteus mirabilis, retroviruses and parvovirus B19 (9).

The inflammatory cascade is well studied in the pathogenesis of RA. Pro-inflammatory cytokines such as interleukin (IL) 1 and tumour necrosis factor (TNF)-α cause macrophage activation and angiogenesis in the synovial membrane. This is further promoted by the differentiation of naïve T cells into T-Helper (TH) 17 cells (66). These cytokines also have systemic effects such as the production of acute phase reactants, anaemia of chronic disease, cardiovascular disease and osteoporosis (66). Other pro-inflammatory cytokines such as IL6, IL15, IL16, IL17, IL18, interferon (IFN)-γ and granulocyte macrophage- colony stimulating factors (GM-CSF) are also found in higher levels in the joints of patients with RA. Anti-inflammatory cytokines (IL2, IL4, IL10, IL11 and IL13) are present, but are overwhelmed by the pro-inflammatory “storm” (66).

The clinical manifestations of RA include a destructive, symmetrical polyarthritis which predominantly affects the small joints. Non-articular muscular structures such as tendons, ligaments and fascia can also be affected. Extra- articular manifestations of RA can affect almost all other systems. They are associated with an increased severity of disease and are predictors of premature mortality in patients with RA (67). Anaemia is recognized as a common extra-articular manifestation of RA and often correlates with disease activity (67). Cardiovascular disease is also considered to be an extra-articular feature of RA and is associated with significant mortality (67).

Disease-modifying antirheumatic drugs (DMARDs) form the backbone of any treatment strategy of RA. These drugs induce immunosuppression which halts disease progression and thus prevents or reduces long term RA joint damage. It is widely accepted that the initiation of DMARDs should be expedited in new onset RA as this comprises the time at which significant benefit in preventing joint destruction is best achieved (10). Amongst DMARDs, MTX forms the anchor drug in any treatment strategy. It is efficacious as monotherapy and has additive effects when combined with newer biologic agents (11). The use of MTX dates back to 1948 when the anti-folate agent aminopterin was discovered to have anti-proliferative effects on connective tissue. Randomized controlled trials using low dose MTX were only performed in the 1980s. These investigations led to the advent of MTX being approved in 1986 by the United States Food and Drug Administration as a treatment for RA (12). Subsequently, MTX has been extensively studied as a monotherapy and as well as in combination with other synthetic DMARDs and biologic agents (13).

Methotrexate is favoured as a first line agent due to its low cost, rapid onset of action, ease of administration and efficacy.

1.2 Methotrexate

1.2.1 Mechanism of action

Methotrexate is a folate analogue which acts as an antagonist by competing with folate for cellular uptake and polyglutamation. It also reversibly inhibits dihydrofolate reductase (DHFR) and other folate dependent enzymes which are required for amino acid (and therefore DNA and RNA) synthesis. In its polyglutamated form it reduces the number of nucleotides (i.e. substrate) available for cellular proliferation. Polyglutamation of MTX helps to prevent its efflux from the cell and thus extends its half-life (13). Methotrexate also inhibits de novo purine synthesis by blocking the activity of 5-amino-imidazole-4-carboxamide ribonucleotide (AICAR) transformylase (13). The above mechanisms are responsible for its anti-proliferative effects.

Methotrexate also exhibits anti-inflammatory activity which is attributed to the release of adenosine. The concentration of adenosine is increased through the inhibition of adenosine deaminase via the AICAR inhibitory pathway. The release of adenosine via A2 receptors results in:

- Inhibition of leucocyte accumulation
- Reduced neutrophil-mediated endothelial injury at sites of inflammation
- Reduced TNF-α production
- Inhibition of natural killer cell, monocytes/macrophage and T-cell activities (14).

It also causes apoptosis of activated CD4 and CD8 lymphocytes –it has no impact on resting lymphocytes (14). The underlying mechanism leading to apoptosis seems to be related to the generation of reactive oxygen species. Methotrexate decreases polyamine (spermine and spermidine) production which reduces tissue injury. It also reduces adhesion molecule and cytokine production. The cytokines predominantly affected are IL1, IL4, IL6, IL13 and TNFα which are responsible for synovial inflammation (15).

1.2.2 Pharmacogenetics of MTX

The response to MTX varies both in terms of efficacy and toxicity. This variability is thought to be due to genetic factors. Pharmacogenetic studies could assist in enabling us to determine optimally tailored therapy for patients (16). Drugs are metabolized differently by patients exhibiting different polymorphisms. Some mutations lead to better efficacy, whilst others predispose to more toxicity (39).

The commonest form of genetic variation involved in MTX activity is the single nucleotide polymorphism (SNP). These polymorphisms represent a difference in a single nucleotide base within a certain region of DNA. This can result in changes in coding regions or non-coding regions of a gene. These changes then cause altered amino acid production or gene transcription. One of the most commonly studied SNP in relation to MTX is methylenetetrahydrofolate reductase (MTHFR). Methotrexate enters target cells through reduced folate carriers (SLC19A1 and RFC-1) and is effluxed from cells via ATP-binding cassettes (ABCC1–2, ABCB1 and ABCG2). In its polyglutamated form it reduces the activity of DHFR (17). Methylenetetrahydrofolate reductase is not directly targeted by MTX. However, as MTX affects the adequacy of the intracellular folate pool through its inhibition of DHFR, the activity of MTHFR can be altered by the drug and vice versa (17). Methylenetetrahydrofolate reductase is needed to produce reduced tetrahydrofolate which acts as a carbon donor for the remethylation of homocysteine to methionine. The two significant polymorphisms of MTHFR are C677T and MTHFR A1298C, both of which have been associated with reduced enzyme activity (18). The C677T variant encodes a thermolabile variant of MTHFR with decreased enzyme activity and increased homocysteine levels. These polymorphisms have been predominantly studied in European and Asian populations and show a similar frequency in these populations (38). The few studies that did include African populations noted a decreased prevalence of the C677T polymorphism (19,38).

Conflicting results were reported on the association of these SNPs with the onset of toxicity (17,18). Van Ede et al (67) specifically noted the association with the mutation and the propensity to develop a transaminitis. It was suggested by Tikly et al that a reduced occurrence of the MTHFR C677T polymorphism in Black populations is responsible for the lower rates of MTX toxicity seen in this group (19). Other genetic markers associated with toxicity include cytochrome P450 genes

(CYP20A1 and CYP39A1), solute carrier genes (SLC22A2 and SLC7A7) and the mitochondrial aldehyde dehydrogenase gene (ALDH2) (16).

A comprehensive meta-analysis by Qiu et al (18) in 2017 found that the solute carrier genotypes SLC19A1, SLC46A1 and SLCO1B1 are associated with toxicity. The SCL19A1 haplotype conferred an increased propensity towards gastrointestinal (GIT) side effects (18). Qui et al did not find the association between MTHFR C667T/ 1298A>C (adenine to cytosine) alleles and toxicity to be significant (OR 0.75, 95% CI: 0.53–1.06, Z=1.61, P=0.107 and OR 1.02, 95% CI: 0.74–1.39, Z=0.10, P=0.923, respectively). It is pertinent to note that this meta-analysis mostly reviewed studies which were conducted on European/Asian populations and thus might not be directly applicable to the South African population. The conflicting data surrounding MTHFR genomics highlights the need for further research and clarity regarding the utility of pharmacogenomics in tailored therapeutics.

1.3 Methotrexate side effects

Methotrexate side effects can be categorized as follows:

- Gastrointestinal: abdominal pain, nausea, vomiting, diarrhea, ulcerative stomatitis, glossitis, anorexia and intestinal perforation
- Hepatic: transient elevation in transaminase concentrations, fibrosis, cirrhosis and hepatic failure
- Pulmonary: interstitial pneumonitis
- Haematologic: bone marrow suppression i.e. leucopenia, thrombocytopenia and/or aplastic anaemia
- Renal: azotemia, nephropathy
- Dermatological: reddening of skin, fatal skin reactions, nodulosis, rash or alopecia
- Central nervous system: headache, dizziness, light headedness, mood alteration and insomnia
- Infections: opportunistic infections(20)

1.3.1 GIT side effects

Gastrointestinal side effects occur commonly with MTX. They are reported more often during the first two years of treatment. They include abdominal discomfort, dyspepsia, nausea and anorexia. The reported incidence of GIT side effects is 52-65%; however, this is noted in a predominantly Caucasian population (21). Gastrointestinal side effects seem to be dose dependent and therefore could be reduced by a dose reduction of MTX. Pharmacogenomic studies suggest that patients with the SCL19A1 haplotype are at an increased risk for developing GIT toxicity.

Folate deficiency occurs frequently in patients with RA and is further compounded by the administration of MTX. This leads to depleted intracellular folate stores in hepatocytes and peripheral blood lymphocytes. Ortiz et al demonstrated that folate supplementation reduced the incidence of GIT side effects, elevated transaminases and stomatitis (22). There is no consensus on the optimal dose and timing of folate administration. Variable regimens have been used by different centers with some giving folate at dose of 1mg/day, some giving it at 5mg/day and some giving it at any dose between 5-25mg/week. There is however agreement that folate should not be given on the same day on which MTX is administered. A recent study by Lui et al (23) in 2018 compared high dose (>25mg/week) folate to low dose (<10mg/week) folate and found no significant difference in the prevalence of toxicities encountered. In addition, folate supplementation appeared to enhance patient compliance to MTX (by virtue of reducing side effects) when compared to placebo (23). This conclusion was based on studies looking at both high and low dose folate supplementation (23).

1.3.2 Hepatic side effects

Elevation of transaminase concentrations account for the most common hepatic toxicity encountered. This may lead to discontinuation of the drug in some patients. Liver enzymes are elevated in 15-50% percent of patients on low dose MTX (doses between 8-15mg/week); however, only about 5% develop a transaminitis which is greater than twice the upper limit of normal (73). Reducing the dose resolves the transaminitis, however, in some cases it resolves without any changes in dosing. This variability maybe related to the timing of blood sampling in relation to the day on which MTX and folate is given. Ideally blood should be sampled six days after the

weekly dose just prior to the next dose. Hepatic folate stores are depleted by MTX. Although a relationship between folate depletion and hepatotoxicity has not been well established, folate supplementation is associated with a reduced incidence of transaminitis (23).

Hepatic fibrosis and cirrhosis are rare complications and are more likely to occur in patients with other risk factors, i.e. alcohol usage, pre-existing liver disease, concomitant use of other hepatotoxic drugs, infection with hepatotropic viruses and obesity-often in association with non-alcoholic fatty liver disease. Hepatic fibrosis/cirrhosis is quantified on liver biopsies. Previously, surveillance biopsies were recommended for patients who had pre-existing liver disease or who had elevated liver enzymes on five serial measurements. These recommendations were based on findings from a dermatological series done in patients with psoriatic arthritis. It estimated the incidence of fibrosis to be up to 26%. Subsequently, they have been discarded, as newer literature suggested a much lower incidence of between 0.9-1.3% (25,26).

Other risk factors for hepatotoxicity include hyperlipidemia, large cumulative doses of MTX, lack of folate supplementation, diabetes and a family history of an inheritable liver disease (24). Numerous studies have demonstrated a strong correlation between alcohol use and hepatotoxicity, suggesting an increased risk of almost 2.5-5 fold when used concurrently with MTX therapy (24,27,28). The mechanism by which MTX causes hepatotoxicity is unclear, but recent studies have suggested that MTX induced hepatic fibrosis is mediated through an adenosine pathway. Hypotheses suggest that MTX activates hepatic stellate cells which then form into myelofibroblasts and secrete collagen and fibronectin (24). The conversion of MTX to a polyglutamated form is also thought to prolong its accumulation in the cell, which in turn results in prolonged folate depletion (24). No SNPs were demonstrated to exclusively cause hepatotoxicity.

1.3.3 Pulmonary side effects

The prevalence of lung toxicities on MTX from previous literature is estimated to be 2-7% (29). Methotrexate associated lung disease can be classified as infective, MTX related interstitial lung disease or MTX induced chronic fibrosis. One of the most severe pulmonary complications is a pneumonitis which is thought to be a hypersensitivity reaction mediated by activated T-cells (30). In recent reports MTX

related pneumonitis is exceedingly uncommon and in fact no longer identified in 13 studies reported after 2002 (29). A lung biopsy or broncho-alveolar lavage is useful in showing interstitial inflammation, fibrosis, giant cells, tissue eosinophils and increased macrophages (31). Methotrexate can also stimulate type 2 alveolar cells to produce cytokines which causes an alveolitis. Patients with RA are more prone to develop Epstein Barr virus secondary to an acquired immunodeficiency and thus may be at an increased risk of developing lymphoproliferative disorders. An analysis done by Thaniyan et al (31) proposed that MTX might be directly toxic to lung parenchyma or that concurrent infection with certain viruses, i.e. Pneumocystis Jiroveci, may predispose to developing toxicity.

Risk factors for developing pulmonary MTX toxicity include low body weight, concomitant interstitial lung disease, hypoalbuminemia, older age, diabetes and renal impairment causing decreased elimination of the drug (69). Smoking has not been implicated in causing increased incidence of lung toxicities (32).

1.3.4 Haematologic side effects

These include leucopenia, thrombocytopenia, megaloblastic anaemia and pancytopenia. The prevalence of cytopenias from previous studies is estimated to be 3-7% (33-35).

The risk factors frequently associated with this toxicity are renal insufficiency, hypoalbuminemia and concurrent infection (36). The concomitant exposure to other drugs such as non-steroidal anti-inflammatories (NSAIDS), angiotensin-converting enzyme (ACE) inhibitors, proton pump inhibitors and beta-blockers has been highlighted as causative factors for developing pancytopenia (37). Pancytopenia typically occurs after long term use, which is suggestive of a cumulative effect. However, it can also occur early in the course of treatment and it is then considered to comprise an idiosyncratic reaction.

Patients who are homozygous for the C677T MTHFR polymorphism (which has a frequency of 0.07 in Sub-Saharan Africans) (38) have a higher incidence of toxicities including elevated ALT values, mucositis and delayed platelet recovery. Urano et al demonstrated that 27% of patients with the polymorphism developed adverse effects ($p<0.05$) (39). Neutropenia following MTX therapy was also described by Toffoli et al as being more common in patients with the 677TT polymorphism (37). The presence of stomatitis was shown to be associated with pancytopenia and accordingly often

accompanies or precedes it. This is probably due to the fact that the mechanism of these toxicities i.e. folate antagonism, are similar (40).

1.3.5 Renal side effects

Methotrexate is excreted predominantly by the kidneys. Glomerular filtration is responsible for about 50-80% of MTX clearance. Impaired clearance is responsible for higher circulating MTX levels and is therefore strongly associated with increased toxicity. In low doses, MTX does not usually cause direct nephrotoxicity, but in high doses it is capable of causing direct tubular injury and vasoconstriction of the afferent arteriole (30). End stage renal disease is a contraindication to MTX use as it results in profound bone marrow suppression due to impaired clearance.

1.3.6 Dermatological side effects

Muco-cutaneous side effects with MTX are rare and range from mild to severe. They comprise of stomatitis, hypersensitivity reactions, pruritus, urticaria, ecchymosis, reversible alopecia, toxic epidermal necrolysis and Stevens-Johnson syndromes (41). Methotrexate also accelerates the development of nodulosis through stimulation of adenosine A1 receptors which enhances giant cell formation. Stomatitis is a common toxicity which occurs in 5 to 30% of patients (33). The mechanism underlying this toxicity is thought to be related to cytotoxic T-lymphocytes and monocytes. These cells induce apoptosis in keratinocytes that express drug derived antigens at their surface (30). Alopecia occurs in about 27% of patients, but is somewhat reversible on discontinuation of the drug (42).

1.3.7 Central nervous system (CNS) side effects

Severe headaches, fatigue and cognitive dysfunction can occur acutely after ingestion of MTX. The prevalence of CNS side effects is estimated to be up to 36% (42). Development of neurotoxicity may be linked to the accumulation of adenosine due to the inhibition of purine synthesis (43). Advanced age and renal impairment are predisposing risk factors for CNS toxicity with MTX. The spectrum of neurotoxicity increases greatly when MTX is administered intravenously or intrathecally. However, these modes of administration are not used in the management of RA.

1.3.8 Infections including opportunistic pathogens

Opportunistic infections can occur more commonly in patients using MTX (44). The musculoskeletal, respiratory and urogenital systems are affected most frequently.

The prevalence of infections secondary to MTX usage is about 25% (42). In a South African context, the high prevalence of tuberculosis (70) is particularly concerning and must be screened for prior to initiation of MTX therapy. The risk of developing an infection is increased with the concomitant usage of other immunosuppressive agents i.e. glucocorticoids, synthetic DMARDs or biologic DMARDs.

1.4 MTX drug monitoring

South African Rheumatism and Arthritis Association (SARAA) recommends a baseline chest x-ray (CXR) and x-rays of the hands and feet prior to commencing DMARDs. The CXR is used to document the presence of RA associated lung disease, exclude tuberculosis and can also be used as a comparator for evaluation of possible pulmonary toxicities. SARAA recommends that a full blood count (FBC) and liver function test (LFT) is done within the first month of therapy and then repeated at three to six month intervals (45). In practice, more frequent testing is done if a dose adjustment has been made or any suggestion of toxicity noted. It is likely that upcoming guidelines will reflect this practice. This would be in line with the European League against Rheumatism (EULAR) guidelines which recommend more frequent testing, particularly in patients with active disease. Human immunodeficiency virus (HIV), hepatitis B and hepatitis C are also screened for prior to initiating DMARDs. Inactivated vaccines are administered (depending on age and risk) at least 14 days prior to starting treatment. Annual lipid and serum creatinine measurements are performed.

1.5 Current practices

Conventional DMARDs remain the mainstay of treatment for RA patients in the South African public sector due to their limited cost and the suboptimal availability of the newer biologic agents. Methotrexate is the first line agent employed in this setting. EULAR recommends an initiation dose of 15mg per week, which is then rapidly escalated (no time period given in literature) to a maximum dose of 25-30mg per week depending on the patient's clinical response and toxicity profile (46). At initiation, MTX is combined with oral glucocorticoids, as a bridging therapy, which is continued for up to six months and then tapered. At six months, patients are re-evaluated to assess if targets, i.e. low disease activity or remission have been achieved. When this is not the case, patients are then escalated on to a second

synthetic or biologic DMARD depending on their prognostic risk factors and drug availability (47). Up to two-thirds of patients on standard combinations of DMARDs fail therapy (45).

It is unusual at this point to increase the dose of MTX further in order to achieve the therapeutic target. However, this option of higher dose MTX has been utilized by rheumatologists in previous studies (48). Since the year 2000, newer insights into the efficacy of higher dose methotrexate therapy have resulted in the utilization of MTX at doses which are more than those recommended in the EULAR recommendations (49-51). Since 2006, patients at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) have been treated with high dose MTX in keeping with this trend. Literature reviews in Black South Africans published thus far have not documented the efficacy or toxicity of this strategy. However, anecdotally, there has been no increase in the prevalence of side effects reported.

Our study aims to document the prevalence of side effects on standard and higher doses of MTX as well as the risk factors that contribute to their development. It is beyond the scope of this study to document the efficacy of high dose MTX in our setting and this will need to be addressed in a further study. We examine the clinical premise in current practice that high dose MTX, in South African patients, does not increase the prevalence of toxicity or side effects when guided by clinician judgement of disease activity and current tolerability/toxicity. If this is proven to be the case, using this strategy could be a viable, cost effective means of managing these RA patients attending public health care facilities. It would likely also reduce the number of patients requiring access to biological therapy which is not readily accessible in the state sector.

Chapter 2: Materials and methods

2.1 Study objectives

The specific objectives of this study comprise:

1) Documenting the nature and prevalence of side effects related to MTX

2) Identifying risk factors for the development of MTX toxicity

3) To determine whether higher doses of MTX are associated with an increase in toxicity

2.2 Study design and site

This was a single centre retrospective chart review of consecutively seen RA patients. The study was conducted at the Department of Rheumatology in CMJAH. This hospital is a quaternary level academic institution, which services a large population of racially and ethnically diverse patients. The predominant ethnicity of the population in the area are Black patients, followed by White and then Indian patients.

2.3 Study population and size

2.3.1 Sample size

A sample size was calculated as an average based on the population sizes of previous, similar studies which reported on the prevalence of MTX side effects (33,36,44,52-54,56,60,77-80). The minimum and maximum numbers from these studies were 70 and 609 respectively. Two hundred charts were subsequently reviewed. These charts were selected in a non-biased fashion. Consecutive charts were chosen upon patient presentation to the Monday, Thursday or Friday RA clinics (no RA clinics on other days). Data collection commenced upon approval of the protocol by the Human Research Ethics Committee (medical) and continued until the sample size was obtained.

2.3.2 Inclusion criteria

Consecutively seen patients, who met the 1987 ACR or 2010 EULAR/ACR criteria for classification of RA, had received MTX for the treatment of RA and were over 18 years of age.

2.3.3 Exclusion criteria

There were no exclusion criteria. All patients screened were receiving/ had received MTX for the treatment of RA.

2.4 Methods

The charts selected from the clinics for data collection were reviewed. All patients selected were on treatment for RA and did not have other overlap/secondary connective tissue diseases. All variables were systematically recorded at three time intervals- initial presentation to the clinic and commencement of MTX therapy, at the time toxicity developed and at the most recent visit to the clinic which coincided with data collection. If the patient screened did not develop toxicity, variables were recorded at the time of presentation to the clinic and at the time of the most recent visit.

The variables which were chosen for analysis were based on previously reported associations of toxicity with MTX. In the files reviewed, toxicity was documented based on the clinical assessment made by the attending doctor at the time of presentation to the clinic.

The following variables were recorded:

1) Patient characteristics:

- Demographics: age, sex and ethnicity

- Lifestyle parameters: smoking, alcohol consumption and exercise (exercise was assessed as a yes/no question based on the patients subjective answer as to whether they participated in exercise regularly or not)

- Metabolic variables: hypertension, diabetes, random glucose (mmol/L), waist circumference (cm), BMI (kg/m^2), total cholesterol (mmol/L), low density lipoprotein (mmol/L), high density lipoprotein (mmol/L) and triglycerides (mmol/L)

  Anthropometric measurements (waist circumference and weight) were measured by trained nursing staff and recorded in files

- Co-morbidities: interstitial lung disease, ischaemic heart disease, cerebrovascular disease, peripheral arterial disease and renal dysfunction (eGFR<60 ml/min/1.73m^2)

2) Concomitant DMARD usage: azathioprine, sulphasalazine (SZP), chloroquine, leflunomide, cyclophosphamide, glucocorticoids and NSAIDs

3) MTX dosing: highest dose of MTX, current dose of MTX and dose at which toxicity occurred

Toxic events screened for included:

- Haematologic: white cell count (WCC) <3.0x10^9/L, haemoglobin <12g/dL in males and haemoglobin < 10g/dL in females and platelets <100x10^9L

A lower WCC cut off for leucopenia was used compared to the National Health Laboratory Service norm of less than 4.0x10^9/L. This value was based on recent literature by Lim et al which sanctioned a lower WCC in healthy African patients due to certain genetic predispositions (55).

- Hepatic: ALT/AST > 2 times upper limit of normal (>70U/L) and ALT/AST > 3 times upper limit of normal (>105U/L)

MTX commonly causes a transaminitis which may be persistent or self-limiting. These cut offs were selected based on previous studies examining MTX hepatotoxicity (26,53,56). Liver enzymes which are elevated more than 3 times the upper limit of normal is an indication to stop therapy (as per the 3E initiative) (57).

- Gastrointestinal: nausea, abdominal pain, stomatitis, dyspepsia and diarrhoea

- Dermatological: rash, alopecia, nodulosis, reddening of skin and fatal skin reactions

- Pulmonary: cough and pneumonitis

- Infections: upper respiratory tract infections, lower respiratory tract infections, soft tissue infections, urinary tract infections and opportunistic infections

  Data pertaining to infections was retrospectively recorded in the patient charts.

- Neurological: dizziness, headache, mood alteration, nightmares and light headedness

- Renal: moderate or chronic kidney disease, i.e. estimated GFR less than or equal to 60ml/min/1.73m^2

A sample data collection sheet reflecting the variables recorded is attached (Appendix 1). All variables, including anthropometric measurements and blood results, were recorded at all three time intervals as per the information available in the clinical charts. Anthropometric measurements were routinely checked by nursing sisters at every visit. If a patient did not develop toxicity, then variables were recorded at entry and exit only. Duration and timing of concomitant DMARDs were systematically captured in some files and had to be calculated manually from others.

2.5 Statistical analysis

Data was collected manually using the data collection sheet and then transcribed onto a Microsoft® Excel spreadsheet. Data was expressed as mean (standard deviation [SD]), median (interquartile range [IQR]) or percentages as appropriate. Age and gender adjusted associations of recorded variables other than MTX with toxicity characteristics were determined in multivariable regression models. Other characteristics were entered into separate models as indicated based on context. Those factors that were associated in age and sex adjusted models with toxicity were entered together with MTX variables in a single model with consideration of collinearity, interactions and cause-effect relations. Statistical analysis was performed using IBM SPSS statistics program (v.23.0, IBM, USA). A p-value of <0.05 was considered significant. Linear variables used in the analysis were all normally distributed, as determined by standard statistical analysis using the afore-mentioned program. Multivariable logistical regression analysis was used to analse the data for variables which were dichotomous.

2.6 Study limitations

Limitations of this study included:

- Incomplete record keeping which resulted in certain variables being omitted from the analysis
- Toxicity was described based on the attending doctors discretion and recorded as such
- Due to the retrospective nature of the study, selection and information bias may have impacted the accuracy of certain elements of the study

- This was a single centre study carried out at a public institution and thus patient demographics might be skewed to reflect the population residing in the hospital drainage area.

Chapter 3: Results

3.1 Patient demographics

A total number of 200 patients were reviewed in this study. Patient demographics are shown in Table 3.1. There were 172 (86%) women and 28 (14%) men. The mean age at entry to the clinic was 50.9 (11.2) years and the mean age at the time of data collection 58.3 (11.8) years. The mean age at the time toxicity developed was 55.1 (9.7) years and the mean duration of treatment at the time toxicity developed was 6 (6.3) years. There were 132 (66%) Black patients, 46 (23%) White patients and 22 (11%) Indian patients captured.

Table 3.1 Demographics of 200 RA patients

	n (%)	Mean age (years) mean ± SD
Total number	200 (100)	
Male	28 (14)	
Female	172 (86)	
Age		
At entry		50.9 (11.2)
At toxicity time		55.1 (9.7)
At exit		58.3 (11.8)
Race		
Black	132 (66)	
White	46 (23)	
Indian	22 (11)	
Years on MTX before toxicity		6 (6.3)
Abbreviations: MTX: Methotrexate		

3.2 Lifestyle parameters and co-morbidities

The lifestyle parameters and co-morbidities present in our cohort are given in table 3.2. Cigarette smoking and alcohol usage were present in less than 20% of the population studied and decreased over time. Hypertension was present in 117 (59%) patients and diabetes in 36 (18%) patients at the time of data collection. The prevalence of comorbidities such as ischaemic heart disease, cerebrovascular disease, peripheral arterial disease or interstitial lung disease was low compared to that of the general population. Renal impairment developed in 16 patients during the course of their treatment. The data pertaining to lifestyle parameters in patients who developed toxicity was omitted from the tables as they were not consistently documented and therefore not suitable for analysis. Missing data relating to the above variables and anthropometric measurements occurred in over 50% of patients and thus was appropriately not included in the analysis.

Table 3.2 Lifestyle parameters and co-morbidities in 200 RA patients

	Entry n (%)	Exit n (%)
Smoking	36 (18)	26 (13)
Alcohol	30 (15)	12 (6)
Interstitial lung disease	0 (0)	4 (2)
Ischaemic heart disease	1 (0.5)	6 (3)
Cerebrovascular accident	4 (2)	4 (2)
Peripheral vascular disease	1 (0.5)	4 (2)
eGFR<60ml/min/1.73m^2	6 (3)	22 (11)
Hypertension	74 (37)	117 (59)
Diabetes	20 (10)	36 (18)
Abbreviations: eGFR: estimated glomerular filtration rate		

3.3 Antirheumatic agents other than MTX

The simultaneous administration of methotrexate with other DMARDs and NSAIDS is depicted in table 3.3. Chloroquine, glucocorticoids and NSAIDs were often co-prescribed at the initiation of treatment (at entry) and no patients were on DMARDS prior to starting treatment with MTX. All patients were MTX naïve at entry. Sulphasalazine (SZP) and leflunomide prescription was increased at exit compared to entry, reflecting either an escalation of therapy or alternate drug use due to toxicity.

Table 3.3 Other antirheumatic drugs in 200 RA patients

	Entry n (%)	Exit n (%)
Azathioprine	2 (1)	4 (2)
Sulphasalazine	16 (8)	53 (26.5)
Chloroquine	140 (70)	88 (44)
Leflunomide	2 (1)	27 (13.5)
Glucocorticoids	31 (15.5)	4 (2)
NSAIDs	79 (39.5)	11 (5.5)
Abbreviations: NSAIDS: non - steroidal anti - inflammatory drugs		

3.4 Methotrexate dosing

The spectrum of MTX dosing is depicted in table 3.4. The mean highest dose of MTX received during treatment was 28.7 (9.6) mg. Doses higher than 30mg were prescribed to 45 (22.5%) patients of whom 12 (26.7%) developed toxicity. The average dose at the time of toxicity was 29.3 (9.3) mg. Of the 200 patients reviewed, 186 (93%) were still receiving MTX at the time of data collection with a mean current dose of 19.5 (7.8) mg. Of the 14 patients who were no longer on MTX, 10 were due to toxicity, 1 developed interstitial lung disease and 3 were unclear due to missing information.

Table 3.4 MTX dosing in 200 RA patients

	n (%)	Mean dose ±(SD)
Average highest dose of MTX		28.7 (9.6)
Average current dose MTX		19.5 (7.8)
Average dose at the time of toxicity		29.3 (9.3)
MTX dose > 30mg/week	45 (22.5)	
Toxicity on doses >30mg/week	12 (26.7)	
Currently still on MTX	186 (93)	
Abbreviations: MTX: Methotrexate		

3.5 Prevalence of side effects

The prevalence of side effects is illustrated in descending order in table 3.5. Overall toxicity was recorded in 50 (25%) patients. However, 60 toxic events were recorded in these 50 patients. The most prevalent side effect was leucopenia, which occurred in 19 (9.5%) patients. Transaminitis occurred in 17 (8.5%) patients. Stomatitis occurred in 9 (4.5%) patients and nausea in 6 (3%) patients. Anaemia was observed in 3 (1.5%) patients. Other side effects were uncommon.

Table 3.5 Prevalence of side effects in 200 RA patients

	n (%)
Leucopenia	19 (9.5)
Transaminitis	17 (8.5)
ALT/AST>2x ULN	15 (7.5)
ALT/AST>3x ULN	2 (1)
Stomatitis	9 (4.5)
Nausea	6 (3)
Anaemia	3 (1.5)
Low platelets	1 (0.5)
Abdominal pain	1 (0.5)
Alopecia	1 (0.5)
Upper respiratory tract infection	1 (0.5)
Lower respiratory tract infection	1 (0.5)
Diarrhoea	0 (0)
Rash	0 (0)
Cough	0 (0)
Pneumonitis	0 (0)
Soft tissue infections	0 (0)
Urinary tract infection	0 (0)
Dizziness	1 (0.5)
Headache	0 (0)
Insomnia	0 (0)
Nightmares	0 (0)
Any side-effect	50 (25)
Abbreviations: ALT: alanine transaminase, AST: aspartate aminotransferase, ULN: upper limit normal	

3.6 Potential risk factors associated with leucopenia

Potential risk factors other than MTX that were associated with leucopenia are given in table 3.6. The only non-modifiable predictor associated with leucopenia was entry age (OR 0.96, 95%CI: 0.91-0.99; p=0.03). Concomitant sulphasalazine use at entry (OR 3.76, 95%CI: 1.08-13.09; p=0.03) or at exit (OR 2.80, 95%CI: 1.07-7.34; p=0.03) was associated with an increased risk of developing leucopenia. Anaemia (OR 21.18, 95%CI: 1.83-245.75; p=0.01) was similarly linked to the development of a leucopenia.

Table 3.6 Associations of characteristics other than MTX with leucopenia in 19 patients with RA

	Mean ± (SD)	n (%)	odds ratio[95%CI]	p-value
Entry age, year	**45.8 (12.8)**		**0.96 [0.91-0.99]**	**0.03**
Exit age, year	55.7 (12.6)		0.93 [0.94-1.02]	0.3
Male		2 (10.5)	0.67 [0.15-3.07]	0.6
Indian race		2 (10.5)	0.95 [0.20-4.47]	0.9
African race		15 (78.9)	2.05 [0.65-6.44]	0.2
Caucasian race		2 (10.5)	0.37 [0.08-1.65]	0.2
Smoking at entry		2 (11.5)	0.80 [0.15-4.23]	0.8
Smoking at exit		1 (5.3)	0.39 [0.05-3.14]	0.4
Alcohol usage at entry		1 (5.3)	0.31 [0.04-2.40]	0.3
eGFR<60ml/min/1.73m^2 at exit		1 (5.3)	0.39 [0.05-3.05]	0.4
Hypertension at entry		4 (21.1)	0.41 [0.13-1.28]	0.1
Hypertension at exit		12 (63.2)	1.17 [0.44-3.11]	0.8
Diabetes at entry		1 (5.3)	0.44 [0.06-3.51]	0.4
Diabetes at exit		2 (10.5)	0.50 [0.11-2.26]	0.4
Azathioprine at exit		1 (5.3)	3.30 [0.33-33.36]	0.3
Sulphasalazine at entry		**4 (21.1)**	**3.76 [1.08-13.09]**	**0.03**
Sulphasalazine at exit		**9 (47.4)**	**2.80 [1.07-7.34]**	**0.03**
Chloroquine at entry		12 (63.2)	0.71 [0.27-1.90]	0.5
Chloroquine at exit		7 (36.8)	0.72 [0.27-1.91]	0.5
Glucocorticoids at entry		4 (21.1)	1.52 [0.47-4.93]	0.5
NSAIDs at entry		9 (47.4)	1.43 [0.55-3.09]	0.5
Anaemia		**2 (10.5)**	**21.18 [1.83-245.75]**	**0.01**
Nausea		2 (10.5)	5.21 [0.89-30.53]	0.07
ALT/AST> 2times ULN		1 (5.3)	0.57 [0.07-4.58]	0.6
Stomatitis		1 (5.3)	1.20 [0.14-10.16]	0.4
Abbreviations: ALT: alanine transaminase, AST: aspartate aminotransferase, ULN: upper limit normal, NSAID: non - steroidal anti – inflammatory drugs, eGFR: estimated glomerular filtration rate				
*highlighted variables are significant				

3.7 Associations of MTX dosing with leucopenia

As shown in table 3.7, in the patients who developed leucopenia, the average highest MTX dose was 30.1 (9.6) mg (OR 1.02, 95%CI: 0.97-1.07; p=0.5). Only 6 (31%) patients in this group were receiving doses of greater 30mg/week. The highest dose of MTX used was not associated with leucopenia. This was assessed in a regression model and adjusted for age at entry, sex and race. Despite developing toxicity, 16 (84%) patients remained on MTX with an average current dose of 14.7 (8.2) mg (OR 0.93, 95%CI: 0.88-0.98; p=0.007). The current MTX dose was lower in patients who had developed leucopenia compared to those who had not developed this side effect.

Table 3.7 Associations of MTX dosing with leucopenia in 19 RA patients

	Mean ± (SD)	n (%)	odds ratio[95%CI]	p-value
Highest MTX dose	30.1 (9.6)	-	1.02 [0.97-1.07]	0.5
Highest MTX dose*		-	1.00 [0.95-1.07]	0.9
MTX dose>30mg/week		6 (31)	1.68 [0.60-4.71]	0.3
MTX dose > 30mg/week*		-	1.48 [0.52-4.24]	0.5
Still on MTX after toxicity		16 (84)	0.35 [0.09-1.37]	0.1
Currently on MTX dose	14.7 (8.2)	-	0.93 [0.88-0.98]	0.007
Abbreviations: MTX: Methotrexate				
*adjusted for age at entry, sex and race				

3.8 Potential risk factors associated with transaminitis

Potential risk factors other than MTX associated with transaminitis are given in table 3.8. Age was the only non-modifiable predictor associated with this, but was not significant (OR 0.96, 95%CI: 0.91-1; p=0.06). Alcohol usage at entry, which is an established risk factor for developing transaminitis, was present in 4 (23.5%) patients (OR 2.25, 95%CI: 0.66-7.6). This association was not significant (p=0.2). Gastrointestinal side-effects that are postulated to have the same underlying mechanism as liver toxicity occurred in 5 patients (nausea in 2 and stomatitis in 3 patients) with odds ratios of 5.97, 95%CI: 1.01-35.29; p=0.04 and 6.32, 95%CI: 1.43-28.2; p=0.01, respectively. Concomitant Leflunomide usage was also significant and occurred in 8 patients (OR 7.67, 95%CI: 2.6-22.2; p=<0.0001). Notably, only one patient developed both a transaminitis and a leucopenia. Accordingly, transaminitis was not associated with leucopenia.

Table 3.8 Associations of characteristics other than MTX with transaminitis in 17 RA patients

	Mean ± (SD)	N (%)	odds ratio[95%CI]	p-value
Entry age, year	45.8 (10.4)	-	0.96 [0.91-1.00]	0.06
Exit age, year	58.5 (11.4)	-	1.00 [0.96-1.05]	0.9
Male		1 (5.9)	0.35 [0.04-2.72]	0.3
Indian race		2 (11.8)	1.09 [0.23-5.10]	0.9
African race		11 (64.7)	0.94 [0.33-2.66]	0.9
Caucasian race		4 (23.7)	1.03 [0.32-3.34]	1.0
Smoking at entry		3 (17.6)	1.04 [0.28-3.9]	0.9
Alcohol usage at entry		4 (23.5)	2.25 [0.66-7.69]	0.2
eGFR<60ml/min/1.73m^2 at exit		1 (5.9)	0.45 [0.06-3.54]	0.4
Hypertension at entry		3 (17.6)	0.33 [0.09-1.18]	0.3
Hypertension at exit		7 (41.2)	0.44 [0.16-1.20]	0.1
Diabetes at exit		3 (17.6)	0.96 [0.26-3.57]	0.9
Leflunomide usage at exit		**8 (47.1)**	**7.67 [2.65-22.24]**	**<0.0001**
Glucocorticoids at entry		2 (11.8)	0.71 [0.15-3.26]	0.7
Glucocorticoids at exit		1 (5.6)	3.75 [0.37-38.17]	0.3
NSAIDs at entry		7 (41.2)	1.08 [0.39-2.96]	0.9
NSAIDs at exit		1 (5.9)	1.07 [0.13-8.89]	1.0
Leucopenia		1 (5.9)	0.57 [0.07-4.58]	0.6
Nausea		**2 (11.8)**	**5.97 [1.01-35.29]**	**0.04**
Stomatitis		**3 (17.6)**	**6.32 [1.43-28.2]**	**0.01**
Abbreviations: eGFR: estimated glomerular filtration rate, NSAID: non - steroidal, anti - inflammatory drugs				
*highlighted variables are significant				

3.9 Associations of MTX dosing with transaminitis

As shown in table 3.9, the approximate highest dose of MTX in patients who developed a transaminitis was 30.6 (7.8) mg with 5 (29.4%) patients receiving doses of greater than 30mg/week. This was assessed in a regression model and adjusted for age at entry, sex, race and alcohol usage at entry. Thirteen patients (OR 0.19, 95%CI: 0.05-0.68; p=0.01) remained on MTX despite toxicity, at an average dose of 14.7 (9.6) mg per week. This weekly dose was lower than in those patients who had not developed a transaminitis (OR 0.93, 95%CI: 0.88-0.98; p=0.01).

Table 3.9 Associations of MTX dosing with transaminitis in 17 RA patients

	Mean ± (SD)	n (%)	odds ratio[95%CI]	p-value
Highest MTX dose	30.6 (7.8)	-	1.02 [0.97-1.07]	0.4
Highest MTX dose*		-	1.02 [0.97-1.07]	0.5
Highest MTX dose**		-	1.04 [0.98-1.10]	0.2
MTX dose>30mg/week		5 (29.4)	1.49 [0.50-4.43]	0.5
MTX dose>30mg/week*		-	1.47 [0.47-4.62]	0.5
MTX dose>30mg/week**		-	2.87 [0.80-10.23]	0.11
Currently on MTX after toxicity		13 (76.5)	0.19 [0.05-0.68]	0.01
Current MTX dose, mg/week	14.7 (9.6)	-	0.93 [0.88-0.98]	0.01
Abbreviations: MTX: Methotrexate				
*adjusted for age at entry, sex and race, ** additionally adjusted for alcohol use at entry				

To increase the statistical power of this study we combined the patients who developed a transaminitis or leucopenia (Table 3.10) and found that none of the MTX dosing characteristics were associated with developing these toxicities.

Table 3.10 Associations of MTX dosing with transaminitis and/or leucopenia in 35 patients with RA

	Odds ratio[95% CI]	p-value
Highest MTX dose	1.02 [0.98-1.06]	0.4
Highest MTX dose*	1.01 [0.97-1.05]	0.5
Highest MTX dose**	1.03 [0.99-1.08]	0.16
MTX dose>30mg/week	1.49 [0.65-3.38]	0.4
MTX dose>30mg/week*	1.36 [0.57-3.21]	0.5
MTX dose>30mg/week**	2.12 [0.84-5.38]	0.11
Abbreviations: MTX: Methotrexate		
*adjusted for age at entry, sex and race, ** additionally adjusted for alcohol use at entry		

Chapter 4: Discussion

The use of MTX plays a pivotal role in arresting disease progression in RA. Historically there have been issues regarding the toxicity profile of this drug. Our study demonstrated a 25% prevalence of toxicity in patients using MTX at doses between 15mg-50mg/week. This was substantially less than the prevalence noted by other studies (21,77) which documented the prevalence to be between 72-90%. It is pertinent to note that most of the literature we reviewed examined prevalence of toxicity in low doses of MTX i.e. doses between 8-15mg/week. A prevalence of 24.5% was also demonstrated by Dubey et al in 2016 in a similar study reviewing hepatic and haematological toxicities on low dose MTX (58). Most noteworthy, is that the prevalence of toxicity was similar (if not less) to that of other studies, despite higher doses being used by the patients in our study.

The frequency of documented toxicities in descending order were; leucopenia (9.5%), transaminitis (8.5%), stomatitis (4.5%) and nausea (3%). This was in contrast to other studies which documented gastrointestinal side effects as being the most prevalent, followed by transaminitis, with leucopenia being the least prevalent (21,33,58). Racial and ethnic variants have been described with regards to the MTHFR SNP (19). A number of MTHFR variants have been identified but the two most researched and understood are 677C>T and 1298A>C. The final expression and influence of these variants are affected by genotype and race/ethnicity. An estimated 50% of a population may inherit one copy (heterozygous C/T) whilst up to 25% may inherit two copies (homozygous T/T). The prevalence of this polymorphism appears to be greater in Italians, Hispanics and Asians than in Africans and Caucasians (38). The frequency in a South African White population is about 28% as opposed to the Black population where it is only present in about 4% (19). A study by Scholtz et al (71) also showed that South African Black patients do not demonstrate homozygosity for this mutation. This polymorphism has been postulated to relate to toxicities on MTX. To date the literature is still conflicting to demonstrate this theory conclusively. It is therefore possible that these genes could account for this discrepancy.

The racial distribution of our study's population was similar to that of the South African population. The predominant ethnicity was Black (66%), followed by White (23%) and Indian (11%) patients. The most recent South African statistics reflect an

average of 80% Black, 10% White and 3% Indian patients. Of note, all data analysed was adjusted for race. Lifestyle choices, particularly smoking and alcohol usage are often implicated in the pathogenesis of disease. Smoking has not been directly implicated in the development of toxicity on MTX; however it could certainly negatively contribute to the increased cardiovascular risk of patients with RA. Smoking has also been associated with ACPA positive disease which is a poor prognostic factor in RA and is associated with more severe disease. Alcohol usage whilst on MTX is a known risk factor for the development of hepatotoxicity (27). Alcohol usage was documented in 15% of patients at entry and 6% at exit. Alcohol usage was distinctly uncommon, in keeping with previous reports by Solomon et al which reviewed cardiovascular risk profiles in black patients with RA (59). Bearing in mind the small numbers, our analysis did not confirm it to be an association with the development of a transaminitis (OR 2.25, 95%CI: 0.66-7.69; p=0.2). The amount of alcohol ingested per day was not quantified in the study and thus inferences about a "safe" daily limit could not be made. A study by Humphreys et al (27) suggested that mild to moderate alcohol usage (<14 units/week) while on MTX was not associated with an increased frequency of transaminitis. This study, however, did not examine the dose of MTX prescribed and it is assumed that the majority of patients were receiving low dose MTX.

Patient co-morbidities often limit therapeutic options or DMARD usage. Renal dysfunction has been well described in previous literature as being a strong predictor of developing toxicity-particularly cytopenias (56). Our cohort comprised of 6 patients with a reduced eGFR<60ml/min at entry and 22 at exit. Only one patient with renal dysfunction developed a leucopenia. This unexpected result could be attributed to the fact that patients who did have renal dysfunction were often subjected to a dose reduction and carefully monitored and thus did not develop toxicity. Another possible explanation is that the majority of patients in our study who had renal dysfunction, did not have an eGFR<30ml/min. Methotrexate is predominantly excreted via glomerular filtration. Its pharmacokinetics can be altered in patients with renal impairment and it must be used cautiously in these patients. Diabetes has been well described in literature as having a strong association with the development of a transaminitis (60). Of the patients we studied, 18 were diabetic, yet this association was not apparent in our results.

4.1 Cytopenias

A WCC less than $3x10^9$/L was used in this study to define leucopenia. This is lower than the traditional cut off used by NHLS of $4x10^9$/L, but in accordance with recent literature sanctioning a lower WCC in healthy African patients (55). Of note, 3 of the patients who developed leucopenia were Indian/Caucasian. Lim et al suggested that healthy African patients were genetically predisposed to lower WCC and absolute neutrophil counts. The mechanism of this is thought to be related to a Duffy antigen receptor for chemokines (DARC) gene mutation which alters chemokine concentrations and thus affects neutrophil movement from the bone marrow (55). The importance of this lower WCC value is in the interpretation of lab results pertaining to toxicity during therapeutic drug monitoring. Using standard reference ranges which are not race specific might lead to erroneous drug withdrawals, dose reductions or even unnecessary diagnostic work-ups. Interestingly, none of the patients in the study who developed leucopenia subsequently developed an infection.

The most prevalent side effect found in this study was leucopenia. This finding was noteworthy, as a higher incidence of leucopenia was found despite the lower cut off used. Of the 19 patients who developed leucopenia, only 6 were on doses greater than 30mg/week with the mean dose being 30.1(±9.6) mg. Sixteen patients remained on MTX after developing a toxic event at a mean dose of 14.7(±8.2) mg/week. Methotrexate was permanently discontinued in only 3 patients. This was in keeping with other toxicity studies which suggested that MTX was unlikely to be completely stopped even after toxicity developed- instead it was usually continued in a reduced dose (33).

Patients at risk for developing cytopenias include the elderly (age>65), patients with renal dysfunction, lack of folate supplementation and concomitant usage of dihydrofolate reductase inhibitors (25). All patients attending the rheumatology clinics at CMJAH are given folate when starting MTX, thus lack of folate supplementation was not evaluated as a risk factor in our study. No association was shown between Black race and the development of a leucopenia.

The usage of more than one DMARD to achieve RA control is standard practice. This confers an increased risk of developing drug toxicities or drug-drug interactions. Sulphasalazine is an agent commonly used in combination therapy with MTX to treat rheumatoid arthritis. Its primary side effects are that of GIT upset and a rash. The

drug can also cause a leucopenia which is thought to be either idiosyncratic or dose related- the prevalence of this seems to be low, ranging from 1-2% (61). Most episodes of neutropenia are self-limiting but rare cases of life threatening agranulocytosis have been reported (0.6%) (72). It is intuitive to surmise that using MTX and SZP in combination would result in a higher incidence of cytopenias. This inference was not supported by the literature reviewed (62,63). The toxicities which seem to be increased by the combination were mostly GIT in nature. However, our study showed that using sulphasalazine in conjunction with MTX at the onset of treatment, increased the risk of developing leucopenia (OR 3.76, 95%CI: 1.08-13.09; p=0.03). None of the other DMARDS evaluated in our study in association with MTX showed an increased risk of leucopenia.

Anaemia was also an association with leucopenia (OR 21.18, 95%CI: 1.83-245.7; p=0.01). This is intuitive as methotrexate can cause bone marrow suppression in the absence of other risk factors (15). This can be counteracted by the usage of folate supplementation (64). All the patients in the study were prescribed folate, but it is possible that compliance was suboptimal and thus these patients developed myelosuppression and subsequent cytopenias. Hypoalbuminaemia is also associated with developing myelotoxicity as MTX and its metabolites are highly albumin bound (81). Serum albumin concentrations were not measured during this study but it is possible that this may have contributed to the above association. Developing a leucopenia was not associated with an increased risk of developing a transaminitis which could in indicate that there are different mechanisms underlying each toxicity. The association between stomatitis and cytopenias was not strongly demonstrated in our study, with only one patient displaying both. Mucositis, when accompanied by a pancytopenia, is often severe and more likely to be fatal as these patients are prone to developing sepsis (37). The prevalence of mucositis reported by other studies ranges between 12-37% (33,42). The low prevalence noted in our study is possibly due to the fact that all our patients received folate supplementation. Another consideration is that none of our patients had significant renal dysfunction which is a known risk factor.

Entry age was associated with the development of a leucopenia. This suggests that older patients were less likely to develop this toxicity. Of note, this is inverse to what has previously been reported, with old age often cited as a risk factor for developing leucopenia (42,56,60). This result may be related to patient weight i.e. older patients

tend to weigh less, and altered volume of distribution of the drug. This was however not investigated in our study due to lack of data regarding BMI being recorded.

4.2 GIT side effects

Liver toxicities are a well-documented side effect of MTX usage and encompass a range of conditions ranging from elevated transaminases to hepatic fibrosis or cirrhosis (24). Routine liver biopsies are not recommended as part of standard MTX usage (65). and thus no histological specimens were reviewed. Our study examined the prevalence of elevated transaminases and found it to be 8.5%. Transaminases greater than twice the upper limit of normal accounted for 15 events and levels greater than thrice the upper limit of normal accounted for 2 events (normal=35u/L). This finding was similar to the CORRONA data registry study which examined toxicity to MTX and MTX and leflunomide combined (73). This data showed that in the MTX monotherapy group the prevalence of transaminitis was 6.8%. Of note, the majority of the liver enzyme elevations both in our study and the CORRONA study did not exceed three times the upper limit of normal.

Of the 17 patients who developed a transaminitis, only 5 were on doses greater than 30mg/week with the average dose being 30.6 (±7.8) mg. Our data did not suggest a synergism between alcohol usage whilst on MTX for the development of a transaminitis. However, this was clearly demonstrated by a study done by Kramer et al (28) which reviewed liver histology results in patients on long term MTX. Thirteen patients remained on MTX after developing a transaminitis at a reduced average dose of 14.7(±9.6) mg/week.

Established risk factors for transaminitis include MTX dose, obesity, lack of folate supplementation and alcohol usage (56). In more recent literature, untreated hyperlipidemia and impaired renal function are also described as risk factors for toxicity (60). According to previous literature, obesity has a significant impact on the development of transaminitis (56,60). The mechanism of this is attributed to obesity causing an increased predisposition to non-alcoholic fatty liver disease which in turn causes an increased susceptibility to toxicity. Unfortunately, due to a paucity of data, we were unable to examine this association in our patients. Transaminitis has also been associated with a longer duration of treatment. This duration is not precisely

specified but on average our patients were on treatment for six years before developing toxicity.

The only non-modifiable predictor which showed an association with transaminitis was entry age, however, this association was not significant (OR 0.96, 95% CI: 0.91-1; p=0.06). No patients who developed a transaminitis were diabetic or had pre-existing renal dysfunction. In terms of concomitant DMARD usage, an association with leflunomide was shown. This has been well described in previous literature (76). Importantly, no patients were on leflunomide (which independently may cause a transaminitis) at entry.

The most prevalent extra-hepatic GIT side effects were nausea and stomatitis. Gastrointestinal side effects usually occur early during the course of treatment and are thought to be dose dependant. Folate supplementation has once again shown to decrease the frequency of this complication (23). Nausea and stomatitis were associated with transaminitis (OR 5.97; p=0.04, OR 6.32; p=0.01 respectively) thus suggesting that the underlying mechanism of toxicity may be the same.

Our study did not identify any patients who developed a pneumonitis which is in keeping with recent literature describing the rare nature of this toxic event (74).

4.3 MTX dosing

EULAR recommends MTX as a first line agent with an initiation dose of 15mg/week. This is then escalated to a maximum dose of 30mg/week depending on the patient's clinical response or the development of toxicity (46). In selected patients with refractory disease, we employed doses of between 30-50 mg/week, hoping to achieve disease control without the usage of biologic agents. Forty-five (22.5%) patients in our study were on doses greater than 30mg/week and 12 of these patients developed toxicity. The average dose at which toxicity developed was 29.3(±9.3) mg/week (within the EULAR recommended range), thus implying that higher doses were not necessarily responsible for an increase in toxicity. We can infer from this result and the fact that most studies documented toxicity at low doses (<15mg/week) that toxicity develops independent of dose of MTX. This finding allows us to continue to cautiously use high dose methotrexate in a select group of patients, provided adequate screening for toxic events is undertaken.

4.4 Limitations

Due to the retrospective nature of the study, incomplete record keeping resulted in certain variables being omitted from the final analysis. Selection and information bias may have impacted on the accuracy of certain elements of the study. Documented toxicity was described based on the attending doctors discretion and was recorded as such. This was a single centre study carried out in the public health sector which might lead to the patient demographics being slightly skewed and not representative of the population at large.

Chapter 5: Conclusion

Despite the limitations of our study, our findings add valuable information regarding the prevalence of side effects in a predominantly Black, South African population. The frequency of individual side effects differ to that of other population groups, but as a whole are in keeping with the toxicities that are well described in previous studies. Leucopenia was the most prevalent toxicity, despite a lower WCC cut off being used. This was surprising as all patients received folate supplementation and very few had significant renal dysfunction. The cause for this finding was not apparent in our study and it is possible that missing data precluded a definitive conclusion being drawn. It is also possible that genetic variations in the MTHFR gene polymorphisms could account for this.

Of the risk factors for developing leucopenia identified exclusively in this study, the only non-modifiable predictor was age at entry. This was in contrast to a study by Bologna et al (75) which concluded that age at initiation of MTX did not influence toxicity. A possible reason for this could be that younger patients are more likely to be treated aggressively from the onset and are thus subjected to higher doses of MTX or aggressive dose escalation. This was not studied in our analysis and would have to be reviewed in a separate study. Modifiable predictors included concomitant sulphasalazine usage. This could be on the basis of both drugs having anti-folate properties. There was an association between developing anaemia simultaneously with leucopenia.

Risk factors for developing transaminitis included the concomitant usage of leflunomide. This association has been noted in previous literature (76). There was also an association between developing a transaminitis in conjunction with other GIT side effects (nausea and stomatitis).

Unconventional doses of MTX i.e. doses greater than 30mg/week are not synonymous with developing toxicity. This strategy may be utilised in the under resourced public sector to attain remission in refractory patients, without using biologic agents. Unfortunately our study was not geared to determine the efficacy of such a strategy and this would need to be evaluated in a separate study. When using higher doses of MTX, stringent monitoring must be adhered to for early detection of possible side effects.

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Appendix 1
DATA COLLECTION SPREADSHEET

	Entry into clinic	At time of developing toxicity	At time of data collection
Demographics			
1) Age			
2) Gender			
3) Ethnicity			
Lifestyle			
1) Smoking			
2) Alcohol			
3) Exercise			
Metabolic Parameters			
1) Hypertension			
2) Diabetes			
3) Random glucose			
4) Waist circumference			
5) Body Mass Index			
6) Total cholesterol			
7) Low density Lipoprotein			
8) High density Lipoprotein			
9) Triglycerides			

	Entry into clinic	At time of developing toxicity	At time of data collection
Co-morbidities			
1) Interstitial Lung disease			
2) Ischaemic Heart disease			
3) Cerebrovascular accident			
4) Peripheral vascular disease			
5) Estimated Glomerular filtration Rate (ml/min/1.73m^2)			
Other DMARDS			
1) Azathioprine			
2) Sulphasalazine			
3) Chloroquine			
4) Leflunomide			
5) Cyclophosphamide			
6) Glucocorticoids			
7) NSAIDS			
Methotrexate dose(mg)			
1) Highest dose MTX			
2) Current dose MTX			
3) Dose at which toxicity occurred			
SIDE EFFECTS			
Haematological			
1) White cell count <3/nl			
2) Haemoglobin <12g/d l(males) <10g/dl (females)			
3) Platelets <100n/l			

Hepatic	
1) ALT/AST > 3times upper limit of normal (>105U/L)	
2) ALT/AST >2times upper limit of normal (>70U/L)	
Gastro-intestinal	
1) Nausea	
2) Abdominal Pain	
3) Stomatitis	
4) Diarrhoea	
Dermatological	
1) Rash/Alopecia	
2) Nodulosis	
Pulmonary	
1) Cough	
2) Pneumonitis	
Infections	
1) Upper respiratory tract infection	
2) Lower respiratory tract infection	
3) Soft tissue infections	
4) Urinary tract infections	
Neurological	
1) Dizziness	
2) Headache	
3) Insomnia	
4) Nightmares	

Appendix 2- TURNITIN report

Plagiarism software, Turn it in, was used to review this dissertation. A similarity index of 18% was reported. This related mostly to the use of standardised definitions and referencing. All other similarities have been appropriately referenced.

TO WHOM IT MAY CONCERN

RE: Turn-it-in report : Dr Nabeela Kajee MMed: " The prevalence of side effects on methotrexate and its associated risk factors in South African patients with rheumatoid arthritis"

I have reviewed the Turn it in report of Dr N Kajee's MMed dissertation. The report identifies a similarity index of 18 %. This mostly relates to the use of standardized definitions and references. Other information that bears similarity has been referenced appropriately.

Yours sincerely,

Professor Ahmed Solomon

HOD Rheumatology

Internal Medicine

Charlotte Maxeke Johannesburg Hospital

Supervisor

Appendix 3- Ethics Clearance Certificate

R14/49 Dr Nabeela Kajee et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170984

NAME: (Principal Investigator)	Dr Nabeela Kajee et al
DEPARTMENT:	Rheumatology Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	The Prevalence of Side Effects Methotrexate and its Associated Risk Factors in South African Patients with Rheumatoid Arthritits
DATE CONSIDERED:	Adhoc
DECISION:	Approved unconditionally
CONDITIONS:	Sub-Study under Primary Study M060733
SUPERVISOR:	Dr Ahmed Solomon
APPROVED BY:	Professor P. Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	20/10/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004,10th floor, Senate House/3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review September and will therefore be due in the month of September each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES