4-13-2015 12:00 AM

Methotrexate and cardiovascular events

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A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

METHOTREXATE AND CARDIOVASCULAR EVENTS

(Thesis format: Monograph)

By

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Master of Science in Epidemiology and Biostatistics

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Abstract

We conducted a systematic review and meta-analysis to assess the association of MTX with cardiovascular morbidity, cardiovascular mortality and all-cause mortality in patients with autoimmune disease. Our primary outcome was incident cardiovascular events. After screening 13,479 citations, we identified a total of 30 eligible studies. We synthesized adjusted risk estimates using a random effects model. MTX was significantly associated with a 25% reduction in cardiovascular events (pooled RR: 0.75, 95% CI: 0.65, 0.86, I^2: 11%), a 55% reduction in cardiovascular mortality (0.45, 95% CI: 0.26, 0.80, I^2: 33%) and a 40% reduction in all-cause mortality (0.60, 95% CI: 0.48, 0.76, I^2: 45%). Low-dose MTX was associated with a stronger effect size for reducing cardiovascular events compared to high-dose MTX (0.61, 95% CI: 0.51, 0.74 versus 0.88, 95% CI: 0.78, 0.99). We concluded a significant associative reduction in cardiovascular events with the use of low-dose MTX in patients with autoimmune disease.

Keywords: Methotrexate, autoimmune disease, cardiovascular events, mortality, rheumatoid arthritis, psoriasis, systematic review, meta-analysis

Acknowledgment

I would like to thank the people who supported me during my Master's study and thesis work.

Firstly, I would like to extend my deepest and sincerest thanks to my supervisor Dr. Dan Hackam for his support, confidence, and mentorship throughout my thesis. I greatly appreciate his timely suggestions and guidance. His mentorship allowed me to grow as a researcher.

I would also like to thank Dr. Lillian Barra and Dr. Grace Parraga for being members of my thesis advisory committee and their suggestions. I would like to thank Ms. Erin Boyce (clinical librarian) for her help in developing the search strategy.

I would like to thank all faculty members of the Department of Epidemiology and Biostatistics for familiarizing me with the exciting and engaging discipline of Epidemiology.

I would like to thank my parents for their support and encouragement throughout my career. I would like to gratefully thank my beloved wife, Kinjal, for her patience, care, unconditional love, encouragement and support during all these years. I would like to thank my daughter, Diya and my son, Ved, for enriching my life with love and happiness.

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Chapter 1: Introduction

1. Overview

The objective of this thesis is to quantify the association of methotrexate (a commonly used therapy for a variety of autoimmune conditions) with cardiovascular morbidity, cardiovascular mortality and all-cause mortality in patients with autoimmune disease. We conducted a systematic review and meta-analysis of trials and observational studies meeting restricted eligibility criteria. We included autoimmune diseases in which methotrexate has been used as one of the treatment options. We used the Newcastle-Ottawa scale for quality assessment of observational studies, and the Cochrane risk of bias instrument to appraise the quality of randomized trials.[1,2]

2. Scope of the problem

Cardiovascular diseases such as coronary artery disease, peripheral arterial disease, stroke, and venous thromboembolism are the leading cause of morbidity and mortality in developed as well as developing countries, accounting for 30% of all deaths worldwide in 2008.[3] Of these deaths, an estimated 42% of deaths were a result of coronary artery disease and 36% of deaths were attributed to stroke.[4] Cardiovascular disease has no geographic, socioeconomic or sex boundaries. According to World Health Organization (WHO) data published in 2011, 80% of deaths among young individuals in low and middle income countries were due to cardiovascular disease.[3] Moreover, costs of treating cardiovascular disease are among the highest for chronic diseases. These costs constituted 17% of all annual medical expenditures in the United States, 12% in the European Union and 17% in the United Kingdom.[5,6] In Canada, while the death rate from cardiovascular disease declined in the last decade, mortality from cardiovascular disease remained the second leading cause of death after cancer (and 25.2% of all deaths).[7] Despite improved management and optimal patient care, cardiovascular disease remains the largest single contributor to mortality worldwide. WHO estimates annual deaths due to cardiovascular disease to reach more than 23 million by 2030.[3]

The global burden of cardiovascular disease will continue to increase due to multi-dimensional effects of cardiovascular risk factors and population aging. Cardiovascular disease is not only caused by major individual cardiovascular risk factors but also by other contributing conditions that alter these risk factors, directly or indirectly. Some novel risk factors for cardiovascular disease include high levels of C-reactive protein, lipoprotein (a), homocysteine, LDL-c particle size and fibrinogen.[8-10] Various chronic medical conditions such as end-stage renal disease, chronic inflammatory connective tissue diseases, and human immunodeficiency virus infection are also considered to be risk factors for cardiovascular disease.[10] In addition, disturbances in tissue plasminogen activator (tPA) levels and low serum testosterone are known to contribute to cardiovascular disease.[11,12] Other situations including hysterectomy before the age of 50 and psychosocial conditions such as mental stress, depression and poor sleep quality are also risk factors for cardiovascular disease.[10]

Some of the above risk factors such as hypertension, diabetes mellitus, dyslipidemia, smoking, obesity and physical activity can be modified and controlled while others cannot. Declining trends in cardiovascular mortality over the past few decades have been observed due to increased awareness, improved management of modifiable risk factors and medical treatment of patients with cardiovascular disease.[13,14] A number of health promotion and prevention programs have also played an important role. Conversely, due to increased prevalence of physical inactivity, obesity, diabetes, high calorie consumption and other risk factors, global morbidity and mortality from cardiovascular disease remains high. Considering the global burden of cardiovascular disease and the health consequences for individual patients, there is a significant need to address other preventive measures for cardiovascular disease that are associated with novel and non-traditional risk factors.

Until now, scant attention has been paid to addressing some of these novel risk factors that may have importance in ameliorating cardiovascular disease. Autoimmune diseases are one of these risk factors. The enhanced risk of cardiovascular disease in major autoimmune disease is a significant clinical problem.

Autoimmune diseases represent a variety of disease manifestations and jointly they affect 5-10% of the population in developed countries.[15,16] Disease activity and severity are associated with high mortality in patients with autoimmune disease; yet with the availability of better therapies, the lifespan of these patients has improved. Hence in part due to improved survival, the long-term consequences of these diseases such as coronary artery disease and stroke are increasingly manifest. Autoimmune diseases cause chronic inflammation and immune dysregulation, which lead to increased autoantibody production, dyslipidemia, platelet dysfunction and vascular pathology, which are consequently responsible for atherosclerosis.[17,18,19] Thus, premature atherosclerosis is more frequent in patients with systemic autoimmune conditions.

A broader concept of therapy targeting inflammation is needed to reduce morbidity and mortality due to cardiovascular as well as autoimmune disease. Different immunotherapeutic agents are in use to treat various autoimmune conditions. The effect of these therapeutic agents on the cardiovascular system and mortality remains a vital issue. One routinely used agent is methotrexate (MTX).

3. Relationship of autoimmune disease with cardiovascular disease, cardiovascular mortality and all-cause mortality

3.1 Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease affecting multiple joints that leads to joint destruction, deformity, loss of function and reduced life expectancy. Due to the inflammatory nature of the disease, RA patients are more prone to develop cardiovascular disease than the general population.[20,21] Several studies also identified a higher cardiovascular and all-cause mortality in RA patients compared to the general population.[22] In a cohort study, Young et al identified a standardized mortality ratio (SMR) of 1.27 (95% CI: 1.04, 1.46) in RA patients compared to the general population in the United Kingdom.[22] In another population based cohort study, Turesson et al reported an age and sex adjusted standardized morbidity ratio of 1.61 (95% CI: 1.21, 2.10) for first ever acute myocardial infarction or stroke in RA patients compared to the Malmo, Sweden general population.[23] Maradit-Kremers et al found a hazard ratio (HR) of

2.41 (95% CI: 1.00, 5.81) for cardiovascular death in RA patients with concomitant vasculitis compared to RA patients without vasculitis.[24]

3.2 Psoriasis

Psoriasis is a chronic inflammatory autoimmune disease of skin characterized by red elevated patches and flaking silvery scales. Severe psoriasis appears to be associated with an increased risk of cardiovascular morbidity and mortality. A study conducted by Gelfand et al reported an incidence rate of 5.13 (95% CI: 4.22, 6.17) per 1000 person years for myocardial infarction in patients with psoriasis in contrast with 3.58 (95% CI: 3.52, 3.65) per 1000 person years in the control group.[25] In a recently published meta-analysis, Horreau et al reported an odds ratio of 1.19 (95% CI: 1.14, 1.24) in cross-sectional studies, 1.20 (95% CI: 1.13, 1.27) in cohort studies, and 1.84 (95% CI: 1.09, 3.09) in case-control studies for the risk of coronary artery disease in psoriasis patients compared to patients without psoriasis.[26] For mortality, Abuabara et al conducted a population-based cohort study and found a higher overall death rate (26, 95% CI: 23, 29 per 1000 patient-years) in psoriasis patients compared to patients without psoriasis (18, 95% CI: 17, 19 per 1000 patient-years).[27]

3.3 Psoriatic Arthritis

Psoriatic arthritis is an inflammatory arthritis associated with psoriasis. Like psoriasis and RA, psoriatic arthritis also carries a high risk of cardiovascular morbidity and mortality. A cohort study from Toronto, Ontario identified high cardiovascular and all-cause mortality in patients with psoriatic arthritis compared to the general population, with a standardized mortality ratio of 1.62 (95% CI: 1.21, 2.12).[28]

3.4 Systemic sclerosis

Systemic sclerosis is a multisystem autoimmune disease characterized by abnormal growth of connective tissue and fibrosis. Barnes and Mayes found higher mortality in systemic sclerosis patients compared to the general population, with a standard mortality ratio of 1.46 (95% CI: 1.28, 1.69).[29] Additionally, they noted that 55% of deaths in systemic sclerosis patients were directly related to the disease itself and 14% of deaths were due to systemic sclerosis-related myocardial disease. A retrospective analysis carried out by Man et al from the General Practitioner database in the United Kingdom

reported a two-fold increase in the risk of myocardial infarction and stroke in systemic sclerosis patients compared to those without systemic sclerosis.[30]

3.5 Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease of various manifestations affecting skin, joints, kidney, brain, and other organs. Petri et al observed that the prevalence rate of cardiovascular disease ranged from 6 to 10% in individuals with SLE.[31] The mortality rate in SLE is 2-5 times higher than that of the general population.[32] Yurkovich et al demonstrated a three-fold increased risk of death in SLE patients compared to the general population, with a pooled standardized mortality ratio of 2.98 (95% CI: 2.32, 3.83).[33] They also showed a standardized mortality ratio of 2.72 (95% CI: 1.83, 4.04) due to cardiovascular disease in SLE patients compared with the general population.

3.6 Dermatomyositis and polymyositis

Dermatomyositis (DM) is a connective tissue disorder characterized by inflammation of muscle and skin. As a systemic disorder, it may affect the joints, esophagus, lungs, and heart. Polymyositis causes muscle inflammation and diffuse weakness of both sides of the body, mainly affects proximal muscles. In Finland, Airio et al carried out a retrospective analysis of dermatomyositis and polymyositis patients from a hospital database.[34] They showed a standardized mortality ratio of 2.92 (95% CI: 2.82, 3.44) in these patients. In this cohort, the main cause of mortality was cardiovascular disease (in 31% of dermatomyositis patients).

3.7 Multiple sclerosis

Multiple sclerosis (MS) is a chronic inflammatory, debilitating disease of the nervous system, resulting in various signs and symptoms. A higher mortality rate is observed in patients with multiple sclerosis compared to the general population.[35] A recently published population-based cohort study observed a 3.5-fold all-cause mortality rate in multiple sclerosis patients compared to the reference population with a hazard ratio of 3.51 (95% CI: 2.63, 4.69).[36] In the same study, the hazard ratio due to cardiovascular death in multiple sclerosis patients was found to be 2.42 (95% CI: 1.47, 3.97).

3.8 Sjogren's syndrome

Sjogren's syndrome is an inflammatory disease of the immune system that can affect different parts of body but most commonly affects the eye and salivary glands. Previous studies have identified evidence of metabolic abnormalities in primary Sjogren's syndrome that may increase the risk of stroke and cardiovascular disease in these patients.[37] However, Chiang et al reported an adjusted hazard ratio of 0.84 (95% CI: 0.62, 1.12) for ischemic stroke in primary Sjogren's patients compared to a non-Sjogren's control group.[38] There was no difference in the survival between Sjogren's syndrome and the healthy population. Theander et al and Nannini et al reported non-significant differences in mortality between patients with Sjogren's syndrome and the general population.[39,40]

3.9 Bullous pemphigoid

Bullous pemphigoid (BP) is a chronic, autoimmune, subepidermal, blistering disease of skin that often affects the lower abdomen, upper thighs or armpits. In Langan et al, patients with bullous pemphigoid had more than twice the all-cause mortality than that of the control group with an adjusted hazard ratio of 2.3 (95% CI: 2.0, 2.7).[41] In Taiwan, Yang et al carried out an analysis from Taiwan's National Insurance Research Database (NHIRD) and determined an adjusted hazard ratio of 2.37 (95% CI: 1.78, 3.15) for stroke in patients with bullous pemphigoid.[42]

3.10 Inflammatory Bowel Disease

Inflammatory Bowel Disease (IBD) is a group of autoimmune inflammatory conditions that affect the digestive system. Crohn's disease (CD) and ulcerative colitis (UC) are the major types of IBD. A study conducted in Canada by Bernstein et al reported an increased risk of ischemic heart disease in IBD patients (both in CD and UC) with an incidence risk ratio of 1.26 (95% CI: 1.11, 1.44).[43] In a cohort study, Jess et al found intermediate and long-term mortality rates increased by 10% (in relative terms) among patients with UC and 50% among patients with CD when compared with the general population.[44] The authors also reported a hazard ratio of 1.20 (95% CI: 1.14, 1.26) in UC and 1.39 (95% CI: 1.28, 1.51) in CD for death due to cardiovascular disease.[44] Cart et al reported similar

results in a study from the United Kingdom with a hazard ratio of 1.54 (95% CI: 1.44, 1.65) for deaths among IBD patients after adjusting for age, sex and smoking status.[45]

3.11 Transverse Myelitis

Transverse myelitis is an inflammatory disease of the spinal cord of varied etiology resulting from loss of spinal cord functions over several hours to weeks. Apart from infections and vaccinations for infectious diseases, causes of transverse myelitis also include several autoimmune conditions such as systemic lupus erythematosus, Sjogren's syndrome, Behcet's disease, antiphospholipid syndrome, multiple sclerosis, neuromyelitis optica and other rheumatic diseases.[46] To the best of our knowledge, no data on cardiovascular risk exist for transverse myelitis.

3.12 Myasthenia Gravis

Myasthenia Gravis (MG) is a chronic autoimmune disease of neuromuscular system characterized by weakness and fatigue of skeletal muscle. Evidence supporting a relationship between myasthenia gravis and cardiovascular disease is limited. However, a study published in 1984 by Hofstad et al observed a relationship of heart disease with myasthenia gravis.[47] The authors in this study observed that 16% of myasthenia gravis patients exhibited signs of heart disease. In contrast, Owe et al examined 1,992,342 deaths from the Norwegian Cause of Death Register from 1951 to 2001 and determined significantly lower cardiac disease in myasthenia gravis patients compared to the controls in the age group 50-69 (19.4% in myasthenia gravis patients versus 52.0% in controls for men, p=0.001, and 14.6% versus 29.6% for women, p=0.036).[48]

3.13 Wegener's Granulomatosis

Wegener's granulomatosis is a chronic inflammatory disease of small and medium size vessels characterized by granuloma formation that affects many organs and requires long term immunosuppression therapy. It is also known as granulomatosis with polyangiitis (GPA). It is a rare disease with varied geographic distribution. Watts et al noted an increasing trend in prevalence in the United Kingdom with a prevalence rate of 62.9 per million in December 1997 and 148 per million at the end of 2010.[49]

3.14 Microscopic Polyangiitis

Microscopic polyangiitis (MPA) is a chronic autoimmune vasculitis characterized by necrosis of small size blood vessels without granulomatous inflammation. In contrast to Wegener's granulomatosis, it usually affects the lower respiratory tract and is associated with anti-neutrophil cytoplasmic autoantibody (ANCA) directed against myeloperoxidase (MPO). Watts et al reported a mean annual incidence of 5.9 per million population (95% CI: 4.4, 7.5) for MPA in the United Kingdom during the period of 1988-2010.[49] They also noted an increasing trend in prevalence for GPA and MPA in the United Kingdom.

3.15 Eosinophilic Granulomatosis with Polyangiitis

Eosinophilic granulomatosis with polyangiitis **(**EGPA) is one of three ANCA-associated vasculitides, predominantly affecting small size blood vessels. It is also known as a Churg-Strauss Syndrome (CSS).

GPA, MPA and EGPA are also known as ANPA-associated vasculitis. In these patients, an increased mortality due to cardiovascular disease is well-documented. The relative risk for coronary heart disease and stroke in ANCA-associated vasculitis is 2 to 4-fold higher than that in control patients.[50] ANCA-related vasculitis patients may experience accelerated atherosclerosis.[50]

3.16 Takayasu's Arteritis

Takayasu's arteritis (TA) is a rare, systemic inflammatory vasculitis of large vessels leading to abnormal stenosis or aneurysm of vessels. Affected patients are more prone to accelerated atherosclerosis. About 10-30% and 10-20% of Takayasu's arteritis patients experience coronary artery disease and stroke respectively due to hemodynamic compromise in large artery stenosis and thromboembolism.[50]

Overall, autoimmune disease are associated with increased risk of cardiovascular events, cardiovascular mortality and all-cause mortality worldwide.[51-54] Autoimmune disease is one of the top ten causes of death and mostly affects women.[51]

4. Mechanisms of cardiovascular disease in autoimmune disease

Development of cardiovascular disease relies on the contributions of both genetic and environmental risk factors. Evidence supports the association of atherosclerosis with chronic inflammation. Shoenfeld et al note the absence of traditional cardiovascular risk factors in approximately 40% of patients with myocardial infarction or stroke and suggest an involvement of inflammatory and immune mechanisms in the rapid development of atherosclerosis.[55] They also identified the participation of several autoantigens and autoantibodies in the process of atherosclerosis.

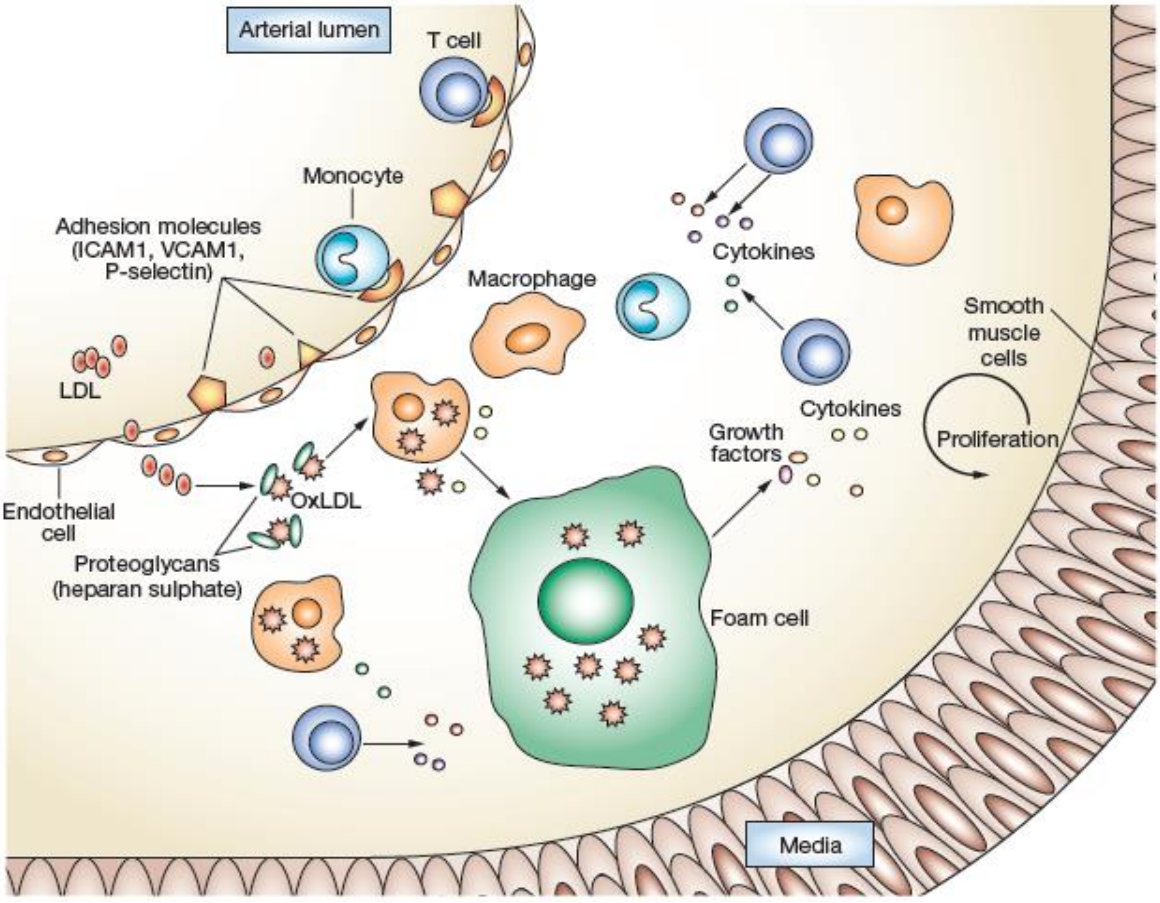


Figure 1. Components of immune system in the process of atherosclerosis.
(Source: Sherer and Shoenfeld)[56]. Reproduced with permission.

4.1 Atherosclerosis in autoimmune disease

Atherosclerotic plaque is characterized by accumulation of lipid particles and immune cells in the artery's subendothelial region. Components of the immune system involved in the process of atherosclerosis include macrophages, T-cells, autoantibodies, autoantigens, LDL particles, cytokines including tumor-necrosis factor (TNF), interleukin 1 (IL-1), IL-2, IL-6, IL-8, IL-10, IL-12, interferon-γ and platelet-derived growth factor (Figure 1).[56]

In RA, several factors accelerate the process of atherosclerosis, including lifestyle, modifiable risk factors, lipid dysregulation, chronic inflammation, immune dysregulation, functional abnormalities of the vascular endothelium, and expansion of CD4+CD28- cells.[17] In SLE, high prevalence of modifiable cardiovascular risk factors, increased inflammatory markers, coronary artery calcification and increased levels of oxidised LDL (oxLDL) are associated with accelerated atherosclerosis.[56]

The exact mechanism of atherosclerosis is not yet clear in systemic sclerosis. However, based on available evidence, it can be inferred that atherosclerosis and vascular disease in systemic sclerosis is caused by several factors such as impaired coronary microcirculation, endothelial injury, intimal thickening, destruction of the internal elastic lamina, transmural lymphocytic cellular infiltration and increased intimal-medial thickness of the major vessels.[17]

GPA, MPA and ECGA are types of primary systemic vasculitides (PSVs) which may trigger atherosclerosis through inflammation and immune reaction. In these diseases, due to a vascular bed lesion, inflammation and in-situ immune reactions activate the endothelial cells in the vessel's intima. This further exposes adhesive molecules to secrete cytokines, chemokines, growth factors and metalloproteinases at the site resulting in rapid atherosclerosis.[17] Several factors such as increased intimal-medial thickness, C-reactive protein, matrix metalloproteinases, several pathological autoantibodies and oxidised LDL are responsible for the accelerated atherosclerotic process in the primary systemic vasculitides.[17]

5. Methotrexate

Methotrexate (MTX) is a disease-modifying anti-rheumatic drug (DMARD) which reduces inflammation in autoimmune disease by suppressing the immune system. While originally designed to treat cancer as a chemotherapeutic agent, it has proven safe and well-tolerated in several autoimmune conditions when used in low doses. It is commonly used as part of the standard of care in first, second or third-line therapy in various autoimmune diseases. Its safety and efficacy have been proved in placebo-controlled trials as well as in active comparator trials using other disease modifying anti-rheumatic drugs.[57,58]

Methotrexate's potential cardioprotective effects may operate through reducing systemic inflammation and by a direct effect on the cellular mechanisms responsible for atherosclerosis. Cutolo et al reviewed different anti-rheumatic and anti-inflammatory mechanisms of MTX.[59] In their review, they noted that low-dose methotrexate exerts both anti-proliferative and anti-inflammatory effects through different pathophysiological mechanisms. Describing the first mechanism, MTX increases extracellular adenosine, which interact with specific cell surface receptors (A2A and A3) and inhibits IL-8, IL-6, and leukotriene B4. Secondly, MTX reduces the production of pro-inflammatory monocytic and macrophagic cytokines, including IL-1, IL-6, and TNF-α. Furthermore, MTX increases the gene expression of anti-inflammatory Th-2 cytokines (IL-4, IL-10) and decreases the gene expression of pro-inflammatory Th-1 cytokines (IL-2, IFNγ).[59]

Coomes et al also describe mechanisms by which MTX reduces atherosclerosis.[60] According to their review, MTX activates A2A and A3 adenosine receptors by releasing adenosine, which consequently up-regulates expression of ABCA1 and 27-hydroxylase. Steps and details of these mechanisms are described in the following Figure 2.

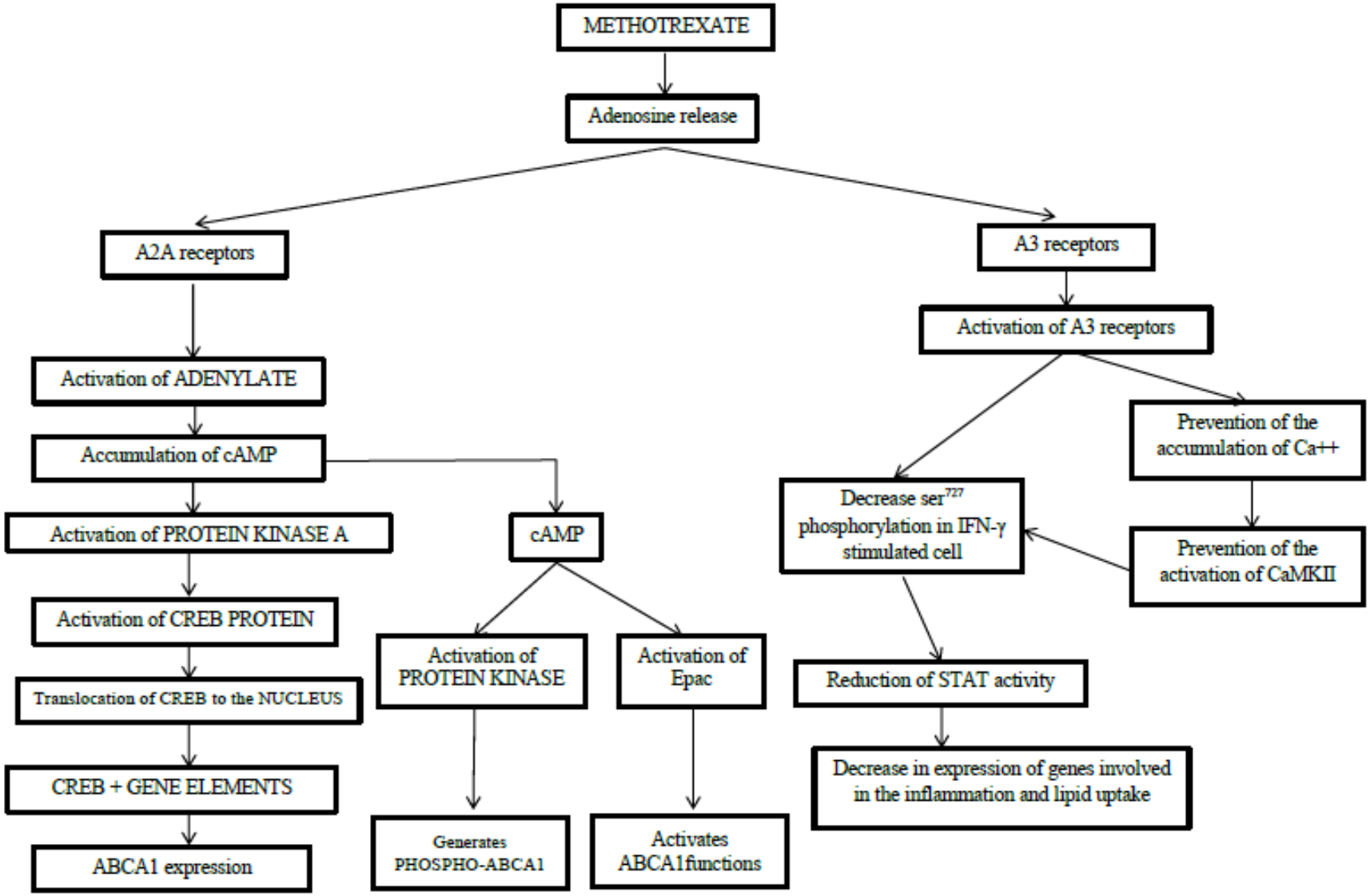


Figure 2. Anti-atherogenic mechanisms of MTX

6. Study rationale

To date, no clinical trial has been conducted addressing the direct association of MTX use and cardiovascular morbidity and mortality in patients with rheumatic and autoimmune disease. Currently, the Cardiovascular Inflammation Reduction Trial (CIRT) is ongoing and recruiting patients to investigate the effect of low-dose MTX on the rate of recurrent cardiovascular events in patients with prior myocardial infarction plus either type 2 diabetes or the metabolic syndrome.[61,62] However, CIRT is focused on an established secondary prevention population while excluding patients with autoimmune disease who are also at risk.

Observational studies including cross-sectional, case-control and cohort studies have addressed the association between the effect of methotrexate and cardiovascular morbidity and mortality in patients with RA, psoriasis, SLE, and psoriatic arthritis.[63-71] These studies are observational and thus not free from the limitations and weaknesses related to their nonrandomized design. Such studies are subject to two major types of bias. The first is 'confounding by indication', whereby in real world clinical practice, sicker patients with a greater indication for treatment would be more likely to get methotrexate than less sick patients. Additionally, these patients may have high inflammation, which leads to increased risk of cardiovascular disease. The second type of bias is 'physician selection bias', where there is a likelihood of selecting methotrexate over other DMARDs in practice, particularly when deciding systemic therapy for psoriasis patients. A non-randomized comparison due to these biases either underestimates or overestimates the effect of methotrexate in reducing cardiovascular disease. To generate a scientifically relevant evidence and understand the relationship of MTX with CVD, we conducted this systematic review, focused on the good quality studies, and tested the results for consistency across various disease endpoints.

Several recent reviews have studied the relationship between MTX and cardiovascular morbidity.[60,72-74] Micha et al reported an overall 21% and 18% reduction in cardiovascular disease and myocardial infarction respectively, in patients treated with MTX compared to those treated with other anti-rheumatic agents.[72] The pooled estimate from this review did not measure an independent effect of methotrexate since the

comparison group consisted of active treatment. Moreover, this meta-analysis assessed only 'hard' cardiovascular events and not 'softer' events such as heart failure. The authors did not assess the association of MTX with mortality. Finally, this meta-analysis included studies published up to June 2010 only. We are expecting to add more studies on the topic which were published after 2010.[63,70,75]

Another systematic review published by Westlake et al in 2010 also found a cardioprotective effect of MTX, but included only RA patients.[73] As well, this review was limited to cardiovascular disease and did not include mortality as a corollary outcome. Finally, the authors did not perform a meta-analysis to quantify the overall association between MTX exposure and cardiovascular disease.

Marks and Edwards studied pathogenesis and risk factors for cardiovascular disease in RA patients.[74] Coomes et al described an overview of the mechanism by which MTX interferes with cholesterol homeostasis and reduces atherogenesis in inflammatory conditions.[60] These narrative reviews used surrogate markers and established biological plausibility for the protective association between MTX and cardiovascular disease.

7. Research questions

7.1 Primary question

Is methotrexate associated with a lower risk of cardiovascular events in patients with autoimmune disease?

Hypothesis: We hypothesize that treatment with methotrexate is associated with a lower risk of cardiovascular events in patients with autoimmune disease, even after adjustment for potential confounders.

7.2 Secondary question

Is methotrexate associated with a lower risk of cardiovascular mortality, all-cause mortality, and other cardiovascular disease endpoints such as coronary events and stroke?

Hypothesis: We hypothesize that methotrexate is associated with a lower risk of cardiovascular mortality, all-cause mortality, and cardiovascular disease endpoints in patients with autoimmune disease.

7.3 Exploratory question (dose-response analysis)

Does the association of methotrexate with cardiovascular events vary by different doses (high or low methotrexate doses)?

Hypothesis: We hypothesize that treatment with high-dose methotrexate is associated with a lower risk of cardiovascular events than treatment with low-dose methotrexate.

Chapter 2: Methods

1. Overview

Using the framework of a systematic review, we quantified the associative risk of MTX for cardiovascular events, cardiovascular mortality and all-cause mortality in patients with autoimmune disease. This review was conducted in accord with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (see Appendix A for PRISMA Checklist).[76]

In terms of study designs, we evaluated cohort studies, case-control studies and randomized controlled trials. We selected cardiovascular events as the primary outcome, given its biological relevance to MTX's mechanism of action. We included autoimmune diseases for which MTX is used as a treatment agent in first, second or third line therapy. We evaluated the association of MTX with events according to a number of parameters including type of autoimmune disease, demographic factors, dose-response, observation period and other potential sources of heterogeneity. We registered our study protocol with the International Prospective Register of Systematic Reviews.

2. Criteria for considering studies

To identify relevant studies, we prespecified the following criteria for types of autoimmune disease, treatment and control groups, and outcomes.

2.1 Study patients

The autoimmune diseases included in this systematic review are rheumatoid arthritis, psoriasis, psoriatic arthritis, dermatomyositis, polymyositis, systemic lupus erythematosus, multiple sclerosis, Sjogren's syndrome, bullous pemphigoid, Crohn's disease, ulcerative colitis, transverse myelitis, systemic sclerosis, myasthenia gravis, Wegener's granulomatosis, microscopic polyangiitis, eosinophilic granulomatosis with polyangiitis and Takayasu's arteritis. We prepared this list of eligible autoimmune diseases by comprehensively searching the online reference "UpToDate", and by reviewing clinical textbooks of rheumatology.[77] Two investigators, a clinical pharmacologist and a practicing rheumatologist (specializing in vasculitis), reviewed this list and finalized the diseases to be included in this meta-analysis. We included

autoimmune diseases for which MTX is used as a treatment agent in first, second or third line therapy.

2.2 Intervention and comparison group

The included study must compare MTX users to non-users (either placebo or a no-MTX comparator group).

2.3 Outcomes measured

We included studies that reported any of the following outcomes: cardiovascular events (including composite cardiovascular endpoints), fatal or non-fatal myocardial infarction, coronary artery disease, acute coronary syndrome, heart failure, cardiac arrest, sudden cardiac death, hospitalization due to cardiovascular events, stroke, cardiovascular mortality and all-cause mortality. We defined all our outcomes using International Classification of Diseases version 10 (ICD-10) criteria.[78]

a. Cardiovascular events (ICD-10: I00-I99): In this category, we considered composite cardiovascular endpoints which include any of the following events: fatal or non-fatal myocardial infarction, angina pectoris, coronary interventions such as coronary bypass grafting or percutaneous coronary intervention, acute heart failure, peripheral arterial disease, vascular surgery, ischemic stroke, transient ischemic attack, and carotid endarterectomy.
b. Coronary events (ICD-10: I20-I25): This includes angina pectoris, myocardial infarction and complications associated with myocardial infarction. We also included any hospitalization or death due to coronary events in this group.
c. Myocardial Infarction (ICD-10: I21-I23): This includes fatal or non-fatal myocardial infarction along with acute (ICD-10: I21) and subsequent (ICD-10: I22) myocardial infarction and its complications (ICD-10: I23).
d. Acute Coronary Syndrome ICD-10: I24.9): This refers to conditions attributed to acute obstruction of a coronary artery. We also included any hospitalization due to acute coronary syndrome in this endpoint.
e. Heart Failure (ICD-10: I50): This refers to chronic or congestive heart failure, left ventricular failure, unspecified heart failure and heart failure requiring hospitalization.

f. Ischemic stroke (ICD-10: I63): This includes both fatal and non-fatal cerebral infarction due to occlusion of cerebral arteries as a result of embolism or thrombosis.
g. Cardiac arrest (ICD-10: I46.9): This comprises hospitalization or death due to cardiac arrest.
h. All-cause mortality: This includes death from any cause.
i. Cardiovascular mortality (ICD-10: I00-I99): Any death due to disease of the circulatory system, including coronary heart disease, stroke, hypertensive diseases, inflammatory heart diseases, rheumatic heart diseases, and other cardiovascular diseases.

2.4 Reporting of adjusted risk estimates

Eligible studies could report the outcome of interest in terms of adjusted relative risk estimates (risk ratios, odds ratios, hazard ratios, standardized morbidity or mortality ratios) and accompanying 95% confidence intervals, p-values, z-scores, or standard errors. Observational studies that reported only crude or unadjusted risk estimates were deemed ineligible. Trials could include unadjusted estimates including dichotomous event data.

3. Literature search

We developed a comprehensive literature search strategy with the help of a clinical librarian. First, we identified all possible terms and their synonyms related to this study's PICO (population, intervention, control and outcome) and then transferred them into a primary search strategy for the Ovid Medline database. We used combinations of medical subject headings (MeSH terms) and free text keywords in this search strategy to identify all relevant articles.

The primary Ovid Medline search strategy identified 1216 articles. We pilot-tested this strategy against relevant key articles; refined and modified it by adding further terms, which were identified from the test articles' mapped keywords; and examined search strategies from related systematic reviews. In Table 1 (at the end of this chapter), we present a final list of MeSH terms and keywords used in our search strategies.

We limited our search to adults and human studies. Once we finalized the Ovid Medline search strategy, we translated it into other online bibliographic databases such as EMBASE, Cochrane library, Web of Science and Google Scholar using analogous terms pertaining to the specific database. We searched records published from the initial available year of indexing up to November 30, 2014 with assistance from weekly auto-alert emails from each of the databases. The full search strategy is presented in Appendix B.

Additionally, we manually searched bibliographies of eligible studies and previous narrative and systematic reviews. We also searched abstracts in recent years from major rheumatology, dermatology and gastroenterology conferences.

4. Screening of studies

For subsequent manipulation and review of citations, we downloaded all retrieved citations into RefWorks, an internet-based reference management tool. We removed all duplicate citations prior to study screening. A total of 13,479 records were then screened.

The principal investigator (AS) initially screened all records by reviewing their titles, abstracts and keywords. Records were excluded upon initial review if they were found to be case reports, cross-sectional studies, reviews, letters, commentaries or guidelines. If the reviewer could not initially determine whether to include or exclude a record by screening the title, keywords and abstract, the full text of the record was reviewed to make a final decision. We identified a total of 187 papers for full text review.

5. Study eligibility assessment

We retrieved the full text of all 187 papers. We developed a standardized eligibility assessment form in Microsoft Excel to rate the eligibility of these records. We pre-tested this sheet in 20 randomly selected studies, modified it based on these results, and finalized it (Appendix C). Two investigators, AS and DH, independently assessed the eligibility of 187 full text reports using the pre-specified inclusion and exclusion criteria (Table 2).

All studies were categorized as either ‘Included’ (if the study met all inclusion criteria) or ‘Excluded’ or ‘Unclear’ in the standardized eligibility form. We recorded reasons for the

excluded and unclear studies. To determine reviewers' agreement, we calculated Cohen's kappa statistic (κ).[79] We interpreted the value of kappa as follows: fair agreement (0.21-0.40), moderate agreement (0.41-0.60), substantial agreement (0.61-0.80) and almost perfect agreement (0.81-1.00).[80] Disagreements between reviewers were resolved by re-evaluation of the studies, followed by discussion and consensus.

6. Data abstraction

We created a comprehensive data abstraction form in Microsoft Excel. The form included the following variables: study number, citation details, study characteristics, sample characteristics, disease particulars, exposure and outcome details, and analysis and results (Appendix D). We pilot-tested this form using five randomly selected studies and refined it based on the results.

One reviewer (AS) abstracted data from the final list of selected studies for the following: study accrual start and end date; inclusion and exclusion criteria; demographic information (mean age, gender distribution); disease details such as diagnostic criteria and disease duration; methotrexate exposure details such as exposure definition, exposure type, exposure data source and dose of methotrexate (mean or median mg/kg); details of outcome (outcome definition, source of outcome data); and results and analysis variables, including number of events in each group, adjusted risk estimates with 95% confidence intervals, variables adjusted for, dose response analyses and subgroup analyses.

7. Quality assessment of included studies

We assessed the quality of included studies using the Newcastle-Ottawa-Scale (NOS) for non-randomized studies and the Cochrane risk of bias tool for randomized trials.[1,2]

7.1 Newcastle-Ottawa Scale (NOS)

The NOS was proposed by Wells et al to assess the quality of observational (non-randomized) studies in meta-analyses. The scale was tested on systematic reviews. Its content validity and inter-rater reliability have been established.[1] Deeks et al evaluated a total of 194 different observational study quality assessment tools, and found the NOS was relatively easy to use, faster to complete and suitable for use in systematic reviews.[81]

The developers of the NOS measured its validity and inter-rater reliability using several cohort and case-control studies and reported an intra-class correlation of 0.88 for cohort studies and 0.62 for case-control studies.[82] In the same study, inter-rater reliability was high for both cohort (ICC=0.94) as well as case-control studies (ICC=0.82). In contrast, Hartling et al noted a varied range of inter-rater reliability, from slight to moderate agreement across the different domains of NOS.[82] A poster presented at the 2010 Cochrane collaboration annual colloquia by Hou et al reported fair to almost perfect reliability for the rating of NOS items and fair to good inter-rater correlation for the total score.[83]

There are two separate Newcastle-Ottawa scales for case-control and cohort studies, respectively. The scale uses a 'star' rating system to adjudicate quality based on three broad domains, namely selection and comparability of study groups, and outcome in cohort studies and exposure in case-control studies (Appendix E).

7.2 Cochrane Risk of Bias tool

The Cochrane risk of bias tool is a widely used tool to assess the internal validity of randomized trials (Appendix F). It addresses seven specific domains, including sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective outcome reporting and other issues.[2] Hartling et al quantified a moderate inter-rater reliability for 'sequence generation' and fair agreement for all other domains.[84]

8. Statistical analysis

To provide descriptive statistics, we prepared a summary data sheet in Excel from the abstracted data. This data sheet included variables such as disease under study, disease duration, outcome of interest, risk estimates and confidence intervals; study design, mean age, gender distribution, exposure details, study region, accrual start date, publication year and variables adjusted in the analysis. We double checked these data against the relevant studies to ensure errorless entry of risk estimates and other variables for the analysis. We used Comprehensive Meta-analysis Version 2.0 (Inglewood, NJ) for the meta-analysis, subgroup analyses and meta-regression analyses. A two-tailed P-value of <0.05 was considered to be statistically significant.

8.1 Primary analysis

The primary outcome was a synthesis of cardiovascular events. We considered MTX exposure in any form or dose for the primary analysis. Dose-response analysis was performed in a secondary analysis.

When a single study presented stratified analyses according to gender, age group, or other risk modifier, where each study group was an autonomous, non-overlapping unit, we used a fixed effects model to combine these stratified results into a single study-specific risk estimate.[85] If a study presented independent effect estimates for more than one disease, we considered each disease as a separate unit of analysis (a separate study). As our outcomes of interest were rare, we considered similarity between different risk estimates (odds ratio, relative risk, hazard ratio and incidence rate ratio).[86] When we found a trial with multiple comparison arms, we used data only for the methotrexate user and methotrexate non-user (or placebo) arms to calculate risk estimates.

We calculated relative risk and its confidence interval from the number of events reported in randomized controlled trials using the online calculator at www.medcalc.org/calc/relative_risk.php. With the exception of randomized trials, we took the maximally adjusted risk ratio from each trial for data synthesis. We synthesized adjusted risk estimates using DerSimonian and Laird random-effects models and computed pooled risk ratios (RR) with 95% confidence intervals for each outcome of interest.[87] The included studies were heterogeneous, representing different autoimmune diseases, various study designs, dissimilar geographical areas, patients with diverse risk of outcomes, and different methotrexate doses. The random effects model integrates these inter-study variations into the analysis, while the fixed effects model does not.

We assessed statistical heterogeneity across the studies using Higgins' I^2 statistic.[88] The I^2 quantity is the proportion of observed variation that is due to real heterogeneity rather than chance between studies. The value of I^2 ranges from 0 to 100%, with zero indicating no heterogeneity and larger values indicating progressively higher heterogeneity. Higgins et al suggested a practical rule for I^2 to classify low, moderate or substantial heterogeneity.[89] According to this rule, $I^2 < 25\%$ denotes low heterogeneity; $I^2 = 25\%$ to 50% denotes moderate heterogeneity, and $I^2 > 50\%$ denotes substantial heterogeneity.

8.2 Publication bias

We used funnel plots to identify publication bias, supplemented by an imputation test.[90] Publication bias is one potential cause of funnel plot asymmetry. In the presence of publication bias, the funnel plot shows an absence of studies in the extreme areas of middle portion as well as missing studies at the bottom of the plot.[91]

To estimate the impact of publication bias on the effect size and obtain a bias-adjusted effect estimate, we used Duval and Tweedie's trim and fill method.[92] This method estimates unbiased effect size by removing or adding studies to the funnel plot, making it symmetrical.

8.3 Secondary analysis

We synthesized studies for the secondary endpoints (all-cause mortality, cardiovascular mortality, coronary events, heart failure and stroke) using a random effects model to obtain pooled risk ratios.

8.4 Heterogeneity

We addressed the issue of heterogeneity for the primary outcome using subgroup analyses and meta-regression.

8.4.1 Subgroup analysis

We pre-specified the following subgroups for the primary analysis of cardiovascular events. We assessed MTX-by-subgroup interactions across the subgroups.

1. Disease under study: Rheumatoid arthritis carries a higher risk of cardiovascular events than any other autoimmune disease. We carried out a subgroup analysis by type of autoimmune disease. We explored how the effect size differed between rheumatoid arthritis, psoriasis and other diseases (dermatomyositis, polymyositis and systemic sclerosis).
2. Study design: All other things being equal, a prospective cohort study is generally considered higher quality evidence than a retrospective cohort or case-control study. Using subgroup analyses, we analyzed how the observed effect size differed between prospective cohorts, retrospective cohorts and case-control studies for cardiovascular events.

3. Study region: In this analysis, we examined the association of MTX with cardiovascular events between North American and non-North American (European and Asian) studies.
4. Patients with cardiovascular disease: The risk of recurrent cardiovascular events is higher in patients with prior cardiovascular disease.[93,94] We performed a subgroup analysis to compare the effect size between studies that excluded patients with prior cardiovascular disease and those that did not.
5. Methotrexate exposure types: We compared the effect of methotrexate in treatment initiators versus ever-users on cardiovascular events.
6. Data source: We performed a subgroup analysis by type of data source. We explored how the effect size differed between studies that used administrative databases as a data source versus those that used patient medical records. Databases included administrative pharmacy records and insurance databases.
7. Adjustment for DMARDs and other anti-rheumatic medications: To examine the independence of the associative risk of methotrexate from concomitant disease-modifying therapies, we computed the effect size for studies that adjusted for non-methotrexate DMARDs and other anti-rheumatic medications in their analysis.
8. Adjustment for cardiovascular risk factors: We explored how the effect size varied between studies that adjusted for hypertension, diabetes and dyslipidemia in their analysis versus those that adjusted for none of these cardiovascular risk factors.
9. Adjustment for cardiovascular disease: We carried out a subgroup analysis for studies that adjusted for cardiovascular disease in their analysis versus studies that did not.
10. Adjustment for smoking: We compared the effect size for studies that adjusted for smoking versus studies that did not.

8.4.2 Meta-regression

We also explored heterogeneity in the primary analysis with respect to several factors using a random effects meta-regression on a log risk ratio scale, weighted by the standard error of the log risk ratio.[95] We performed this univariate random effects meta-regression using the unrestricted maximum likelihood estimation method. We chose this method because it provides conservative confidence interval coverage for the point estimate.[96,97] Factors assessed for heterogeneity were:

1. Mean age of patients: Due to the differences in cardiovascular risk factor distribution, coronary disease is two to five times as common in middle-aged men as women.[98] Similarly, age is the strongest predictor of cardiovascular disease.[99] Risk of cardiovascular disease increases with advancing age.[98,100] We carried out a meta-regression analysis for mean age distribution across the studies. For each study, we collected mean age for the whole cohort. If a study reported median age, we used that as the mean age. In case-control studies, where a study reported mean age in cases and controls separately, we calculated the weighted average of the means.
2. Sex distribution: Autoimmune rheumatic diseases are typically more common in women than men.[101,102] Conversely men tend to be at greater risk for cardiovascular disease than women.[102] We collected the proportion of women from each study and modelled it as a continuous variable in the meta-regression.
3. Study accrual (start year): For each study, we collected data on the accrual period. We modelled the initial year of the accrual period as a continuous variable.
4. Publication year: We modelled the year of publication to assess whether the association of MTX with cardiovascular events changed over time.
5. Observation time (in person-years): Several studies have suggested that the association between autoimmune disease and cardiovascular disease is sensitive to disease duration, study follow-up epoch and cohort type.[103,104] We obtained a study observation period in terms of cumulative person-years exposure for cohort studies. Case-control studies were excluded from this analysis.
6. Quality of studies according to Newcastle-Ottawa Scale (NOS): We assessed quality of observational studies using the NOS. The score ranged from 1-9, with greater scores representing higher quality.
7. Analysis adjusted for hypertension, diabetes and dyslipidemia: Obesity, cigarette smoking, elevated blood pressure, dyslipidemia and diabetes mellitus are considered traditional risk factors for cardiovascular disease.[10] Using meta-regression, we modelled the studies that adjusted for hypertension, diabetes and dyslipidemia versus those that adjusted for none of these variables.
8. Analysis adjusted for smoking: We performed meta-regression among the studies that adjusted for smoking versus those that did not.

9. Power score of included studies: We calculated the power for the included studies using the formula: (1-φ (Cα-Z score) + φ (-Cα-Z score)), where Cα is the critical value of Z associated with significance level α (for α=0.05, Cα=1.96) and Z score=effect size/SE. This can be calculated in Excel by: 1- NORMSINV (1.96-Z score) + NORMSINV (-1.96-Z score). We used these power scores in the meta-regression to assess the effects of varying power on effect size.

We did not have sufficient studies to run a meta-regression by disease duration, as only three studies with primary outcome data reported disease duration.

8.5 Dose-response analysis

To test for a dose-response relationship, we explored the associative risk of high and low dose MTX on cardiovascular events. We considered the cut-point for high and low dose MTX as defined by each study. We performed separate syntheses for high and low dose MTX.

9. Overall quality of the evidence

We used the GRADE approach to rate the overall quality and strength of the estimated association with cardiovascular events.[105] Two investigators (AS and DH) independently assessed the quality of the evidence. We presented the results of this assessment in a GRADE 'summary of findings' table. We then reported the overall GRADE score.

10. Presentation of results

We reported this systematic review according to the PRISMA guidelines. A flow chart was presented for the study identification and selection process. We summarized the study characteristics, disease under study, outcome characteristics, exposure details, and summary statistics in tabular format. We presented forest plots for the overall association of MTX with primary as well as secondary outcomes. We used funnel plots with trim-and-fill analyses to assess for publication bias.

11. Tables

Table 1. Search terms used in the Ovid Medline search strategy

Topic	MeSH terms	Key words
Autoimmune diseases	Autoimmune Diseases, Rheumatic diseases, Arthritis, Rheumatoid Psoriasis Myositis Polymyositis Dermatomyositis Lupus Erythematosus, Systemic Multiple Sclerosis Sjogren's Syndrome Pemphigoid, Bullous Inflammatory Bowel Diseases Myelitis, Transverse Scleroderma, Systemic Myasthenia Gravis Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis Polyarteritis nodosa Takayasu arteritis	Autoimmune, rheumatic, rheumatoid, psoriatic, arthritis, psoriasis, polymyositis, dermatomyositis, inflammatory myopathies, systemic lupus erythematosus, SLE, multiple sclerosis, Sjogren's, bullous pemphigoid, inflammatory bowel disease, ulcerative colitis, Crohn's, IBD, systemic sclerosis, myasthenia gravis, Wegener's granulomatosis, microscopic polyangiitis, polyarteritis, allergic and eosinophilic granulomatosis, Churg-strauss syndrome, ANCA, anti-neutrophil cytoplasmic antibody associated vasculitis
Methotrexate	Methotrexate	methotrexate, amethopterin, MTX and all possible trade names of methotrexare identified from online search and EMBASE database
Mortality outcomes	mortality, cause of death, fatal outcome, hospital mortality, death, sudden cardiac death, sudden death, death certificate, life expectancy, life tables, vital statistics	Mortality, death, died, die, fatal, life expectancy, life table, Cox model, Kaplan Meier
Cardiovascular outcomes	cardiovascular diseases cerebrovascular diseases	cardiovascular, cerebrovascular, cardiac, myocardial, heart, coronary, morbidity, stroke, IHD, CHF, CVA, CVD, MI, CHD, CAD, infarct, arrest, disease, ischemic, failure, event, bypass, revascularization, disorders

Abbreviations-IHD: Ischaemic heart disease, CAD: Coronary artery disease, CHD: Coronary heart disease, CHF: Congestive heart failure, CVA: Cerebrovascular accident, CVD: Cardiovascular disease, MI: Myocardial infarction.

Table 2. Study inclusion and exclusion criteria

	Inclusion criteria	Exclusion criteria
Study design	Cohort studies, case-control studies, randomized controlled trials	Cross-sectional studies, letters, commentaries, editorials, case reports, reviews, cross-over trials, in vitro studies
Disease under study	Any of the autoimmune diseases listed in the text	Studies without one of the listed autoimmune diseases
Outcomes of interest	- Studies reporting cardiovascular events, cardiovascular mortality and/or all-cause mortality - Trials reporting mortality or CV events in MTX users and a placebo group	Studies without any of these endpoints
Country of origin	Any	
Study duration	Any	
Language	English articles	Non-English articles
Comparison	- Studies comparing outcome of interest in MTX users versus non-users or placebo group, using multivariate regression to adjust for potential confounders - Study using topical agents or phototherapy as a control group	- Studies assessing exposure other than MTX such as anti-TNF agents, other conventional DMARDs, glucocorticoids, cytotoxic and non-cytotoxic agents - Studies comparing outcome of interest in MTX users versus an active control group
Risk measures	- Observational studies reporting any of the following adjusted risk measures along with its 95% confidence interval or standard error or p-value: incidence rate ratio, odds ratio, relative risk, hazard ratio. - Trials reporting binary event data	- Observational studies reporting unadjusted numbers or percentages of events without adjusted relative risk measures - Trials without binary event data
Other consideration	In case of multiple publications from the same patient population, we selected the study with the largest sample size	

Chapter 3: Results

1. Citation screening

We initially identified a total of 14,042 potential records from bibliographic databases and grey literature (Figure 3). We removed 563 duplicate records. We then screened 13,479 records by title, abstract and keywords to identify relevant studies. We excluded 13,292 irrelevant records. What remained were 187 potentially relevant records, for which we retrieved full text to better assess their eligibility.

We excluded 157 studies for the reasons described in Figure 3. A total of 30 studies met all selection criteria and were included in the meta-analysis. The kappa statistic for inter-rater agreement was 0.77, indicating substantial agreement.

2. INCLUDED STUDIES

2.1 Study characteristics

Appendix G presents characteristics of the included studies. There were 20 cohort studies, 7 case-control studies and 3 randomized controlled trials. The majority of the studies were from North America (n=17); a number were from Europe (n=10) and relatively few from Asia (n=3). The earliest published study was by van Den Hoogen et al in 1996 and the latest one was by Norton et al in 2014.[106,107] Our review included a total of 122,113 patients; 98,295 from cohort studies, 23,400 from case-control studies and 418 from randomized trials.

2.2 Characteristics of disease

As per Appendix H, the majority of studies examined the effect of MTX in patients with RA (n=21). Other autoimmune diseases studied were psoriasis (n=3), systemic sclerosis (n=3), inflammatory polyarthritis (n=1), and myositis (n=1). Prodanowich et al studied patients with RA as well as psoriasis.[108] Two studies, Chin et al and Wu et al, included patients with psoriatic arthritis along with psoriasis.[67,109] Among the studies using RA patients, 11 studies used American College of Rheumatology criteria for RA diagnosis, three studies used ICD-9 diagnosis codes and four studies used rheumatologist diagnosis as a diagnostic criterion. Three studies did not specify diagnostic criteria for the disease

under study.[68,107,110] Only 15 studies specified disease duration, which ranged from a maximum of 12 months in Ajeganova et al to a mean of 14.5 years in Davis et al.[63,75]

2.3 Exposure characteristics

Appendix I describes the exposure characteristics for MTX. The included studies used differing definitions for MTX exposure. Use of MTX for more than six months during the observation period was considered MTX exposure in four studies.[63,66,111,112] Choi et al used the intention-to-treat concept for defining MTX therapy.[113] MTX-user comparisons included ever-users versus never-users (n=13), current-users versus non-users (n=8) and initiators versus non-initiators (n=8). All randomized trials compared MTX users with a placebo group.[106,114,115] In these trials, we categorized MTX exposure as initiators versus non-initiators. Exposure status was not clear in Mantel et al.[110] The majority of studies extracted MTX exposure data from patient medical records (n=16); whereas others used administrative databases (n=11) or self-administered questionnaires (n=3) to capture MTX exposure.

2.4 Outcome characteristics

Outcome characteristics are described in Appendix J. Most of the studies reported composite cardiovascular events (n=7).[63,70,75,108,116-118] Others reported myocardial infarction (n=5), acute coronary syndrome (n=1), coronary artery disease (n=2), stroke (n=4), and heart failure (n=2). Eleven studies reported all-cause mortality and only 3 studies reported cardiovascular mortality. Choi et al and Goodson et al assessed both all-cause and cardiovascular mortality.[111,113] Ajeganova et al and Davis et al evaluated both cardiovascular events and all-cause mortality.[63,75] All randomized controlled trials reported mortality as an adverse event.

The following are study-specific definitions for cardiovascular events in each of seven studies reporting the primary outcome.

Study name	Cardiovascular events
Ajeganova et al., 2013[63]	Fatal or non-fatal myocardial infarction (MI), angina pectoris, coronary artery bypass grafting (CABG) or percutaneous coronary intervention (PCI), peripheral artery disease (PAD), vascular surgery, ischemic stroke, transient ischemic attack (TIA)
Davis et al., 2013[75]	Fatal or non-fatal MI, stroke, PCI, CABG
Gonzalez-Gay et al., 2007[116]	Ischemic heart disease (IHD), acute coronary syndrome (ACS), MI, heart failure, cerebrovascular accident (CVA), peripheral arteriopathy
Meek et al., 2014[70]	Fatal or non-fatal MI, Percutaneous transluminal coronary angioplasty (PTCA), CABG, angina pectoris, acute heart failure, CVA, death due to cardiac causes and sudden death
Prodanowich et al., 2005[108]	Cardiovascular disease, cerebrovascular disease, atherosclerosis
Tisseverasinghe et al., 2009[118]	Stroke, IHD, PAD, MI
van Halm et al., 2006[117]	MI, CABG, PTCA, ischemic abnormality on ECG, CVA, TIA, carotid endarterectomy and PAD

2.5 Number of events

There were a total of 4380 cardiovascular events (primary endpoints), 958 strokes, 612 diagnoses of heart failure, 2081 myocardial infarctions, 29 episodes of ischemic heart disease, 1829 deaths and 186 cardiovascular deaths. Three studies presented only relative risk measures without reporting the number of events.[65,107,110] Prodanowich et al reported the highest number of cardiovascular events; Wasko et al reported the highest number of all-cause deaths; and Goodson et al reported the highest number of cardiovascular deaths.[108,111,119]

2.6 Characteristics of studies reporting primary outcome (n=7)

Seven studies included cardiovascular events as the outcome. Prodanowich et al studied the effect of MTX in RA and psoriasis patients separately; therefore these were considered as two separate studies. Thus, there were a total of eight studies in the meta-analysis for the primary outcome, with an aggregate sample size of 17,796 patients.

2.7 Results of individual studies

Results from individual studies are presented in Table 3 at the end of this chapter. Most of the studies (n=16) used hazard ratios (95% confidence intervals) to report the effect of MTX on the outcomes; two studies used rate ratios, two studies used relative risks, one study reported incidence rate ratio and six studies used odds ratios. From the number of events reported in three randomized trials, we calculated the relative risk and 95% confidence interval.

A study by Meek et al[70] presented a hazard ratio of 3.436 (95% CI: 1.553, 7.576) for the protective effect of MTX against incident first cardiovascular event in RA patients. To interpret this hazard ratio as a risk of the first cardiovascular event in MTX users versus non-users, we took the reciprocal of it. We calculated the standard error from 95% confidence interval, inverted it and re-calculated the 95% confidence interval to be used in the meta-analysis.

Chin et al reported two outcomes: cerebrovascular and cardiovascular events.[67] To avoid doubling the control group by combining these outcomes into a single measure, we only considered cerebrovascular outcomes for the analysis on stroke.

Troelsen et al assessed ischemic heart disease (IHD) and myocardial infarction (MI) as two separate outcomes.[120] There was a chance of overrepresentation of the study if we had combined these two outcomes in a single measure. As MI is a subset of IHD, and IHD is a more diverse outcome, we used only IHD in this meta-analysis.

Walfe and Michaud presented risk estimates for first observed MI and all MI separately.[112] We considered all MI as the outcome of interest.

3. PRIMARY ANALYSES

3.1 Cardiovascular events

Figure 4 gives the associations from individual studies and the overall pooled estimate for the association of MTX with cardiovascular events, analysed using random effects meta-analysis. MTX was significantly associated with a decreased risk of cardiovascular events with a pooled risk ratio of 0.75 (95% CI: 0.65, 0.86).

The greatest effect size was observed in a study by Meek et al with a risk ratio of 0.29 (95% CI: 0.13, 0.65) and the smallest effect in Tisseverasinghe et al (RR: 0.90, 95% CI: 0.33, 2.49). Although the individual study effects were numerically protective, three studies presented statistically non-significant effects.[116-118] I^2 for the overall pooled estimate was 11%, indicating minimal heterogeneity.

4. SECONDARY ANALYSES

4.1 All-cause mortality

MTX was associated with significantly decreased all-cause mortality with a pooled risk ratio of 0.60 (95% CI: 0.48, 0.76). In Figure 8, we have presented separate analyses for cohort studies and randomized trials. The overall effect was statistically significant in cohort studies (RR: 0.60, 95% CI: 0.47, 0.76), while non-significant in randomized trials (RR: 0.69, 95% CI: 0.24, 2.00). There was moderate heterogeneity for the overall pooled effect estimate with an I^2 of 45%.

4.2 Cardiovascular mortality

Only three studies examined the association of MTX with cardiovascular mortality.[111,113,116] As shown in Figure 9, MTX was associated with reduced cardiovascular mortality. The overall pooled risk ratio was 0.45 (95% CI: 0.26, 0.80). I^2 for the pooled estimate was 33%, indicating moderate heterogeneity.

4.3 Specific cardiovascular events (as a secondary analysis)

MTX was associated with a lower risk of coronary events with a pooled risk ratio of 0.78 (95% CI: 0.67, 0.91), and a non-significantly lower risk of heart failure (RR: 0.61, 95% CI: 0.32, 1.19) and stroke (RR: 0.67, 95% CI: 0.42, 1.09) (Figure 11).

5. Subgroup analysis for cardiovascular events

Table 4 reports the results from subgroup analyses. Tests of interaction across the specified subgroups were all non-significant at the $p<0.05$ level of significance.

6. Meta-regression for cardiovascular events

We carried out univariate random effects meta-regression for the pre-specified variables. The results are shown in Table 5. All predictors were non-significant in the analysis.

7. Dose-response analysis

A total of three studies reported an MTX-dose analysis for cardiovascular events.[69,108,117] In Figure 6, we compared the effect of high- and low- cumulative dose MTX. Low cumulative dose MTX showed a more sizeable reduction in cardiovascular events (RR: 0.61, 95% CI: 0.51, 0.74) compared to the association for high cumulative dose MTX (RR: 0.88, 95% CI: 0.78, 0.99). The test of significance across dose was statistically significant (p-value: 0.001).

8. Publication bias

We assessed publication bias using funnel plots, and its impact using Duval and Tweedie's trim and fill method (Figures 7 for cardiovascular events and Figure 10 for all-cause mortality).

For cardiovascular events, the method imputed two studies to the right of the null line to make the plot symmetrical. The publication bias adjusted risk ratio was almost the same as the observed risk ratio: RR 0.75 (95% CI: 0.65, 0.86) versus RR 0.76 (95% CI: 0.63, 0.92). Similarly, for all-cause mortality, the observed risk ratio remained unchanged after adjustment for publication bias.

9. Methodological quality of included studies

We assessed the methodological quality of observational studies using the Newcastle-Ottawa-Scale. Appendix L and M displays the score of NOS for cohort and case-control studies respectively. The cohort studies' NOS score ranged from 6 to 9. Median (IQR) of the NOS score for cohort studies was 8 (1). All seven case-control studies had an NOS score from 6 to 8. Median (IQR) of the NOS score for case-control studies was 7 (0.5).

Among the studies reporting the primary outcome, median (IQR) of the NOS score was 8 (1.5).

We used the Cochrane risk of bias tool to assess the methodological quality of randomized controlled trials. In Appendix N, we have presented the review author's (AS) judgement for each quality item.

10. Overall quality of evidence

We used the GRADE 'summary of findings' table to present the overall quality of evidence for each outcome.[105] Table 7 represents the rating of the evidence. In the GRADE system, randomized controlled trials are graded as high-quality and observational studies as low-quality evidence. However, if there is evidence of a large effect size, dose response gradient or implausible confounding, the quality of observational studies should be rated up.[105]

All 8 studies reporting the primary outcome were observational studies. We rated this evidence as moderate evidence due to the reported dose response gradient for low and high dose MTX. For all-cause mortality and coronary endpoints, the evidence was labelled as very low-quality as there was moderate heterogeneity with I^2 of 45% and 30% respectively. The evidence for cardiovascular mortality was rated very low-quality evidence due to inconsistency (I^2: 33%) and suspected publication bias. For stroke, the evidence has both imprecision and inconsistency, and thus was labelled as very low grade evidence. Results from heart failure studies were assessed as inconsistent and imprecise, resulting in very low grade evidence.

11. Tables and Figures

Figure 3. Screening and selection process for studies

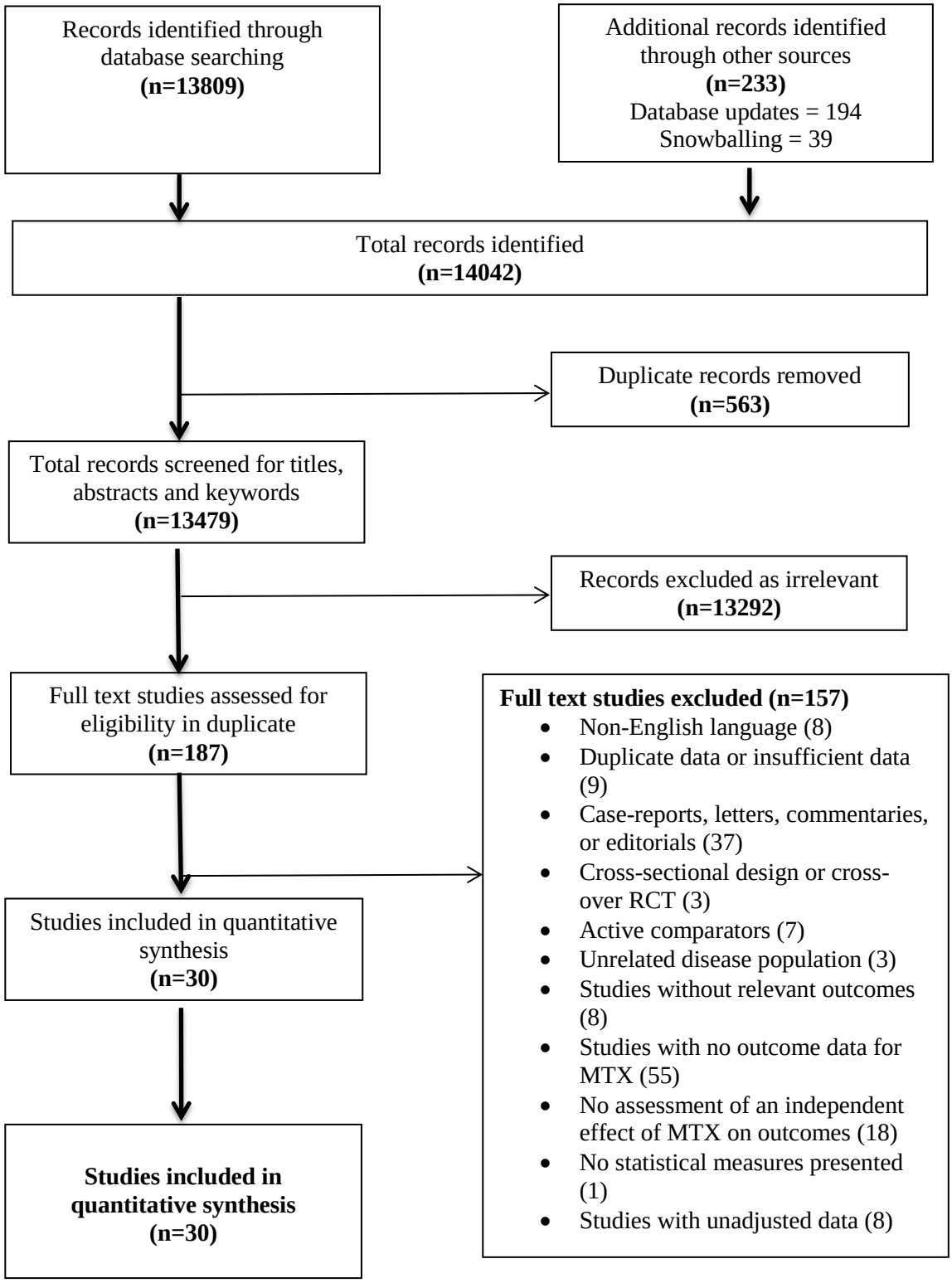


Table 3. Results of individual studies

Study	Observation time	Outcome	Number of events	Effect measure	Risk estimates (95% CI)	Variables adjusted for
Ajeganova et al., 2013	9405 person-years	Cardiovascular events All-cause mortality	177 151	HR	0.72 (0.53 - 0.99) 0.99 (0.71 - 1.38)	Age, sex, smoking status at inclusion, HTN, DM, hyperlipidemia
Bernatsky et al., 2005	51301 person-years	CHF requiring hospitalization	520	Rate ratio	0.80 (0.60 - 1.00)	Age, sex, cohort, comorbidities, current DMARDs, use of NSAIDs, COX-2 inhibitors, glucocorticoids
Bozaite-Gluosniene et al.,2011	Not specified	CAD	Not specified	HR	0.54 (0.37 - 0.77)	Age, sex, HTN, hyperlipidemia, DM, RF, BMI, blood pressure, LDL, ESR, Hydroxychloroquine, MTX, corticosteroid and NSAID use
Chiang et al., 2013	Median 4.7 years	Ischemic stroke	86	HR	1.47 (0.64 - 3.42)	Age, sex (male), HTN, DM, dyslipidemia, chronic kidney disease, CAD and AF
Chin et al., 2013	Cerebrovascular event: psoriasis: 3428.5 ± 11.2 and PsA: 3235.2 ± 60.4 days, CV events: psoriasis:3275.0 ± 14.5 and PsA: 3085.1 ± 70.5 days	Cerebrovascular events Cardiovascular events	406 688	HR	0.45 (0.23 - 0.85) 0.48 (0.29 - 0.81)	Age, sex, HTN, diabetes, dyslipidemia and phototherapy
Choi et al., 2002	91007 person-months	All-cause mortality Cardiovascular mortality	191 84	HR	0.40 (0.20 - 0.80) 0.30 (0.20 - 0.70)	Age, sex, RF, calendar year, disease duration, smoking, education, HAQ score, PtGA, joint counts, ESR, prednisone use, no. of other DMARDs
Cohen et al., 2001	24 months	All-cause mortality	3	RR	1.34 (0.12 - 14.7)	
Davis et al., 2013	3743 person-years	Cardiovascular events All-cause mortality	97 252	HR	0.66 (0.44 - 1.00) 0.75 (0.58 - 0.97)	Multiple patient demographic and RA severity
Edwards et al., 2008	Not specified	MI	966	IRR	0.86 (0.74 – 1.00)	Age, sex, BMI, HTN, DM, smoking

Study	Observation time	Outcome	Number of events	Effect measure	Risk estimates (95% CI)	Variables adjusted for
Gonzalez-Gay et al., 2007	Mean (IQR): 13.4, (10-16) years	Cardiovascular events Cardiovascular mortality	39 17	HR	0.86 (0.39 - 1.80) 0.86 (0.28 - 2.69)	Age at disease onset and sex
Goodson et al., 2008	10 years	All-cause mortality Cardiovascular mortality	203 85	OR	0.59 (0.35 - 0.97) 0.53 (0.25 - 1.14)	Age, sex, joint counts, RF, nodules, RA, NSAIDs, steroids, CRP, smoking, HAQ, number of comorbid medications used
Lan et al., 2012	Not specified	Cerebrovascular event	399	HR	0.50 (0.27 - 0.92)	HTN, DM, dyslipidemia, age and sex
Levesque et al., 2013	2,386.4 person-years (MTX exposure)	MI	53	HR	0.85 (0.40 - 1.84)	Age, sex, history of MI, DM, HTN, hyperlipidemia and use of corticosteroid
Mantel et al., 2014	Average 5.3 years	Acute coronary syndrome	Not specified	OR	1.10 (0.60 – 2.20)	Age, sex, year of RA diagnosis and study center*****
Meek et al., 2014	1380 person-years	Cardiovascular event	29	HR	0.29 (0.13, 0.65)	CV risk factors, inflammatory parameters, disease duration, presence of IgM RF and/or anti-CCP antibodies, use of anti-inflammatory immunosuppressive therapy
Mikuls et al., 2011	2314 person-years	All-cause mortality	138	HR	0.63 (0.42 - 0.96)	Age, race, BMI, comorbidities
Myasoedova et al., 2011	7692 person-years	Heart failure	92	HR	0.40 (0.20 – 0.80)	Age, sex, calendar year, CV risk factors, CHD, RF positivity, RA duration, ESR and severe ExRA
Nadareishvili et al., 2008	Mean (IQR): 3.9 (2.0 – 6.0) Years	Ischemic stroke	67 cases	OR	0.77 (0.39-1.54)	(Age, sex, calendar time)***** HAQ, total joint replacement, RA duration, low dose aspirin, comorbidity index (0-9 for 11 comorbidities)
Norton et al., 2014	Not specified	All-cause mortality	Not specified	HR	0.40 (0.25 - 0.64)	Demographic and clinical features at baseline, confounding by indication of treatment effect
Pope et al., 2001	Median 1.5 years	All-cause mortality	10	RR	0.44 (0.12-1.56)	

Study	Observation time	Outcome	Number of events	Effect measure	Risk estimates (95% CI)	Variables adjusted for
Prodanowich et al., 2005	Not specified	Cardiovascular events (psoriasis) Cardiovascular events (RA)	1869 2017	RR	0.73 (0.55-0.98) 0.83 (0.71-0.96)	Age, sex, DM, HTN, dyslipidemia, other medications (FA, B6, B12)
Suissa et al., 2006	166194 person-years	MI	558	Rate ratio	0.81 (0.60 - 1.08)	Age, DMARDs, other anti-RA drugs, IHD, PAD, other CVD, DM, respiratory illness
Tisseverasinghe et al., 2009	Mean (SD): 4 (3.7) years	Cardiovascular events	80	RR	0.90 (0.30 – 2.30)	Comorbidities, steroid, NSAID, Cox-2 inhibitors, immunomodulators, data source for MI diagnosis, h/o coagulopathy and/or exposure to aspirin, warfarin or Low molecular weight heparin
Troelsen et al., 2007	1799 person-years	IHD MI	29 12	HR	0.60 (0.20 – 1.80) 0.70 (0.10 – 5.00)	Age and sex
van den Hoogen et al., 1996	48 weeks	All-cause mortality	3	RR	1.41 (0.14 -13.85)	
van Halm et al., 2006	5649 person-years	Cardiovascular events	Cases: 72	OR	0.47 (0.07-3.23)	Age, sex, smoking, RA duration, HTN, DM, hypercholesterolemia
Wasko et al., 2013	40,722 patient-years	All-cause mortality	666	HR	0.30 (0.09 – 1.03)	Age, education level, sex, BMI, HAQ score, ethnicity, RA duration, HTN, CAD, DM, stroke, prednisone, TNF inhibitors, non-MTX DMARDs, NSAIDs, and cox-2 inhibitors and other comorbidities
Wolfe and Michaud, 2008	Mean (IQR): 3 (0.5 – 8.5) Years	MI (All) MI (First MI)	283 223	OR	0.90 (0.70 - 1.20) 0.90 (0.70 - 1.20)	Education, ethnicity, smoking, DM, aerobic exercise, HTN, comorbidity index from 11 present and past conditions, low-dose aspirin, BMI, baseline MI status, PAS score, joint replacement status, RA duration
Wolfe et al., 2003	88063 person-months	All-cause mortality	212	OR	0.51 (0.37-0.72)	Age, sex, HAQ score, MTX (time-varying), RA and disease factors

Study	Observation time	Outcome	Number of events	Effect measure	Risk estimates (95% CI)	Variables adjusted for
Wu et al., 2012	42424 person-years	MI	221	HR	0.52 (0.31-0.85)	Age, sex, person-years among the cohorts, CV risk factors, medications that are known to reduce MI risk

Abbreviations: HTN: hypertension, DM: diabetes mellitus, BMI: Body Mass Index, RF: rheumatoid factor, LDL: low density lipoprotein, ESR: erythrocyte sedimentation rate, NSAID: non-steroidal anti-inflammatory drug, CAD: coronary artery disease, AF: atrial fibrillation, HAQ: Health Assessment Questionnaire, PtGA: patient global assessment of disease activity, IRR: incident rate ratio, CRP: C-reactive protein, ExRA: extra-articular manifestations of RA, RCT: randomized controlled trials

* Variables matched in study design

Figure 4. Effect of MTX on primary outcome (cardiovascular events)

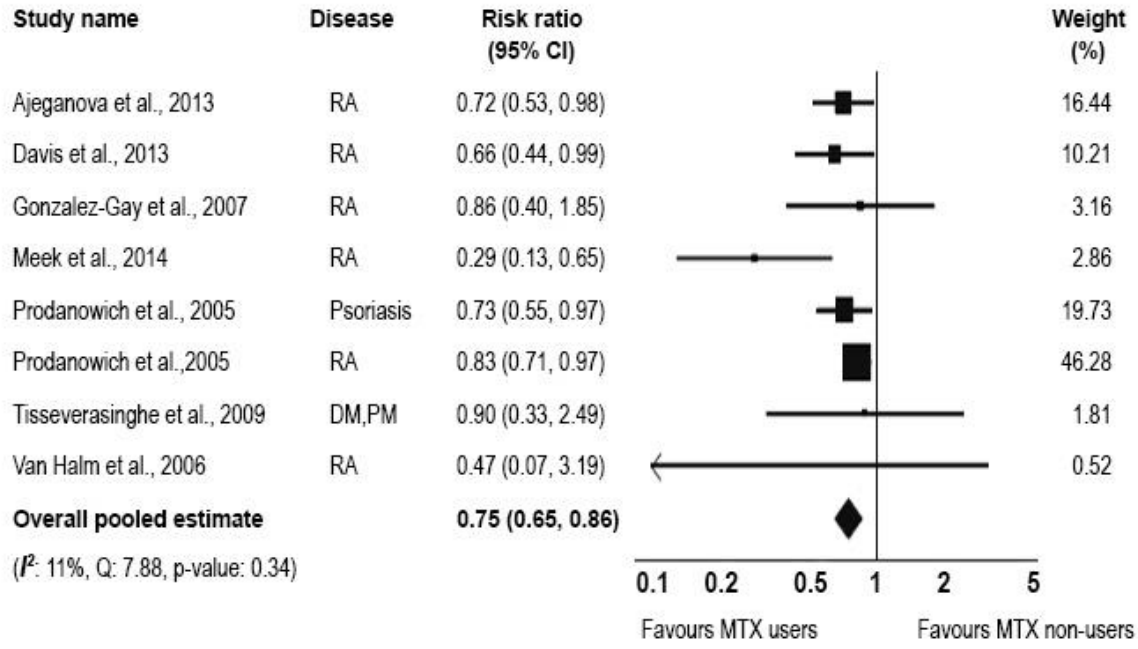


Table 4. Subgroup analysis for the primary outcome (cardiovascular events)

Subgroup	# of studies	Risk ratio (95% CI)	I^2	Q statistics (P-value) (Test of interaction)
Disease				
RA	6	0.72 (0.58, 0.89)	34.71	0.17 (0.92)
Psoriasis	1	0.73 (0.48, 1.10)	0.00	
Others (PM, DM)	1	0.90 (0.31, 2.60)	0.00	
Study design				
Prospective cohort	4	0.66 (0.51, 0.84)	37.09	1.60 (0.45)
Retrospective cohort	2	0.80 (0.67, 0.96)	0.00	
Case-control	2	0.78 (0.31, 1.90)	0.00	
Study region				
America	4	0.79 (0.68, 0.91)	0.00	1.27 (0.26)
Europe	4	0.66 (0.50, 0.87)	38.66	
Study excluded patients with history of CVD				
Yes	6	0.75 (0.63, 0.88)	30.91	0.10 (0.75)
No	2	0.69 (0.46, 1.05)	0.00	
MTX exposure type				
Initiators	2	0.56 (0.39, 0.80)	68.59	3.27 (0.07)
Ever-users	6	0.79 (0.70, 0.90)	0.00	
Data source for MTX exposure				
Database	3	0.71 (0.54, 0.93)	0.00	0.13 (0.72)
Medical records	5	0.75 (0.62, 0.91)	42.69	
Adjusted for DMARDs/other anti-rheumatic medications in the analysis				
Yes	2	0.45 (0.24, 0.84)	65.80	2.88 (0.09)
No	6	0.78 (0.70, 0.88)	0.00	
Adjusted for hypertension, diabetes and dyslipidemia in the analysis				
Yes	6	0.74 (0.63, 0.88)	31.02	0.06 (0.80)
No	2	0.71 (0.47, 1.05)	0.00	
Adjusted for CVD in the analysis (omitted studies which excluded patients with CVD)				
Yes	1	0.90 (0.32, 2.54)	0.00	0.14 (0.71)
No	7	0.74 (0.63, 0.86)	22.93	
Adjusted for smoking in the analysis				
Yes	3	0.63 (0.48, 0.85)	54.13	1.98 (0.16)
No	5	0.80 (0.70, 0.90)	0.00	

Table 5. Meta-regression results for primary outcome (cardiovascular events)

Covariate	Estimated β coefficient (95% CI)	p- Value	τ^2
Mean age (years)	-0.008 (-0.065, 0.048)	0.77	0.000
% Female	-0.002 (-0.007, 0.002)	0.28	0.000
Accrual start (year)	-0.030 (-0.071, 0.010)	0.15	0.000
Publication year	-0.028 (-0.061, 0.005)	0.10	0.000
Observation time (person-years)	0.000 (-0.000, 0.000)	0.31	0.000
Quality of studies according to NOS	0.072 (-0.062, 0.208)	0.29	0.000
Analysis adjusted for HTN, DM and dyslipidemia	0.101 (-0.279, 0.483)	0.60	0.000
Analysis adjusted for smoking	-0.225 (-0.539, 0.088)	0.16	0.000
Analysis adjusted for hypertension	0.101 (-0.279, 0.483)	0.60	0.000
Analysis adjusted for diabetes	0.101 (-0.279, 0.483)	0.60	0.000
Power score of included studies	0.708 (-0.002, 1.419)	0.05	0.000

Figure 5. Association of MTX with the primary outcome by study design

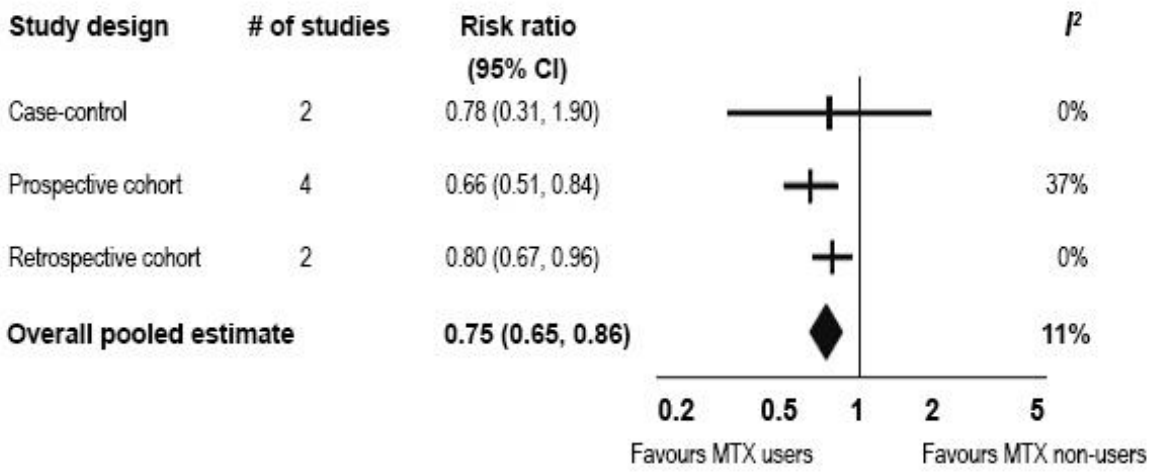
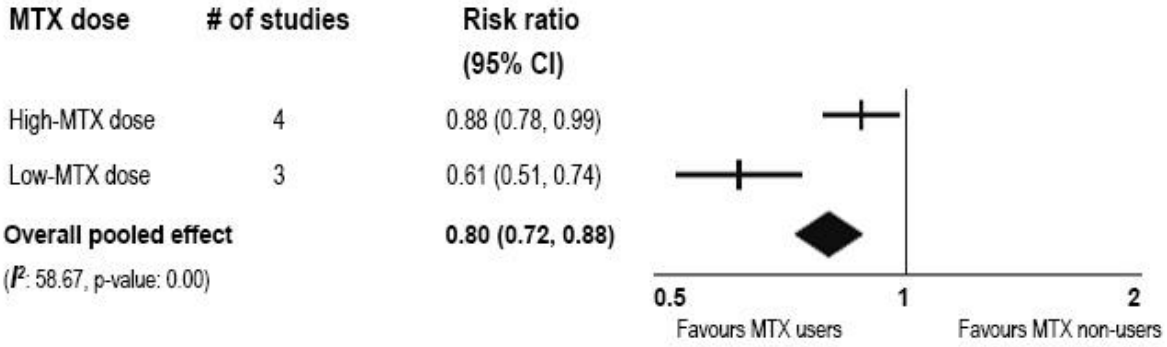


Figure 6. MTX dose response analysis for the primary outcome (cardiovascular events)



(Low cumulative MTX dose: cumulative dose <1.56 g (in Lan et al), or less than median dose (in Prodanowich et al); **High cumulative MTX dose:** cumulative dose >1.56 g (in Lan et al), or more than median dose (in Prodanowich et al and van Halm et al))

Figure 7. Funnel plot: Effect of MTX on primary outcome (cardiovascular events)

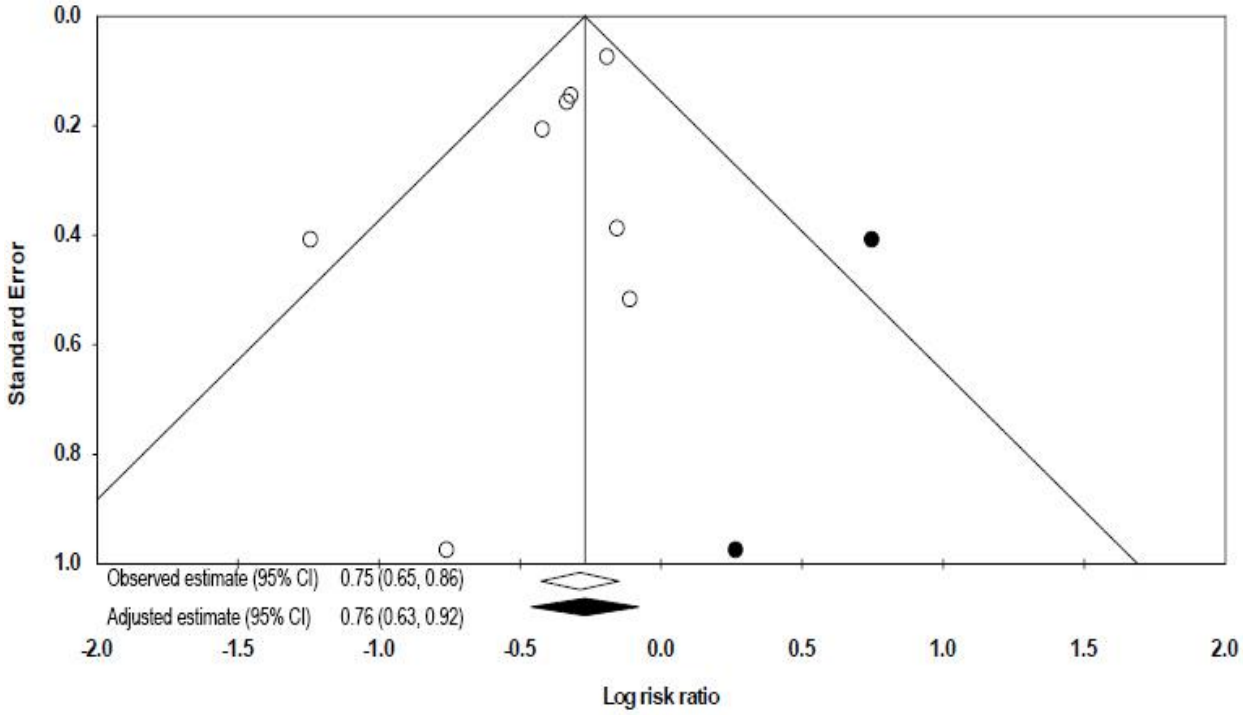


Figure 8. Effect of MTX on all-cause mortality (secondary outcome)

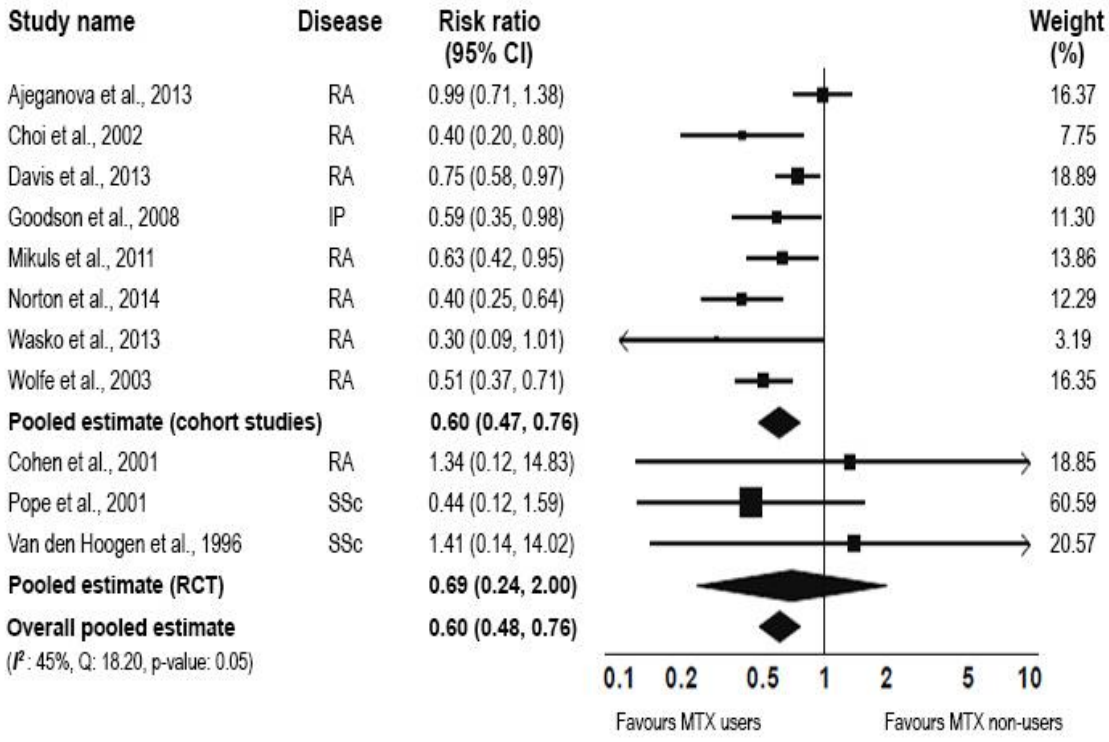


Figure 9. Effect of MTX on cardiovascular mortality (secondary outcome)

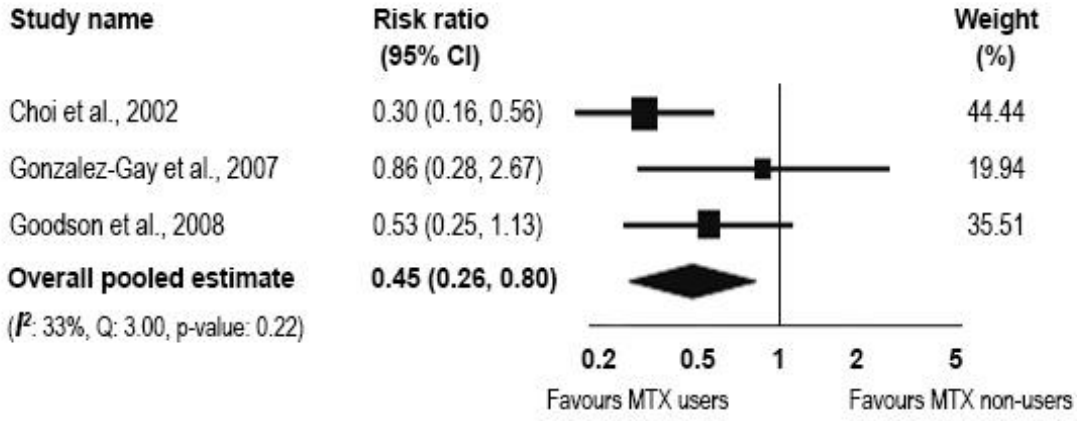


Figure 10. Funnel plot: Effect of MTX on all-cause mortality (secondary outcome)

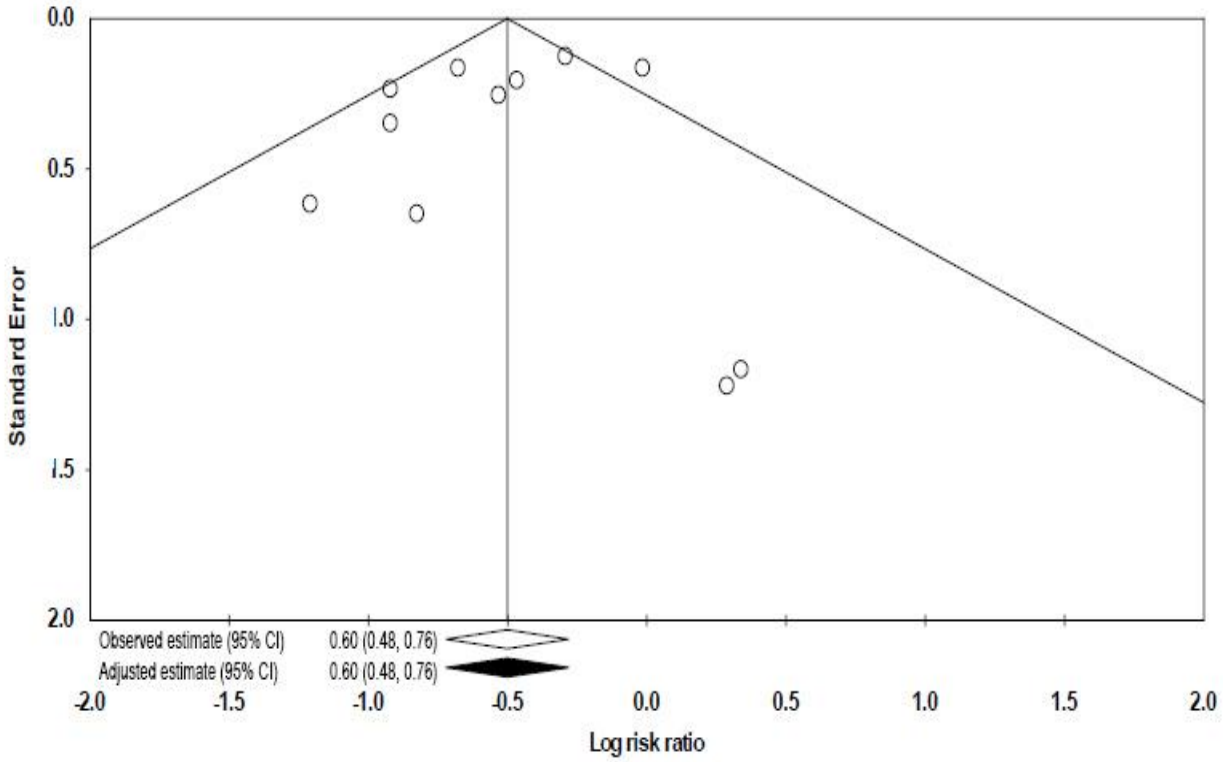


Figure 11. Effect of MTX on cardiovascular diseases (secondary outcome)

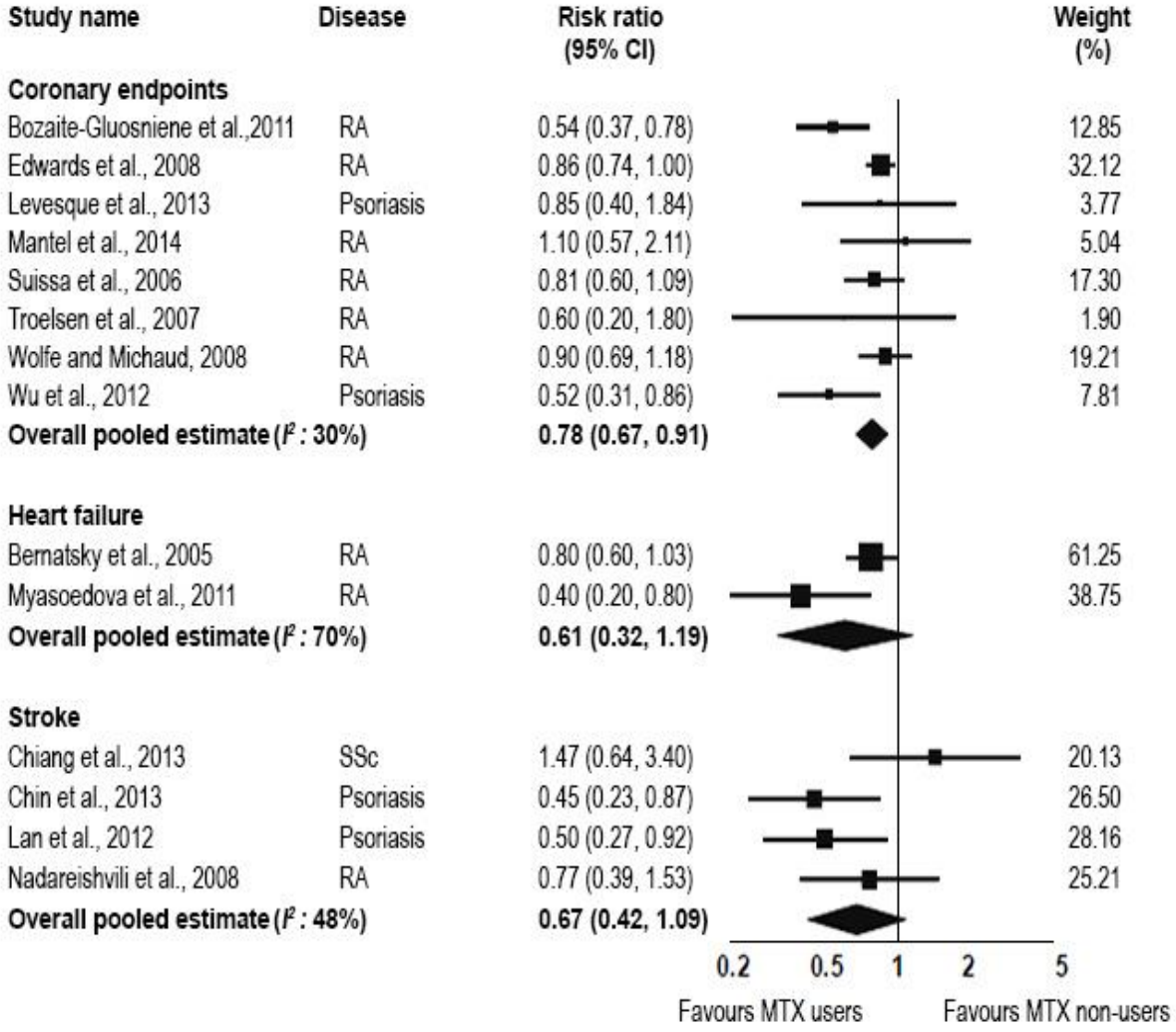


Table 6. Summary table of effect of MTX on different outcomes

Outcome	# of studies	Random effects RR (95% CI)	I-squared
Primary outcome			
Cardiovascular events	8	0.75 (0.65, 0.86)	11%
Secondary outcomes			
All-cause mortality	11	0.60 (0.48, 0.76)	41%
Cardiovascular mortality	3	0.45 (0.26, 0.80)	33%
Coronary endpoints	8	0.78 (0.67, 0.91)	30%
Stroke	4	0.67 (0.42, 1.09)	48%
Heart failure	2	0.61 (0.32, 1.19)	70%

Table 7. Summary of findings

Question:	**Is methotrexate associated with a lower risk of cardiovascular events in patients with autoimmune disease?**
Population:	Patients with autoimmune disease (RA, psoriasis, psoriatic arthritis, systemic sclerosis, myositis, inflammatory polyarthritis)
Intervention:	MTX
Comparison:	MTX non-user group
Outcome:	Cardiovascular events, all-cause mortality, cardiovascular mortality, coronary endpoints, and stroke

Outcome	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Number of participants (studies)	Relative effect (95% CI)	Quality of evidence (GRADE)
Cardiovascular events	likely[1]	no serious inconsistency[2]	no serious indirectness[3]	no serious imprecision[4]	not likely[5]	17796 (8)	0.75 (0.65, 0.86)	moderate[1,2,3,4,5,6] ⨁⨁⨁◯
All-cause mortality	likely[7]	inconsistency[8]	no serious indirectness[3]	no serious imprecision[4]	not likely[5]	15160 (11)	0.60 (0.48, 0.76)	very low[3,4,5,7,8] ⨁◯◯◯
Cardiovascular mortality	likely[9]	inconsistency[10]	no serious indirectness[3]	no serious imprecision[11]	strongly suspected[12]	2345 (3)	0.45 (0.26, 0.80)	very low[3,9,10,11,12] ⨁◯◯◯
Coronary endpoints	likely[14]	inconsistency[13]	no serious indirectness[3]	no serious imprecision[4]	not likely[5]	66248 (8)	0.78 (0.67, 0.91)	very low[3,4,5,13,14] ⨁◯◯◯
Stroke	likely[17]	inconsistency[15]	no serious indirectness[3]	imprecision[16]	not likely[5]	18182 (4)	0.67 (0.42, 1.09)	very low[3,5,15,16,17] ⨁◯◯◯

Heart failure	likely[18]	inconsistency[19]	no serious indirectness[3]	imprecision[16]	Undetected[20]	6515 (2)	0.61 (0.32, 1.19)	very low[3,16,18,19,20] ⨁◯◯◯

[1]median score of Newcastle-Ottawa scale was 8 (interquartile range (IQR): 1.5), however, all included studies were observational
[2]overlaping CIs, non-significant p for heterogeneity and I^2*:* 11%, suggesting minimal heterogeneity
[3]patients (autoimmune disease), intervention (MTX) and outcome were consistent
[4]CI of the overall effect does not include null value. Also, a number of events and total patients were large enough to achieve adequate power
[5]publication bias adjusted estimate didn't change considerable from the observed estimate
[6]dose-response gradient was observed in the subgroup analysis, showing higher effect with low dose MTX
[7]median score of New-castle-Ottawa scale was 9 (IQR: 1.25) for cohort studies; and the overall assessment for RCTs suggests a low risk of bias, however, the majority of included studies were observational
[8]significant p value for heterogeneity and I^2*:* 45%, suggesting moderate heterogeneity
[9]median score of New-castle-Ottawa scale was 8 (IQR: 0.5), however, all included studies were observational
[10]non-overlapping CIs, non-significant p value for heterogeneity and I^2:33%, suggesting moderate heterogeneity
[11]CI of the overall estimate does not include null value. A total number of events was 433 with >30% of relative risk reduction
[12]publication bias adjusted estimate differed from the observed estimate
[13]non-overlapping CIs with I^2: 30%
[14]median score of New-castle-Ottawa scale was 8 (IQR: 1.25), however, all included studies were observational
[15]non-overlapping CIs, non-significant p value for heterogeneity and I^2:48%, suggesting moderate heterogeneity
[16]wide CI for the overall estimate which includes null value; and point estimate showed an extreme benefit
[17]median score of New-castle-Ottawa scale was 8 (IQR: 0), however, all included studies were observational
[18]median score of New-castle-Ottawa scale was 8 (IQR: 1), however, all included studies were observational
[19]non-overlapping CIs and I^2:70%, suggesting substantial heterogeneity
[20]publication bias could not be tested for n = 2 studies for heart failure

Chapter 4: Discussion

1. Summary of findings

Our systematic review and meta-analysis suggests that MTX is associated with a 25% reduction in cardiovascular events in patients with autoimmune disease. GRADE assessment labelled this as moderate evidence, meaning that the true effect is close to the observed effect but there is a possibility that it is considerably different. In our search, we included all autoimmune diseases for which MTX is recommended as therapy. However, we found studies in RA, psoriasis, psoriatic arthritis and myositis only. The statistical heterogeneity for the pooled effect estimate was 11%. We explored this heterogeneity in subgroup analyses and meta-regression according to a variety of patient and study characteristics. None of the subgroup analyses or meta-regression predictors were statistically significant.

A similar direction of effect was observed for all-cause mortality. MTX was associated with a 40% reduction in all-cause mortality in patients with RA, systemic sclerosis and inflammatory polyarthritis. This finding was accorded very low GRADE evidence. We included three RCTs reporting mortality as an adverse event in the synthesis of this effect estimate. These trials were of short duration and small sample size, so the confidence intervals for individual trials as well as the pooled effect were wide. These trials were underpowered for reporting mortality outcomes. Therefore, this result should be treated with caution. The pooled effect from cohort studies also indicated 40% lower risk for mortality with a narrow confidence interval. This result from the cohort studies seems adequately powered, with the total number of patients studied and observed events equal to 14,742 and 1684 respectively.

There was a clear association of MTX with cardiovascular mortality with a 55% lower risk in patients with RA and inflammatory polyarthritis. This was very low GRADE evidence due to inconsistency (I^2 = 33%) and publication bias, suggesting that the true effect is likely considerably different from the observed effect.

MTX was associated with a lower risk of coronary events in patients with RA, psoriasis and psoriatic arthritis. A statistically significant 22% lower risk was observed for coronary endpoints in patients receiving MTX. However, the evidence was labelled as very low GRADE evidence because of the inconsistency in effect across studies (I^2 = 33%).

There was no clear effect of MTX on stroke despite the overall large sample size of 18,182. Similarly there was not clear association for heart failure. For stroke, this may be due to the diverse etiology of cerebrovascular disease, with atherosclerosis accounting for only 20% of cases. Heart failure is also diverse and may be due to different causes (e.g. viral, idiopathic, valvular), some of which may not be amenable to MTX exposure.

Due to the low prevalence of ANCA-associated vasculitis, studies assessing MTX and cardiovascular disease are lacking. Additionally, cardiac involvement may be different in vasculitis than in common rheumatic conditions.[121]

2. Exploratory findings

The included studies reported varied types of MTX exposure. It is difficult to identify exposure status with certainty in real-world practice, particularly given such issues as non-compliance and temporary treatment discontinuation due to remission or side effects. Several included studies compared the cardioprotective effect of MTX in initiators versus non-initiators and ever-users versus never-users. To assess the association as per "intention-to-treat" analysis, we limited our subgroup analysis to initiators versus non-initiators and reported a 44% lower risk of cardiovascular events. However, this pooled analysis is not free from heterogeneity (I^2 = 68%). We also assessed the effect of MTX in studies adjusted for other non-MTX disease-modifying anti-rheumatic drugs and found a 55% lower risk of cardiovascular events in association with MTX exposure. But again, substantial heterogeneity (I^2 = 66%) should be considered before interpreting this result.

We found a consistent effect of MTX across different strata. The effect was constant in patients from American and European countries where different health care systems exist. The effect was also consistent across mean age, suggesting its applicability to different patients regardless of age. However, the results from this meta-regression produce an

ecological fallacy. A decrease in the risk of cardiovascular events with one unit increase in mean age is an association observed between two group-level variables, which may not resembles the individual level association. These results would not be as robust as that of regression analysis using individual patient data. Finally, the results were consistent across publication and accrual years.

3. Dose-response

Both low and high cumulative dose MTX treatments are associated with significant reductions in cardiovascular events. However, low cumulative dose MTX treatment showed more than three times the cardioprotective effect of high cumulative dose MTX treatment (39% versus 12% reduction). The difference in effect between the two groups was statistically significant ($p = 0.001$).

The difference in the cardioprotective effect of low and high dose MTX might be explained by differences in their mechanism of action, safety profile, tolerability and treatment durability. High-dose MTX is commonly used in cancer treatment, while low-dose is recommended for the treatment of systemic inflammatory rheumatic diseases.[77,122] High-dose MTX acts on rapidly growing cells through anti-proliferative and cytotoxic mechanisms. It inhibits dihydrofolate reductase enzyme and stops *de novo* synthesis of DNA, RNA, thymidylates and proteins.[122,123] As discussed in Chapter 1, low-dose MTX mainly exerts an anti-inflammatory effect.[123] Increased systemic inflammation is known to accelerate atherosclerosis; therefore, the anti-inflammatory mechanism of low-dose MTX may play a crucial role in preventing cardiovascular disease. An alternate explanation is that patients requiring high-dose MTX were sicker than patients requiring low-dose MTX, and therefore there may be confounding by disease severity.

4. Strengths

In this systematic review, we searched for studies with populations having those autoimmune diseases for which MTX is used as either first, second or third line therapy; Micha et al included patients with only RA and psoriasis.[72] In addition to RA and psoriasis, we identified studies assessing MTX in systemic sclerosis, myositis and inflammatory polyarthritis.

We executed a systematic literature search of more than 13,000 citations using a comprehensive search strategy; we identified studies from major databases such as Ovid Medline, EMBASE, Cochrane library and Web of Science, as well as different grey literature sources. Using detailed pre-specified inclusion and exclusion criteria, we adjudicated studies independently and in duplicate to increase the validity of the results.

We identified observational studies for the primary outcome, representing patients treated in real world clinical settings. In addition to cardiovascular events, we assessed the association of MTX with cardiovascular mortality and all-cause mortality, as well as coronary events, stroke and heart failure. To assess an independent and less confounded effect of MTX, we only included studies that reported adjusted risk estimates. MTX can cause severe adverse events such as hepatotoxicity, pulmonary toxicity, severe infection, lymphoproliferative disorders and nephrotoxicity which, consequently, could lead to death.[124] To properly weigh the risks and benefits of MTX on mortality, we included randomized controlled trials that reported mortality as an adverse event.

Despite the minimal heterogeneity of the overall pooled estimate (11%), we checked for consistency across study design, study region, type of autoimmune disease, and presence of adjustment for cardiovascular risk factors.

We used the established GRADE system to rate the overall quality of evidence for the association of MTX with the reported outcomes. Previous meta-analyses have not rated overall quality for their reported outcomes.[72,73]

5. Limitations

We found only observational studies for the primary outcome. These studies are not free from confounding by selection bias and indication bias. As we discussed earlier in chapter-1, these may either underestimate or overestimate the association. Thus, the results should be interpreted with caution. We included only adjusted risk estimates in the meta-analysis to reduce the impact of confounding. However, residual confounding by unmeasured confounders could give rise to biased effect estimates.

The majority of studies did not adjust for the severity of the underlying disease, which could cause a protective association of MTX to be underestimated. Conversely, by

decreasing folate levels, MTX can cause hyperhomocysteinemia, a known risk factor for cardiovascular disease.[125,126] Most of the included studies did not adjust for concomitant folic acid therapy in their analyses. As well, we identified only four studies reporting associations of MTX for high and low doses.

Some studies used insurance claim data to assess MTX exposure.[63,75,118] Therefore, detailed information regarding treatment duration, doses and compliance were not available. Further, none of the studies assessing the primary outcome included MTX as time-varying covariate in their analysis. Thus, authors assumed that patients had complied with prescribed treatment. This may present dilution bias in the results; real effects may be considerably stronger.

We were unable to carry out meta-regression for disease duration, an important covariate predicting cardiovascular risk in rheumatic diseases;[127] only three studies reported disease duration for the primary outcome. Different studies adjusted for different covariates in their statistical models. However, all studies showed a similar direction of effect with minimal statistical heterogeneity. Most of the studies failed to report the route of administration for MTX treatment. Thus, our results cannot distinguish between oral and subcutaneous administration.

Only one reviewer screened the articles for titles, abstracts and keywords, thus some subjectivity and the risk of incorrectly discarding relevant reports cannot be neglected.[128] A further limitation is that this review was restricted to English language studies. Excluding non-English publications may introduce bias and reduce the precision of estimates of treatment effects. It has been shown that trials with positive results are more likely to be published in English.[129] However, Morrison et al found no evidence of bias from the use of language restrictions in systematic reviews.[130]

6. Implications for practice

MTX in autoimmune diseases (RA, psoriasis, psoriatic arthritis and myositis) is associated with a reduced incidence of cardiovascular events. Low-dose MTX offers more protective effect than high-dose MTX and is known to cause less toxicity.[123] Treatment with MTX may improve physical activity in patients with rheumatic

autoimmune disease, and subsequently reduce the risk of diabetes, hypertension and obesity. Associations with all-cause mortality and cardiovascular mortality were clear, but evidence was graded as very low quality. MTX was not clearly protective in associations with stroke and heart failure. Results of this meta-analysis cannot be generalized to all MTX treated autoimmune diseases, because of lack of potential evidence for ulcerative colitis, Crohn's disease, systemic lupus erythematosus, transverse myelitis, multiple sclerosis, myasthenia gravis, vasculitis and other autoimmune diseases.

7. Implications for research

As noted, MTX may be associated with several adverse effects. Assessment of the true extent of MTX therapy on cardiovascular outcomes is needed. Currently, the Cardiovascular Inflammation Reduction Trial (CIRT) is investigating the anti-inflammatory effect of low-dose MTX in patients with prior myocardial infarction.[61,62] CIRT is testing MTX in patients with high cardiovascular risk, representing a small subpopulation of cardiovascular patients who do not have autoimmune disease. Regardless of this caveat, results from this well-powered trial could address the efficacy and safety of low-dose MTX as noted in our meta-analysis.

We found numerically protective but statistically non-significant effects of MTX on heart failure and stroke. Further high quality research is needed to assess any protective effect of MTX on heart failure and stroke.

Other autoimmune diseases such as systemic lupus erythematosus, inflammatory bowel disease, bullous pemphigoid, ANCA associated vasculitis and Takayasu's arteritis carry high risks of cardiovascular events and death.[31,33,41,42,43,44,50] As we noted, MTX is a treatment option for these diseases. At present, almost no evidence exists on the association of MTX with cardiovascular disease and mortality for these autoimmune diseases. Further research is needed to define the association of MTX with cardiovascular disease and mortality in these diseases. Such research, in juxtaposition with our meta-analysis, would broaden the MTX knowledge base across a wide range of diseases.

8. Conclusion

In conclusion, early intervention with low-dose MTX together with careful monitoring in patients with autoimmune disease such as RA, psoriasis, psoriatic arthritis or myositis is recommended. This may not only control the underlying disease but hopefully also reduce the risk of cardiovascular disease by 25% (if the association we have detected is causal). MTX can cause adverse effects, and in such cases, benefits need to be weighed against risks.

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Appendices

Appendix A. Preferred Reporting Item for Systematic Reviews and Meta-analysis (PRISMA) guidelines

Section/Topic	Item No.	Checklist item	Page number
Title	1	Identify the report as a systematic review, meta-analysis, or both.	ii
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	ii
Rationale	3	Describe the rationale for the review in the context of what is already known.	13
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	13
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	16
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	16
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	18
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	18, 72
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	18
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	20

Section/Topic	Item No.	Checklist item	Page number
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	--
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	20
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	21
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	22
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	23
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	23
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	36
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	29
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	34
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	41
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	32

Section/Topic	Item No.	Checklist item	Page number
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	42
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	42
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	49
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	54
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	56
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	--

Appendix B. Search strategy

Ovid MEDLINE (1946 to Present)

1. Autoimmune Diseases/
2. Rheumatic Diseases/
3. Arthritis, Rheumatoid/
4. exp Psoriasis/
5. exp Polymyositis/
6. Myositis/
7. exp Lupus Erythematosus, Systemic/
8. exp Multiple Sclerosis/
9. Sjogren's Syndrome/
10. Pemphigoid, Bullous/
11. exp Inflammatory Bowel Diseases/
12. Myelitis, Transverse/
13. exp Scleroderma, Systemic/
14. Myasthenia Gravis/
15. exp Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis/
16. Polyarteritis Nodosa/
17. Takayasu Arteritis/
18. (autoimmune adj (disease* or disorder*)).tw.
19. (rheumatic adj (disease* or disorder*)).tw.
20. rheumatoid arthritis.tw.
21. psoriasis.tw.
22. psoriatic arthritis.tw.
23. polymyositis.tw.
24. dermatomyositis.tw.
25. (inflammatory adj2 myopath*).tw.
26. systemic lupus erythematosus.tw.
27. (SLE and lupus).tw.
28. multiple sclerosis.tw.
29. sjogren*.tw.
30. (bullous adj2 pemphigoid).tw.
31. inflammatory bowel disease*.tw.
32. (IBD and (inflammatory and bowel)).tw.
33. Crohn*.tw.
34. ulcerative colitis.tw.
35. transverse myelitis.tw.
36. systemic sclerosis.tw.
37. systemic scleroderma.tw.
38. myasthenia gravis.tw.
39. wegener* granulomatosis.tw.

40. microscopic polyangiitis.tw.
41. microscopic polyarteritis.tw.
42. (granulomatosis adj2 polyangiitis).tw.
43. allergic granulomatosis.tw.
44. eGPA.tw.
45. eosinophilic granulomatosis.tw.
46. churg strauss syndrome*.tw.
47. takayasu arteritis.tw.
48. (ANCA adj2 vasculitis).tw.
49. anti neutrophil cytoplasmic antibody associated vasculitis.tw.
50. or/1-49
51. Methotrexate/
52. methotrexate*.tw.
53. MTX.tw.
54. (abitrexate or amethopterine or ametopterine or antifolan or artrait or atrexel or bendatrexat or biotrexate or carditrex or canceren or dermotrex or ebetrex or emtexate or emthexat or emthexate or emtrexate or enthexate or farmitrexat or farmitrexate or farmotrex or folex or ifamet or imeth or lantarel or ledertrexate or maxtrex or metex or methoblastin or methohexate or methotrate or methotrexat or methotrexato or methoxtrexate or methrotrexate or meticil or metoject or metothrexate or metotrexat or metotrexate or metotrexin or metrex or mexate or mpi 5004 or mpi5004 or neotrexate or novatrex or nsc 740 or nsc740 or otrexup or reumatrex or rheumatrex or texate or texorate or trexall or trexan or xaken or zexate).tw.
55. amethopterin.tw.
56. or/51-55
57. Mortality/
58. Cause of Death/
59. Fatal Outcome/
60. Hospital Mortality/
61. Mortality, Premature/
62. Death/
63. Death, Sudden/
64. Death, Sudden, Cardiac/
65. Life Expectancy/
66. Life Tables/
67. Vital Statistics/
68. mortality.fs.
69. mortalit*.tw.
70. (death or dead or die or died or dies).tw.
71. ((hazard* or cox) adj2 (model* or regression*)).tw.
72. (sudden adj2 death).tw.
73. kaplan meier*.tw.
74. (life table* or lifetable*).tw.

75. or/57-74
76. exp Cardiovascular diseases/
77. exp Cerebrovascular Disorders/
78. (cardiac adj2 (event* or arrest* or failure)).tw.
79. (cardiovascular adj2 (disease* or event* or disorder*)).tw.
80. (cerebrovascular adj2 (disease* or event* or disorder* or accident*)).tw.
81. (myocardi* adj2 (infarct* or revascular* or isch?emi*)).tw.
82. (morbid* adj2 (heart* or coronar* or ischaem* or ischem* or myocard*)).tw.
83. (heart adj (infarct* or arrest* or attack* or failure or event* or bypass*)).tw.
84. (coronary adj (disease* or event* or bypas* or graft*)).tw.
85. (Coronary adj (heart or artery) adj disease*).tw.
86. stroke*1.tw.
87. (acute coronary adj2 syndrome*).tw.
88. apoplexy.tw.
89. isch?emic heart disease*.tw.
90. or/76-89
91. 75 or 90
92. 50 and 56
93. 91 and 92
94. exp animals/ not humans.sh.
95. 93 not 94
96. (adolescent or children).tw. or child, preschool.sh.
97. 95 not 96

EMBASE (1947 to present)

1. autoimmune disease/
2. rheumatic disease/
3. rheumatoid arthritis/
4. psoriasis/
5. psoriatic arthritis/
6. polymyositis/
7. myositis/
8. polymyositis/
9. dermatomyositis/
10. systemic lupus erythematosus/
11. lupus erythematosus nephritis/
12. brain vasculitis/
13. multiple sclerosis/
14. Sjoegren syndrome/
15. bullous pemphigoid/
16. inflammatory bowel disease/
17. ulcerative colitis/

18. Crohn disease/
19. myelitis/
20. exp systemic sclerosis/
21. myasthenia gravis/
22. ANCA associated vasculitis/
23. Churg Strauss syndrome/
24. microscopic polyangiitis/
25. Wegener granulomatosis/
26. polyarteritis nodosa/
27. aorta arch syndrome/
28. (autoimmune adj (disease* or disorder*)).tw.
29. (rheumatic adj (disease* or disorder*)).tw.
30. rheumatoid arthritis.tw.
31. psoriasis.tw.
32. psoriatic arthritis.tw.
33. polymyositis.tw.
34. dermatomyositis.tw.
35. (inflammatory adj2 myopath*).tw.
36. systemic lupus erythematosus.tw.
37. (SLE and lupus).tw.
38. multiple sclerosis.tw.
39. sjogren*.tw.
40. (bullous adj2 pemphigoid).tw.
41. inflammatory bowel disease*.tw.
42. (IBD and (inflammatory and bowel)).tw.
43. Crohn*.tw.
44. ulcerative colitis.tw.
45. transverse myelitis.tw.
46. systemic sclerosis.tw.
47. systemic scleroderma.tw.
48. myasthenia gravis.tw.
49. wegener* granulomatosis.tw.
50. microscopic polyangiitis.tw.
51. microscopic polyarteritis.tw.
52. (granulomatosis adj2 polyangiitis).tw.
53. allergic granulomatosis.tw.
54. eGPA.tw.
55. eosinophilic granulomatosis.tw.
56. churg strauss syndrome*.tw.
57. takayasu arteritis.tw.
58. (ANCA adj2 vasculitis).tw.
59. antineutrophil cytoplasmic antibody associated vasculitis.tw.
60. or/1-59

61. methotrexate/
62. methotrexate.tw.
63. MTX.tw.
64. (abitrexate or amethopterine or ametopterine or antifolan or artrait or atrexel or bendatrexat or biotrexate or carditrex or canceren or dermotrex or ebetrex or emtexate or emthexat or emthexate or emtrexate or enthexate or farmitrexat or farmitrexate or farmotrex or folex or ifamet or imeth or lantarel or ledertrexate or maxtrex or metex or methoblastin or methohexate or methotrate or methotrexat or methotrexato or methoxtrexate or methrotrexate or meticil or metoject or metothrexate or metotrexat or metotrexate or metotrexin or metrex or mexate or mpi 5004 or mpi5004 or neotrexate or novatrex or nsc 740 or nsc740 or otrexup or reumatrex or rheumatrex or texate or texorate or trexall or trexan or xaken or zexate).tw.
65. amethopterin.tw.
66. or/61-65
67. mortality/
68. "cause of death"/
69. fatality/
70. premature mortality/
71. death/
72. exp sudden death/
73. life expectancy/
74. life table/
75. Vital Statistics/
76. vital statistics/
77. mortalit*.tw.
78. (death or dead or die or died or dies).tw.
79. ((hazard* or cox) adj2 (model* or regression*)).tw.
80. (sudden adj2 death).tw.
81. kaplan meier*.tw.
82. (life table* or lifetable*).tw.
83. or/67-82
84. exp cardiovascular disease/
85. exp cerebrovascular disease/
86. cardiovascular mortality/
87. (cardiac adj2 (event* or arrest* or failure)).tw.
88. (cardiovascular adj2 (disease* or event* or disorder*)).tw.
89. (cerebrovascular adj2 (disease* or event* or disorder* or accident*)).tw.
90. (myocardi* adj2 (infarct* or revascular* or isch?emi*)).tw.
91. (morbid* adj2 (heart* or coronar* or isch?em* or myocard*)).tw.
92. (heart adj (infarct* or arrest* or attack* or failure or event* or bypass*)).tw.
93. (coronary adj (disease* or event* or bypas* or graft*)).tw.
94. (coronary adj (heart or artery) adj disease*).tw.
95. stroke*1.tw.

96. (acute coronary adj2 syndrome*).tw.
97. apoplexy.tw.
98. isch?emic heart disease*.tw.
99. or/84-98
100. 83 or 99
101. 60 and 66
102. 100 and 101
103. (animal$ not human$).sh,hw.
104. 102 not 103
105. exp pediatrics/
106. child/
107. adolescent/
108. (adolescent* or child* or preschool* or pre school*).tw.
109. or/105-108
110. 104 not 109

Web of Science

1. TS=(((autoimmune OR rheumatic) NEAR/1 disease*) OR rheumatoid arthritis OR psoriasis OR polymyositis OR myositis OR "systemic lupus erythematosus" OR "multiple sclerosis" OR sjogren* OR "bullous pemphigoid" OR dermatomyositis OR (inflammatory NEAR/1 myopath*) OR ("inflammatory bowel" NEAR/1 disease*) or crohn* or "ulcerative colitis")
   DocType=All document types; Language=All languages;
2. TS=(methotrexate OR MTX OR abitrexate OR amethopterine OR ametopterine OR antifolan OR artrait OR atrexel OR bendatrexat OR biotrexate OR carditrex OR canceren OR dermotrex OR ebetrex OR emtexate OR emthexat OR emthexate OR emtrexate OR enthexate OR farmitrexat OR farmitrexate OR farmotrex OR folex OR ifamet OR imeth OR lantarel OR ledertrexate OR maxtrex OR metex OR methoblastin OR methohexate OR methotrate OR methotrexat OR methotrexato OR methoxtrexate OR methrotrexate OR meticil OR metoject OR metothrexate OR metotrexat OR metotrexate OR metotrexin OR metrex OR mexate OR mpi 5004 OR mpi5004 OR neotrexate OR novatrex OR nsc 740 OR nsc740 OR otrexup OR reumatrex OR rheumatrex OR texate OR texorate OR trexall OR trexan OR xaken OR zexate OR amethopterin)
   DocType=All document types; Language=All languages;
3. (#2 AND #1)
   DocType=All document types; Language=All languages;
4. TS=(mortalit* OR fatal* OR death OR dead OR die OR died OR dies OR "life table" OR "life tables" OR lifetable* OR "hazard model" OR "kaplan meier" OR "cox model")
   DocType=All document types; Language=All languages;
5. TS=((cardiac NEAR/2 (event* OR arrest* OR failure)) OR ((cardiovascular OR

cerebrovascular) NEAR/2 (disease* OR event* OR disorder* OR accident*)) OR (myocardi* NEAR/2 (infarct* OR revascular* OR isch?em*)) OR (morbid* NEAR/2 (heart* OR coronar* OR isch?em* OR myocard*)) OR (heart NEAR/1 (infarct* OR arrest* OR attack* OR failure OR event*)) OR (coronary NEAR/1 (disease* OR event OR bypas* OR graft*)) OR coronary heart disease* OR coronary artery disease* OR stroke OR strokes)
DocType=All document types; Language=All languages;

6. (#5 OR #4)
   DocType=All document types; Language=All languages;
7. (#3 AND #6)
   DocType=All document types; Language=All languages;

The Cochrane Library

1. MeSH descriptor: [Autoimmune Diseases] this term only
2. MeSH descriptor: [Rheumatic Diseases] this term only
3. MeSH descriptor: [Arthritis, Rheumatoid] this term only
4. MeSH descriptor: [Psoriasis] explode all trees
5. MeSH descriptor: [Myositis] this term only
6. MeSH descriptor: [Polymyositis] this term only
7. MeSH descriptor: [Dermatomyositis] this term only
8. MeSH descriptor: [Lupus Erythematosus, Systemic] 1 tree(s) exploded
9. MeSH descriptor: [Multiple Sclerosis] 1 tree(s) exploded
10. MeSH descriptor: [Sjogren's Syndrome] this term only
11. MeSH descriptor: [Pemphigoid, Bullous] this term only
12. MeSH descriptor: [Inflammatory Bowel Diseases] explode all trees
13. MeSH descriptor: [Myelitis, Transverse] 3 tree(s) exploded
14. MeSH descriptor: [Myasthenia Gravis] this term only
15. MeSH descriptor: [Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis] 1 tree(s) exploded
16. MeSH descriptor: [Polyarteritis Nodosa] this term only
17. MeSH descriptor: [Takayasu Arteritis] this term only
18. MeSH descriptor: [Scleroderma, Systemic] explode all trees
19. (AUTOIMMUNE near/2 (DISEASE* or DISORDER*)):ti,ab,kw
20. (RHEUMATIC near/2 (DISEASE* or DISORDER*)):ti,ab,kw
21. (RHEUMATOID next ARTHRITIS)
22. (PSORIASIS)
23. (PSORIATIC next ARTHRITIS)
24. (POLYMYOSITIS or DERMATOMYOSITIS)
25. (INFLAMMATORY near/2 MYOPATH*)
26. (SYSTEMIC next LUPUS next ERYTHEMATOSUS)
27. (SLE and LUPUS)
28. (MULTIPLE next SCLEROSIS)

29. (SJOGREN*)
30. (BULLOUS near/2 PEMPHIGOID)
31. (INFLAMMATORY next BOWEL next DISEASE*)
32. (IBD and (INFLAMMATORY and BOWEL))
33. (CROHN*)
34. (ULCERATIVE next COLITIS)
35. (TRANSVERSE next MYELITIS)
36. (SYSTEMIC near/2 SCLEROSIS)
37. (SYSTEMIC near/2 SCLERODERMA)
38. (MYASTHENIA next GRAVIS)
39. (WEGENER* next GRANULOMATOSIS)
40. (MICROSCOPIC next POLYANGIITIS)
41. (MICROSCOPIC next POLYARTERITIS)
42. (GRANULOMATOSIS near/2 POLYANGIITIS)
43. (ALLERGIC next GRANULOMATOSIS)
44. (EGPA)
45. (EOSINOPHILIC next GRANULOMATOSIS)
46. (CHURG next STRAUSS next SYNDROME)
47. (ANCA near/2 VASCULITIS)
48. (#1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #17 or #19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or #27 or #28 or #29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37 or #38 or #39 or #40 or #41 or #42 or #43 or #44 or #45 or #46 or #47)
49. MeSH descriptor: [Methotrexate] this term only
50. MeSH descriptor: [Aminopterin] this term only
51. (METHOTREXATE)
52. (AMINOPTERIN)
53. (MTX)
54. (ABITREXATE or AMETHOPTERIN or AMETHOPTERINE or AMETOPTERINE or ANTIFOLAN or ARTRAIT or ATREXEL or BENDATREXAT or BIOTREXATE or CARDITREX or CANCEREN or DERMOTREX or EBETREX or EMTEXATE or EMTHEXAT or EMTHEXATE or EMTREXATE or ENTHEXATE or FARMITREXAT or FARMITREXATE or FARMOTREX or FOLEX or IFAMET or IMETH or LANTAREL or LEDERTREXATE or MAXTREX or METEX or METHOBLASTIN or METHOHEXATE or METHOTRATE or METHOTREXAT or METHOTREXATO or METHOXTREXATE or METHROTREXATE or METICIL or METOJECT or METOTHREXATE or METOTREXAT or METOTREXATE or METOTREXIN or METREX or MEXATE or MPI 5004 or MPI5004 or NEOTREXATE or NOVATREX or NSC 740 or NSC740 or OTREXUP or REUMATREX or RHEUMATREX or TEXATE or TEXORATE or TREXALL or TREXAN or XAKEN or ZEXATE)
55. (#49 or #50 or #51 or #52 or #53 or #54)

56. (#48 and #55)
57. MeSH descriptor: [Mortality] this term only
58. MeSH descriptor: [Cause of Death] this term only
59. MeSH descriptor: [Fatal Outcome] this term only
60. MeSH descriptor: [Hospital Mortality] this term only
61. MeSH descriptor: [Mortality, Premature] this term only
62. MeSH descriptor: [Death] this term only
63. MeSH descriptor: [Death, Sudden] this term only
64. MeSH descriptor: [Death, Sudden, Cardiac] this term only
65. MeSH descriptor: [Death Certificates] this term only
66. MeSH descriptor: [Life Expectancy] this term only
67. MeSH descriptor: [Life Tables] this term only
68. MeSH descriptor: [Vital Statistics] this term only
69. ((HAZARD* or COX) near/2 MODEL*)
70. (SUDDEN near/2 DEATH)
71. (MORTALIT* or DIED or DIE or DEATH or DEAD)
72. (KAPLAN next MEIER*)
73. (LIFE next TABLE*) or (LIFETABLE*)
74. (#57 or #58 or #59 or #60 or #61 or #62 or #63 or #64 or #65 or #66 or #67 or #68 or #69 or #70 or #71 or #72 or #73)
75. MeSH descriptor: [Cardiovascular Diseases] explode all trees
76. MeSH descriptor: [Cerebrovascular Disorders] explode all trees
77. (CARDIAC near/2 (EVENT* or ARREST* or FAILURE))
78. (CARDIOVASCULAR near/2 (DISEASE* or EVENT* or DISORDER*))
79. (CEREBROVASCULAR near/2 (DISEASE* or EVENT* or DISORDER* or ACCIDENT*))
80. (HEART near/2 (INFARCT* or ARREST* or DISEASE* or ATTACK* or FAILURE or EVENT* or BYPAS*))
81. (CORONARY near/2 (DISEASE* or EVENT* or BYPAS* or GRAFT*))
82. (MYOCARDIAL* near/2 (INFARCT* or RE?VASCULAR* or ISCH?EMI*))
83. (MORBID* near (HEART* or CORONARY* or ISCH?EMI* or MYOCARD*))
84. (CORONARY next (DISEASE* or EVENT* or BYPAS* or GRAFT*))
85. (STROKE or STROKES)
86. (ISCH?EMIC next HEART next DISEASE*)
87. (APOPLEXY)
88. (#75 or #76 or #77 or #78 or #79 or #80 or #81 or #82 or #83 or #84 or #85 or #86 or #87)
89. (#74 or #88)
90. (#56 and #89)

Google scholar

Methotrexate AND "rheumatoid arthritis" AND (coronary OR cardiovascular OR myocardial OR ischemic OR infarction OR infarct OR stroke OR cerebrovascular OR ischemia OR vascular OR angina OR mortality OR morbidity OR death OR dead OR chd OR cad OR ihd)

Methotrexate AND "Psoriasis" AND (coronary OR cardiovascular OR myocardial OR ischemic OR infarction OR infarct OR stroke OR cerebrovascular OR ischemia OR vascular OR angina OR mortality OR morbidity OR death OR dead OR chd OR cad OR ihd)

Methotrexate AND "Psoriatic arthritis" AND (coronary OR cardiovascular OR myocardial OR ischemic OR infarction OR infarct OR stroke OR cerebrovascular OR ischemia OR vascular OR angina OR mortality OR morbidity OR death OR dead OR chd OR cad OR ihd)

Methotrexate AND "dermatomyositis" AND (coronary OR cardiovascular OR myocardial OR ischemic OR infarction OR infarct OR stroke OR cerebrovascular OR ischemia OR vascular OR angina OR mortality OR morbidity OR death OR dead OR chd OR cad OR ihd)

Methotrexate AND "polymyositis" AND (coronary OR cardiovascular OR myocardial OR ischemic OR infarction OR infarct OR stroke OR cerebrovascular OR ischemia OR vascular OR angina OR mortality OR morbidity OR death OR dead OR chd OR cad OR ihd)

Methotrexate AND "systemic lupus erythematosus" AND (coronary OR cardiovascular OR myocardial OR ischemic OR infarction OR infarct OR stroke OR cerebrovascular OR ischemia OR vascular OR angina OR mortality OR morbidity OR death OR dead OR chd OR cad OR ihd)

Methotrexate AND "multiple sclerosis" AND (coronary OR cardiovascular OR myocardial OR ischemic OR infarction OR infarct OR stroke OR cerebrovascular OR ischemia OR vascular OR angina OR mortality OR morbidity OR death OR dead OR chd OR cad OR ihd)

Methotrexate AND "Sjogren's syndrome" AND (coronary OR cardiovascular OR myocardial OR ischemic OR infarction OR infarct OR stroke OR cerebrovascular OR ischemia OR vascular OR angina OR mortality OR morbidity OR death OR dead OR chd OR cad OR ihd)

Methotrexate AND "bullous pemphigoid" AND (coronary OR cardiovascular OR

myocardial OR ischemic OR infarction OR infarct OR stroke OR cerebrovascular OR ischemia OR vascular OR angina OR mortality OR morbidity OR death OR dead OR chd OR cad OR ihd)

Methotrexate AND “crohn’s disease” AND (coronary OR cardiovascular OR myocardial OR ischemic OR infarction OR infarct OR stroke OR cerebrovascular OR ischemia OR vascular OR angina OR mortality OR morbidity OR death OR dead OR chd OR cad OR ihd)

Methotrexate AND “ulcerative colitis” AND (coronary OR cardiovascular OR myocardial OR ischemic OR infarction OR infarct OR stroke OR cerebrovascular OR ischemia OR vascular OR angina OR mortality OR morbidity OR death OR dead OR chd OR cad OR ihd)

Methotrexate AND “transverse myelitis” AND (coronary OR cardiovascular OR myocardial OR ischemic OR infarction OR infarct OR stroke OR cerebrovascular OR ischemia OR vascular OR angina OR mortality OR morbidity OR death OR dead OR chd OR cad OR ihd)

Methotrexate AND “systemic sclerosis” AND (coronary OR cardiovascular OR myocardial OR ischemic OR infarction OR infarct OR stroke OR cerebrovascular OR ischemia OR vascular OR angina OR mortality OR morbidity OR death OR dead OR chd OR cad OR ihd)
Methotrexate AND “myasthenia gravis” AND (coronary OR cardiovascular OR myocardial OR ischemic OR infarction OR infarct OR stroke OR cerebrovascular OR ischemia OR vascular OR angina OR mortality OR morbidity OR death OR dead OR chd OR cad OR ihd)

Methotrexate AND “Wegener’s granulomatosis” AND (coronary OR cardiovascular OR myocardial OR ischemic OR infarction OR infarct OR stroke OR cerebrovascular OR ischemia OR vascular OR angina OR mortality OR morbidity OR death OR dead OR chd OR cad OR ihd)

Methotrexate AND “microscopic polyangitis” AND (coronary OR cardiovascular OR myocardial OR ischemic OR infarction OR infarct OR stroke OR cerebrovascular OR ischemia OR vascular OR angina OR mortality OR morbidity OR death OR dead OR chd OR cad OR ihd)

Methotrexate AND “eosinophilic granulomatosis with polyangitis” AND (coronary OR cardiovascular OR myocardial OR ischemic OR infarction OR infarct OR stroke OR cerebrovascular OR ischemia OR vascular OR angina OR mortality OR morbidity OR death OR dead OR chd OR cad OR ihd)

Methotrexate AND “takayasu’s arteritis” AND (coronary OR cardiovascular OR myocardial OR ischemic OR infarction OR infarct OR stroke OR cerebrovascular OR ischemia OR vascular OR angina OR mortality OR morbidity OR death OR dead OR chd OR cad OR ihd)

Methotrexate AND “ANCA” AND “vasculitis” AND (coronary OR cardiovascular OR myocardial OR ischemic OR infarction OR infarct OR stroke OR cerebrovascular OR ischemia OR vascular OR angina OR mortality OR morbidity OR death OR dead OR chd OR cad OR ihd)

Appendix C. Study Eligibility Assessment Form

Study No.: **RefWorks ID:**
Study title:
Last name of first author: **Year of publication:**
Reviewer's Initial:

<u>All criteria must met</u>

1	Is the study published in English language?	Yes☐	No☐
2	Study design (Cohort, Case-control or RCT)	Yes☐	No☐
3	Did the study report any of the following events in its analysis including adverse event (AE) analysis? 1. Cardiovascular events 2. Fatal/non-fatal MI 3. ACS/IHD/CAD 4. Sudden cardiac death 5. Heart failure 6. Cardiac arrest 7. Stroke 8. Hospitalization due to cardiac event 9. All-cause mortality 10. Cardiovascular mortality	Yes☐	No☐
4	Did the study report the association of MTX with any of the outcomes listed above in its analysis including AE analysis?	Yes☐	No☐
5	Did the study produce an analysis (including AE analysis) that compares the outcome of interest between MTX users versus non-users or placebo group?	Yes☐	No☐
6	Did the study report any of the following statistical measuresfor the association of MTX with the outcomes of interest? 1. Number of patients experiencing event (n) 2. Incidence rates 3. Cumulative incidence 4. OR (95% CI) 5. RR (95% CI) 6. Risk ratio (95% CI)	Yes☐	No☐

7 Did the study include patients with any of the following autoimmune diseases? Yes☐ No☐

1. Rheumatoid arthritis
2. Psoriasis
3. Psoriatic arthritis
4. Dermatomyositis
5. Polymyositis
6. Myositis
7. Systemic Lupus Erythematosus
8. Multiple sclerosis
9. Sjogren's syndrome
10. Bullous pemphigoid
11. Crohn's disease
12. Ulcerative colitis
13. Transverse Myelitis
14. Systemic sclerosis (Scleroderma)
15. Myasthenia Gravis
16. ANCA associated vasculitis
17. Takayasu's arteritis

8 Additional comments _____

9 Reviewer's final assessment.

1. Include Include ☐
2. Exclude Exclude ☐
3. Unclear Unclear ☐

10 Reason for exclusion/unclear study_____

Appendix D. Data Abstraction Form

1. Citation details

Study No.	
RefWorks ID.	
Data abstraction date	
Abstracter's initial	
First author	
Second author (if only two authors on the study	
Publication year	

2. Study characteristics

Publication type	☐ Full text ☐ Abstract ☐ Other (Specify) _____
Country of study	
Study design	☐ Randomized Controlled Trial (RCT) ☐ Case-control ☐ Cohort
Data collection	☐ Prospective ☐ Retrospective
Study setting	☐ Hospital based ☐ Out-patient ☐ Unclear
Accrual start date (dd/mm/yyyy)	
Accrual end date (dd/mm/yyyy)	
Follow start date (dd/mm/yyyy)	
Follow end date (dd/mm/yyyy)	
Total follow up period (months)	
Inclusion criteria	
Exclusion criteria	
Source of information for study sample	

3. Additional information for RCT

Trial design	☐ Parallel ☐ Cross-over ☐ Factorial ☐ Other (Specify) _____
Treatment arms	

4. Characteristics of underling disease

Disease	
Diagnostic criteria	
Disease duration (mean, SD, median IQR, range)	
Severity of underlying disease	

5. Sample characteristics

Sample size (cohort)	
Sample size (cases or MTX group)	
Sample size (controls or non-MTX group)	
Age (cohort) (mean, SD, median, IQR)	
Age (cases or MTX group) (mean, SD, median, IQR)	
Age (controls or non-MTX group)	
% Female or Gender (cohort)	
% Female (cases or MTX group)	
% Female (controls or non-MTX group)	
% with baseline CVD (cohort)	
% with baseline CVD (cases or MTX group)	
% with baseline CVD (controls or non-MTX group)	
Does the sample contain patients with RA?	☐ Yes ☐ No

6. Exposure characteristics (MTX)

MTX exposure definition	
Dose of MTX reported	☐ Yes ☐ No
Dose in mg/week (mean, median, SD)	

Duration of MTX use (months) (mean, median, SD)	
Exposure type	☐ Initiators vs. non-initiators ☐ Ever-users vs. never-users ☐ Current-users vs. non-current users ☐ Unclear
Data source of MTX exposure	

7. Outcome characteristics

Outcome	Definition	Data source
1.		
2.		
3.		
Did the study report any of the CVD or mortality outcomes as adverse event?	☐ Yes ☐ No	
Did the study exclude patients with prior or current CVD in analysis?	☐ Yes ☐ No	

8. Analysis and results

Total observation time (mean-SD, median- range)	
Person-years or months exposure of whole cohort	

Outcome of interest	Person-years exposure for particular outcome	Events in cohort	Events in cases or MTX group	Events in control group	Sample size (cohort) (n)	Sample size (cases or MTX group) (n2)	Sample size (control or non-MTX group) (n3)

Outcome of interest	Effect measures (RR, OR, HR, rate ratio, IRR)	Adjusted effect estimate (MTX vs. no-MTX)	95% CI of adjusted estimate (MTX vs. no-MTX)	p-value of adjusted estimate (MTX vs. no-MTX)	Adjusted variables in the analysis	Methods used to adjust confounding	Source of outcome in article 1. Page # 2. Table # 3. Fig.#

Did the analysis include MTX as time-varying covariate?	☐ Yes ☐ No
List all other time varying covariates in the model	

9. Subgroup analysis

Subgroups	Operational definition	Adjusted effect estimate (MTX vs. no-MTX)	95% CI of adjusted estimate (MTX vs. no-MTX)	p-value of adjusted estimate (MTX vs. no-MTX)	Adjusted variables in the analysis	Formal test of interaction	Source of outcome in article 1. Page # 2. Table # 3. Fig.#

10. Dose response analysis

MTX dose group	Definition	Adjusted effect estimate (MTX vs. no-MTX)	95% CI of adjusted estimate (MTX vs. no-MTX)	p-value of adjusted estimate (MTX vs. no-MTX)	Adjusted variables in the analysis	Source of outcome (article) 1.page # 2. Table # 3. Fig.#:

11. Additional comment

Appendix E. Newcastle-Ottawa Quality Assessment Scale

CASE CONTROL STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?
   a) Yes, with independent validation *
   b) Yes, e.g. record linkage or based on self-reports
   c) No description

2) Representativeness of the cases
   a) Consecutive or obviously representative series of cases *
   a) Potential for selection biases or not stated

3) Selection of Controls
   a) Community controls *
   b) Hospital controls
   c) No description

4) Definition of Controls
   a) No history of disease (endpoint) *
   b) No description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analysis
   a) Study controls for _____ (Select the most important factor) *
   b) Study controls for any additional factor (This criteria could be modified to indicate specific control for a second important factor)

Exposure

1) Ascertainment of exposure
   a) Secure record (e.g. surgical records) *
   b) Structured interview where blind to case/control status *
   c) Interview not blinded to case/control status
   d) Written self-report or medical record only
   e) No description

2) Same method of ascertainment for cases and controls
   a) Yes *
   b) No

3) Non-Response rate
   a) Same rate for both groups *
   b) Non respondents described
   c) Rate different and no designation

COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

Selection

1) Representativeness of the exposed cohort
   a) Truly representative of the average _____ (describe) in the community *
   b) Somewhat representative of the average _____ in the community *
   c) Selected group of users e.g. nurses, volunteers
   d) No description of the derivation of the cohort
2) Selection of the non-exposed cohort
   a) Drawn from the same community as the exposed cohort *
   b) Drawn from a different source
   c) No description of the derivation of the non-exposed cohort

3) Ascertainment of exposure
   a) Secure record (e.g. surgical records) *
   b) Structured interview *
   c) Written self-report
   d) No description

4) Demonstration that outcome of interest was not present at start of study
   a) Yes *
   b) No

Comparability

1) Comparability of cohorts on the basis of the design or analysis
   a) Study controls for _____ (select the most important factor) *
   b) Study controls for any additional factor * (This criteria could be modified to indicate specific control for a second important factor)

Outcome

1) Assessment of outcome
   a) Independent blind assessment *
   b) Record linkage *

c) Self-report
d) No description

2) Was follow-up long enough for outcomes to occur
   a) Yes (select an adequate follow up period for outcome of interest) *
   b) No

3) Adequacy of follow up of cohorts
   a) Complete follow up - all subjects accounted for *
   b) Subjects lost to follow up unlikely to introduce bias - small number lost - > ____ % (select an adequate %) follow up, or description provided of those lost) *
   c) Follow up rate < ____% (select an adequate %) and no description of those lost
   d) No statement

Appendix F. The Cochrane Collaboration's tool for assessing risk of bias

Domain	Description	Review authors' judgement
Sequence generation	Describe the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups.	Was the allocation sequence adequately generated?
Allocation concealment	Describe the method used to conceal the allocation sequence in sufficient detail to determine whether intervention allocations could have been foreseen in advance of, or during, enrolment.	Was allocation adequately concealed?
Blinding of participants, personnel and outcome assessors *Assessments should be made for each main outcome (or class of outcomes)*	Describe all measures used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. Provide any information relating to whether the intended blinding was effective.	Was knowledge of the allocated intervention adequately prevented during the study?
Incomplete outcome data *Assessments should be made for each main outcome (or class of outcomes)*	Describe the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis. State whether attrition and exclusions were reported, the numbers in each intervention group (compared with total randomized participants), reasons for attrition/exclusions where reported, and any re-inclusions in analyses performed by the review authors.	Were incomplete outcome data adequately addressed?
Selective outcome reporting	State how the possibility of selective outcome reporting was examined by the review authors, and what was found.	Are reports of the study free of suggestion of selective outcome reporting?
Other sources of bias	State any important concerns about bias not addressed in the other domains in the tool. If particular questions/entries were pre-specified in the review's protocol, responses should be provided for each question/entry.	Was the study apparently free of other problems that could put it at a high risk of bias?

Possible approach for *summary assessments* outcome (across domains) within and across studies

Risk of bias	Interpretation	Within a study	Across studies
Low risk of bias	Plausible bias unlikely to	Low risk of bias for all key	Most information is from studies at low risk

	seriously alter the results.	domains.	of bias.
Unclear risk of bias	Plausible bias that raises some doubt about the results	Unclear risk of bias for one or more key domains.	Most information is from studies at low or unclear risk of bias.
High risk of bias	Plausible bias that seriously weakens confidence in the results.	High risk of bias for one or more key domains.	The proportion of information from studies at high risk of bias is sufficient to affect the interpretation of the results.

Criteria for judging risk of bias in the 'Risk of bias' assessment tool

SEQUENCE GENERATION **Was the allocation sequence adequately generated? [Short form: *Adequate sequence generation*?]**	
Criteria for a judgement of 'YES' (i.e. low risk of bias).	The investigators describe a random component in the sequence generation process such as: • Referring to a random number table; Using a computer random number generator; Coin tossing; Shuffling cards or envelopes; Throwing dice; Drawing of lots; Minimization*. *Minimization may be implemented without a random element, and this is considered to be equivalent to being random.
Criteria for the judgement of 'NO' (i.e. high risk of bias).	The investigators describe a non-random component in the sequence generation process. Usually, the description would involve some systematic, non-random approach, for example: • Sequence generated by odd or even date of birth; • Sequence generated by some rule based on date (or day) of admission; • Sequence generated by some rule based on hospital or clinic record number. Other non-random approaches happen much less frequently than the systematic approaches mentioned above and tend to be obvious. They usually involve judgement or some method of non-random categorization of participants, for example: • Allocation by judgement of the clinician; • Allocation by preference of the participant; • Allocation based on the results of a laboratory test or a series of tests; • Allocation by availability of the intervention.
Criteria for the judgment of 'UNCLEAR' (uncertain risk of	Insufficient information about the sequence generation process to permit judgement of 'Yes' or 'No'.

bias).	
ALLOCATION CONCEALMENT **Was allocation adequately concealed? [Short form: *Allocation concealment*?]**	
Criteria for a judgement of 'YES' (i.e. low risk of bias).	Participants and investigators enrolling participants could not foresee assignment because one of the following, or an equivalent method, was used to conceal allocation: • Central allocation (including telephone, web-based, and pharmacy-controlled, randomization); • Sequentially numbered drug containers of identical appearance; • Sequentially numbered opaque, sealed envelopes.
Criteria for the judgement of 'NO' (i.e. high risk of bias).	Participants or investigators enrolling participants could possibly foresee assignments and thus introduce selection bias, such as allocation based on: • Using an open random allocation schedule (e.g. a list of random numbers); • Assignment envelopes were used without appropriate safeguards (e.g. if envelopes were unsealed or non-opaque or not sequentially numbered); • Alternation or rotation; • Date of birth; • Case record number; • Any other explicitly unconcealed procedure.
Criteria for the judgement of 'UNCLEAR' (uncertain risk of bias).	Insufficient information to permit judgement of 'Yes' or 'No'. This is usually the case if the method of concealment is not described or not described in sufficient detail to allow a definite judgement – for example if the use of assignment envelopes is described, but it remains unclear whether envelopes were sequentially numbered, opaque and sealed..
BLINDING OF PARTICIPANTS, PERSONNEL AND OUTCOME ASSESSORS **Was knowledge of the allocated interventions adequately prevented during the study? [Short form: *Blinding*?]**	
Criteria for a judgement of 'YES' (i.e. low risk of bias).	Any one of the following: • No blinding, but the review authors judge that the outcome and the outcome measurement are not likely to be influenced by lack of blinding; • Blinding of participants and key study personnel ensured, and unlikely that the blinding could have been broken; • Either participants or some key study personnel were not blinded, but outcome assessment was blinded and the non-blinding of others unlikely to introduce bias.
Criteria for the judgement of	Any one of the following:

'NO' (i.e. high risk of bias).	• No blinding or incomplete blinding, and the outcome or outcome measurement is likely to be influenced by lack of blinding; • Blinding of key study participants and personnel attempted, but likely that the blinding could have been broken; • Either participants or some key study personnel were not blinded, and the non-blinding of others likely to introduce bias.
Criteria for the judgement of 'UNCLEAR' (uncertain risk of bias).	Any one of the following: • Insufficient information to permit judgement of 'Yes' or 'No'; • The study did not address this outcome.
INCOMPLETE OUTCOME DATA **Were incomplete outcome data adequately addressed? [Short form: *Incomplete outcome data addressed*?]**	
Criteria for a judgement of 'YES' (i.e. low risk of bias).	Any one of the following: • No missing outcome data; • Reasons for missing outcome data unlikely to be related to true outcome (for survival data, censoring unlikely to be introducing bias); • Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups; • For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate; • For continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes not enough to have a clinically relevant impact on observed effect size; • Missing data have been imputed using appropriate methods.
Criteria for the judgement of 'NO' (i.e. high risk of bias).	Any one of the following: • Reason for missing outcome data likely to be related to true outcome, with either imbalance in numbers or reasons for missing data across intervention groups; • For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk enough to induce clinically relevant bias in intervention effect estimate; • For continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes enough to induce clinically relevant bias in observed effect size; • 'As-treated' analysis done with substantial departure of the intervention received from that assigned at randomization;

	• Potentially inappropriate application of simple imputation.
Criteria for the judgement of 'UNCLEAR' (uncertain risk of bias).	Any one of the following: • Insufficient reporting of attrition/exclusions to permit judgement of 'Yes' or 'No' (e.g. number randomized not stated, no reasons for missing data provided); • The study did not address this outcome.
SELECTIVE OUTCOME REPORTING **Are reports of the study free of suggestion of selective outcome reporting? [Short form: *Free of selective reporting*?]**	
Criteria for a judgement of 'YES' (i.e. low risk of bias).	Any of the following: • The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported in the pre-specified way; • The study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified (convincing text of this nature may be uncommon).
Criteria for the judgement of 'NO' (i.e. high risk of bias).	Any one of the following: • Not all of the study's pre-specified primary outcomes have been reported; • One or more primary outcomes is reported using measurements, analysis methods or subsets of the data (e.g. subscales) that were not pre-specified; • One or more reported primary outcomes were not pre-specified (unless clear justification for their reporting is provided, such as an unexpected adverse effect); • One or more outcomes of interest in the review are reported incompletely so that they cannot be entered in a meta-analysis; • The study report fails to include results for a key outcome that would be expected to have been reported for such a study.
Criteria for the judgement of 'UNCLEAR' (uncertain risk of bias).	Insufficient information to permit judgement of 'Yes' or 'No'. It is likely that the majority of studies will fall into this category.
OTHER POTENTIAL THREATS TO VALIDITY **Was the study apparently free of other problems that could put it at a risk of bias? [Short form: *Free of other bias*?]**	
Criteria for a judgement of 'YES' (i.e. low risk of bias).	The study appears to be free of other sources of bias.
Criteria for the judgement of	There is at least one important risk of bias. For example, the study:

'NO' (i.e. high risk of bias).	• Had a potential source of bias related to the specific study design used; or • Stopped early due to some data-dependent process (including a formal-stopping rule); or • Had extreme baseline imbalance; or • Has been claimed to have been fraudulent; or • Had some other problem.
Criteria for the judgement of 'UNCLEAR' (uncertain risk of bias).	There may be a risk of bias, but there is either: • Insufficient information to assess whether an important risk of bias exists; or • Insufficient rationale or evidence that an identified problem will introduce bias.

Appendix G. Characteristics of included studies

Study	Design	Disease studied	Setting/Data source	Accrual period	Sample size	Inclusion criteria (Exclusion criteria)
Ajeganova et al., 2013[63]	Prospective cohort	RA	Better Anti-Rheumatic Pharmaco Therapy (BARFOT) (Sweden)	1993-1999	741	RA patients according to ACR criteria, age ≥18 years, disease duration ≤12 months (Patients with prevalent CVD at the time of RA diagnosis)
Bernatsky et al., 2005[64]	Nested Case-control	RA	Protocare longitudinal health benefit claims database and PharMetrics Integrated Outcomes Database (North America)	1998-2001	5720	RA patients, age ≥18 years, without history of CHF at the time of database entry, to have >3 months of eligibility in the health insurance plan prior to main cohort entry Cases: RA with CHF (ICD-9, code 428), Controls: RA without CHF
Bozaite-Gluosniene et al.,2011[65]	Retrospective cohort	RA	Medical Centers, Danville, PA, USA	2001-2008	1829	RA patients without pre-existing CAD
Chiang et al., 2013[66]	Retrospective cohort	Systemic sclerosis (SSc)	Longitudinal Health Insurance Database2005, Taiwan	1997-2006	1238	SSc patients (ICD-9-CM, code 710.1), age ≥18 years at the time of SSc diagnosis, without prior history of cerebrovascular disease
Chin et al., 2013[67]	Retrospective cohort	Psoriasis, psoriatic arthritis (PsA)	Longitudinal Health Insurance Database 2005, Taiwan	1997-2006	7932	Newly diagnosed psoriasis patients (ICD-9, code without arthritis: 696.1, 696.8, with arthritis: 696.0), born between 1930 and 1990 (Patients with severe vascular disease prior to psoriasis diagnosis, patients received both MTX and retinoid)
Choi et al., 2002[113]	Prospective cohort	RA	Wichita Arthritis Center, USA	1981-1999	1240	RA patients fulfilling the 1958-1987 ACR criteria, age ≥18 years, without use of MTX before their first visit to the Center (Patients with contraindications for MTX use)
Cohen et al., 2001[114]	RCT	RA	Multicenter, North America	Not specified	318	RA patients diagnosed by ACR criteria for ≥6 months, age 18-75 years, not previously received MTX, and could not have been receiving other DMARDs for ≥30 days prior to trial entry

Study	Design	Disease studied	Setting/Data source	Accrual period	Sample size	Inclusion criteria (Exclusion criteria)
Davis et al., 2013[75]	Prospective cohort	RA	Veterans Affairs RA (VARA) registry, Veterans Affairs medical centers, USA	2003-unclear end date	1047	RA patients meeting 1987 ACR criteria and available for genotyping data for MTHER C677T and/or A1298C polymorphisms
Edwards et al., 2008[68]	Retrospective cohort	RA	The UK General Practice Research Database (GPRD)	1987-2002	34364	Adult patients with RA
Gonzalez-Gay et al., 2007[116]	Prospective cohort	RA	Rheumatology outpatient clinic of Hospital Xeral-Calde, Lugo, Spain	Mar 1996-Sept 1996	182	RA patients diagnosed as per ACR 1987 criteria, consecutive unselected patients attending OPD between Mar-Sep 1996
Goodson et al., 2008[111]	Prospective cohort	Inflammatory polyarthritis	The UK Norfolk Arthritis Register (NOAR), UK	1990-1994	923	Patients with Inflammatory Polyarthritis (IP)
Lan et al., 2012[69]	Retrospective cohort	Psoriasis	Longitudinal Health Insurance Database 2005, Taiwan	1997-2006	8180	Psoriasis patients(ICD-9 696.0, 696.1, 696.8), born between 1930 and 1990 (patients with CVD before their first psoriasis diagnosis)
Levesque et al., 2013[131]	Retrospective cohort	Psoriasis	RAMQ database, The public health plan, Province of Quebec, Canada	2005-2010	5157	Newly diagnosed psoriasis patients between 2005-2010, age ≥20 years and used phototherapy, oral or injectable psoriasis treatment
Mantel et al., 2014[110]	Nested case-control	RA	Epidemiological Investigation of Rheumatoid Arthritis (EIRA) study, Sweden	Not specified	533	Cases: RA patients experienced ACS following RA diagnosis. Controls: RA patients without ACS and matched with cases for sex, year of diagnosis and EIRA center
Meek et al., 2014[70]	Prospective cohort	RA	The Arthritis Center Twente Cardiovascular Disease(ACT-CVD) project, The Netherlands	2009-2011	480	RA patients, diagnosed by attending rheumatologist, without prior CVD and completed the CV screening protocol
Mikuls et al., 2011[132]	Prospective cohort	RA	Veterans Affairs Rheumatoid Arthritis (VARA) registry, USA	2002-2009	1015	RA patients meeting 1987 ACR criteria, RA onset > 18 years of age (Women and RA patients with limited follow-up data)

Study	Design	Disease studied	Setting/Data source	Accrual period	Sample size	Inclusion criteria (Exclusion criteria)
Myasoedova et al., 2011[71]	Retrospective cohort	RA	Rochester Epidemiology Project (REP) medical records linkage system, Minnesota, USA	1980-2008	795	Incident RA patients meeting 1987 ACR criteria, age ≥18 years (patients with personal history of heart failure)
Nadareishvili et al., 2008[103]	Nested case-control	RA	National Database for Rheumatic Diseases (NDB), USA	Not specified	832	RA diagnosed by rheumatologist Cases: RA with Ischemic stroke Controls: RA without stroke (Cases with Intracerebral, subarachnoid, subdural, epidural hemorrhages, TIA)
Norton et al., 2014[107]	Retrospective cohort	RA	32 rheumatology centres in the UK	1986-2012	2763	DMARD naïve RA patients
Pope et al., 2001[115]	RCT	Systemic sclerosis (SSc)	Multicenter, North America	Not specified	71	SSc patients, age ≥18 years, diagnosed SSc within the past three years of study entry (Overlap syndrome, mixed CTD, morphea, linear scleroderma, contraindications to MTX treatment, current or past use of MTX)
Prodanowich et al., 2005[108]	Retrospective cohort	RA, psoriasis	Veterans Health Administration Data Source, Miami, Florida, USA	1998-2003	Psoriasis: 7615 RA: 6707 (total: 14322)	Patients with psoriasis (ICD-9, code 696.1) or RA (ICD-9 code 714.0) or both diagnosis (Vascular diagnosis before the diagnosis of RA or psoriasis, MTX prescription after the diagnosis of vascular disease)
Suissa et al., 2006[133]	Nested case-control	RA	PharMetrics Patient-Centric Outcomes Database, Insurance claims database, North America	1999-2003	6138	RA patients (ICD-9, code 714.0), age ≥18, no prior history of MI Cases: RA with AMI hospitalization (ICD-9, code 410) Controls: RA without AMI
Tisseverasinghe et al., 2009[118]	Nested case-control	Dermatomyositis,polymyositis	Quebec provincial physician billing, hospitalization, and pharmacy database, Canada	1994-2003	411	Patients with DM or PM (ICD-9: 710.3-710.4) Cases: DM/PM with thrombotic event, Controls: DM/PM without thrombotic event (Patients with thrombotic events prior to the diagnosis of DM or PM)

Study	Design	Disease studied	Setting/Data source	Accrual period	Sample size	Inclusion criteria (Exclusion criteria)
Troelsen et al., 2007[120]	Prospective cohort	RA	Clinical sites in Denmark	1995-1998	229	RA patients meeting ACR 1987 criteria
van den Hoogen et al., 1996[106]	RCT	Systemic sclerosis (SSc)	Multicenter trial, The Netherlands	Not specified	29	SSc patients meeting American Rheumatism Association criteria, disease duration <3 years (age<16 years, presence of other CTD, contraindications to MTX use)
van Halm et al., 2006[117]	Case-control	RA	Jan van Breemen Institute, an outpatient clinic in Amsterdam, The Netherlands	Not specified	613	RA patients fulfilling ACR criteria, without prior CV event Cases: RA with first CV event Controls: RA without CV event
Wasko et al., 2013[119]	Prospective cohort	RA	10 rheumatology practices, North America	1981-2005	5626	RA patients fulfilling ACR 1987 criteria, age ≥18 years
Wolfe and Michaud, 2008[112]	Nested case-control	RA	National Databank for Rheumatic Diseases (NDB) longitudinal Study, USA	Not specified	9153	RA diagnosed by rheumatologist Cases: RA with incident myocardial infarction (MI) Controls: RA without MI
Wolfe et al., 2003[134]	Retrospective cohort	RA	Wichita Arthritis Center, an outpatient rheumatology clinic, USA	1981-1999	1387	RA patients fulfilling 1958 or 1987 ACR criteria (patients not seen for 2 years of their death)
Wu et al., 2012[109]	Retrospective cohort	Psoriasis, psoriatic arthritis	Kaiser Permanente Southern California (KPSC) health plan, USA	2004-2010	8845	Psoriasis (ICD-9-CM code 696.1) or psoriatic arthritis (ICD-9-CM code 696.0) (prior history of MI (ICD-9-CM code 410.XX or 412))

Abbreviations: ACR: American College of Rheumatology, CHF: Congestive heart failure, CAD: Coronary artery disease, ICD: International Classification of Disease, MTX: Methotrexate, RCT: Randomized Controlled Trial, ACS: Acute coronary syndrome, TIA: Transient ischemic attack, DMARD: Disease Modifying Anti-Rheumatic Drugs, CTD: Connective tissue disease, AMI: Acute myocardial infarction, DM: Dermatomyositis, PM: Polymyositis

Appendix H. Characteristics of disease in each study

Study	Disease under study	Diagnostic criteria	Disease duration
Ajeganova et al., 2013	RA	ACR criteria	≤ 12 months
Bernatsky et al., 2005	RA	Patients with ICD-9, code 714	Not specified
Bozaite-Gluosniene et al.,2011	RA	Diagnosed by treating physician	Not specified
Chiang et al., 2013	Systemic sclerosis	Patients with ICD-9-CM code 710.1	Not specified
Chin et al., 2013	Psoriasis, psoriatic arthritis	Patients with ICD-9 code 696.1, 696.8, and 696.0	Newly diagnosed Patients
Choi et al., 2002	RA	1958-1987 ACR criteria	Mean (SD): 9.0 (9.4) years
Cohen et al., 2001	RA	ACR criteria	Mean 6.5 years
Davis et al., 2013	RA	1987 ACR criteria	Mean (SD): 14.5 (12.2) years
Edwards et al., 2008	RA	Criteria not specified	Not specified
Gonzalez-Gay et al., 2007	RA	1987 ACR criteria	Mean, Median (IQR): 10.5, 8, (4-14) years
Goodson et al., 2008	Inflammatory polyarthritis	Patients with polymyositis and Dermatomyositis	Not specified
Lan et al., 2012	Psoriasis	Patients with ICD-9 code 696.0, 696.1, 696.8	Not specified
Levesque et al., 2013	Psoriasis	Newly diagnosed patients, Criteria not specified	Not specified
Mantel et al., 2014	RA	Not specified	Not specified
Meek et al., 2014	RA	Diagnosed by attending Rheumatologist	Median (IQR): 4.2 (1.5-11.3) years
Mikuls et al., 2011	RA	1987 ACR criteria	Mean (SD): 12 (12) years
Myasoedova et al., 2011	RA	1987 ACR criteria	Not specified
Nadareishvili et al., 2008	RA	Diagnosed by treating Rheumatologist	Mean (SD): 15.9 (13.5) years
Norton et al., 2014	RA	Not specified	Not specified
Pope et al., 2001	Systemic sclerosis	ACR preliminary criteria for Scleroderma	Mean (SEM) months: 6.3 (1.0) (MTX), 7.3 (1.1) (placebo)

Study	Disease under study	Diagnostic criteria	Disease duration
Prodanowich et al., 2005	RA, psoriasis	Patients with ICD-9 code 696.1 for psoriasis or ICD-9 code 714.0 for RA	Not specified
Suissa et al., 2006	RA	Patients with ICD-9 code 714.0	Not specified
Tisseverasinghe et al., 2009	Dermatomyositis, Polymyositis	Patients with ICD-9 codes 710.3-710.4	Not specified
Troelsen et al., 2007	RA	1987 ACR criteria	Median (range): 6.3 (0.1-54) years
van den Hoogen et al., 1996	Systemic sclerosis	American Rheumatism Association criteria	Mean (SD): 3.2 (6.3) years
van Halm et al., 2006	RA	ACR criteria	Median Cases: 7.7 years Controls: 10.6 y.
Wasko et al., 2013	RA	1987 ACR criteria	Mean (SD): 10.58 (10.26) years
Wolfe and Michaud, 2008	RA	Diagnosed by treating rheumatologist	Median: 12.2 years
Wolfe et al., 2003	RA	1958 or 1987 ACR criteria	Mean (SD): 7.06 (8.52) years
Wu et al., 2012	Psoriasis, psoriatic arthritis	ICD-9-CM code 696.1, 696.0	Not specified

Abbreviations: RA: Rheumatoid arthritis, ACR: American College of Rheumatology, ICD: International Classification of Diseases

Appendix I. Exposure characteristics

Study	Data source of MTX exposure	MTX Exposure	Exposure definition
Ajeganova et al., 2013	Better Anti-Rheumatic Pharmaco Therapy registry	Ever-users vs. never-users (67.2% MTX users)	Regular use: >6 months during observation period
Bernatsky et al., 2005	Insurance claim database	Current-users vs. non-users MTX users: cases (29%), controls (36%)	A prescription dispensed during the 45 days period prior to the outcome (CHF)
Bozaite-Gluosniene et al.,2011	Medical records	Ever-users vs. never-users Ever users: 61% Never users: 39%	Time-varying use of MTX, medication start and stop date before CAD diagnosis or censor date
Chiang et al., 2013	Longitudinal Health Insurance Database (LHID2000), Taiwan	Ever-users vs. never-users	> 6 months of therapy before reaching primary endpoint, death, or end of follow-up
Chin et al., 2013	Longitudinal Health Insurance Database (LHID2000), Taiwan	Ever-users vs. never-users	Prescription drug claims of MTX
Choi et al., 2002	Wichita Arthritis Center medical records, USA	Initiators vs. non-initiators (mean dose 13 mg per week maximum dose 25 mg per week)	Once a patient starts MTX therapy, he or she was considered on therapy for the rest of the follow-up (intension-to-treat approach)
Cohen et al., 2001	Medical records	Initiators vs. placebo Dose: 15 to 17.5 or 20 mg/week.	MTX naïve patients were randomized to MTX or placebo
Davis et al., 2013	VARA clinical database, USA	Initiators vs. non-initiators MTX initiators: 51.2%	MTX exposure at the time of study enrollment (yes/no)
Edwards et al., 2008	General Practice Research Database, UK	Ever-users vs. never-users	Prescription of DMARDs compared to no prescription during study period
Gonzalez-Gay et al., 2007	Medical records, Rheumatology clinic, Spain	Ever-users vs. never-users	Medication prescribed at study start and changes noted during follow-up period
Goodson et al., 2008	Medical records, UK Norfolk Arthritis Register	Current-users vs. never-users MTX users: 23%	Current medication was recorded annually for 6 years & then every 2-3 years, Time-varying use of MTX in analysis
Lan et al., 2012	Longitudinal Health Insurance Database 2005, Taiwan	Ever-users vs. never-users	Prescription claims of MTX were identified from the database
Levesque et al., 2013	RAMQ Health plan database, Canada	Ever-users vs. never-users MTX users: 23.7%	Treatment identified from the database

Study	Data source of MTX exposure	MTX Exposure	Exposure definition
Mantel et al., 2014	Medical charts, patient register, National Prescribed Drug Register	Unclear	MTX use in cases and controls was identified
Meek et al., 2014	Medical records, The Arthritis Center Twente, The Netherlands	Initiators vs. non-initiators MTX: 60.6%	Baseline MTX users at study start
Mikuls et al., 2011	Medical records, VARA registry USA	Current-users vs. never-users	MTX use at baseline and follow-up visits, analyzed as a time-varying use
Myasoedova et al., 2011	Medical records, REP medical record linkage system, USA	Current users vs. never-users	Time-dependent variable represented the time each patient was taking medication
Nadareishvili et al., 2008	Patient self-report in questionnaire	Initiators vs. non-initiators	Baseline use of MTX reported by patients
Norton et al., 2014	Medical records	Initiators vs. non-initiators	Time-varying use of MTX in DMARD naïve patients
Pope et al., 2001	Medical records	Initiators vs. Placebo	Patients were randomized to MTX or placebo treatment
Prodanowich et al., 2005	Computerized medical records	Ever-users vs. never-users	MTX prescriptions vs. no-MTX prescriptions before the development of vascular disease
Suissa et al., 2006	Dispensed prescription data	Current users vs. non-current users	Prescription dispensed during the 30-day period prior to the AMI in cases
Tisseverasinghe et al., 2009	Pharmacy database and physician billing data	Ever-users vs. never-users MTX: 26.3%	≥ 1 prescription for the given drug, any time between cohort entry and index date
Troelsen et al., 2007	Medical charts	Current users vs. non-current users, MTX: 81% patients	Use of MTX reported in clinical charts
van den Hoogen et al., 1996	Medical records	Initiators vs. placebo	Patients were randomized to MTX and placebo
van Halm et al., 2006	Medical records	Ever-users vs. never-users, MTX (Cases): 72% MTX (controls): 44%	Medication use was identified either as monotherapy or in combinations
Wasko et al., 2013	Patient self-report in semi-annual questionnaire	Current-users vs. non-users	MTX use was assessed as time-varying variable in the analysis
Wolfe and Michaud, 2008	Patient self-report in questionnaire	Current-users vs. never-users, Average MTX dose: 14mg/week	Patients receiving MTX within 6 months prior to their first MI
Wolfe et al., 2003	Medical records	Ever-users vs. never-users	MTX use was assessed as time-varying variable in the analysis
Wu et al., 2012	KPSC pharmacy database	Ever-users vs. never-users	The date of the first dispensation of any non–TNF inhibitor systemic agent after the third psoriasis diagnosis

Abbreviations: CHF: Congestive heart failure, VARA: Veterans Affairs Rheumatoid Arthritis, REP: Rochester Epidemiology Project, AMI: acute myocardial infarction, KPSC: Kaiser Permanente Southern California

Appendix J. Outcome characteristics

Study	Data source of outcomes	Outcome	Definition	Excluded patients with prior or current CVD
Ajeganova et al., 2013	Swedish Hospital Discharge Registry National Cause of Death Registry, Sweden	CVD All-cause mortality	First ever CVD (ICD-9 and ICD-10 codes for CVD: 410, 411, 413, 427F, 433-436, 440-444, 3066-3067, 3080, 3092, 3105, 3127, 3141, 3158, 88, 0961-0964, I20-21, Y832, I46, I63-I66, G45, I70-I72, I73.9, and I74), Mortality regardless of cause	Yes
Bernatsky et al., 2005	Hospitalization records	CHF requiring hospitalization	First ever hospitalization due to CHF (ICD-9 code 428)	No
Bozaite-Gluosniene et al.,2011	Medical center records	Coronary artery disease Cardiac revascularization procedure	First ever CAD (ICD-9 code 410-419.99)	Yes
Chiang et al., 2013	Insurance claim database, Hospital records, outpatient visit records, prescriptions claims for stroke medications	Ischemic stroke	ICD-9-CM codes 433 - 435	No
Chin et al., 2013	Physician claims	Cerebrovascular events Cardiovascular events	Cardiovascular events: ICD9 codes 410 - 414.05, 414.10, 414.11, 414.19, 414.8 - 414.9, 429.79 Cerebrovascular events: ICD9 codes 430.0 - 438.9	Yes
Choi et al., 2002	Medical records, Death certificates, National Death Index, USA	All-cause mortality Cardiovascular mortality	Cause of death according to ICD-9 codes, for Cardiovascular mortality: ICD-9 codes 390 - 449	No
Cohen et al., 2001	Medical records	All-cause mortality	Death as an adverse event	Not specified
Davis et al., 2013	Inpatients and outpatients treatment files	Cardiovascular events All-cause mortality	First occurrence of any of the CV events: MI (ICD-9 code 410.x), Stroke (ICD-9 codes 433.11, 434.91, 435.x, 438.x), PCI (ICD-9 codes 36.06, 36.07, 0.66; CPT: 92973, 92980, 92995), CABG (ICD-9 codes 36.1x)	No
Edwards et al., 2008	Outpatient records, GPRD, UK	Myocardial infarction	Incidence of MI	Not specified

Study	Data source of outcomes	Outcome	Definition	Excluded patients with prior or current CVD
Gonzalez-Gay et al., 2007	Medical records	Cardiovascular events Cardiovascular mortality	Any CV event diagnosed at the hospital in a patient without previous history of CVD. IHD included ACS with or without persistent ST-segment elevation and chronic CHD. Cerebrovascular accident included stroke/TIAs	Yes
Goodson et al., 2008	Medical records	All-cause mortality Cardiovascular mortality	Mortality regardless of cause and cardiovascular mortality	Not specified
Lan et al., 2012	Inpatients and outpatients physician claims in Longitudinal Health Insurance Database 2005, Taiwan	Cerebrovascular event	First occurrence of cerebrovascular event (ICD-9 codes 430.0–438.9)	Yes
Levesque et al., 2013	Health plan database, Quebec, Canada	Myocardial infarction	Acute MI diagnosis consistent with an ICD-9 code for MI	No
Mantel et al., 2014	National Patient Register and Cause of Death Register, Sweden	Acute coronary syndrome	Hospitalization or cause-of-death listing ACS following RA diagnosis	No
Meek et al., 2014	Hospital Electronic Registration System, Medical chart review, Dutch National Registry of Death Certificates, The Netherlands	Cardiovascular event	CV events (fatal/non-fatal) included MI, PTCA, CABG, angina pectoris, acute heart failure, CVA, death due to cardiac causes and sudden death, diagnosis was confirmed by a cardiologist	Yes
Mikuls et al., 2011	Veterans Affairs Computerized Patient Record System (CPRS), USA	All-cause mortality	Identified through systematic review of the Veterans Affairs CPRS	No
Myasoedova et al., 2011	Rochester Epidemiology Project (REP) medical records linkage system, USA	Heart failure	Based on the Framingham criteria* for diagnosis of heart failure	No
Nadareishvili et al., 2008	Hospitalization records, physician reports and death records, confirmed by medical review or death records.	Ischemic stroke	Included ICD-9 codes 433.01 - 433.80 and 434 - 434.91. Excluded intracerebral, subarachnoid, subdural, and epidural hemorrhages and TIA	No

Study	Data source of outcomes	Outcome	Definition	Excluded patients with prior or current CVD
Norton et al., 2014	National Health Service (NHS) central register, UK	All-cause mortality	Confirmed by death certificates from NHS register	Not specified
Pope et al., 2001	Medical records	All-cause mortality	Death as an adverse event	Not specified
Prodanowich et al., 2005	Computerized outpatient medical records	CVD	CVD (ICD-9-CM codes 410.0-410.02, 410.1-411.0, 411.89, 413.0-413.9, 414.0-414.9, 429.2), Cerebrovascular disease (ICD-9-CM codes 433.0-433.9, 434-436), Atherosclerosis (ICD-9-CM codes 440.0-440.9)	Yes
Suissa et al., 2006	Hospitalization physician records	Myocardial infarction	First occurrence of AMI requiring hospitalization (ICD-9 code 410)	No
Tisseverasinghe et al., 2009	Hospital records and physician billing data	CVD	≥1 hospital diagnosis or ≥2 relevant billing codes, ≥8 weeks apart for stroke (ICD-9 code 433.5.x), IHD (410-1.x, 413.x), PAD (444-5.x) or AMI (≥1 hospitalization)	No
Troelsen et al., 2007	Discharge diagnosis from hospitalization records	IHD MI	Verified diagnosis by reviewing clinical charts, IHD (ICD-10 code I20-I25), MI (ICD-10: I21-I22)	Not specified
Van den Hoogen et al., 1996	Medical records	All-cause mortality	Death as an adverse event	No
Van Halm et al., 2006	Medical records from rheumatology outpatient clinic, Amsterdam	CVD	First CV event. A verified medical history of coronary (MI, CABG, PTCA, ischemic abnormality on ECG), cerebral (CVA (confirmed by neurologist), TIA, Carotid endarterectomy) or peripheral arterial disease	Yes
Wasko et al., 2013	National Death Index , North America	All-cause mortality	Death was ascertained by communication with next of kin or by searching the National Death Index	No
Wolfe and Michaud, 2008	Study questionnaire, hospital records, physician reports, and death records	Myocardial infarction	MI confirmed by medical or death records review by independent physician	No
Wolfe et al., 2003	Medical records, death certificates and the National Death Index, USA	All-cause mortality	Deaths were confirmed by review of medical records and death certificates, specific causes of death were classified using ICD-9 codes	Not specified

Study	Data source of outcomes	Outcome	Definition	Excluded patients with prior or current CVD
Wu et al., 2012	Kaiser Permanente Southern California EMR, USA	Myocardial infarction	First occurrence of fatal or non-fatal MI (ICD-9-CM code 410.XX or 412)	Yes

Abbreviations: GPRD: General Practitioner Research Database, CVD: Cardiovascular disease, CHF: Congestive heart failure, CV: Cardiovascular, IHD: Ischemic heart disease, CHD: Coronary heart disease, ACS: Acute coronary syndrome, TIA: Transient ischemic attack, PTCA: Percutaneous transluminal coronary angioplasty, CABG: Coronary artery bypass graft, AMI: Acute myocardial infarction, ECG: Electrocardiogram

*Framingham criteria: HF diagnosis requires ≥ 2 of the major criteria [i.e., paroxysmal nocturnal dyspnea or orthopnea, neck vein distention, rales, radiographic cardiomegaly (i.e., increasing heart size on chest radiograph), acute pulmonary edema, S3 gallop, increased central venous pressure ≥ 16 cm of water at the right atrium, circulation time ≥ 25 seconds, hepatojugular reflux, weight loss > 4.5 kg in 5 days in response to treatment of congestive HF)], or the presence of 1 major criterion and ≥ 2 minor criteria (i.e., bilateral ankle edema, nocturnal cough, dyspnea on ordinary exertion, hepatomegaly, pleural effusion, decrease in vital capacity by 33% from maximal value recorded, and tachycardia rate ≥ 120 beats/min). Minor criteria were counted only if they could not be attributed to another medical condition. Ejection fraction (EF) was determined by echocardiography and classified as preserved EF (≥ 50%) or reduced EF (< 50%)

Appendix K. Data for subgroup analysis and meta-regression for primary outcome (cardiovascular events)

Study	Publication year	Disease	Study design	Study region	Sample size	Mean age (years)	% Female	Observation period (person-years)	MTX exposure type
Ajeganova	2013	RA	Prospective	Europe	741	55	67.5	9405	Ever-users
Davis	2013	RA	Prospective	America	1047	63.7	9.07	3743	Initiators
Gonzalez-Gay	2007	RA	Prospective	Europe	182	59.7	72	NA	Ever-users
Meek et al	2014	RA	Prospective	Europe	480	59	72.3	1380	Initiators
Prodanowich	2005	Psoriasis	Retrospective	America	7615	NA	5.39	NA	Ever-users
Prodanowich	2005	RA	Retrospective	America	6707	NA	10.15	NA	Ever-users
Tisseverasinghe	2009	DM,PM	Case-control	America	411	62.4	70	0	Ever-users
Van Halm	2006	RA	Case-control	Europe	613	64.72	70.35	0	Ever-users

Study	Exposure source	Excluded patients with CVD	MTX as time-varying in analysis	Adjusted DMARDs in analysis	Number of CV events	Adjusted for CV risk factors	Adjusted for smoking	Adjusted for CVD	Quality score	Power score
Ajeganova	Database	yes	no	no	177	yes	yes	no	9	0.99
Davis	Database	no	no	no	97	no	no	no	6	0.88
Gonzalez-Gay	Medical records	yes	no	no	39	no	no	no	8	0.60
Meek et al	Medical records	yes	no	yes	29	yes	yes	no	8	0.11
Prodanowich	Medical records	yes	no	no	1869	yes	no	no	9	1.00
Prodanowich	Medical records	yes	no	no	2017	yes	no	no	9	1.00
Tisseverasinghe	Database	no	no	yes	80	yes	no	yes	7	0.41
Van Halm	Medical records	yes	no	no	72	yes	yes	no	7	0.08

Appendix L. Methodological quality of cohort studies according to the Newcastle Ottawa Scale

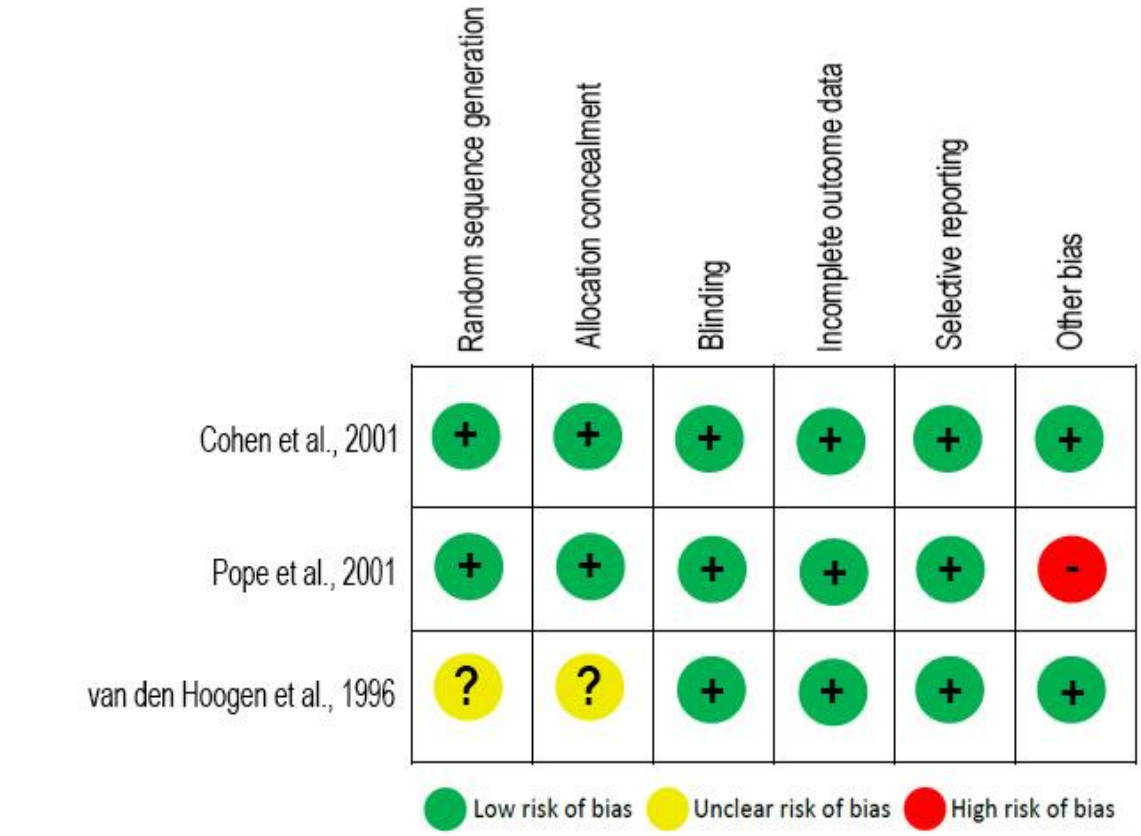
Study	Representativeness of exposed cohort (1 point)	Selection of non-exposed cohort (1 point)	Ascertainment of exposure (1 point)	Demonstration that outcome of interest was not present at baseline (1 point)	Comparability of cohorts on the basis of the design or analysis (2 points)	Assessment of outcome (1 point)	Follow-up long enough for outcomes to occur (1 point)	Adequacy of follow up of cohorts (1 point)	Total score (9 pts)
Ajeganova	1	1	1	1	2	1	1	1	9
Bozaite-Gluosniene	1	1	1	1	2	1	1	1	9
Chiang	1	1	1	1	1	1	1	1	8
Chin	1	1	1	1	1	1	1	1	8
Choi	1	1	1	0	2	1	1	1	8
Davis	1	1	1	0	1	1	0	1	6
Edwards	1	1	1	0	1	1	1	1	7
Gonzalez-Gay	1	1	1	1	1	1	1	1	8
Goodson	1	1	1	0	2	0	1	1	7
Lan	1	1	1	1	1	1	1	1	8

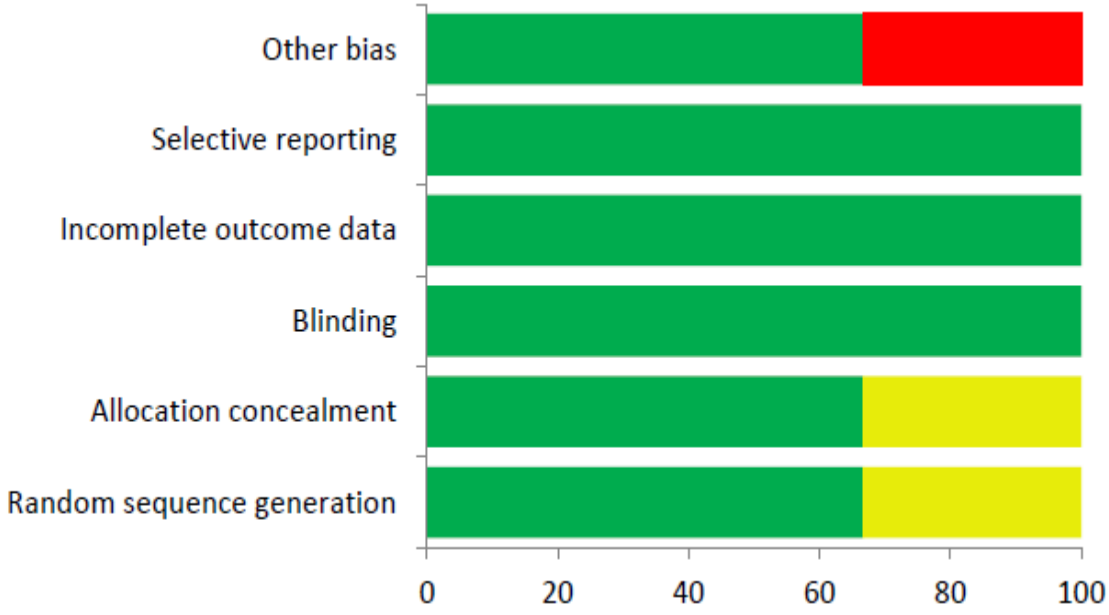
Levesque	1	1	1	0	2	1	1	1	8
Meek	1	1	1	1	2	1	0	1	8
Mikuls	1	1	1	1	2	1	1	1	9
Myasoedova	1	1	1	1	2	1	1	1	9
Norton	1	1	1	1	2	1	1	1	9
Prodanowich	1	1	1	1	2	1	1	1	9
Troelsen	1	1	1	0	2	1	1	1	8
Wasko	1	1	1	1	2	1	1	1	9
Wolfe	1	1	1	1	2	1	1	1	9
Wu	1	1	1	1	2	1	1	1	9

Appendix M. Methodological quality of case-control studies according to the Newcastle Ottawa Scale

Study	Adequate case definition (1 point)	Representa-tiveness of the cases (1 point)	Selection of controls (1 point)	Definition of controls (1 point)	Comparability of cases and controls on the basis of the design or analysis (2 points)	Ascertainment of exposure (1 point)	Same method Of ascertainment for cases and controls (1 point)	Non-Response rate (1 point)	Total score (9 pts)
Bernatsky	0	1	1	1	2	0	1	1	7
Mantel	0	1	1	1	1	0	1	1	6
Nadareishvili	1	1	1	1	2	0	1	1	8
Suissa	0	1	1	1	2	0	1	1	7
Tisseverasinghe	0	1	1	1	2	0	1	1	7
van Halm	0	1	1	1	2	0	1	1	7
Wolfe and Michaud.	1	1	1	1	2	0	1	1	8

Appendix N. Risk of bias assessment of randomized controlled trials (n=3)





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