SUPPLEMENTARY FILE

Investigating Changes in Disease Activity as a Mediator of Cardiovascular Risk Reduction with Methotrexate use in Rheumatoid Arthritis

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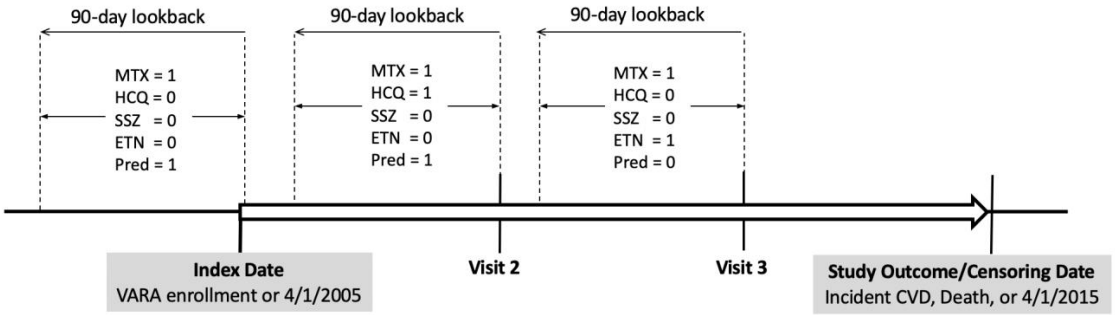

Supplementary Figure 1. Study design for an illustrative RA patient on MTX + prednisone at enrollment, who was subsequently switched to: MTX + HCQ + prednisone prior to visit 2, and MTX + ETN prior to visit 3. Patients are defined as exposed (1) or not exposed (0) at each visit, looking back 90 days (180 days for rituximab) for an active medication course to define exposure.
Abbreviations: CVD = cardiovascular disease, ETN = etanercept, HCQ = hydroxychloroquine, MTX = methotrexate, Pred = prednisone, SSZ = sulfasalazine, VARA = Veterans Affairs Rheumatoid Arthritis registry

Supplementary Table 1. Diagnostic codes used to define comorbid conditions at index date in patients with RA

Condition	ICD-9 Code
Cerebrovascular disease	362.34, 430.x–438.x
Heart failure	398.91, 402.01, 402.11, 402.91, 404.01, 404.03, 404.11, 404.13, 404.91, 404.93, 425.4–425.9, 428.x
Coronary artery disease	410.x-414.x, 429.7, V45.81, V45.82
Diabetes	250.x
Hyperlipidemia	272.0, 272.2-272.4
Hypertension	401.x, 402.x-405.x
Liver disease	070.22, 070.23, 070.32, 070.33, 070.44, 070.54, 070.6, 070.9, 456.0–456.2, 570.x, 571.x, 572.2–572.8, 573.3, 573.4, 573.8, 573.9, V42.7
Renal disease	403.01, 403.11, 403.91, 404.02, 404.03, 404.12, 404.13, 404.92, 404.93, 582.x, 583.0–583.7, 585.x, 586.x, 588.0, V42.0, V45.1, V56.x

Supplementary Table 2. Diagnostic codes used to define non-fatal cardiovascular events in patients with RA

Condition	ICD-9 code	ICD-9 procedure code	ICD-10 procedure code	CPT code
Diagnostic codes queried in both inpatient and outpatient encounters				
CAD	410.x, excluding 410.x2			
PCI		0.66, 36.01-36.09	0270-0273.xxx	92980-92996, 92920-21, 92924-25, 92928-29, 92933-34, 92937-38, 92941, 92943-44, 92973, 92975, 92977
CABG		36.1x	0210-0213.xxx	33510-33536
Stroke	430.x, 431.x, 433.x1, 434.x1, 435.x, 436			
HF	428.x [excluding 428.22, 428.32, 428.42], 402.x1, 404.x1, 415.0			
Diagnostic codes queried in outpatient encounters only				
Stable angina	413.x			
History of CAD	410.x2, 412.x			
Stroke sequelae	438.x			

Abbreviations: CABG = coronary artery bypass graft, CAD = coronary artery disease, HF = congestive heart failure, CPT = Current Procedural Terminology, ICD = International Classification of Disease, PCI = percutaneous coronary intervention, RA = rheumatoid arthritis

Supplementary Appendix. Analytic methods for marginal structural models and mediation analysis

Marginal Structural Models

Marginal structural models were utilized to examine the association of MTX with CVD events, incorporating inverse probability of treatment weights (IPTW) for MTX exposure to account for the influence of time-varying and time-fixed variables on the likelihood to receive treatment with MTX . Stabilized IPTW at each visit were calculated using the equation: *IPTW = Pmtx / Pmtx**, where *Pmtx* represents the propensity for treatment with MTX at the current visit based on MTX use at the previous visit and *Pmtx** represents the propensity for MTX treatment at the current visit based on MTX use at the previous visit and given confounders that may influence this probability. *Pmtx* was estimated using logistic regression models for MTX exposure at the current visit with MTX exposure at the prior visit as the independent variable. *Pmtx** was estimated from similar logistic regression models, which also included DAS28-CRP from the prior visit and the following additional variables (hereafter referred to as "additional covariates"): time-varying variables from the current and prior visit (age, BMI, non-MTX conventional DMARDs, biologic DMARDs, prednisone, aspirin, and NSAIDs) and time-invariant variables obtained at baseline (sex, race, smoking status, RA duration, hypertension, hyperlipidemia, diabetes, kidney disease, liver disease, prior CVD, statin use, and the Rheumatic Disease Comorbidity Index score). Both Pmtx and Pmtx* were estimated cumulatively to reflect the complete treatment history. After incorporating IPTW, standardized mean differences were calculated for each variable between MTX-exposure groups to ensure covariate balance. IPTWs

(median 0.98, range 0.06-50.07) were examined to ensure extreme weights were not present and ensure that the positivity assumption of this model was not violated. Cox proportional hazards models were used to assess the association of MTX use with future CVD events, using the estimated IPTW for MTX.

Mediation Analysis

To estimate the direct effect of MTX on CVD risk in RA, we conducted a mediation analysis of MTX and CVD events using marginal structural models similar to that described above by treating both MTX and disease activity as an exposure in the MSM. Stabilized IPTW were calculated using the equation: *IPTW = (Pmtx1 / Pmtx1*) * (Pmtx2 / Pmtx2*)*. *Pmtx1* was estimated using logistic regression models for MTX exposure at the current visit with MTX exposure and DAS28-CRP from the prior visit as independent variables. *Pmtx1** was estimated from similar logistic regression models also incorporating the "additional covariates" listed above. *Pmtx2* was estimated using the normal density with mean predicted from linear regression models for DAS28-CRP at the current visit with DAS28-CRP from the prior visit and MTX exposure from the current and prior visit as the independent variables. *Pmtx2** was estimated by additionally incorporating the "additional variables" listed above into this linear regression model. *Pmtx1*, *Pmtx1**, *Pmtx2*, and *Pmtx2** were estimated cumulatively to reflect the complete treatment history. Cox proportional hazards models with current MTX exposure and current DAS28-CRP as independent variables were used to assess the association of MTX use with future CVD events, using the estimated joint IPTW for MTX and DAS28-CRP. Indirect effects were subsequently estimated as *Indirect Effect = Total Effect - Direct Effect*, using beta

coefficients from the Cox proportional hazards models which were then exponentiated to estimate hazard ratios. For each model, standard errors were estimated using 200 bootstrapped samples with sampling and replication done at the subject level.

Supplementary Table 3. Association of MTX use with CVD events, stratified by baseline disease activity and hsCRP

DAS28-CRP*	Moderate/High		Remission/Low	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Composite	**0.70 (0.49-0.99)**	**0.04**	0.83 (0.56-1.24)	0.37
HF hospitalization	**0.32 (0.13-0.82)**	**0.02**	0.56 (0.20-1.57)	0.27
MACE	0.78 (0.56-1.11)	0.17	0.86 (0.55-1.34)	0.51
CAD	0.79 (0.45-1.40)	0.42	1.01 (0.51-2.00)	0.97
Stroke	0.67 (0.26-1.69)	0.39	0.62 (0.20-1.99)	0.43
CVD Death	0.75 (0.46-1.21)	0.24	0.87 (0.45-1.67)	0.68
hsCRP	hsCRP >2 mg/L		hsCRP ≤2 mg/L	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Composite	0.72 (0.41-1.27)	0.26	0.76 (0.56-1.02)	0.07
HF hospitalization	**0.18 (0.05-0.65)****	**0.01**	0.52 (0.26-1.05)	0.07
MACE	0.83 (0.46-1.49)	0.50	0.80 (0.57-1.11)	0.18
CAD	1.37 (0.60-3.12)	0.45	0.73 (0.45-1.19)	0.21
Stroke	0.87 (0.25-3.01)**	0.83	0.58 (0.23-1.46)	0.25
CVD Death	0.57 (0.27-1.22)	0.15	0.84 (0.56-1.26)	0.39

Marginal structural models for the total effect of MTX on CVD events as described in **Supplementary Appendix.**

* A DAS28-CRP cutoff of <3.2 was used to define low disease activity or remission. 3.2 or greater was considered moderate to high disease activity.

**Bootstrap estimates were unstable and not used. Reported estimates are calculated using observed data only.

Abbreviations: CAD = coronary artery disease, CVD = cardiovascular disease, HF = heart failure, LD = low disease activity, MACE = major adverse cardiovascular event, mod = moderate, MTX = methotrexate, rem = remission

Supplementary Table 4. Association of MTX use with CVD events among patients without a history of MACE or HF

	No pre-existing MACE		No pre-existing HF	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Composite	0.76 (0.53-1.09)	0.13	**0.75 (0.57-1.00)**	**0.05**
HF hospitalization	**0.26 (0.09-0.70)**	**0.01**	0.52 (0.23-1.18)	0.12
MACE	0.82 (0.55-1.23)	0.34	0.78 (0.58-1.06)	0.11
CAD	0.65 (0.35-1.20)	0.17	0.85 (0.55-1.31)	0.46
Stroke	0.99 (0.42-2.36)	0.98	0.63 (0.29-1.34)	0.23
CVD Death	0.89 (0.48-1.63)	0.70	0.68 (0.46-1.02)	0.06

Marginal structural models for the total effect of MTX on CVD events as described in **Supplementary Appendix**.

Abbreviations: CAD = coronary artery disease, CVD = cardiovascular disease, HF = heart failure, MACE = major adverse cardiovascular events, MTX = methotrexate

Supplementary Table 5. Association of MTX use with CVD events among patients with baseline RA duration <5 years

Event Category	HR (95% CI)	p-value
Composite	0.70 (0.43, 1.14)	0.15
HF Hospitalization	**0.24 (0.08, 0.67)***	**0.01**
MACE	0.75 (0.49, 1.16)	0.20
CAD	0.91 (0.48, 1.71)	0.76
Stroke	0.45 (0.12, 1.72)*	0.24
CVD Death	0.56 (0.29, 1.08)	0.08

Marginal structural models for the total effect of MTX on CVD events as described in **Supplementary Appendix**.

Abbreviations: CAD = coronary artery disease, CVD = cardiovascular disease, HF = heart failure, MACE = major adverse cardiovascular events, MTX = methotrexate

*Bootstrap estimates were unstable and not used as there were only 24 HF hospitalizations and 21 strokes. Reported estimates are calculated using observed data only.

Supplementary Table 6: Association of MTX use with CVD events, incorporating education level, lung disease, and MDHAQ

Event Category	HR (95% CI)	p-value
Composite	0.79 (0.60-1.05)	0.10
HF hospitalization	0.45 (0.24-0.84)	0.01
MACE	0.84 (0.65-1.10)	0.21
CAD	0.92 (0.58-1.45)	0.71
Stroke	0.69 (0.30-1.61)	0.39
CVD Death	0.82 (0.56-1.19)	0.29

Marginal structural models for the total effect of MTX on CVD events as described in **Supplementary Appendix**.

Abbreviations: CAD = coronary artery disease, CVD = cardiovascular disease, HF = heart failure, MACE = major adverse cardiovascular events, MTX = methotrexate