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Systematic Review and Meta-analysis of Methotrexate Use and Risk of Cardiovascular Disease

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Abstract

Inflammation predicts risk of cardiovascular disease (CVD) events, but the relation of drugs that directly target inflammation with CVD risk is not established. Methotrexate is a disease-modifying anti-rheumatic drug broadly used for treatment of chronic inflammatory disorders. We performed a systematic review and meta-analysis of evidence for relationships of methotrexate with CVD occurrence. Cohorts, case-control studies or randomized trials were included if they reported an association between methotrexate and CVD risk. Inclusions/exclusions were independently adjudicated, and all data were extracted in duplicate. Pooled effects were calculated using inverse-

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Dr Ridker is listed as a co-inventor on patents held by the Brigham and Women's Hospital that related to the use of inflammatory biomarkers in cardiovascular disease. All other authors declare no conflicts of interest.

Author Contributions

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Fumiaki Imamura: Conception and design, data collection, interpretation of the data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, and approval of final manuscript for submission.

Moritz Wyler von Ballmoos: Conception and design, data collection, interpretation of the data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, and approval of final manuscript for submission.

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variance-weighted meta-analysis. Of 694 identified publications, 10 observational studies, in which methotrexate was administered among patients with rheumatoid arthritis, psoriasis, or polyarthritis met inclusion criteria. Methotrexate was associated with 21% lower risk of total CVD (n=10 studies, 95% CI=0.73-0.87, $p<0.001$), and 18% lower risk of myocardial infarction (n=5, 95% CI=0.71-0.96, $p=0.01$), without evidence for statistical between-study heterogeneity ($p=0.30$, $p=0.33$, respectively). Among prespecified sources of heterogeneity explored, stronger associations were observed in studies that adjusted for underlying disease severity (RR=0.64, 95% CI=0.43-0.96, $p<0.01$), and for other concomitant medication (RR=0.73, 95% CI=0.63-0.84, $p<0.001$). Publication bias was potentially evident (funnel plot, Begg's test, $p=0.06$); excluding studies with extreme risk estimates did not, however, alter results (RR=0.81, 95% CI=0.74-0.89). In conclusion, methotrexate use is associated with lower risk of CVD among patients with chronic inflammation. These findings suggest that a direct treatment of inflammation may reduce CVD risk.

Keywords

systematic review; meta-analysis; methotrexate; inflammation; cardiovascular disease

INTRODUCTION

Systemic inflammation is strongly linked to increased risk of cardiovascular disease (CVD) ^{1, 2}. However, whether this relationship is causal or simply an association is not established; no randomized trials have directly addressed whether targeted anti-inflammatory agents that do not have concomitant lipid-lowering or anti-platelet effects also reduce CVD event rates. Methotrexate has received particular interest in that regard, as it is a disease-modifying anti-rheumatic drug broadly used for the treatment of systemic inflammatory disorders, such as rheumatoid arthritis (RA) and psoriasis. Although the underlying mechanisms are not fully understood, methotrexate is known to ameliorate inflammatory responses by altering nucleotide metabolisms and, at least in part, mitigating cytokine signaling ³. The anti-inflammatory properties of methotrexate have been hypothesized to be beneficial in reducing CVD risk among patients with chronic inflammatory disorders (e.g., RA) ⁴ or even among patients with persistent inflammatory responses (e.g., increased CRP levels) ⁵. Recently, the evidence for relationships between methotrexate use in patients with RA and CVD outcomes has been systematically reviewed ⁴. However, no systematic review and meta-analysis has been performed to critically and statistically evaluate heterogeneity among published studies in this field, and to quantify the effects of methotrexate on CVD, which would help elucidate whether direct treatment of inflammation could potentially reduce CVD risk. To address this important question, we performed a systematic review and meta-analysis of the evidence for relationships of methotrexate use with risk of CVD.

METHODS

The Meta-Analysis of Observational Studies in Epidemiology guidelines ⁶ were used as reference for all stages of design, implementation, and reporting of this systematic review and meta-analysis.

We searched for all clinical trials, or observational studies (prospective or retrospective or case-control studies) in which adults received methotrexate, the duration of follow-up was at least 3 months, and reported effect estimates on occurrence of "hard" CVD events (myocardial infarction (MI), coronary heart disease (CHD), sudden death, and/or stroke). We performed searches using multiple online databases, including MEDLINE (*see*

Supplemental Methods), EMBASE, AGRIS, AMED, Web of Knowledge, CINAHL, CAB abstracts, Cochrane library, conference abstracts (ZETOC), Faculty of 1000, and grey literature sources (SIGLE). We additionally reviewed related articles, hand-searched reference lists, and performed direct author contact. For each database, the years searched included the earliest available online year of indexing up to June 2010, without language restrictions. Key words were *methotrexate*, *mexate*, *amethopterin*, and *cardiovascular diseases*. We excluded *a priori* studies that only had information on intermediate secondary endpoints (e.g., lipid or glucose levels) or “soft” CHD outcomes (e.g., angina, heart failure), studies in which methotrexate was administered as part of a combination therapy, and studies in which users were very different from non-users (e.g., diseased vs. non-diseased participants). We also excluded *a priori* ecological or cross-sectional studies; commentaries, general reviews, or case reports; duplicate publications from the same study; and studies reporting only crude risk estimates.

Of 694 identified articles, 621 were excluded based on review of the title and abstract (Figure 1). The remaining 73 manuscripts were reviewed in detail independently and in duplicate to determine inclusion/exclusion (95% concordant); differences were resolved by consensus or, if necessary, group consultation among all investigators. Sixty-three studies were excluded because they were reviews (n=6), commentaries (n=1), letters (n=1), cross-sectional (n=2), or *in vitro* (n=1); included methotrexate users which were very different from non users (n=1); assessed exposure other than methotrexate (n=19); did not have an appropriate control group (n=3); did not assess “hard” CVD endpoints (n=27); or reported only crude risk estimates (n=2) (*see* Supplemental Methods). In the end, 10 studies were included in this meta-analysis.

For each study, data were extracted independently and in duplicate by 2 investigators, including year study was published and performed, study location, study design, sample size and number of events, inclusion and exclusion criteria, duration of follow-up, underlying disease (e.g., RA), duration of underlying disease, assessment of underlying disease severity, methotrexate comparison groups (e.g., initiators vs. non-initiators, ever users vs. never users), ascertainment of methotrexate use, treatment dose, disease outcome, disease incidence vs. recurrence, folate use, whether the reported analysis was primary or secondary and prespecified or posthoc in each paper, covariates adjusted for in the analysis, and adjusted risk estimates and confidence intervals (CIs). Accepted standardized quality scores are not available for observational studies. We performed quality assessment as previously described and used ⁷, by evaluating and scoring 6 design criteria on an integer scale (0 or 1, with 1 being better), including review of study design and inclusion and exclusion criteria, assessment of exposure, assessment of outcome, control of confounding, assessment of underlying disease severity, and evidence of bias. These scores were summed, and quality scores from 0 to 3 were considered lower quality, and scores from 4 to 6 higher quality. Differences in data extracted and quality assessment were very unusual, and if present, were resolved by group discussion and consensus. Missing data were obtained by direct author contact for only 1 of 10 studies, as described above.

All included studies were observational and reported rate ratios or odds ratios; because of the low incidence of CVD in all studies, we collectively refer to these measures using the general term “relative risk” (RR). Between-study heterogeneity of RRs was assessed using the Dersimonian and Laird Q-statistic, the I² statistic, and meta-regression ^{8,9}. To calculate the overall pooled RR we used both a fixed effects and a random effects meta-analysis using the methods of Dersimonian and Laird ⁸, and reported the former if the point estimates were virtually equal. In one study ¹⁰, methotrexate was considered as the reference group to estimate the RRs of other RA medication on risk of MI or stroke hospitalization, including biologics, other cytotoxic agents, noncytotoxic agents, and glucocorticoids. Subsequently, to

estimate the RR of methotrexate vs. other RA medication (the latter as the reference group) on CVD risk we pooled the inverse RRs of all other RA medication. Potential for publication bias was explored by visually inspecting a funnel plot of the effect size versus SE¹¹, and statistically using the Begg adjusted-rank correlation test¹². We explored potential prespecified sources of heterogeneity using stratified inverse-variance weighted fixed and random effects meta-analysis (and reported the latter if significant between-study heterogeneity was present, $p < 0.1$) and inverse-variance weighted meta-regression, including study design (prospective vs. retrospective cohorts), study location (America vs. Europe), years of follow-up, methotrexate comparison groups (initiators vs. non-initiators; ever vs. never users; current vs. non-current users), ascertainment of methotrexate use (physician vs. self-reported), underlying disease (RA; psoriasis; polyarthritis), disease outcome (CVD, MI, stroke), event (incident vs. recurrent), degree of covariate adjustment (most studies adjusted for sociodemographics and CVD risk factors, so further adjusting for underlying disease severity, other medication for underlying disease, or folate use), overall quality score (0-3 vs. 4-6), and whether the reported analysis was primary or secondary and prespecified or posthoc (to address potential publication bias of “positive” findings) in each publication. Analyses were performed using STATA 10.0 (StataCorp, College Station, Tex), with 2-tailed $\alpha < 0.05$.

RESULTS

The 10 identified investigations included 8 prospective and 2 retrospective cohort studies in America ($n=6$) and Europe ($n=4$), and included 66,334 individuals in whom 6,235 CVD events were identified^{10, 13–21} (Table 1). We did not identify any randomized controlled trials that assigned methotrexate and assessed the occurrence of “hard” CVD events. One study¹⁹ provided 2 separate estimates for patients receiving methotrexate with RA and polyarthritis as the underlying disease (total of 11 estimates). Therefore, 9 studies evaluated methotrexate use in RA as the underlying disease, 1 in psoriasis and 1 in polyarthritis.

Five studies^{10, 13, 14, 16, 17} reported median duration of underlying disease, which ranged from 6 to 16 years. The majority of the studies did not report dose of methotrexate treatment, and for those which did^{13, 14, 18} median dose ranged from 13–15 mg/week. With the exception of one study¹⁹, all studies reported duration of follow-up; median duration across studies was 5.84 years. In the majority of the studies methotrexate use was ascertained by a physician (including prescription files and chart review); in only two studies^{17, 18} methotrexate use was self-reported. Half of the studies^{10, 13–15, 20} utilized health claims databases to ascertain methotrexate exposure. Most studies compared methotrexate ever users vs. never users ($n=6$ estimates), followed by current vs. non-current users ($n=3$ estimates), and initiators vs. non-initiators ($n=2$ estimates). Seven studies ascertained total or fatal CVD, 3 MI, and 1 ischemic stroke; 2 studies that evaluated CVD further provided separate estimates for MI^{10, 16} and total stroke¹⁰. Reported events were incident in 7 studies; case incidence vs. recurrence was not specified for 3 studies^{14, 16, 21}. Quality scores were in the lower range (0-3) for most studies, reflecting mainly study design limitations, such as lack of control for potentially important confounders and lack of assessment of underlying disease severity. Degree of covariate adjustment also varied among studies; all studies adjusted for CVD risk factors (e.g., blood pressure, blood cholesterol, smoking), and all but 1 study adjusted for socio-demographics (e.g., age, sex, socio-economic status, race); 6 adjusted for underlying disease severity; 4 for other medications used to treat the underlying disease; and 2 for folate use. For all studies the reported exposure-outcome assessment was a prespecified primary^{10, 13, 15, 19, 21} or secondary^{14, 16–18, 20} aim.

The p-value for between-study heterogeneity was 0.30 ($I^2=15\%$). Figure 2 presents the RR of CVD events associated with methotrexate use. Combining all studies, methotrexate use was associated with 21% lower CVD risk (95% CI=0.73-0.87); results from the random-effects meta-analysis were very similar (RR=0.79, 95% CI=0.71-0.88). Visual inspection of the funnel plot (*see* Supplemental Figure) suggested possible publication bias ($p=0.06$), but excluding the 4 smallest studies^{13, 14, 16, 21} with extreme risk estimates did not substantially alter the pooled estimate (RR=0.81, 95% CI=0.74-0.89). All p-values for heterogeneity for potential prespecified sources were >0.14 in separate meta-regression models (Table 2).

When prespecified sources of heterogeneity were explored in stratified meta-analyses models, the pooled RR (95% CI) was 0.71 (0.51-0.98) for studies conducted in Europe, 0.52 (0.22-1.23) for studies that compared methotrexate initiators vs. non-initiators, 0.68 (0.40-1.15) for studies with a higher quality score, and 0.97 (0.76-1.24) for studies with self-reported methotrexate use. Stronger associations were observed in studies that adjusted for underlying disease severity (RR=0.64, 95% CI=0.43-0.96), and studies that adjusted for concomitant medication use (RR=0.73, 95% CI=0.63-0.84). Figure 3 presents the forest plot for each of the latter 2 stratified meta-analyses performed. The pooled RR was 0.76 (95% CI=0.69-0.84) in studies that assessed only CVD, and 0.70 (95% CI=0.56-0.87) in those that assessed only stroke.

DISCUSSION

In this meta-analysis of observational studies, methotrexate use among patients with systemic inflammation (mainly rheumatoid arthritis) was associated with 21% lower CVD risk, with little evidence of between-study heterogeneity. A similar inverse association was observed for MI and stroke separately.

In general, findings in each of the sensitivity analyses performed were consistent and similar to the overall pooled estimate. For a few sources of heterogeneity, which in fact reflect potentially important study design limitations, results did differ. Lack of adjustment for underlying disease severity and other medication for underlying disease could lead to bias; sicker people would be more likely to get methotrexate and to also develop CVD, and thus methotrexate would appear less beneficial. This type of bias, “confounding by indication”, is a well recognized limitation of observational studies of treatment effects. Indeed, studies that controlled for underlying disease severity and medication for underlying disease (most of which had been carried out in Europe) were associated with 36% and 27% lower CVD risk, respectively compared with studies that did not. Furthermore, methotrexate comparison groups differed among studies, suggesting that combining them may be problematic. Ideally, the comparison should be among those who initiated vs. those who did not initiate methotrexate originally, which limits potential bias of studies of people who remain on the drug long-term, who could be doing that because they tolerate the drug (closer to an intention to treat analysis). When we restricted the analysis to the only two studies that assessed initiators vs. non-initiators, methotrexate use was associated with a trend towards 48% lower CVD risk (almost double that seen with all other comparison groups), but the CIs were wide. Ideally, such analyses should also adjust for underlying disease severity and use of other RA drugs, as described above. Ascertainment of methotrexate use could also be a potential study design limitation, as it could lead to exposure misclassification and attenuation of the observed relationship; in fact, when methotrexate use was self-reported it was not associated with CVD risk. The overall study design quality score reflects such design limitations; studies that were assigned a higher quality score were associated with lower CVD risk. In fact, the study by Choi et al.¹⁴, which showed the largest reduction in RR, had taken into account such important study design limitations, further reflected by the highest attained quality score among the studies reviewed.

Rheumatoid arthritis is a chronic inflammatory degenerative disease characterized by substantial loss of functioning and mobility over time. Furthermore, patients with RA are at increased risk for CVD, and have substantially shorter life expectancy compared with the general population, mainly attributable to death due to CVD^{22, 23}. Methotrexate is the most commonly prescribed disease-modifying antirheumatic drug, which has serious side effects, but its long-term safety has been established among patients with RA^{3, 24}. Methotrexate improves the mobility of RA patients, as assessed for example by health assessment questionnaires and other global measures of life quality^{25, 26}. Methotrexate has also been shown to reduce inflammatory biomarkers, such as CRP, IL-6, and tumor necrosis factor (TNF)- α in patients with RA and psoriasis, without major concomitant effects on platelet function^{27, 28}, as well as in animal models²⁹. Regarding methotrexate's potential effects on other intermediate risk factors for CVD, including lipid levels and insulin resistance, these have been recently systematically reviewed⁴, and limited current evidence suggests lack of an association between methotrexate use and changes in either lipid profiles or insulin resistance. Therefore, it could be argued that methotrexate may potentially reduce CVD risk mainly through its anti-inflammatory properties, and not by acting on other traditional CVD risk factors. Our findings of a significant inverse association between methotrexate use in systemic inflammation and occurrence of CVD support the need for randomized clinical trials to test the effects of MTX on CVD events, such as the planned Cardiovascular Inflammation Reduction Trial (CIRT)⁵, as well as additional experimental studies to elucidate possible underlying mechanisms of effect.

There were several strengths to our analysis. We performed a systematic literature search and directly contacted several authors making it likely that we identified all major published relevant reports. Study review (inclusion/exclusion) and data extraction was performed independently and in duplicate by at least two investigators increasing validity of results. We carefully identified several potential prespecified sources of heterogeneity, which were formally explored, including methotrexate comparison groups, study design, and degree of covariate adjustment, highlighting the importance of the consistency in reporting findings from observational studies of treatment effects. The 10 identified publications were performed in several countries increasing generalizability.

As in all meta-analyses, analyses are restricted to available data and their inherent limitations. No randomized controlled trials were identified that evaluated effects of methotrexate use in systemic inflammation on CVD events. Each of the included observational studies has potential limitations, which should be kept in mind in the interpretation of the findings. To evaluate the possible impact of these important limitations, we performed multiple sensitivity analyses to explore whether findings differed by study design (prospective vs. retrospective cohort), geographical location (America vs. Europe), methotrexate comparison groups, ascertainment of methotrexate use, control for potential confounders, and other identified prespecified sources of heterogeneity. Findings from each of the sensitivity analyses performed were generally consistent with the overall pooled estimate (inverse association). Although every effort was made in directly contacting the authors to request for either additional data or clarifications with regard to the identified sources of heterogeneity, responses were received for only 1¹³ of 10 identified publications resulting in missing data. Because of that, some of the identified prespecified sources of heterogeneity, such as methotrexate dose and duration of underlying disease could not be explored statistically. In all but one study the underlying disease was rheumatoid arthritis; compared with very high doses that can be used for cancer treatment, the range of methotrexate dose is relatively narrower for RA treatment (i.e., 10-25 mg per week). Since all studies were observational residual confounding by poorly measured or unmeasured confounders remains a possibility. Most studies did not adjust for folate use that could protect against harms and side effects of methotrexate use³⁰, and several studies did not

adjust for underlying disease severity; these would generally cause underestimation of protective associations. Furthermore, the majority of the studies did not adjust for steroid use that might also attenuate the observed risk estimates; methotrexate has steroid-sparing properties (lower steroid use among methotrexate users and thus potentially lower CVD risk), and this could possibly be one of the mechanisms of effect. Publication bias cannot be completely excluded.

In summary, our findings provide support for the inflammatory hypothesis of atherothrombosis. Given the heterogeneous methodology identified, future observational studies should ideally compare methotrexate initiators vs. non-initiators and also adjust for confounding by underlying disease severity. Our findings also support the need for further experimental studies to elucidate likely mechanisms and randomized clinical trials to establish causality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Screening and selection process of studies of methotrexate use and cardiovascular disease risk.

Figure 2. Risk of cardiovascular disease associated with methotrexate use, including 8 prospective and 2 retrospective cohort studies, 66,334 participants and 6,235 events. Random effects meta-analysis was used to calculate the overall pooled relative risk (RR), in the presence of statistical between-study heterogeneity ($p>0.1$). Solid diamonds and lines are study-specific RRs and 95% CI, respectively; the size of each box is weighted by the inverse-variance of each study. Dashed line and open diamond are pooled RR and 95% CI, respectively, combining each study-specific RR.

* Assessed other RA medication vs. methotrexate as the reference group. The RR of methotrexate vs. other RA medication was calculated by pooling the inverse RRs of all other RA medication, using fixed effects meta-analysis.

Abbreviations: CI, Confidence interval; CVD, Cardiovascular disease; IHD; Ischemic heart disease; MI, Myocardial infarction; RA, Rheumatoid arthritis; RR, Relative risk.

Figure 3.

Risk of cardiovascular disease associated with methotrexate use, among studies that adjusted for underlying disease severity (top; 5 cohort studies and 1 retrospective study, 7,760 participants and 509 events), and among studies that adjusted for other medication used for underlying disease (bottom; 3 cohort studies and 1 retrospective study, 12,051 participants and 1,043 events). Fixed effects meta-analysis was used to calculate the overall pooled relative risk (RR), in the absence of statistical between-study heterogeneity ($p > 0.1$). Solid diamonds and lines are study-specific RRs and 95% CI, respectively; the size of each box is weighted by the inverse-variance of each study. Dashed line and open diamond are pooled RR and 95% CI, respectively, combining each study-specific RR.

* Assessed other RA medication vs. methotrexate as the reference group. The RR of methotrexate vs. other RA medication was calculated by pooling the inverse RRs of all other RA medication, using fixed effects meta-analysis.

Abbreviations: CI, Confidence interval; CVD, Cardiovascular disease; RR, Relative risk.

Table 1
Identified studies evaluating methotrexate use among patients with systemic inflammation and occurrence of cardiovascular disease.

First Author (Year)s	Country	Study Name	Under- lying disease	Ascertainment of MTX use	MTX comparison groups	Disease outcome	Disease ascertainment	Sample size	N of events	Follow- up (y)	Mean Age (y)	Adjust- ments [*]	Quality score [†]
Prospective Cohort Studies													
Choi, HK <i>et al.</i> (2002) ¹⁴	US	Wichita Arthritis Center Cohort	RA	Arthritis medical record database	Initiators vs. non-initiators	CVD mortality ^{//}	Medical records, death certificates, or national death registry	1,240	84	6.0	57	1, 2, 3, 4	6
Solomon, DH <i>et al.</i> (2006) ¹⁰	US	Pharmaceutical Assistance Contract for the Elderly	RA	Health-care utilization database	Initiators vs. non-initiators	MI or stroke hospitalization	Healthcare utilization database	4770	398	2.0	82	1, 2, 4	3
Suisa, S <i>et al.</i> (2006) ¹⁵	North America	PharMetrics Patient-Centric Outcomes Database Cohort	RA	Health-care utilization database	Current users vs. non-current users	MI hospitalization	Medical records	4770	Not reported	2.0	82	1, 2, 4	3
						Stroke hospitalization		4770	Not reported	2.0	82	1, 2, 4	3
Troelsen, LN <i>et al.</i> (2007) ¹⁶	Denmark	N/A	RA	Clinical chart	Current users vs. non-current users	AMI hospitalization [§]	Medical records	5,118	476	1.2	65	1, 2, 4	2
Nadareishvili, Z <i>et al.</i> (2008) ¹⁷	US	National Data Bank for Rheumatic Disease longitudinal study	RA	Patients' self-report	Ever-users vs. never-users	IHD hospitalization ^{//}	Medical records, death and patients registry databases	178	29	9.5	62	1, 2, 3	5
						MI hospitalization	Medical records and death certificates	178	12			1, 2, 3	5
Wolfe, F <i>et al.</i> (2008) ¹⁸	US	National Data Bank for Rheumatic Disease longitudinal study	RA	Patients' self-report	Ever-users vs. never-users	Ischemic stroke [§]		832	41	4.0	70	2, 3	4
Edwards, CJ <i>et al.</i> , (2008) ²⁰	UK	UK General Practice Research Database	RA	General practice database	Ever-users vs. never-users	MI [§]	Study questionnaires, Medical records	3,974	198	3.0	41	1, 2, 3	4
						MI [§]	General practice database	34,364	966	7	53.5	1, 2	1
Goodson, NJ <i>et al.</i> , (2008) ²¹	UK	UK Norfolk Arthritis Register	Polyarthritis	Not reported	Current users vs. never users	CVD mortality ^{//}	Not reported	923	85	10.7	55	1, 2, 3, 4	3
Retrospective Cohort Studies													
Prodanowich, S <i>et al.</i> (2005) ¹⁹	US	Miami Veterans cohort	Psoriasis	Pharmacy database	Ever-users vs. never-users	CVD [§]	Medical records	7,615	1,869	Not reported [#]	65	1, 2, 5	2
			RA					6,707	2,017	Not reported [#]	66	1, 2, 5	2

First Author (Year)s	Country	Study Name	Under- lying disease	Ascertainment of MTX use	MTX comparison groups	Disease outcome	Disease ascertainment	Sample size	N of events	Follow- up (y)	Mean Age (y)	Adjust- ments *	Quality score [†]
van Halm, VP <i>et al.</i> (2006) ¹³	Netherlands	Jan van Breemen Institute	RA	Medical record	Ever-users vs. never-users	CVD [§]	Medical records	613	72	9.2	64	1, 2, 3	3

* Control of confounding: 1. Sociodemographic indicators; 2. Cardiovascular risk factors; 3. Severity of underlying disease; 4. Medications for underlying disease; 5. Use of folate.

[†] Quality assessment was performed by review of study design, including inclusion and exclusion criteria, assessment of exposure, assessment of outcome, assessment of underlying disease severity, control of confounding (any two plus disease severity was given a score of 1; otherwise, 0), and evidence of bias. Each of the six quality criteria was evaluated and scored on an integer scale (0 or 1; 1 being better) and summed; quality scores from 0 to 3 were considered lower quality, and 4-6 higher quality.

[‡] Incident events.

[§] Not specified whether incident or recurrent event.

// Upon independent review by 4 authors duration of follow-up was felt to exceed 3 months given the design and number of incident events. CVD, cardiovascular diseases; IHD, ischemic heart disease; MI, myocardial infarction; MTX, methotrexate; RA, rheumatoid arthritis.

Table 2

Potential prespecified sources of heterogeneity explored among the studies evaluating methotrexate treatment among patients with systemic inflammation and risk of CVD.

Prespecified source of heterogeneity	No of estimates	Stratified fixed effects meta-analysis RR (95% CI)	Meta-regression P-value for heterogeneity
Study design			
Prospective cohort	8	0.79 (0.70-0.89)	0.97
Retrospective cohort	3	0.80 (0.70-0.91)	
Study location			
America	7	0.80 (0.73-0.88)	0.51
Europe	4	0.71 (0.51-0.98)	
Duration of follow-up (yrs)	9	0.79 (0.70-0.89)	0.25
MTX comparison groups			
Initiators vs. non-initiators	2	0.52 (0.22-1.23)*	0.22
Ever vs. never users	6	0.84 (0.75-0.93)	
Current vs. non-current users	3	0.76 (0.59-0.99)	
Ascertainment of MTX use			
Physician	8	0.78 (0.71-0.85)	0.14
Self-reported	2	0.97 (0.76-1.24)	
Underlying disease			
Rheumatoid arthritis	9	0.81 (0.73-0.88)	
Disease outcome			
CVD (including IHD)	7	0.76 (0.69-0.84)	0.15
MI [†]	3	0.82 (0.71-0.96)	
Stroke [‡]	1	0.70 (0.56-0.87)	
Event			
Incident	7	0.83 (0.75-0.92)	
Degree of covariate adjustment			
Underlying disease severity			
Yes	6	0.64 (0.43-0.96)*	0.88
No	5	0.79 (0.72-0.87)	
Other medication for underlying disease			
Yes	4	0.73 (0.63-0.84)	0.22
No	7	0.83 (0.75-0.93)	
Folate use			
Yes	2	0.81 (0.71-0.92)	0.83
No	9	0.78 (0.69-0.88)	
Quality score[‡]			
Lower (0-3)	7	0.78 (0.71-0.86)	0.59
Higher (4-6)	4	0.68 (0.40-1.15)*	
Reported analysis			
Primary	6	0.78 (0.71-0.86)	

Prespecified source of heterogeneity	No of estimates	Stratified fixed effects meta-analysis RR (95% CI)	Meta-regression P-value for heterogeneity
Secondary	5	0.87 (0.71-1.06)	0.50

We identified 10 observational studies that provided 11 separate estimates for investigating the association between methotrexate use and CVD risk. When reported total number of estimates is <11 this represents either missing information or 1 study in other subgroups. Potential prespecified sources of heterogeneity were explored by using stratified inverse-variance weighted fixed effects meta-analysis and inverse-variance weighted meta-regression.

* Significant between-study heterogeneity was present ($p < 0.1$) in stratified meta-analysis, and random effects meta-analysis is reported.

[†] Two studies that evaluated CVD further provided separate estimates for MI (n=2) and stroke (n=1). These additional estimates were included in the stratified meta-analysis, resulting in 5 estimates for MI and 2 estimates for stroke.

[‡] Quality assessment was performed by review of study design, including inclusion and exclusion criteria, assessment of exposure, assessment of outcome, assessment of underlying disease severity, control of confounding, and evidence of bias. Each of the six quality criteria was evaluated and scored on an integer scale (0 or 1; 1 being better) and summed; quality scores from 0 to 3 were considered lower quality, and 4 to 6 higher quality.

CVD, cardiovascular disease; MI, myocardial infarction; MTX, methotrexate; RR, relative risk; CI, confidence interval.