Protective effects of methotrexate against ischemic cardiovascular disorders in patients treated for rheumatoid arthritis or psoriasis: novel therapeutic insights coming from a meta-analysis of the literature data

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ABSTRACT

Objective: The association between chronic use of methotrexate and decreased risk of ischemic cardiovascular events (CVE) among patients with psoriatic or rheumatoid arthritis (RA) was investigated using a systematic review and meta-analysis.

Methods: The studies should have recruited adults receiving methotrexate, followed up for at least one year. Moreover, studies should have reported "hard" cardiovascular endpoints, by evaluating the cardiovascular outcomes of the habitual users of the drug or of new users compared with patients with the same disease who had never used methotrexate. The outcome of interest was the overall pooled odds ratio (OR) of major adverse cardiovascular events, i.e., a composite of new-onset angina, acute coronary syndrome, need for percutaneous or surgical coronary revascularization, stroke, and cardiovascular death. The study was performed according to the PRISMA statement.

Results: Seven observational studies, mostly engaging patients with RA, were included in the meta-analysis. The pooled odds ratio (OR) was 0.73 (95% CI=0.70- 0.77 p<0.001). When stratified meta-analysis models were assessed, the pooled OR was 0.80 (95% CI=0.66-0.97; p=0.022) for studies adjusting for clinical severity of RA. Furthermore, the OR was even more significant after adjustment for concomitant use of other drugs specific for RA (OR=0.71, 95% CI=0.67-0.75, p<0.001).

Conclusion: Methotrexate at low doses, such those used for maintenance therapy of RA, predicted a decreased risk of CVE. Since methotrexate doesn't interfere with blood lipids, platelet aggregation or insulin resistance, the protective association may originate from mechanisms other than those exerted by antiplatelet drugs or statins. *(Anatol J Cardiol 2016; 16: 2-9)*

Keywords: methotrexate, cardiovascular risk, meta-analysis

Introduction

Systemic inflammation is likely to be involved in causing the destabilization of atherosclerotic plaques in patients with coronary atherosclerosis or ischemic cerebrovascular disease. According to this interpretation, inflammatory oedema of atherosclerotic plaques may lead to intra-plaque sub-intimal haemorrhage, that in turn results in their erosion and rupture. At this point, the intimal erosion may become the seat where circulating platelets accumulate, resulting in conglomerates that are super-imposed to the eroded plaque, which generate a fibrin net and constitute the precursor of thrombus. Thrombosis superimposed to the inflamed plaque causes a narrowing of the vessel lumen, provoking its sub-occlusion or complete obstruction and it is responsible for the critical reduction or complete interruption of blood supply in the myocardial area served by the diseased vessel. Therefore, the atheroma inflammation may be the *primum movens* capable of starting the cascade of the events that leads to the acute coronary syndrome and myocardial necrosis. The theory that the systemic inflammatory state subsequently makes the plaques more vulnerable and prone to the erosion and thrombosis, so as to decisively impact on the dynamic evolution of coronary atherosclerosis, is also supported by the fact that episodes of spontaneous angina pectoris at rest are frequently accompanied by parallel concomitant elevation of the C-reactive protein, i.e. a well-known marker of systemic inflammation (1). Studies by several authors (2-4) have highlighted that exacerbation of anginal symptoms in patients with known ischemic heart disease frequently occurs coupled with elevation of serum inflammatory markers, especially high-sensitivity C-reactive protein. These observations should have led to the development of therapeutic strategies aimed to prevent or reduce the systemic inflammatory activity as a tool to avoid the genesis of thrombotic events, related to plaque inflam-

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mation (5). However, the pharmacologic strategies adopted so far have predominantly pursued either the prevention of lipid intimal accumulation, through a reduction of circulating levels of atherogenic lipoproteins (in the case of statins) (6, 7) or the prophylaxis of platelet accumulation at the level of the atheroma (with antiplatelet drugs) (8). By contrast, immunosuppressive agents have been exploited only in conjunction with coronary stents (the case of sirolimus, everolimus, etc.) (9) in order to counter the traumatic inflammation of the coronary vascular segments, when manipulated with the use of balloons and expandable prostheses. Thus, at present there are no encoded recommendations regarding the use of drugs with anti-inflammatory effects in coronary artery disease. In fact, the low doses of acetylsalicylic acid, used as an anti-aggregant medication, are devoid of anti-inflammatory effect (8). In addition, both non-steroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase-2 (COX-2) selective inhibitors (COXIBs) don't seem to be suitable for reducing the ischemic-thrombotic risk; on the contrary an increased risk of myocardial infarction has been attributed to the latter (10). Finally, according to some authors, the corticosteroids would have proved to favour vascular atherogenesis (11, 12). A scenario of conflicting evidence therefore emerges from the sum of current knowledge. On the one hand, there is the evidence of an increase in some inflammatory indices, in particular the C-reactive protein, which often occurs simultaneously with the phases of clinical destabilization of ischemic heart disease. On the other hand, a total lack of cardioprotective effects of various classes of anti-inflammatory drugs, such as corticosteroids, NSAIDs and COXIBs has been found.

In this context, some researchers have evidenced a significantly decreased risk of cardiovascular events in patients (5) as well as in laboratory animals (13) undergoing prolonged therapy with low doses of methotrexate (5, 13), an immunosuppressant drug whose administration at low doses is used to effectively antagonize joint inflammation and deformation of some articular diseases such as rheumatoid or psoriatic arthritis (14).

Data from these studies on the cardiovascular risk of patients with rheumatoid arthritis(RA) undergoing methotrexate therapy compared with other patients with the same disease treated with different anti-inflammatory therapies are precisely the material that was used as a basis for the elaboration of the meta-analysis illustrated in the present study.

Methods

This meta-analysis aimed to assess whether the chronic use of methotrexate is associated with a significantly decreased risk of experiencing one or more major acute cardiovascular events. For this purpose, the data of various studies were pooled and employed as a source of information for comparison between methotrexate users and nonusers.

For inclusion in the meta-analysis, the following criteria had to be met: the studies must have included adults who received methotrexate, with a follow up duration of at least 3 months; and they must have reported the estimates of hard cardiovascular events occurring during the observation period, i.e., new-onset angina, acute coronary syndrome (including unstable angina or acute myo-

cardial infarction), need for percutaneous or surgical coronary revascularization, stroke, or cardiovascular death. Moreover, each of the studies should have assessed the cardiovascular outcomes of habitual or new users of methotrexate compared to patients with the same disease who had never used this drug.

In contrast, studies in which a comparison had been made between current and previous users were to be excluded. Studies offering information only about intermediate secondary endpoints (e.g., serum LDL-cholesterol or glucose levels), or intermediate "soft" cardiovascular disease outcomes (such as recent impairment in tolerance to workload, number of tablets to be assumed every day etc.), and studies in which methotrexate was administered only and exclusively as part of an anti-inflammatory combination therapy (without a group with methotrexate given as a monotherapy) were also excluded from the meta-analysis. A systematic search using related terms was conducted using the PubMed and Embase electronic archives. We limited our search to adults (>18 years old) and, in the absence of randomized controlled trials, to comparative observational studies. The study was performed according to the guidelines and recommendations expressed in the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) statement (15).

Search terms included "methotrexate", "mexate", "amethopterin", and "cardiovascular diseases". All authors participated in determining the eligibility of candidate studies. Differences in interpretation were resolved through discussion. The search included publications up to June 2013 and no lower date limit was applied. The titles and abstracts of all identified citations were reviewed independently by two authors (R.D.V. and C.B.), and any candidate study meeting the inclusion criteria was selected for further screening of the full text.

Outcome of interest

The outcome of interest that we assumed was the overall pooled odds ratio (OR) of major adverse cardiovascular events, i.e., a composite endpoint of new- onset angina, acute coronary syndrome (including unstable angina or acute myocardial infarction), need for percutaneous or surgical coronary revascularization, stroke, and cardiovascular death.

Data extraction

The authors, time periods, patients, and methods of each study were scrutinized to ensure the most complete possible data set. From the reports of the included studies, the following data was extracted: year of study, study design, number of patients treated with methotrexate, number of patients treated with drugs other than methotrexate, number of events in both methotrexate users and non-users for each of the considered studies, duration of follow-up, underlying disease (e.g., RA), duration of underlying disease, assessment of underlying disease severity, and covariates employed in the regression models respectively adopted by each study.

Quality assessment of the studies

The Newcastle-Ottawa quality assessment scale (16) was used for quality evaluation of the studies to be incorporated in the meta-analysis. Eligibility was based on the following criteria: the selection of the study groups (0-4 points), the comparability

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of the groups (0-2 points), and the ascertainment of either the exposure or outcome of interest (0-3 points), with a total score of 9. Quality scores from 0 to 4 were considered lower quality, and 5-9 higher quality.

Statistical analysis

Statistical analysis was performed using Review Manager 5.0.4 software (available from Cochrane Collaboration at http://www.cochrane.org) and Stata version 10 (Stata Corp LP, College Station, TX, USA). The effect of methotrexate on the occurrence of adverse cardiac outcomes was presented as the overall pooled odds ratio (OR) with 95% confidence intervals (CIs), using a random-effects model. Inverse variance was employed as the weighting method. Heterogeneity was evaluated using Cochran's Q test and I^2 statistic (I^2 was assumed to represent the percentage of variability due to between-study variability). We rated an I^2 of less than 25%, 25-50%, and >50% as a low, moderate, and high amount of heterogeneity, respectively. Results were regarded as statistically significant if $p<0.05$.

Results

556 studies were collected from the PubMed and Embase databases. After preliminary screening, 483 papers were excluded because of inconsistency with our inclusion criteria, on the basis of the abstracts (Fig. 1). Of the remaining 73 papers, 8 were excluded due to the fact that they were not experimental observational studies but rather review articles, and 49 were excluded due to the inadequate quality of the information provided, i.e., unavailable or incomplete data concerning possible exposure to methotrexate (n=19), lack of an appropriate control group (n=3) and use of intermediate or surrogate cardiovascular endpoints (n=27) which did not conform to hard endpoints (major cardiac adverse events or the need for coronary revascularization) required by our study protocol.

Of the remaining 16 studies, five were eliminated because they did not provide for methotrexate as an isolated anti-inflammatory therapy but admitted only patients whose use of methotrexate was systematically combined with simultaneous use of other adjunctive anti-inflammatory drugs, such as other immunosuppressant agents, glucocorticoids, etc.; moreover two studies were excluded because they reported only crude risk estimates and another two studies were eliminated because they made a comparison between current and previous methotrexate users. Thus seven articles were included in the present meta-analysis. Separate meta-analyses were performed for studies that adjusted for a concomitant use of other drugs specific for RA as well as for studies that adjusted for underlying disease severity.

Among the selected studies, we did not detect any randomized controlled trials that evaluated the impact of methotrexate on the "hard" CVD events. One study of Prodanowich et al. (17) provided two separate estimates for patients receiving methotrexate with RA or psoriatic arthritis as the underlying disease. In our meta-analysis, therefore, seven studies assessed methotrexate use in patients with RA as the underlying disease, and one study also dealt with methotrexate given to patients with psoriatic arthritis. A nested case-control approach within the cohort was frequently used as an alternative to the cohort design, considering also that the nested procedure increases

the ease of analysis with inconsequential loss of power (18). This choice was made by the respective authors because of the complexity and time-varying nature of the exposures to the various medications used.

Qualitative findings

All studies adjusted for cardiovascular disease risk factors (e.g., blood pressure, blood cholesterol, smoking), and all except one study adjusted for socio-demographics (e.g., age, sex, socio-economic status, race). By contrast, only three studies adjusted for medications used to treat the underlying disease (19-21), and only four studies adjusted for underlying disease severity (Table 1) (19, 22-24). In addition, as already said, a nested case-control design was exploited in some of the studies (20, 21, 23, 24) incorporated into the meta-analysis. Some information about the criteria used to judge about the RA severity is reported below, concerning the 4 studies (19, 22-24) that included the specific indices of clinical severity of disease among their covariates. In the case of the study by Choi et al. (19), the RA-related variables consisted of rheumatoid arthritis duration, tender joint count, patient's global assessment of disease status, erythrocyte sedimentation rate, health assessment questionnaire score, grip strength, pain scale, depression, and presence of rheumatoid nodules. In the case of the study by Van Halm et al. (22), covariates related to the disease clinical severity were the presence or absence of a positive rheumatoid factor test and erosion on radiographs.

For the study by Nadaireishvili et al. (23), prior total joint replacements and the Health Assessment Questionnaire (HAQ) disability index score were assumed as specific covariate measures of RA severity, together with RA duration. For the study by Wolfe et al. (24), clinical variables specific to RA severity and outcome included duration of RA, total joint arthroplasties, the HAQ disability index and visual analogue scales for pain intensity and global severity.

The decision by some authors (19-21) to include pharmacologic regimens other than methotrexate alone, among the exposure variables, is another important issue. In all studies that used this approach, by building a logistic regression model in which all possible pharmacologic treatments for RA were represented as exposure variables, a favorable profile of cardiovascular safety was found for MTX, proven by the fact that this drug appeared to be consistently associated with a lower risk of developing major cardiovascular events, when compared with the other drugs that are currently used in the RA management: non-steroidal anti-inflammatory drugs (NSAIDs), cyclooxygenase 2 inhibitors (COXIBs), glucocorticoids, cytotoxic immunosuppressive agents, etc.

Quantitative analysis (meta-analysis)

On the basis of our meta-analysis, methotrexate use was associated with significantly reduced cardiovascular disease risk (overall pooled odds ratio: 0.73; 95% CI=0.70-0.77 p<0.001) (Fig. 2).

In the present investigation, stratified meta-analyses were performed by considering studies that adjusted for underlying disease severity (Fig. 3) as well as those that adjusted for use of other drugs for underlying disease (Fig. 4). For studies adjusting for AR clinical severity, a significant association between methotrexate use and decreased cardiovascular risk was proven

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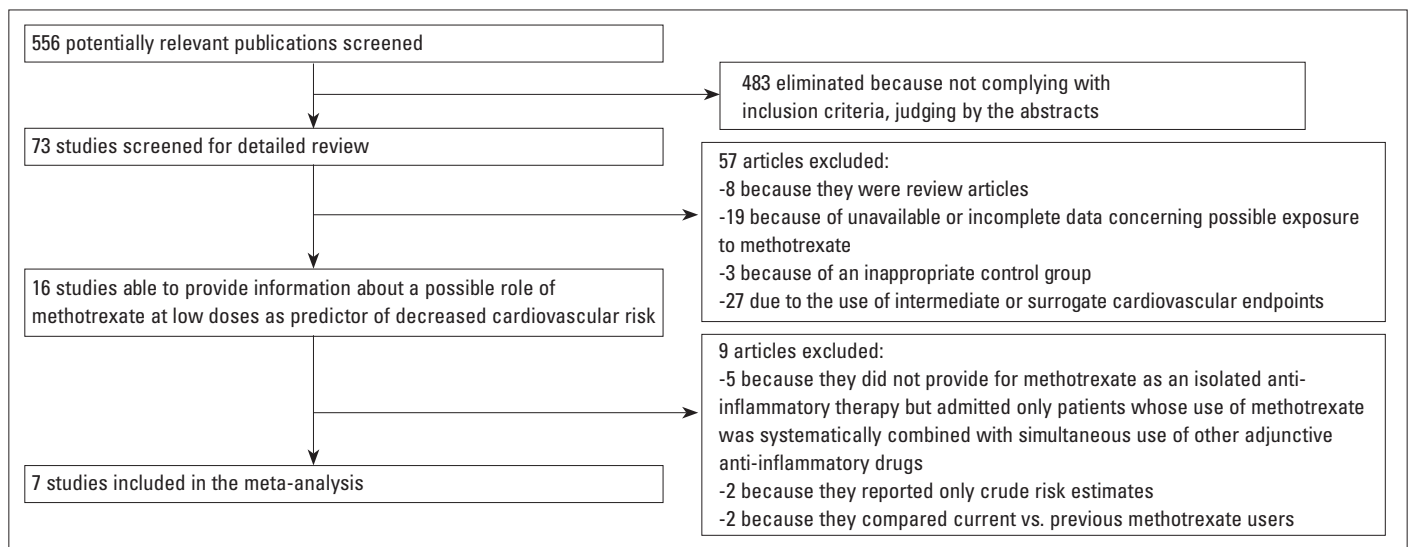
Figure 1. Flow diagram for selection of literature articles suitable for exploring the relationship between methotrexate use and cardiovascular disease risk

(overall pooled OR=0.80; 95% CI=0.66-0.97; p=0.022); similarly, in studies adopting use of other RA-related drugs as exposure variable, methotrexate was also found to be associated with a reduction in cardiovascular disease risk (overall pooled OR=0.71; 95% CI=0.67-0.75; p<0.001).

We performed sensitivity analysis to determine the effect of plausible changes in assumptions on the inverse association between methotrexate therapy and occurrence of major adverse cardiac events (Fig. 5 and Table 2). The significance of the inverse relationship between MTX therapy and the frequency of cardiovascular adverse events was not affected by the exclusion of each of the seven studies originally pooled in the meta-analysis. In other words, the point estimates for cardiovascular (CV) disease events were stable under a range of assumptions, so that significance of the protective effect exerted by methotrexate was retained under any scenario.

Discussion

In the present study of meta-analysis, methotrexate was shown to be associated with a decrease of 27% (Fig. 2) in the cardiovascular disease risk (expressed as a composite endpoint of new-onset angina, acute coronary syndrome, need for percutaneous or surgical coronary revascularization, stroke, and cardiovascular death) within a study population where RA turned out as the largely predominant underlying disease. Moreover, in another meta-analysis we conducted, based on studies that had inserted the degree of clinical severity of arthritis between the covariates within their regression models (Fig. 3), use of methotrexate predicted a reduction of 20% in the risk of cardiovascular events. Similarly, in a subsequent stratified meta-analysis (Fig. 4) that included the studies that had considered also the various drug regimens used as an alternative to monotherapy with methotrexate, the latter was found to be associated with a reduction in cardiovascular risk of 29%.Our meta-analysis shows some similarities and substantial agreement of results with respect to the only other meta-analysis on the subject reported so far in the lit-

erature (27), where, however, the effect size was measured by adopting the relative risk and not with the use of the odds ratio and that included 11 studies in place of the 7 studies comprised in the present meta-analysis, because of less restrictive inclusion criteria compared to those used in our search.

Some relevant differences are noticeable when evaluating the exposure variables comprised within the logistic regression models (or within the Cox models) of the various studies incorporated into the meta-analysis.

In particular, all analyses adjusted for major cardiovascular risk factors (i.e., blood pressure, serum LDL-cholesterol, tobacco smoking and diabetes); instead, among the studies admitted to the meta-analysis, only four (19, 20, 22, 23) reported the average duration of the underlying disease. This was represented by rheumatoid arthritis, found in seven studies, and by psoriatic arthritis in a part of the data collated by the study of Prodanowich et al. (17). In this study two separate estimates of the effects of methotrexate therapy vs. other therapies were made, namely one derived from a group of RA patients and another from a group of patients with psoriatic arthritis.

Overall, RA represented the underlying disease in almost the entire examined case-record of patients taking methotrexate. Patients with RA are known to be burdened with an increased risk of cardiovascular disease events; they have also been shown to have a life expectancy significantly lower than the general population, mainly due to death from cardiovascular causes (25, 26). Among the disease-modifying anti-rheumatic drugs, methotrexate is the most commonly prescribed medication. Moreover, although the side effects of methotrexate can be serious, its long-term safety has been established in patients with RA. Methotrexate improves the mobility of patients with RA, judging from questionnaires for health assessment and other global measures of quality of life. This drug has also been shown to reduce inflammatory biomarkers, such as CRP, IL-6, and the tumour necrosis factor (TNF) - α in patients with RA or psoriasis, without particular concurrent effects on platelet function. As regards the potential effects of methotrexate on other risk fac-

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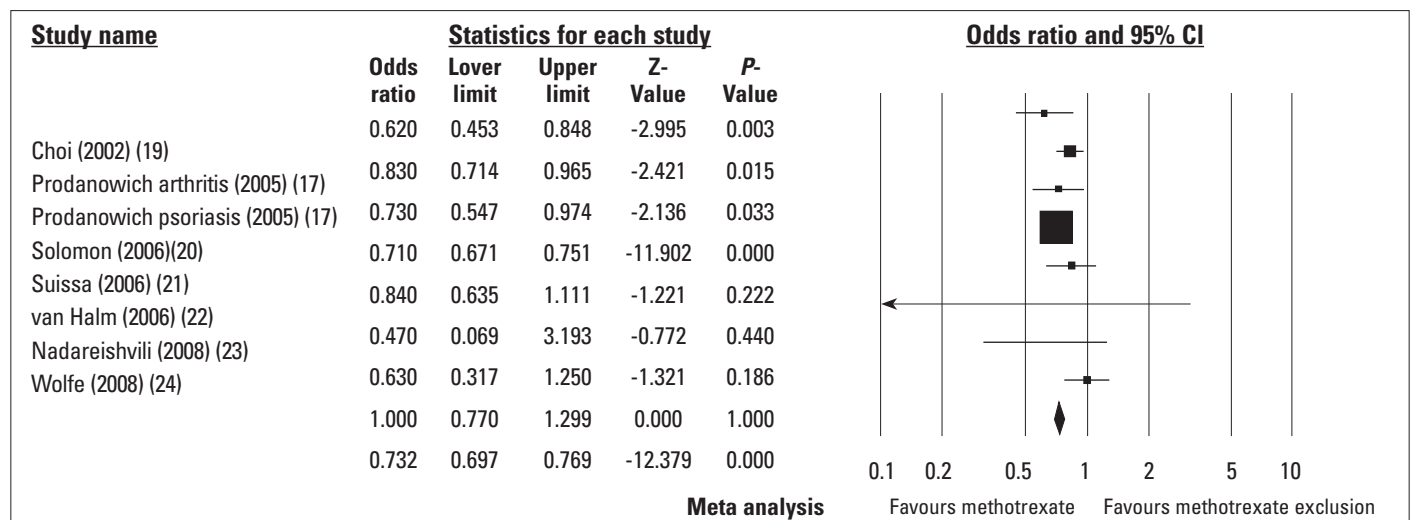
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Table 1. Overview of the studies evaluating patients with inflammatory disease (rheumatoid arthritis in 7 studies and psoriatic arthritis in one study) that were incorporated in our meta-analysis conceived to assess the occurrence of cardio-vascular disease events in patients who used methotrexate compared to those who did not

First author, year	Study design	Underlying disease	Endpoint of interest	Sample size	No. of events	Follow-up, years	Mean age, years	Adjustments*	Quality score°
Choi et al. (19)	Prospective cohort study	RA	mortality from cardiovascular causes	1240	84	6.0	57	1, 2, 3,4	8
Prodanowich et al. (17)	Retrospective cohort study	RA	cardiovascular disease events	6707	2017	not available	66	1, 2, 5	4
Prodanowich et al. (17)	Retrospective cohort study	psoriasis	cardiovascular disease events	7615	1869	not available	65	1, 2, 5	4
Solomon et al. (20)	Prospective cohort study	RA	MI or stroke hospitalization	4770	398	2.0	82	1, 2, 4	5
Suissa et al. (21)	Prospective cohort study	RA	MI hospitalization	5118	476	2.0	65	1, 2, 4	4
van Halm et al. (22)	Retrospective cohort study	RA	cardiovascular disease events	613	72	9.2	64	1, 2, 3	5
Nadareishvili et al. (23)	Prospective cohort study	RA	Ischemic stroke	832	41	4.0	70	2, 3	6
Wolfe et al. (24)	Prospective cohort study	RA	MI	3974	198	3.0	41	1, 2, 3	6

Legenda. MI - myocardial infarction; RA - rheumatoid arthritis.
*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 of the studies was assessed based on the following criteria: the selection of the study groups (0-4 points), the comparability of the groups (0-2 points), and the ascertainment of either the exposure or outcome of interest (0-3 points), with a total score of 9. Quality scores from 0 to 4 were considered lower quality, and 5-9 higher quality

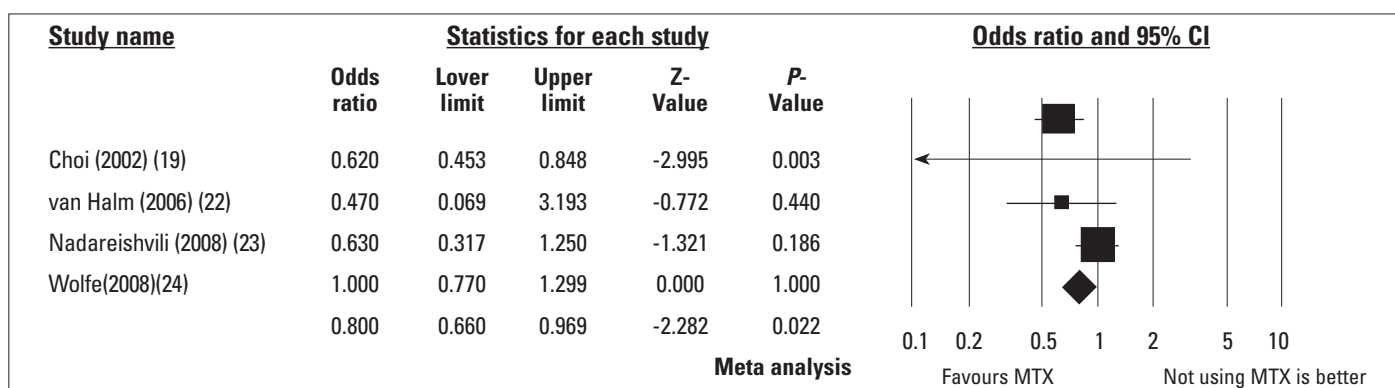| Study name		Statistics for each study					Odds ratio and 95% CI
		Odds ratio	Lover limit	Upper limit	Z-Value	P-Value	
		0.620	0.453	0.848	-2.995	0.003	
Choi (2002) (19)		0.830	0.714	0.965	-2.421	0.015	
Prodanowich arthritis (2005) (17)							
Prodanowich psoriasis (2005) (17)		0.730	0.547	0.974	-2.136	0.033	
Solomon (2006)(20)		0.710	0.671	0.751	-11.902	0.000	
Suissa (2006) (21)		0.840	0.635	1.111	-1.221	0.222	
van Halm (2006) (22)							
Nadareishvili (2008) (23)		0.470	0.069	3.193	-0.772	0.440	
Wolfe (2008) (24)		0.630	0.317	1.250	-1.321	0.186	
		1.000	0.770	1.299	0.000	1.000	
		0.732	0.697	0.769	-12.379	0.000	
						Meta analysis	0.1 0.2 0.5 1 2 5 10
							Favours methotrexate Favours methotrexate exclusion

Figure 2. Forest plot of odds ratios for cardiovascular disease (CVD) events among patients with rheumatoid arthritis (RA) undergoing methotrexate (MTX) therapy at low doses compared with RA patients who received other drugs. MTX at low doses was associated with significantly reduced CVD risk [overall pooled odds ratio =0.732; 95% CI=0.697-0.769; *P*<0.001 (fixed effects meta-analysis)]. Test for heterogeneity: Q=11.64 on 7 degrees of freedom (*P*=0.11); percentage of variability due to between- study variability (I²)=39.8%
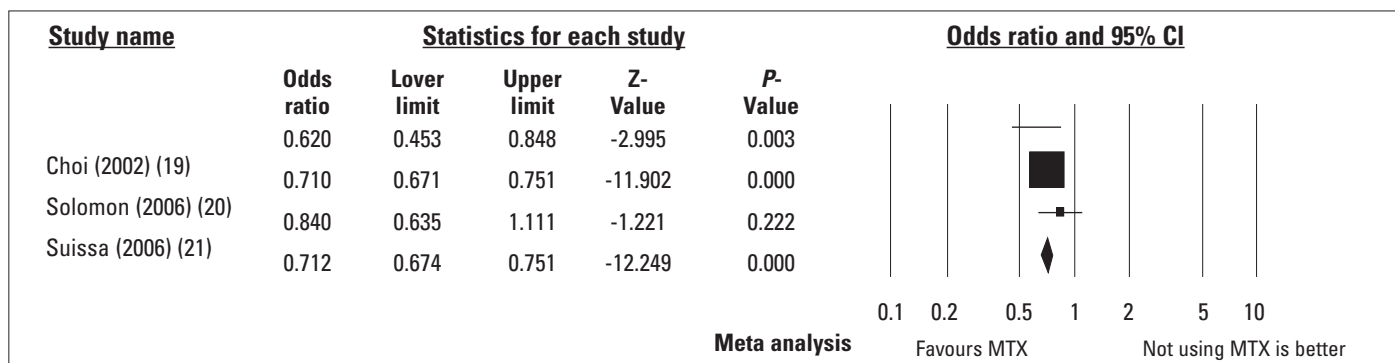
tors for cardiovascular intermediate end-points, including lipid levels and insulin resistance, these have been systematically examined, and the limited current documentation seems to argue for a lack of association between the use of methotrexate and changes in lipid profile or attenuation of insulin resistance (5). Therefore, it could be inferred that methotrexate can potentially reduce cardiovascular risk mainly through its anti- inflammatory effects, without acting on other traditional cardiovascular risk factors.

It is important to note that, in order to avoid a possible bias by indication, it would have been appropriate to include the level of clinical severity of the underlying disease, however only four studies (19, 22-24) provided for this covariate (Fig. 3). Similarly, adjustment for RA-specific drugs other than methotrexate was made in only three studies (Fig. 4) (19-21).

In spite of these methodological limitations, which have lowered the quality of some studies included in the meta-analysis (Table 1), the findings in each of the performed sensitivity analy-

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Study name	Odds ratio	Lover limit	Upper limit	Z-Value	P-Value
Choi (2002) (19)	0.620	0.453	0.848	-2.995	0.003
van Halm (2006) (22)	0.470	0.069	3.193	-0.772	0.440
Nadareishvili (2008) (23)	0.630	0.317	1.250	-1.321	0.186
Wolfe(2008)(24)	1.000	0.770	1.299	0.000	1.000
	0.800	0.660	0.969	-2.282	0.022

Figure 3. Forest plot of odds ratios for cardiovascular disease events among patients with rheumatoid arthritis (RA) who received methotrexate (MTX) compared with those who did not, within studies that adjusted for RA clinical severity. MTX use was found associated with significantly reduced cardio- vascular risk [overall pooled odds ratio =0.80; 95% CI=0.66-0.97; *P*=0.022 (fixed effects meta-analysis)]. Test for heterogeneity: Q=6.1 on 3 degrees of freedom (*P*=0.107); percentage of variability due to between-study variability (I^2) =30%

Study name	Odds ratio	Lover limit	Upper limit	Z-Value	P-Value
	0.620	0.453	0.848	-2.995	0.003
Choi (2002) (19)	0.710	0.671	0.751	-11.902	0.000
Solomon (2006) (20)	0.840	0.635	1.111	-1.221	0.222
Suissa (2006) (21)	0.712	0.674	0.751	-12.249	0.000

Figure 4. Forest plot of odds ratios for cardiovascular disease events among patients with rheumatoid arthritis (RA) who received methotrexate (MTX) compared with those who did not, within studies that adjusted for use of the other RA- specific drugs. MTX use was demonstrated to predict significant decrease in cardiovascular disease events [overall pooled odds ratio =0.71; 95% CI=0.67-0.75; *P*<0.001 (fixed effects model)]. Test for heterogeneity: Q=2.1 on 2 degrees of freedom (*P*=0.35); percentage of variability due to between-study variability (I^2)=4.8%

ses were consistent and similar to the overall pooled estimate (Fig. 5).

This would mean that the association that we detected, the significant inverse association between the use of methotrexate in systemic inflammation and the occurrence of CV disease events, was so robust as to remain unchanged under any scenario, irrespective of the removed study.

In addition, in the studies in which a more effective removal of the confounding was achieved, methotrexate's favourable effects on cardiovascular outcomes were also consistently maintained by taking into account the underlying disease severity (Fig. 3) as well as the possible exposure to other drugs modifying the disease status (Fig. 4).

As already said, in all except one study, the underlying disease was rheumatoid arthritis (RA). In this regard, it is important to emphasize that the range of methotrexate dose is relatively narrower for RA treatment (i.e. 10-25 mg per week), when compared with the very high doses that can be used for cancer treatment. Since all studies were observational, residual confounding by unknown or poorly measured confounders cannot be ruled out.
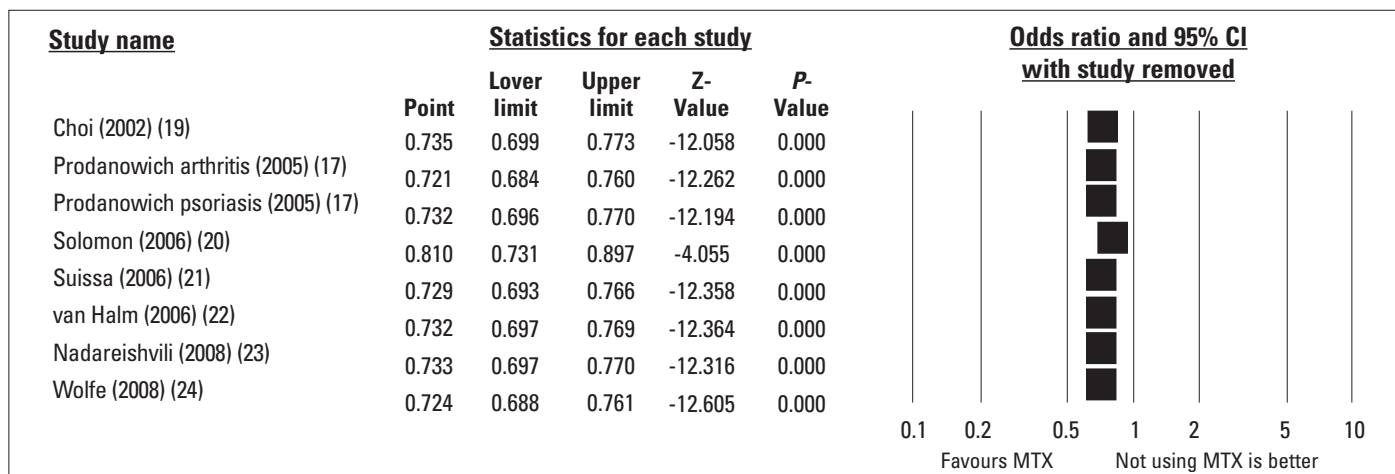
Moreover, most studies did not adjust for folate use (Table 1), which could protect against harm from, and side effects of, methotrexate use, and several studies did not adjust for underlying disease severity. According to some authors (27), this should be emphasized because the choice of omitting these covariates generally causes an underestimation of protective associations.

Furthermore, the majority of the studies did not adjust for steroid use, which 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 can also be included among the possible mechanisms of methotrexate's favourable cardiovascular effects in RA patients. According to this interpretation, the beneficial effect on cardiovascular outcomes observed for methotrexate in patients with RA may simply be an epiphenomenon of the effects of savings on the dosing of glucocorticoids which can be obtained with the inclusion of methotrexate into the therapeutic scheme. In other words, methotrexate could act simply as a confounder in the relationship between the chronic use of glucocorticoids (exposure variable) and the endpoint "cardiovascular disease events "(outcome variable).

In line with this interpretation, the alleged cardiovascular protective effects of methotrexate would be evident in patients with RA simply because in this disease the harmful effects on the cardiovascular system due to other classes of drugs administered as an alternative to methotrexate (glucocorticoids, COXIBs) may play an important role.

In fact, it is plausible that in the absence of methotrexate or other immunosuppressive agents capable of acting as disease modifying anti-rheumatic drugs (DMARDs) for suppressing the

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Study name	Statistics for each study					Odds ratio and 95% CI with study removed
	Point	Lover limit	Upper limit	Z-Value	P-Value	
Choi (2002) (19)	0.735	0.699	0.773	-12.058	0.000	
Prodanowich arthritis (2005) (17)	0.721	0.684	0.760	-12.262	0.000	
Prodanowich psoriasis (2005) (17)	0.732	0.696	0.770	-12.194	0.000	
Solomon (2006) (20)	0.810	0.731	0.897	-4.055	0.000	
Suissa (2006) (21)	0.729	0.693	0.766	-12.358	0.000	
van Halm (2006) (22)	0.732	0.697	0.769	-12.364	0.000	
Nadareishvili (2008) (23)	0.733	0.697	0.770	-12.316	0.000	
Wolfe (2008) (24)	0.724	0.688	0.761	-12.605	0.000	

0.1   0.2   0.5   1   2   5   10
Favours MTX   Not using MTX is better

Figure 5. Exclusion sensitivity plot concerning the forest plot of odds ratios for cardiovascular disease events shown in Fig. 2. The exclusion sensitivity plot represents the hypothetical values that the pooled odds ratio (OR) would assume after removal in turn of each of the eight studies previously incorporated in the meta-analysis. In the exclusion sensitivity plot, each of the eight black squares represents the value of the overall OR arising from a pooled analysis of only seven of the eight studies originally included in the meta-analysis. Note that the point estimates for cardiovascular disease events were stable through the entire range of assumptions, i.e. the statistical significance of the association between methotrexate use and decreased risk of major cardiovascular events in patients with rheumatoid arthritis or psoriasis was kept unchanged under any scenario

Table 2. Sensitivity analysis of the effect of methotrexate therapy on the cardiovascular disease events* in rheumatoid arthritis patients (7 studies) or patients with psoriasis (one study)

Study removed	Studies, no.	Patients evaluated, no.	Major cardiovascular events, OR	P
Choi et al. (19)	7	29629	0.735	<0.001
Prodanowich arthritis et al. (17)	7	24162	0.721	<0.001
Prodanowich psoriasis et al. (17)	7	23254	0.732	<0.001
Solomon et al. (20)	7	25919	0.810	<0.001
Suissa et al. (21)	7	25751	0.729	<0.001
Van Halm et al. (22)	7	30256	0.732	<0.001
Nadareishvili et al. (23)	7	30037	0.733	<0.001
Wolfe et al. (24)	7	26895	0.724	<0.001

*cardiovascular disease events: this term refers to the occurrence of one or more of the following cardiovascular events: new onset angina, acute coronary syndrome (including unstable angina or acute myocardial infarction), need for mechanical or surgical coronary revascularization, stroke, and cardiovascular death

inflammatory and disfiguring process of rheumatoid arthritis, the use of symptomatic drugs such as glucocorticoids, NSAIDs and/or COXIBs is inevitably more frequent with a more probable need for relatively high doses. This results in a higher probability of major adverse cardiovascular events propitiated or triggered by the chronic use of these molecules, whose cardiovascular safety profile is less favourable than that of the methotrexate (10-12).

According to this view, in the case of patients with rheumatoid or psoriatic arthritis who are assigned to background therapy with methotrexate, the putative protective role of methotrexate would simply derive from the reduced consumption of other drugs exerting toxic effects on the cardiovascular system. At

this point the choice of planning a randomized controlled trial to determine whether a causal relationship exists between the use of methotrexate and the reduction of cardiovascular events could be highly appropriate.

Such a trial would hopefully be implemented in a context of already diagnosed ischemic heart disease, with methotrexate administered in addition to other drugs already used for the secondary prevention of ischemic cardiac events among patients free from rheumatoid arthritis or other systemic inflammatory disease. In such a scenario, it would of course be necessary to take care to avoid the simultaneous use of steroids as well as COXIBs or other agents suspected of exerting an unfavourable influence on cardiovascular outcomes.

In fact, such a trial, called CIRT (Cardiovascular Inflammation Reduction Trial) has already been begun by Ridker et al. (28), in 2013, and the results are expected in a few years (29). Nevertheless, further experimental verification of the potential beneficial cardiovascular properties of low-dose methotrexate may be appropriate, even by extending it to other centres in Europe and Asia or by preparing further trials of this type.

Study limitations

Because all of the studies comprised in the meta-analysis were observational, residual confounding by poorly measured or unknown confounders cannot be excluded. The association of methotrexate with a reduced risk of cardiovascular events may have been underestimated in studies that did not include the indices of RA clinical severity among the covariates of their logistic regression models. It is also important to remember that the information value of a meta-analysis depends on the quality of the studies incorporated in it. Indeed, among the patients with only slight inflammatory activity, the protective value of methotrexate might be not evident for the low expected number of events in such a cohort of patients. Moreover, in patients with

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severe RA persisting for many years, methotrexate may not be associated with statistical evidence of cardiac protection because it is very likely that these patients are treated with a polypharmacy with methotrexate in combination with steroids, NSAIDs or COXIBs, the latter being able to invalidate the cardiovascular protective effect of methotrexate. Thus the combined use of methotrexate with other drugs should have been inserted between the covariates of the respective regression models, but this was done in only three studies.

Conclusion

Methotrexate use is associated with a lower risk of CVD among patients with chronic inflammation, such as those suffering from rheumatoid arthritis. These findings suggest that a direct treatment of inflammation using suitable drugs may reduce CVD risk. The choice of planning a randomized controlled trial to establish whether any causality relationship exists between methotrexate use and reduction in cardiovascular events even among patients free from rheumatoid arthritis and/or concurrent steroid or COXIB therapy appears warranted as a new desirable research direction in the cardiovascular field.

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References

1. Haverkate F, Thompson SG, Pyke SD, Gallimore JR, Pepys MB. Production of C-reactive protein and risk of coronary events in stable and unstable angina. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. Lancet 1997; 349: 462-6.
2. Biasucci LM. C - reactive protein and secondary prevention of coronary events. Clin Chim Acta 2001; 311: 49-52.
3. Li JJ, Jiang H, Huang CX, Fang CH, Tang QZ, Xia H, et al. Elevated level of plasma C-reactive protein in patients with unstable angina: its relations with coronary stenosis and lipid profile. Angiology 2002; 53: 265-72.
4. Ursella S, Mazzone M, Portale G, Testa A, Pignataro G, Covino M, et al. How to use the C-reactive protein in cardiac disease? Minerva Cardioangiol 2005; 53: 59-68.
5. Ridker PM. Targeting inflammatory pathways for the treatment of cardiovascular disease. Eur Heart J 2014; 35: 540-3.
6. Rosenson RS, Tangney CC. Antiatherothrombotic properties of statins: implications for cardiovascular event reduction. JAMA 1998; 279: 1643-50.
7. Koh KK. Effects of statins on vascular wall: vasomotor function, inflammation, and plaque stability. Cardiovasc Res 2000; 47: 648-57.
8. Hennekens CH, Dyken ML, Fuster V. Aspirin as a therapeutic agent in cardiovascular disease: a statement for healthcare professionals from the American Heart Association. Circulation 1997; 96: 2751-3.
9. Elezi S, Dibra A, Schömig A, Kastrati A. Current drug-eluting stents in complex patients and lesions. Minerva Cardioangiol 2006; 54: 5-22.
10. Kearney PM, Baigent C, Godwin J, Halls H, Emberson JR, Patrono C. Do selective cyclo-oxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomized trials. BMJ 2006; 332: 1302-8.
11. Nashel DJ.Is atherosclerosis a complication of long-term corticosteroid treatment? Am J Med 1986; 80: 925-9.
12. Frostegård J. Atherosclerosis in patients with autoimmune disorders. Arterioscler Thromb Vasc Biol 2005; 25: 1776-85.
13. Bulgarelli A, Martins Dias AA, Caramelli B, Maranhão RC. Treatment with methotrexate inhibits atherogenesis in cholesterol-fed rabbits. J Cardiovasc Pharmacol 2012; 59: 308-14.
14. Cronstein BN. Low-dose methotrexate: a mainstay in the treatment of rheumatoid arthritis. Pharmacol Rev 2005; 57: 163-72.
15. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ 2009; 339: b2535.
16. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010; 25: 603-5.
17. Prodanovich S, Ma F, Taylor JR, Pezon C, Fasihi T, Kirsner RS. Methotrexate reduces incidence of vascular diseases in veterans with psoriasis or rheumatoid arthritis. J Am Acad Dermatol 2005; 52: 262-7.
18. Suissa S. Novel approaches to pharmaco epidemiological study design and statistical analysis. In: Strom B, editor. 4th ed. Pharmaco epidemiology. New York: John Wiley & Sons; 2005. p. 812-29.
19. Choi HK, Hernán MA, Seeger JD, Robins JM, Wolfe F. Methotrexate and mortality in patients with rheumatoid arthritis: a prospective study. Lancet 2002; 359: 1173-7.
20. Solomon DH, Avorn J, Katz JN, Weinblatt ME, Setoguchi S, Levin R, et al. Immunosuppressive medications and hospitalization for cardiovascular events in patients with rheumatoid arthritis. Arthritis Rheum 2006; 54: 3790-8.
21. Suissa S, Bernatsky S, Hudson M. Antirheumatic drug use and the risk of acute myocardial infarction. Arthritis Rheum 2006; 55: 531-6.
22. van Halm VP, Nurmohamed MT, Twisk JW, Dijkmans BA, Voskuyl AE. Disease-modifying antirheumatic drugs are associated with a reduced risk for cardiovascular disease in patients with rheumatoid arthritis: a case control study. Arthritis Res Ther 2006; 8: R151.
23. Nadareishvili Z, Michaud K, Hallenbeck JM, Wolfe F. Cardiovascular, rheumatologic, and pharmacologic predictors of stroke in patients with rheumatoid arthritis: a nested, case-control study. Arthritis Rheum 2008; 59: 1090-6.
24. Wolfe F, Michaud K. The risk of myocardial infarction and pharmacologic and nonpharmacologic myocardial infarction predictors in rheumatoid arthritis: a cohort and nested case-control analysis. Arthritis Rheum 2008; 58: 2612-21.
25. Maradit-Kremers H, Crowson CS, Nicola PJ, Ballman KV, Roger VL, Jacobsen SJ, et al. Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a population-based cohort study. Arthritis Rheum 2005; 52: 402-11.
26. Kaplan MJ. Cardiovascular disease in rheumatoid arthritis. Curr Opin Rheumatol 2006; 18: 289-97.
27. Micha R, Imamura F, Wyler von Ballmoos M, Solomon DH, Hernán MA, Ridker PM, et al. Systematic review and meta-analysis of methotrexate use and risk of cardiovascular disease. Am J Cardiol 2011; 108: 1362-70.
28. Ridker PM. Testing the inflammatory hypothesis of atherothrombosis: scientific rationale for the cardiovascular inflammation reduction trial (CIRT). J ThrombHaemost 2009; 7(Suppl 1): 332-9.
29. Everett BM, Pradhan AD, Solomon DH, Paynter N, Macfadyen J, Zaharris E, et al. Rationale and design of the Cardiovascular Inflammation Reduction Trial: a test of the inflammatory hypothesis of atherothrombosis. Am Heart J 2013; 166: 199-207.e15.