Systematic Review and Meta-Analysis of *Methotrexate* Use and Risk of Cardiovascular Disease

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Inflammation predicts risk for cardiovascular disease (CVD) events, but the relation of drugs that directly target inflammation with CVD risk is not established. Methotrexate is a disease-modifying antirheumatic drug broadly used for the treatment of chronic inflammatory disorders. A systematic review and meta-analysis of evidence of relations of methotrexate with CVD occurrence were performed. Cohorts, case-control studies, and randomized trials were included if they reported associations between methotrexate and CVD risk. Inclusions and exclusions were independently adjudicated, and all data were extracted in duplicate. Pooled effects were calculated using inverse variance–weighted meta-analysis. Of 694 identified publications, 10 observational studies in which methotrexate was administered in patients with rheumatoid arthritis, psoriasis, or polyarthritis met the inclusion criteria. Methotrexate was associated with a 21% lower risk for total CVD (n = 10 studies, 95% confidence interval [CI] 0.73 to 0.87, p <0.001) and an 18% lower risk for myocardial infarction (n = 5, 95% CI 0.71 to 0.96, p = 0.01), without evidence for statistical between-study heterogeneity (p = 0.30 and p = 0.33, respectively). Among prespecified sources of heterogeneity explored, stronger associations were observed in studies that adjusted for underlying disease severity (relative risk 0.64, 95% CI 0.43 to 0.96, p <0.01) and for other concomitant medication (relative risk 0.73, 95% CI 0.63 to 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 (relative risk 0.81, 95% CI 0.74 to 0.89). In conclusion, methotrexate use is associated with a lower risk for CVD in patients with chronic inflammation. These findings suggest that a direct treatment of inflammation may reduce CVD risk. © 2011 Elsevier Inc. All rights reserved. (Am J Cardiol 2011; 108:1362–1370)

Systemic inflammation is strongly linked to increased risk for cardiovascular disease (CVD).[1,2] However, whether this relation 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 antiplatelet effects also reduce CVD event rates. Methotrexate has received particular interest in that regard, as it is a disease-modifying antirheumatic 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 in patients with chronic inflammatory disorders (e.g., RA)[4] or even in patients with persistent inflammatory responses (e.g., increased C-reactive protein levels).[5] Recently, the evidence for relations between methotrexate use in patients with RA and CVD outcomes has been systematically reviewed.[4] However, no systematic review and meta-analysis have 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

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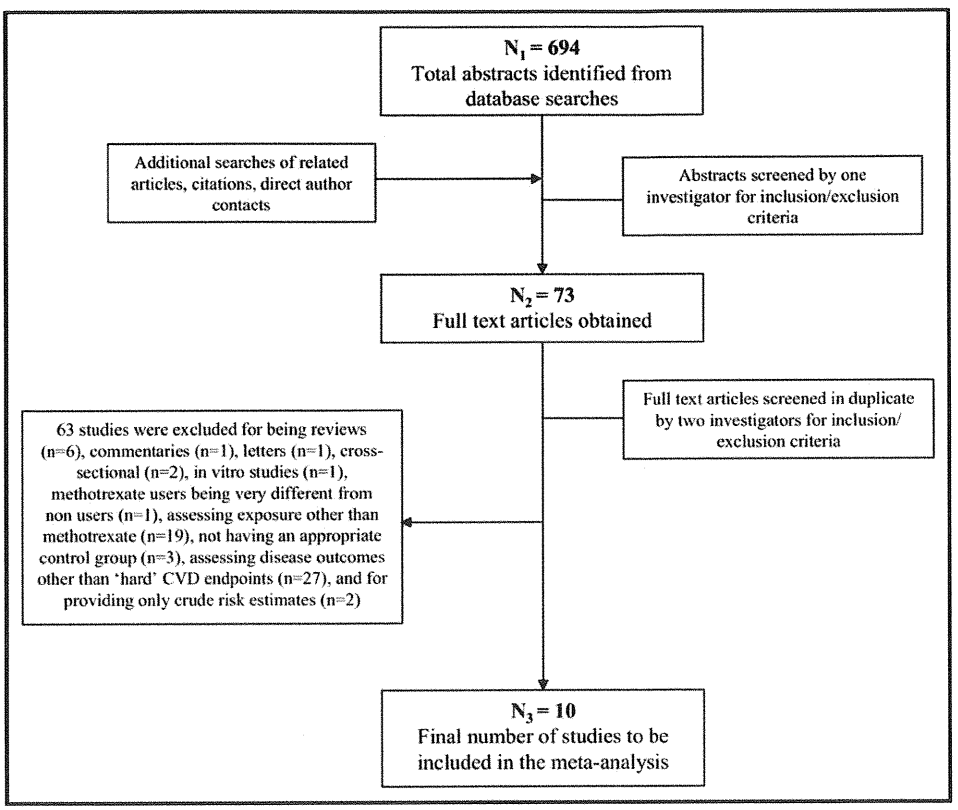

Figure 1. Screening and selection process of studies of methotrexate use and CVD risk.

this important question, we performed a systematic review and meta-analysis of the evidence for relations of methotrexate use with risk for CVD.

Methods

The Meta-Analysis of Observational Studies in Epidemiology guidelines[6] were used as a 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 ≥3 months, and reported effect estimates on occurrence of "hard" CVD events (myocardial infarction [MI], coronary heart disease), sudden death, and/or stroke). We performed searches using multiple online databases, including MEDLINE (see "Supplemental Methods"), Embase, AGRIS, the Allied and Complementary Medicine Database, the Web of Knowledge, the Cumulative Index to Nursing and Allied Health Literature, CAB Abstracts, the Cochrane Library, conference abstracts (Zetoc), Faculty of 1000, and gray literature sources (System for Information on Grey Literature in Europe). We additionally reviewed related reports, hand-searched reference lists, and performed direct investigator 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 had information only on intermediate secondary end points (e.g., lipid or glucose levels) or "soft" coronary heart disease 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 nonusers (e.g., diseased vs nondiseased participants). We also excluded a priori ecologic 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 reports, 621 were excluded on the basis of review of the title and abstract (Figure 1). The remaining 73 reports were reviewed in detail independently and in duplicate to determine inclusion or exclusion (95% concordance); 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 who were very different from nonusers (n = 1); assessed exposure other than methotrexate (n = 19); did not have appropriate control groups (n = 3); did not assess "hard" CVD end points (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 the year the 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 noninitiators, ever users vs never users), ascertainment of methotrexate use, treatment dose, disease outcome, disease incidence versus recurrence, folate use, whether the reported analysis was primary or secondary and prespecified or post hoc in each report, 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 investigator contact for only 1 of 10 studies, as described previously.

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 I^2 statistic, and meta-regression.[8,9] To calculate the overall pooled RR, we used 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 1 study,[10] methotrexate was considered as the reference group to estimate the RRs of other RA medications on risk for MI or stroke hospitalization, including biologics, other cytotoxic agents, noncytotoxic agents, and glucocorticoids. Subsequently, to estimate the RR of methotrexate compared to other RA medications (the latter as the reference group) on CVD risk, we pooled the inverse RRs of all other RA medications. Potential for publication bias was explored by visually inspecting a funnel plot of the effect size versus the SE[11] and statistically using the Begg adjusted-rank correlation test.[12] 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, $p < 0.10$) 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 noninitiators, ever vs never users, current vs noncurrent 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 sociodemographic characteristics and CVD risk factors, so further adjusting for underlying disease severity, other medication for underlying disease, or folate use), overall quality score (0 to 3 vs 4 to 6), and whether the reported analysis was primary or secondary and prespecified or post hoc (to address potential publication bias of "positive" findings) in each publication. Analyses were performed using Stata version 10.0 (StataCorp LP, College Station, Texas) with a 2-tailed α value <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 subjects 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 study[19] provided 2 separate estimates for patients receiving methotrexate with RA and polyarthritis as the underlying disease (a 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. Most of the studies did not report dose of methotrexate treatment, and for those that did,[13,14,18] the median dose ranged from 13 to 15 mg/week. With the exception of 1 study,[19] all studies reported duration of follow-up; the median duration across studies was 5.84 years. In most of the studies, methotrexate use was ascertained by a physician (including prescription files and chart review); in only 2 studies,[17,18] methotrexate use was self-reported. Half of the studies[10,13–15,20] used health claims databases to ascertain methotrexate exposure. Most studies compared methotrexate ever users versus never users (n = 6 estimates), followed by current versus noncurrent users (n = 3 estimates) and initiators versus noninitiators (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 stroke.[10] Reported events were incident in 7 studies; case incidence versus recurrence was not specified for 3 studies.[14,16,21] Quality scores were in the lower range (0 to 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 sociodemographic characteristics (e.g., age, gender, socioeconomic 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,

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

Study	Country	Study Name	Underlying Disease	Ascertainment of MTX Use	MTX Comparison Groups
Prospective cohort studies					
Choi et al (2002)[14]	United States	Wichita Arthritis Center Cohort	RA	Arthritis medical record database	Initiators vs noninitiators
Solomon et al (2006)[10]	United States	Pharmaceutical Assistance Contract for the Elderly	RA	Health care utilization database	Initiators vs noninitiators
Suissa et al (2006)[15]	North America	PharMetrics Patient-Centric Outcomes Database Cohort	RA	Health care utilization database	Current users vs noncurrent users
Troelsen et al (2007)[16]	Denmark	NA	RA	Clinical chart	Current users vs noncurrent users
Nadareishvili et al (2008)[17]	United States	National Data Bank for Rheumatic Disease Longitudinal Study	RA	Patients' self-report	Ever users vs never users
Wolfe et al (2008)[18]	United States	National Data Bank for Rheumatic Disease Longitudinal Study	RA	Patients' self-report	Ever users vs never users
Edwards et al (2008)[20]	United Kingdom	United Kingdom General Practice Research Database	RA	General practice database	Ever users vs never users
Goodson et al (2008)[21]	United Kingdom	United Kingdom Norfolk Arthritis Register	Polyarthritis	Not reported	Current users vs never users
Retrospective cohort studies					
Prodanowich et al (2005)[19]	United States	Miami Veterans Cohort	Psoriasis RA	Pharmacy database	Ever users vs never users
van Halm et al (2006)[13]	The Netherlands	Jan van Breemen Institute	RA	Medical record	Ever users vs never users

* 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 2 plus disease severity was given a score of 1; otherwise 0), and evidence of bias. Each of the 6 quality criteria was evaluated and scored on an integer scale (0 or 1, with 1 being better) and summed; quality scores ranging from 0 to 3 were considered lower quality and those ranging from 4 to 6 higher quality.

‡ Incident events.

§ Not specified whether incident or recurrent event.

‖ Upon independent review by 4 investigators, duration of follow-up was believed to exceed 3 months, given the design and the number of incident events.

IHD = ischemic heart disease; MTX = methotrexate.

methotrexate use was associated with 21% lower CVD risk (95% CI 0.73 to 0.87); results of the random-effects meta-analysis were very similar (RR 0.79, 95% CI 0.71 to 0.88). Visual inspection of the funnel plot (see Supplemental Figure 1) 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 to 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 RRs were 0.71 (95% CI 0.51 to 0.98) for studies conducted in Europe, 0.52 (95% CI 0.22 to 1.23) for studies that compared methotrexate initiators versus noninitiators, 0.68 (95% CI 0.40 to 1.15) for studies with higher quality scores, and 0.97 (95% CI 0.76 to 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 to 0.96) and studies that adjusted for concomitant medication use (RR 0.73, 95% CI 0.63 to 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 to 0.84) in studies that assessed only CVD and 0.70 (95% CI 0.56 to 0.87) in those that assessed only stroke.

Discussion

In this meta-analysis of observational studies, methotrexate use among patients with systemic inflammation (mainly RA) was associated with 21% lower CVD risk, with little evidence of between-study heterogeneity. Similar inverse associations were 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 patients would be more likely to receive methotrexate and to also de-

Table 1
(*continued*)

Disease Outcome	Disease Ascertainment	n	Events	Follow-Up (yrs)	Mean Age (yrs)	Adjustments*	Quality Score†
CVD mortality§	Medical records, death certificates, or national death registry	1,240	84	6.0	57	1, 2, 3, 4	6
MI or stroke hospitalization	Health care utilization database	4,770	398	2.0	82	1, 2, 4	3
MI hospitalization		4,770	Not reported	2.0	82	1, 2, 4	3
Stroke hospitalization		4,770	Not reported	2.0	82	1, 2, 4	3
AMI hospitalization‡	Medical records	5,118	476	1.2	65	1, 2, 4	2
IHD hospitalization§	Medical records, death and patients registry databases	178	29	9.5	62	1, 2, 3	5
MI hospitalization		178	12			1, 2, 3	5
Ischemic stroke‡	Medical records and death certificates	832	41	4.0	70	2, 3	4
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
CVD mortality§	Not reported	923	85	10.7	55	1, 2, 3, 4	3
CVD‡	Medical records	7,615	1,869	Not reported‖	65	1, 2, 5	2
		6,707	2,017	Not reported‖	66	1, 2, 5	2
CVD‡	Medical records	613	72	9.2	64	1, 2, 3	3

velop 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 to 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 versus those who did not initiate methotrexate originally, which limits potential bias of studies of patients who remain on the drug long term, who could be doing so because they tolerate the drug (closer to an intention-to-treat analysis). When we restricted the analysis to the only 2 studies that assessed initiators versus noninitiators, methotrexate use was associated with a trend toward 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 previously. Ascertainment of methotrexate use could also be a potential study design limitation, because it could lead to exposure misclassification and attenuation of the observed relation; 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 higher quality scores 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.

RA 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 to the general population, mainly attributable to death from 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 in patients with RA.[3,24] Methotrexate improves the mobility of patients with RA, 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 C-reactive protein, interleukin-6, and tumor necrosis factor–α in patients with RA and psoriasis, without major concomitant effects on platelet function,[27,28] as well as in animal models.[29] Regarding the potential effects of methotrexate on other intermediate risk factors for CVD, including lipid levels and insulin resistance, these have been recently systematically reviewed,[4] and limited current evidence suggests

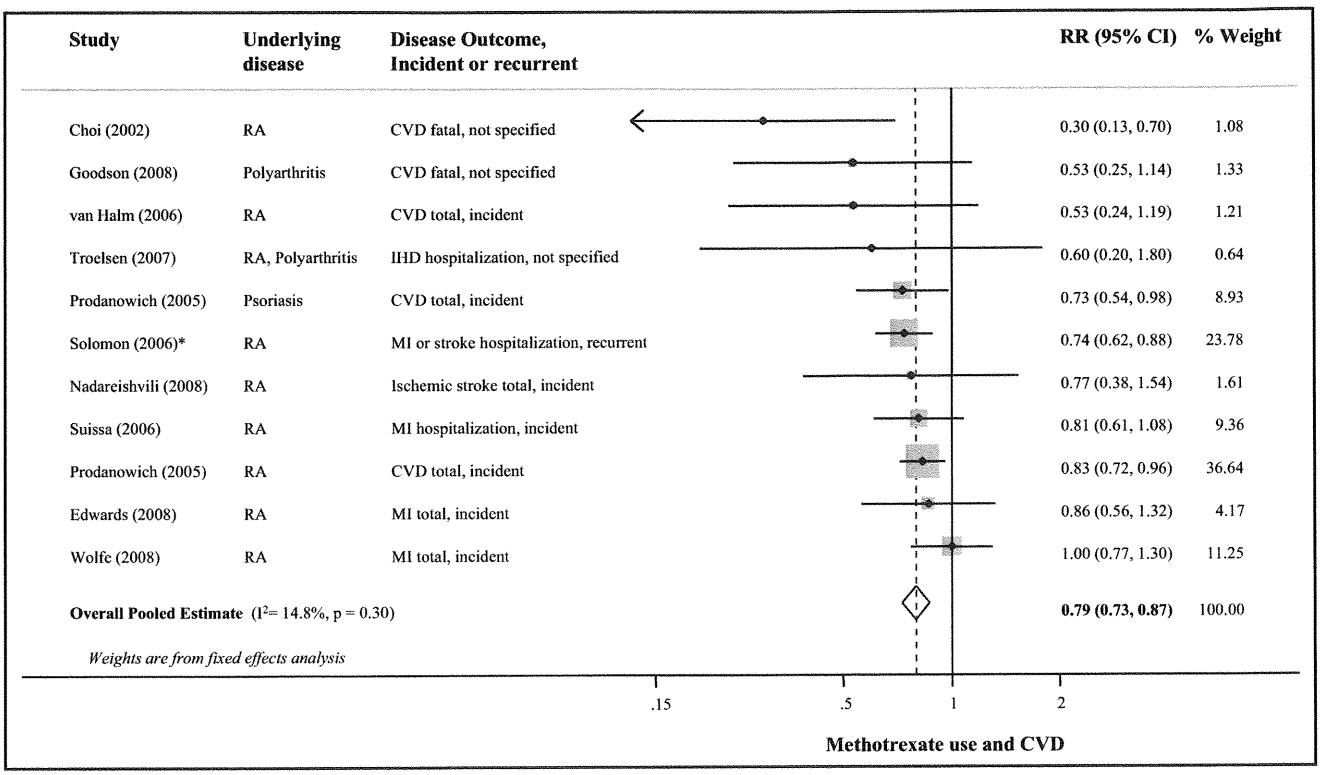

Figure 2. Risk for CVD associated with methotrexate use, including 8 prospective and 2 retrospective cohort studies, 66,334 participants, and 6,235 events. Random-effects meta-analysis was used to calculate the overall pooled RR, in the presence of statistical between-study heterogeneity (p >0.10). *Solid diamonds and lines* are study-specific RRs and 95% CIs, 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 compared to methotrexate as the reference group. The RR of methotrexate versus other RA medications was calculated by pooling the inverse RRs of all other RA medications, using fixed-effects meta-analysis. IHD = ischemic heart disease.

the 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, 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 methotrexate 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 search of published research and directly contacted several investigators, making it likely that we identified all major published relevant reports. Study review (inclusion and exclusion) and data extraction were performed independently and in duplicate by ≥2 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), geographic 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 investigators to request either additional data or clarifications with regard to the identified sources of heterogeneity, responses were received for only 1 of 10 identified publications,[13] 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

Table 2
Potential prespecified sources of heterogeneity explored among the studies evaluating methotrexate treatment in patients with systemic inflammation and risk for cardiovascular disease

Prespecified Source of Heterogeneity	Number 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 (years)	9	0.79 (0.70–0.89)	0.25
MTX comparison groups			
Initiators vs noninitiators	2	0.52 (0.22–1.23)*	
Ever vs never users	6	0.84 (0.75–0.93)	0.22
Current vs noncurrent users	3	0.76 (0.59–0.99)	0.70
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			
RA	9	0.81 (0.73–0.88)	
Disease outcome			
CVD (including IHD)	7	0.76 (0.69–0.84)	
MI†	3	0.82 (0.71–0.96)	0.15
Stroke†	1	0.70 (0.56–0.87)	0.94
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)	0.50
Secondary	5	0.87 (0.71–1.06)	

Ten observational studies that provided 11 separate estimates for investigating the association between MTX use and CVD risk were identified. When the 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 ($p < 0.10$) 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 6 quality criteria was evaluated and scored on an integer scale (0 or 1, with 1 being better) and summed; quality scores ranging from 0 to 3 were considered lower quality and those ranging from 4 to 6 higher quality.

IHD = ischemic heart disease; MI = myocardial infarction; MTX = methotrexate.

statistically. In all but 1 study, the underlying disease was RA; compared to very high doses that can be used for cancer treatment, the range of methotrexate dose is relatively narrower for RA treatment (i.e., 10 to 25 mg per week). Because all studies were observational, residual confounding by poorly measured or unmeasured confounders remains a possibility. Most studies did not adjust for folate use, which could protect against the harms and side effects of methotrexate use,[30] and several studies did not adjust for underlying disease severity; these would generally cause underestimation of protective associations. Furthermore, most 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 in methotrexate users and thus potentially lower CVD risk), and this could possibly be 1

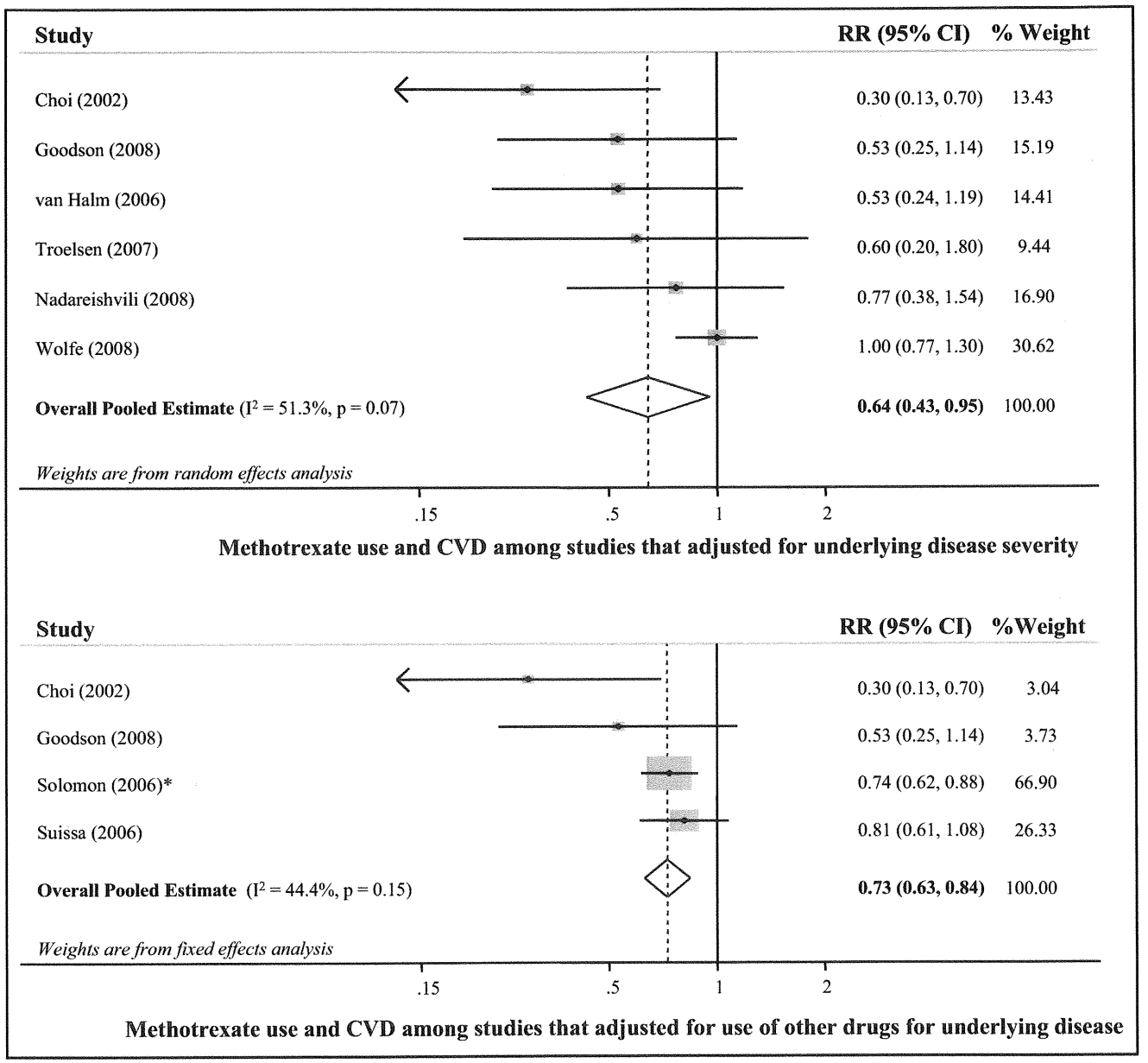

Figure 3. Risk for CVD associated with methotrexate use among studies that adjusted for underlying disease severity *(top)* 5 cohort studies and 1 retrospective study, 7,760 participants, 509 events) and among studies that adjusted for other medications used for underlying disease *(bottom)* (3 cohort studies and 1 retrospective study, 12,051 participants, 1,043 events). Fixed-effects meta-analysis was used to calculate the overall pooled RR, in the absence of statistical between-study heterogeneity (p >0.10). *Solid diamonds and lines* are study-specific RRs and 95% CIs, 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 medications compared to methotrexate as the reference group. The RR of methotrexate versus other RA medications was calculated by pooling the inverse RRs of all other RA medication, using fixed-effects meta-analysis.

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 heterogenous methods identified, future observational studies should ideally compare methotrexate initiators versus noninitiators 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.

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Supplementary Data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.amjcard.2011.06.054.

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