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OBSERVATIONAL RESEARCH

Methotrexate associates with ischemic cardiovascular risk reduction in males but not females: a transatlantic cardiovascular consortium for people with rheumatoid arthritis observational study

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Abstract

Objective Patients with rheumatoid arthritis (RA) experience higher cardiovascular risk. Methotrexate may decrease this risk, although it is unclear whether males and females similarly benefit. We explored the influence of sex on the effect of methotrexate use on cardiovascular risk in RA.

Methods An observational cohort of 4362 patients, 3223 (73.9%) females, without cardiovascular disease were included from an international cardiovascular consortium for RA. Outcomes were (a) major adverse cardiovascular events (MACE) including cardiovascular death, myocardial infarction, or stroke and (b) any ischemic cardiovascular events (iCVE) including MACE, angina, revascularization, transient ischemic attack, and peripheral arterial disease. The effects of sex, prevalent methotrexate use at enrollment visit and their interaction on MACE and iCVE were assessed with multivariable Cox regression models, reporting adjusted hazard ratios (HRs) and 95% confidence intervals (CIs).

Results There were 237 first MACE and 358 first iCVE. The sex by methotrexate interaction was significant for MACE ($p=0.005$) and iCVE ($p=0.006$), suggesting the effect of methotrexate use on cardiovascular risk differed among males and females. In males, methotrexate use associated with lower risk of MACE (HR 0.32, [95% CI 0.12–0.83]) and iCVE (HR 0.43 [95% CI 0.21–0.85]). In females, methotrexate use was not associated with MACE ($p=0.267$) or iCVE ($p=0.407$). In sensitivity analyses, models with inverse probability of treatment weighting and models additionally adjusting for inflammation yielded similar results.

Conclusion Methotrexate use associated with cardiovascular benefit in males but not females with RA and the effect was independent of inflammation.

Keywords Rheumatoid arthritis · Cardiovascular disease · Methotrexate · Sex

Introduction

Patients with rheumatoid arthritis (RA) exhibit greater atherosclerosis burden and cardiovascular risk compared to age and gender-matched individuals without autoimmune disease [1, 2]. Residual RA activity and cumulative inflammation predict plaque progression and cardiovascular risk [3, 4]. In contrast, suppression of disease activity and systemic inflammation attenuates both [3, 5–7]. Methotrexate is the main conventional synthetic disease modifying antirheumatic drug (DMARD) endorsed by guidelines for initial treatment in RA [8]. It decreases proinflammatory cytokines instrumental in disease pathogenesis [9] and was shown to decrease cardiovascular risk [10]. This effect may be dose-dependent, with higher doses yielding greater benefit [11]. Moreover, the beneficial effects of methotrexate on

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cardiovascular risk may involve pathways beyond control of systemic inflammation [9, 12].

Methotrexate treatment reduced atherosclerosis in animals [13–15] and inhibited transformation of human macrophages to foam cells—the main cell population in atherosclerotic plaques [16]. Several mechanisms may underlie the beneficial effects of methotrexate, including adenosine production. Adenosine promotes cholesterol efflux by engaging adenosine A2 receptor on macrophages, which upregulates synthesis of ATP-binding cassette A1-membrane transporter protein (ABCA1) that exports cholesterol to apolipoprotein A1 (ApoA1) and lipid-poor pre-β HDL particles [17, 18]. Lipid removal from lesional macrophages attenuates atherosclerosis progression and was shown to reduce cardiovascular risk [19]. Adenosine may also bind adenosine A3 receptor on endothelial cells within visceral adipose tissue and promote microRNA-181b synthesis, a potent anti-inflammatory factor which attenuates endothelial cell activation, decreases macrophage accumulation and inflammation, and increases insulin signal activity within visceral fat [20]. Both endothelial cell activation and visceral fat inflammation have been linked to cardiometabolic risk in RA [6, 21].

Prior studies showed that males with RA have a better response to methotrexate than females [22, 23] and better response to methotrexate is linked to reduced mortality [24, 25]. Testosterone enhanced the in vitro anti-inflammatory and antiproliferative effects of methotrexate [26]. In psoriasis, another autoimmune disease with enhanced ischemic cardiovascular risk, methotrexate treatment reduced erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and proatherogenic apoB/apoA1 ratio exclusively in males [27, 28]. We previously reported that males with RA experienced higher cardiovascular risk compared to females [29]. However, it is unknown whether males and females derive similar cardioprotective benefit from methotrexate treatment.

In the present study we evaluated the influence of sex on major adverse cardiovascular events (MACE) and all ischemic cardiovascular events in methotrexate users and nonusers. We further explored potential differences in cardioprotective benefits from methotrexate among males and females with RA.

Methods

Patient recruitment

The sample included RA patients from 13 academic centers in 10 countries (Canada, Greece, Mexico, Netherlands, Norway, South Africa, Spain, Sweden, United Kingdom, and United States) enrolled into An inTernationAl Cardiovascular Consortium for people with Rheumatoid Arthritis (ATACC-RA) between 1985 and 2012. Details of the sample have been previously reported [30]. Inclusion criteria upon enrollment entailed patient ages between 18 and 85 years and fulfillment of 1987 classification criteria for RA. Exclusion criteria were presence of concurrent autoimmune syndromes (except Sjogren's), and prior diagnosis or suspicion of cardiovascular disease including stable angina, acute coronary syndrome, transient ischemic attack, stroke, peripheral arterial disease, revascularization, or heart failure. Participants were followed either prospectively through regular study visits or retrospectively through chart review. The study was approved by the local institutional review boards of all participating centers and complied with the declaration of Helsinki. Of 4537 enrolled participants, individuals were excluded from the analysis for the following reasons: missing methotrexate data ($n=54$), over 85 years old ($n=25$), and missing all RA disease activity data ($n=96$). The final sample included 4362 individuals.

Covariates and outcomes

Cardiac risk factors recorded at the enrollment visit included hypertension, systolic and diastolic blood pressure, diabetes, family history of coronary artery disease, smoking status, body mass index, total cholesterol, HDL-c, low-density lipoprotein cholesterol, and triglycerides. Antihypertensive and lipid-lowering medication use at the time of enrollment was also documented. Rheumatoid factor (RF) and anticitrullinated peptide antibody (ACPA) status was extracted from the patients' medical record. Tender and swollen joint counts, ESR and CRP levels, and 28-joint disease activity scores with ESR (DAS28-ESR) and with CRP (DAS28-CRP) at enrollment visit were recorded. Information on use of non-steroidal anti-inflammatory drugs, corticosteroids, conventional synthetic DMARDs (csDMARDs), and biologic DMARDs (bDMARDs) at the time of enrollment was also collected, however no information on dosing was catalogued. Management strategies were at the discretion of the treating physician. Data on all predictors and covariates were only available at baseline.

Prespecified clinical outcomes were (a) time to first major adverse cardiovascular event (MACE) defined as cardiovascular death, non-fatal myocardial infarction, or non-fatal stroke; and (b) time to first any ischemic cardiovascular event (iCVE) defined as cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, unstable or stable angina, coronary revascularization, transient ischemic attack, or symptomatic peripheral arterial disease with or without revascularization. Standardized definitions were used during data collection, however cardiovascular events

were not centrally adjudicated. Patients incurring both MACE and non-MACE iCVE events were considered in the MACE analysis if the MACE event occurred first and censored thereafter. If the non-MACE iCVE event occurred first, then the same patients were censored after the index event and considered in the iCVE analysis.

Methotrexate exposure

Patients taking methotrexate upon enrollment were considered prevalent methotrexate users. Information about methotrexate use or duration before and after enrollment visit was not available. Patients were classified as methotrexate users if they received methotrexate monotherapy or in combination with other csDMARDs and/or bDMARDs at enrollment. Patients not taking methotrexate at enrollment visit were considered nonusers, even in the case of potential prior exposure. Similar assumptions and exposure definitions were used for all medications used upon enrollment.

Statistical analysis

Participant characteristics were summarized overall and by sex as means with standard deviations (SD) for continuous variables and numbers with percentages for categorical variables. Males and females were compared using the chi-square test and t test for categorical and continuous variables, respectively. Non-normally distributed variables were natural logarithm transformed. The primary analyses evaluated the effect of sex, methotrexate use and their interaction on risk of MACE and iCVE using multivariable Cox regression models, reporting adjusted hazard ratios (HRs) and 95% confidence intervals (CIs). The sex by methotrexate use interaction was assessed with likelihood ratio tests. Covariates included in all Cox models were age, sex, hypertension, diabetes, family history of cardiovascular disease, smoking, total cholesterol/HDL-c ratio, and RA duration. Cox models were stratified by center cardiovascular event rate (high and low risk groups), as previously described [29, 30]. For the Cox models, missing data were imputed using multiple imputation by chained equations with 10 iterations.

Four sets of sensitivity analyses assessed the robustness of the primary analyses. First, to balance the differences between methotrexate users and nonusers, the imputed multivariable Cox regression models were weighted using inverse probability of treatment weighting weights. Stabilized weights were calculated using propensity score values. The individual propensities for methotrexate treatment were estimated with a logistic regression model that included all covariates (i.e. age, sex, hypertension, diabetes, family history of cardiovascular disease, smoking, total cholesterol/HDL-c ratio, and RA duration) plus RF or ACPA positivity, non-methotrexate csDMARD use, bDMARD use, and DAS28-ESR. A standardized mean difference <0.10 for each covariate indicated balance between methotrexate user and nonuser groups. Second, the unweighted Cox models were evaluated adjusting only for age. Third, unweighted fully adjusted primary Cox models were tested with sample enrollment limited to after 1/1/2000 when methotrexate use became more prevalent in the cohort. Fourth, we explored if RA disease activity or inflammation influenced the effect of methotrexate use on MACE and iCVE in males and females by evaluating multivariable Cox models additionally adjusting for DAS28-ESR, DAS28-CRP or CRP. Stata 15.0 was used for all analyses and two-tailed P values <0.05 were considered statistically significant.

Results

Patients were predominantly female ($n=3223$, 73.9%), seropositive for RF ($n=2885$, 66.9%) or ACPA ($n=2356$, 60.1%), and with moderate to severe disease activity (DAS28-ESR $=4.2\pm1.5$). At enrollment visit 1378 (31.6%) used methotrexate, 1169 (26.8%) received corticosteroids, and 515 (11.8%) received bDMARDs. Among 1211 methotrexate users with complete medication data, 540 (44.6%) patients received methotrexate monotherapy, 326 (26.9%) combined with additional csDMARDs, 260 (21.5%) combined with bDMARDs, and 85 (7.0%) combined with additional csDMARDs and bDMARDs. Among males, 304 (26.7%) were methotrexate users, while 1074 females (33.3%) used methotrexate. Patient characteristics by sex and methotrexate use and information about missing data are shown in Table 1. Compared to male nonusers, male methotrexate users had longer disease duration, lower disease activity, and greater concomitant alternate csDMARD, bDMARD, and steroid use (all $p<0.001$). Compared to female nonusers, females using methotrexate had older average age ($p<0.001$), longer disease duration ($p<0.001$), lower disease activity ($p<0.001$), lower smoking ($p=0.001$) and hypertension prevalence ($p=0.02$), greater diabetes prevalence ($p=0.047$), and greater concomitant alternate csDMARD, bDMARD, steroid, and statin use (all $p<0.001$).

Mean follow-up duration was 6.0 ± 4.5 years. Overall, 358 patients suffered any cardiovascular events. Forty-six patients suffered both MACE and non-MACE iCVE; in 14 MACE was the first event, in 24 non-MACE iCVE was the first event, and in eight the events were concurrent. There were 237 first MACE events (40 cardiovascular deaths, 113 myocardial infarctions, and 84 strokes) over 26,286 patient-years of follow-up. There were 358 first iCVEs (31 cardiovascular deaths, 109 myocardial infarctions, 82 strokes, 54 new unstable or stable angina diagnoses, 23 transient

Table 1 Participant characteristics at enrollment stratified by sex and methotrexate use ($N=4362$)

	Available *n*	Female MTX Nonuser ($n=2149$)	Female MTX User ($n=1074$)	Male MTX Nonuser ($n=835$)	Male MTX User ($n=304$)
Age, years	4362	53.6±14.8	56.2±13	56.3±12.5	57.6±12.8
RA duration, years	4340	3.4±6.6	8.5±8.5	2.1±5.0	8.0±8.1
Age at RA diagnosis, years	4339	50.3±14.8	47.9±13.7	54.2±13	49.8±13.0
ACPA positive	3921	1087 (58.3%)	635 (61.8%)	451 (61.3%)	183 (62.9%)
RF positive	4310	1400 (65.7%)	704 (66.7%)	579 (69.9%)	202 (68.5%)
ESR, mm/hr	4320	27.0±21.6	21.2±18.8	26.3±23.8	17.6±17.9
CRP, mg/L	3349	19.2±29.4	10.7±18.3	26.4±37.5	13.2±18.4
Swollen joint count	3123	7.1±5.9	3.5±4.5	7.7±6.0	3.6±4.5
Tender joint count	3123	6.5±6.2	4.2±5.5	5.9±5.7	3.8±5.3
DAS28-ESR	3106	4.5±1.4	3.6±1.4	4.4±1.5	3.3±1.6
DAS28-CRP	2935	4.1±1.4	3.3±1.3	4.2±1.4	3.3±1.3
Hypertension	4361	933 (43.4%)	419 (39.0%)	399 (47.8%)	119 (39.1%)
Systolic BP, mmHg	4211	138.0±23.7	133.6±20.6	143.4±21.5	140.0±19.2
Diastolic BP, mmHg	4210	79.7±11.3	78.7±10.7	83.7±11.0	82.3±10.0
Total cholesterol, mmol/L	3951	5.2±1.1	5.3±1.1	5.1±1.1	5.0±1.0
LDL-c, mmol/L	3881	3.1±1.0	3.1±0.9	3.1±1.0	3.1±0.9
HDL-c, mmol/L	3900	1.5±0.4	1.6±0.5	1.2±0.3	1.3±0.4
Triglycerides, mmol/L	3933	1.4±0.8	1.3±0.7	1.5±0.9	1.5±0.9
Current smoker	4232	499 (24.2%)	204 (19.0%)	253 (31.8%)	86 (28.5%)
Ever smoker	4232	1006 (48.8%)	488 (45.5%)	555 (69.8%)	223 (73.8%)
Diabetes mellitus	4362	137 (6.4%)	89 (8.3%)	60 (7.2%)	29 (9.5%)
Family history of CVD	3353	360 (23.9%)	247 (24.6%)	130 (22.8%)	80 (28.9%)
Body mass index, kg/m^2	3973	26.9±5.6	27.3±5.5	26.9±4.4	27.5±4.4
Other csDMARD	4196	422 (19.6%)	322 (33.9%)	112 (13.4%)	89 (33.8%)
Biologic DMARD	4355	125 (5.8%)	304 (28.4%)	26 (3.1%)	60 (19.7%)
Corticosteroid	4359	497 (23.1%)	390 (36.3%)	176 (21.1%)	106 (34.9%)
Antihypertensive therapy	4360	439 (20.4%)	268 (25.0%)	159 (19.0%)	69 (22.7%)
Lipid-lowering therapy	4356	182 (8.5%)	164 (15.3%)	77 (9.2%)	40 (13.2%)

Values in table are mean±standard deviation or n (%). MTX: methotrexate; RA: rheumatoid arthritis; ACPA: anti-citrullinated protein antibody; RF: rheumatoid factor; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; DAS28: 28-joint disease activity score; BP: blood pressure; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; CVD: cardiovascular disease; csDMARD: conventional synthetic disease modifying anti-rheumatic drug

ischemic attacks, 32 new peripheral arterial disease diagnoses, and 27 revascularizations) over 26,029 patient-years. Overall crude incidence rate per 1000 patient-years was 9.0 (95% CI 7.9–10.2) for MACE and 13.8 (95% CI 12.4–15.3) for iCVE. Incidence rates were higher in males compared to females for MACE (15.2 [95% CI 12.6–18.4] versus 6.8 [95% CI 5.7-8.0], p for difference < 0.001) and iCVE (21.6 [95% CI 18.4–25.3] versus 10.9 [95% CI 9.6–12.5], p for difference < 0.001). Among males, crude incidence rates per 1000 patient-years were higher in methotrexate nonusers compared to users for both MACE (17.6 [95% CI 14.5–21.3] versus 4.2 [95% CI 1.7–10.0], p for difference < 0.001) and iCVE (24.5 [95% CI 20.7–28.9] versus 7.6 [95% CI 4.0-14.6], p for difference < 0.001). Among females, incidence rates were not significantly different in methotrexate nonusers compared to users for MACE (6.9 [95% CI 5.7–8.4] versus 6.4 [95% CI 4.4–9.2], p for difference = 0.72) or iCVE (11.4 [95% CI 9.8–13.3] versus 9.4 [95% CI 7.0-12.8], p for difference = 0.28). The cumulative incidence of MACE and iCVE differed according to methotrexate use stratified by sex (Fig. 1).

In multivariable Cox models, male sex predicted greater overall risk of MACE (HR 1.75 [95% CI 1.35–2.27]) and any iCVE (HR 1.53 [95% CI 1.24–1.89]). Methotrexate use was not associated with MACE ($p=0.57$) or iCVE ($p=0.50$) overall (Fig. 2). The interaction between sex and methotrexate was significant for MACE ($p=0.005$) and iCVE ($p=0.01$), suggesting the effect of methotrexate use on cardiovascular risk differed among males and females (Fig. 2). Specifically, among males, methotrexate use associated with a lower risk of MACE (HR 0.32, [95% CI 0.12–0.83]) and iCVE (HR 0.43 [95% CI 0.21–0.85]]). In females, methotrexate use was not associated with either MACE ($p=0.267$) or iCVE ($p=0.41$). When the effect of sex is considered stratified by methotrexate use, male nonusers incurred greater risk of both MACE (HR 2.03 [95%

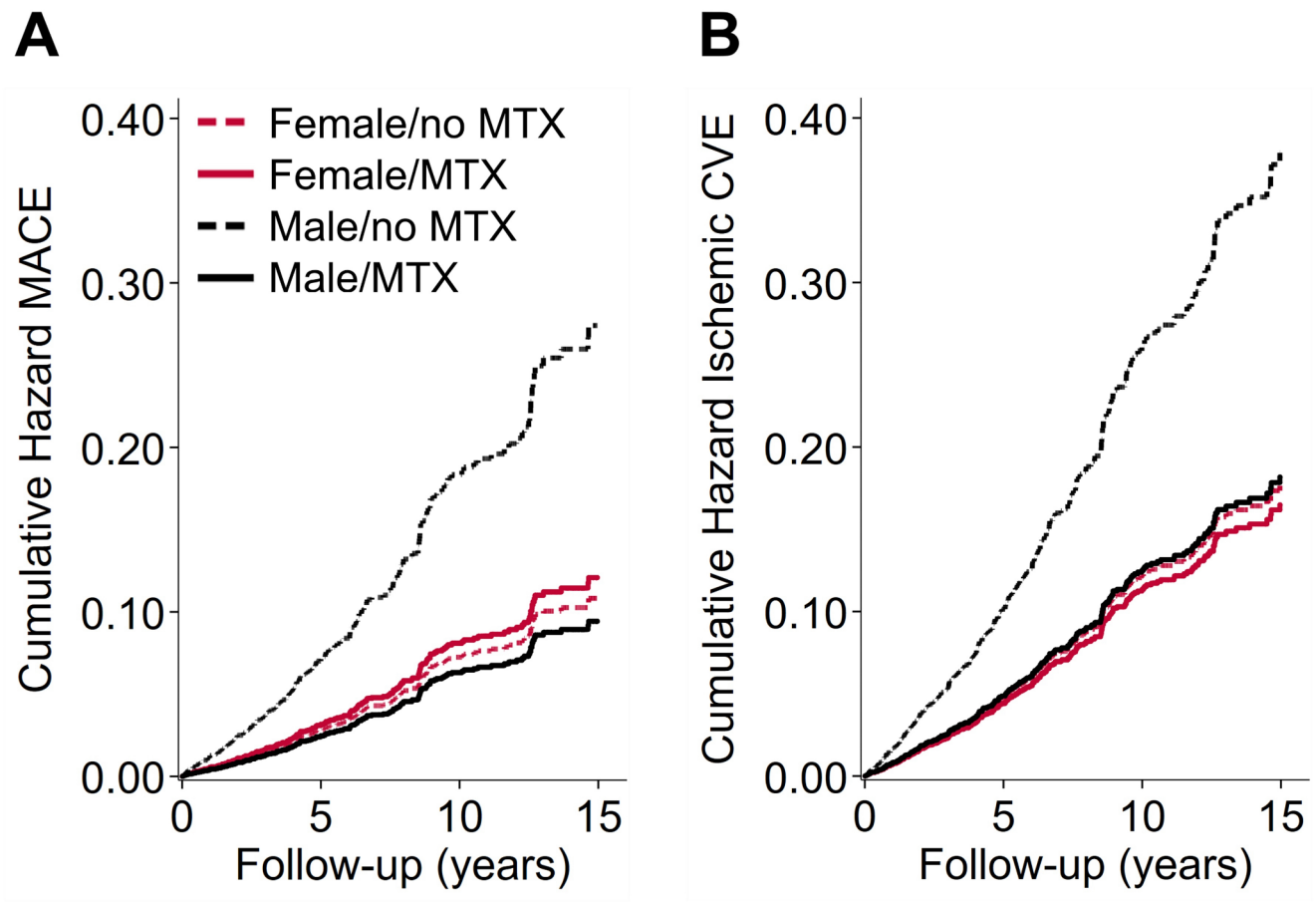

Fig. 1 Cumulative hazard of **A** MACE and **B** any ischemic CVE stratified by methotrexate use and sex. *MTX* methotrexate; *MACE* major adverse cardiovascular event; *CVE* cardiovascular event

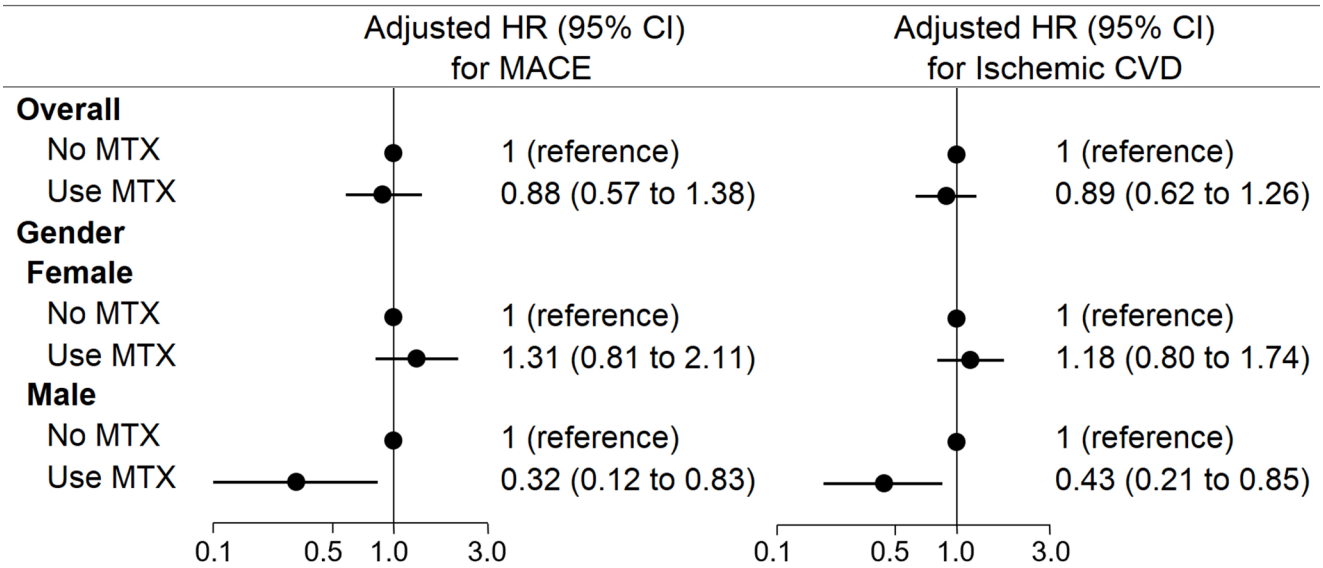

Fig. 2 Effect of methotrexate use on cardiovascular events overall and stratified by sex. All models adjust for age, hypertension, diabetes, family history of cardiovascular disease, smoking, total cholesterol/high-density lipoprotein cholesterol ratio, and rheumatoid arthritis duration. *HR* hazard ratio; *MACE* major adverse cardiovascular event; *CVE* cardiovascular event; *MTX* methotrexate

Table 2 Sensitivity analyses for the effect of methotrexate use on cardiovascular event risk in males and females

	MACE		Any Ischemic CVE	
	HR (95% CI)	P-int.	HR (95% CI)	P-int.
Inverse probability weighting[a]				
Female	1.23 (0.71–2.13)	0.02	1.14 (0.73–1.80)	0.01
Male	0.30 (0.10–0.90)		0.41 (0.20–0.85)	
Adjusted for age only				
Female	1.26 (0.79–2.02)	0.01	1.16 (0.79–1.69)	0.01
Male	0.34 (0.13–0.88)		0.44 (0.22–0.91)	
Follow-up after 01/01/2000[a]				
Female	1.29 (0.76–2.17)	0.02	1.16 (0.77–1.76)	0.02
Male	0.36 (0.13–0.96)		0.45 (0.22–0.92)	
Adjusted model[a] +DAS-ESR				
Female	1.33 (0.83–2.14)	0.01	1.21 (0.81–1.79)	0.01
Male	0.34 (0.13–0.88)		0.45 (0.23–0.90)	
Adjusted model[a] +DAS-CRP				
Female	1.34 (0.83–2.15)	0.01	1.21 (0.82–1.79)	0.01
Male	0.34 (0.13–0.87)		0.45 (0.23–0.89)	
Adjusted model[a] + CRP				
Female	1.34 (0.83–2.16)	0.01	1.20 (0.81–1.77)	0.01
Male	0.33 (0.13–0.85)		0.43 (0.22–0.86)	

[a]Adjusted for age, hypertension, diabetes, family history of cardiovascular disease, smoking, total cholesterol/high-density lipoprotein cholesterol ratio, and rheumatoid arthritis duration

MACE: major adverse cardiovascular event; CVE: cardiovascular event; HR: hazard ratio for the effect of methotrexate use; CI: confidence interval; P-int.: *P* value for sex by methotrexate interaction; DAS28: 28-joint disease activity score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein

CI 1.54–2.68]) and iCVE (HR 1.70 [95% CI 1.35–2.13]) compared to female nonusers. In contrast, male sex did not predict MACE (HR 0.50 [95% CI 0.19–1.30]) or iCVE risk (HR 0.61 [95% CI 0.30–1.23]) among methotrexate users.

In sensitivity analyses, results from age-adjusted only models, fully adjusted models limited to follow-up after January 1, 2000, and adjusted models weighted using inverse probability of treatment weights were similar to the primary models (Table 2). Unweighted multivariable models additionally adjusting for DAS28-ESR, DAS28-CRP and CRP also yielded similar results (Table 2).

Discussion

A number of metanalyses of widely overlapping studies examined the association of methotrexate use with various composite cardiovascular outcomes over the years [10, 31–34]. All except one [10] were published prior to 2015 and included reports on populations studied prior to 2005. The individual studies considered included variable predictors, covariates and limited adjustments for concurrent therapies. The overarching message was that methotrexate was overall beneficial, especially when co-administered along with other DMARDs and the observed benefit was primarily driven by the same three studies recirculated across all reported metanalyses [24, 35, 36]. Despite prior reports that males treated with methotrexate experience better responses than females [22, 23], none of the aforementioned studies evaluated potential differences in cardiovascular benefits of methotrexate use between males and females. Moreover, none explicitly assessed whether the effect of methotrexate on cardiovascular risk was mediated by control of inflammation.

Our study specifically examined the influence of sex on cardiovascular risk in methotrexate users and nonusers and the potential differences in benefits from methotrexate among males and females with RA. Consistent with our prior report, males experienced greater overall event rates compared to females independently of classical risk factors or disease activity [37]. Methotrexate use was not associated with cardiovascular risk in our cohort at large. This finding differs from some of the aforementioned studies but is in agreement with three studies reporting on methotrexate monotherapy, included in the latest metanalysis, where no cardiovascular benefit was observed [10]. Importantly, our finding was robust and confirmed in inverse probability weighted sensitivity analyses accounting for the use of both non-methotrexate csDMARDs as well as bDMARDs and therefore reflecting the specific contribution of methotrexate use.

We showed that methotrexate was linked to MACE and iCVE risk reduction among males but not females. Similarly, in a recent report, methotrexate associated with reduction

in a composite cardiovascular outcome including ischemic events and heart failure in a large, overwhelmingly male (90%) RA population [12]. Since methotrexate was shown to suppress proinflammatory cytokines [9], we explored whether adjusting for disease activity or systemic inflammation influenced the effect of methotrexate on cardiovascular risk in males. We observed that additionally adjusting for the effects of DAS28-ESR, DAS28-CRP or CRP only minimally impacted model estimates. This finding suggested that reduction in cardiovascular risk associated with methotrexate was not simply explained by suppression of inflammation, consistently with recent reports [9, 12].

The question then arises as to how might methotrexate use associate with cardiovascular benefits exclusively in males and independently of inflammation. Methotrexate was shown to promote extracellular adenosine release which upon binding adenosine A2 receptors on macrophages upregulates expression of ABCA1 membrane transporter, in turn facilitating cholesterol efflux to ApoA1 or pre-b HDL particles [17, 18]. Cholesterol efflux via the ABCA1 transporter is 31% higher in males versus females matched for age, body mass index, HDL, ApoA1, and triglycerides [38]. This was likely because males displayed 2.4 times higher preb-HDL concentration than females, serving as main acceptors—along with ApoA1—for cholesterol efflux through the ABCA1 transporter [38]. Consequently, a methotrexate-mediated amplification in ABCA1 expression may disproportionately promote cholesterol efflux through this transporter/ acceptor system in lesional macrophages of males and attenuate both atherosclerosis progression and cardiovascular risk.

Secondly, males with RA displayed greater expansion of visceral adipose tissue compared to females [21, 39] and this was linked to metabolic syndrome [21] and cardiovascular risk in RA [6, 21, 40]. Notably, the prevalence of metabolic syndrome varied among males and females stratified by methotrexate use in our cohort in models adjusted for age, disease activity, prednisone, bDMARD and hydroxychloroquine use (p-for-interaction = 0.015). Among baseline methotrexate users, metabolic syndrome was more prevalent in males than females and this was not the case among nonusers. Hence, methotrexate treatment over time may yield relatively greater anti-inflammatory microRNA-181b output in male visceral fat and its vascular endothelium, a stronger increase in insulin sensitivity, and therefore preferentially mitigate metabolic syndrome and cardiovascular risk in males [20].

Earlier studies suggested that androgens may potentiate the anti-inflammatory properties of methotrexate [26] and sex-specific pharmacogenetic effects on methotrexate response were described in RA [41]. Indeed, our male methotrexate users exhibited lower disease activity and inflammation (ESR) compared to female users (both $p < 0.001$). In fact, differences in both disease activity and systemic inflammation between males and females were greater among methotrexate users than nonusers. Likewise, suppression of ESR, CRP and apoB/apoA1 ratio was seen only in males but not females with psoriasis upon treatment with methotrexate [27, 28].

Our female methotrexate nonusers—like all females—experienced lower cardiovascular risk compared to male nonusers. This may reflect an atheroprotective benefit of estrogens in females. HDL-associated estradiol esters, especially in premenopausal females, enhanced the ability of HDL to perform cholesterol efflux compared to male HDL lacking estradiol [42]. Indeed, data from a US participating cohort indicated that females displayed significantly greater macrophage cholesterol efflux through the ABCG1 membrane transporter than males, independently of methotrexate use [43]. Moreover, ABCG1-mediated cholesterol efflux associated with endogenous estrone levels in premenopausal but not postmenopausal patients (p-for-interaction = 0.032) and inversely associated with cardiovascular risk [43].

Neointimal expression of estrogen receptors is higher in female than male aortas. Moreover, concentrations of enzymes locally converting estrogens into bioactive forms are significantly higher in female than male aortas, irrespective of menopause [44]. These conditions may therefore optimize the local availability, biologic activity and receptor burden estrogens can engage and exert their atheroprotective effects specifically in females. Accordingly, data from the aforementioned US participating center indicated that endogenous estrone levels inversely associated with event risk after adjustments for atherosclerotic cardiovascular risk score and coronary atherosclerosis burden [45]. Moreover, this inverse association was stronger in premenopausal patients [45]. In summary, in the context of an estrogen-mediated optimization of HDL efflux capacity through ABCG1 transporter, lower preb HDL and Apo A1 acceptor particle levels, and enhanced action of estrogens on the vessel wall, the relative contribution of a tentative methotrexate-associated upregulation of ABCA1 expression in females is likely limited.

Our study has notable strengths. It is a large, multinational, real-life, long-term, observational study carried out in an unselected, ethnically diverse sample representative of clinical practice and therefore conceptually poised to yield results with greater external validity and generalizability. However, several limitations should be acknowledged. Patients originated in referral and academic centers with a special interest in RA-associated cardiovascular comorbidities; this may have led to an earlier recognition and mitigation of traditional risk factors and disease activity therefore introducing referral bias and potentially limiting

the generalizability of our findings. Surveillance methods differed across cohorts; some centers prospectively followed patients and others retrospectively gathered data. Despite the use of standardized definitions, it is still conceivable that there were differences in measurement of cardiovascular risk factors and events. Additionally, events were not centrally adjudicated; rather, they were locally arbitrated at the respective centers and by the treating specialist.

RA-related characteristics, cardiovascular risk factors, and medication use data were available only at enrollment visit. No information on those predictors was available beyond baseline. Despite stratifying our analyses by center, our results may be still confounded by secular and regional guideline variation in RA-specific, comorbidity and preventive therapies among the various centers. Adjustment for posttreatment factors like disease activity may result in overadjustment; however, estimates from both our age only-adjusted and fully-adjusted models were similar, mitigating this concern. Although our models adjusted for a number of RA-related and cardiovascular risk factors, unmeasured confounders or time-varying factors may have influenced the observed effect. In an effort to address potential confounding bias, we reran analyses using inverse probability of treatment weighting and results were similar. Nevertheless, disease activity, which is influenced by methotrexate, was used to calculate inverse probability weights which can contribute to collider stratification bias. Like many observational studies there is also risk of prevalent user bias. However, this bias is most relevant when the risk of an outcome of interest is highest in the early stages of treatment [46, 47]. Literature suggests this is not the case for cardiovascular risk in RA [48, 49]. Lastly, despite rigorous statistical analysis, the observed findings may be affected by differential follow-up or other selection biases that are possible in any observational study. Further research involving a randomized controlled trial would be useful to validate our findings.

In conclusion, methotrexate treatment at baseline associated with lower risk of MACE and iCVE in males but not females with RA and this effect appeared to be independent of inflammation. The precise mechanisms underlying the observed benefits require further study.

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Author contributions Conceptualization: GAK; Methodology: GAK and SRO; Investigation: GAK, PVR, EM, MAGG, AC, SRD, PS, PD, LT, CAH, VPR, ICY, ICP, DGD, JAL, AGS, DPM, GDK, and EMH; Data Curation: GAK and SRO; Formal analysis: GAK and SRO; Visualization: GAK and SRO; Writing– Original Draft: GAK and SRO; Writing– Review & Editing: PVR, EM, MAGG, AC, SRD, PS, PD, LT, CAH, VPR, ICY, ICP, DGD, JAL, AGS, DPM, GDK, and EMH; Supervision: GAK; Project administration: GAK; Funding acquisition: GAK. All authors were involved in building and maintaining the study cohort and read and approved of the final manuscript. All authors take full responsibility for the integrity and accuracy of all aspects of the work.

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Data availability The data that support the findings of this study are not openly accessable due to restrictions on availability and may be obtained from the corresponding author, GAK, upon reasonable request.

Declarations

Ethics approval and consent to participate This study was approved by the institutional review boards of all participating centers and performed in accordance with the 1964 Declaration of Helsinki and its later amendments. These included the Mayo Clinic and Olmsted Medical Center Institutional Review Boards, CMO Arnhem Nijmegen, Dudley Local Research Ethics Committee, Office of the Oslo University Hospital's Privacy and Data Protection Officer, Ethics Committee at the University Hospital of Umeå, John F Wolfe MD. Human Subjects Committee at Harbor-UCLA (project number of 13564-01, approval date 8/26/2009), Laiko Hospital Institutional Review Board (Athens, Greece), Human Research Ethics Committee (Medical) from the University of the Witwatersrand in Johannesburg, South Africa, University of Manitoba Health Research Ethics Board and the Manitoba Health Information Privacy Committee, Ethics Committee of Cantabria for Hospital Universitario de Valdecilla in Santander (Spain), Comites de Ética e Investigación del Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, and Arthritis Center Twente Institutional Review Board.

Consent to participate Informed consent was obtained from participants.

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