Benefits of Methotrexate Use on Cardiovascular Disease Risk among Rheumatoid Arthritis Patients Initiating Biologic Disease-Modifying Antirheumatic Drugs

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Declarations

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Abstract

Objectives: Methotrexate has been associated with reduced risk for cardiovascular disease (CVD) events among rheumatoid arthritis (RA) patients not exposed to biologic disease-modifying antirheumatic drugs (bDMARD). The effect of concomitant methotrexate on CVD risk among RA patients initiating bDMARDs remains unknown.

Methods: A retrospective cohort study was conducted to assess the effect of methotrexate on CVD risk using 2006–2015 Medicare claims data for RA patients initiating bDMARDS. The main exposure was current use of methotrexate, updated in a time varying fashion. The primary outcome was a composite of incident myocardial infarction, stroke, and fatal CVD. Secondary outcomes were each event that comprised the primary outcome. Incidence rates (IR) and 95% confidence intervals (CI) were calculated using Poisson regression. Associations between methotrexate and risk of CVD were assessed using Cox regression.

Results: A total of 88,255 bDMARD initiations and 1,861 CVD events were included in this study. Mean age was 64.6 (12.3) years, 84.0% female, 68.2% non-Hispanic white. The crude IRs for CVD were 17.9 (16.9–18.8) and 12.1 (95%CI: 11.1–13.2) per 1,000 patient-years among methotrexate unexposed and exposed, respectively. The multivariable adjusted HR for CVD events associated with methotrexate was 0.76 (0.68–0.85). Multivariable adjusted HRs were 0.78 (0.66, 0.91), 0.74 (0.62, 0.88), 0.77 (0.68, 0.86), and 0.82 (0.73, 0.93) for MI, stroke, MI or stroke, and a composite CVD outcome. Results were robust in sensitivity and subgroup analyses.

Conclusion: Among RA patients receiving biologics, concomitant methotrexate use was associated with a 24% lower risk for CVD events.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory polyarthritis affecting about 0.5–2% of the population.[1-3] Without effective treatment, RA often leads to joint destruction, deformity, loss of function, disability, poor quality of life, and shortened life expectancy.[4-10] Inflammatory features of RA are characterized by elevated production of cytokines and inflammatory markers (interleukin 1 (IL-1), IL-6, C-reactive protein (CRP), and tumor necrosis factor (TNF)). Cytokines play an important role in the increased risk for cardiovascular disease (CVD) in RA patients, and they also are shown to promote atherosclerosis.[11-13] Accelerated atherosclerosis remains important to rheumatologists given that CVD related deaths account for a 50% excess mortality in RA patients.[14]

Methotrexate is the first line disease-modifying antirheumatic drug (DMARDS) recommended for the treatment for RA.[15,16] RA patients with intolerance or with inadequate response to methotrexate typically add or switch to biologic DMARDS (bDMARDS). While many bDMARDS (e.g. tocilizumab, sarilumab) have appreciable efficacy for RA treatment even in the absence of concomitant methotrexate use, other bDMARDS (e.g. infliximab, adalimumab) are clearly more effective when administered with concomitant methotrexate.[17]

A number of studies and meta-analyses have demonstrated that methotrexate is associated with reduced risk for CVD.[18-28] Most studies concluded mechanistically that this effect is mediated primarily by controlling systemic inflammation or reducing serum cytokines.[29] A large scale NIH-funded trial -Cardiovascular Inflammation Reduction trial (CIRT) showed no benefit of methotrexate on CVD prevention in patients with diabetes or metabolic syndrome with known coronary disease.[30,31] However, there was no requirement that participants have any meaningful degree of systemic inflammation (e.g. as measured by elevated CRP), and there may have been other reasons (e.g. dosing) underlying the negative finding in this population. For patients with autoimmune or inflammatory conditions who are receiving other immunomodulatory

therapies that may reduce systemic inflammation (e.g. bDMARDS), the incremental benefit of concomitant methotrexate on CVD risk is not clear. Therefore, the objective of this study was to assess the association between methotrexate and CVD risk when administered concomitantly with bDMARDS to patients with RA.

METHODS

Population and data source: This study utilized Medicare claims data for all RA patients from 1/1/2006-9/30/2015. Medicare is a government sponsored health plan provided to >90% of US residents age ≥65. Medicare also covers individuals age<65 if disabled (e.g. due to RA) or with end-stage renal disease.

RA Cohort Definition and Biologic Exposure: A retrospective cohort study was conducted among adult RA patients who initiated a bDMARD between 1/1/2007-9/30/2015. Injection bDMARDS were identified from Medicare pharmacy data using national drug codes (NDC) and infusion therapies identified using Healthcare Common Procedure Coding System (HCPCS) codes. Infusion drugs in the first year after regulatory approval and before a permanent HCPCS code was available were identified using a published algorithm with sensitivity 94%, specificity 100% and PPV 97%.[32] To be considered a new user for each bDMARD, RA patients had to have a minimum of 365 days of prior data with no previous claim for the specific therapy. These 365 days were referred to the baseline period for assessing co-morbidities. RA patients initiating a bDMARD who did not meet this criterion would have been prevalent users and, while excluded from analysis of that specific bDMARD, might still have qualified for initiation of other bDMARDS. RA was identified using ≥2 two claims with International Classification of Disease, 9th Revision, Clinical Modification (ICD-9-CM) codes for RA assigned by a physician, separated by 7 days and occurring within one year. To make sure the treatment was for RA, patients diagnosis codes for other autoimmune/inflammatory disease in baseline were excluded. Patients with diagnosis codes for malignancy other than non-melanoma skin cancer, HIV, or organ transplantation before bDMARD initiation (using all available data) were also excluded. Initiations for RA patients with a diagnosis code for myocardial infarction (MI: ICD-9-CM 410.xx), history of MI (412.xx) or

stroke (430.xx, 431.xx, 433.xx, 434.xx and 436.xx) before initiation date (using all available data) were excluded to allow for the study of incident CVD events. For a broader composite CVD event, we included all of the above, plus angina, coronary artery bypass grafting (CABG), and percutaneous coronary intervention (PCI) as outcomes. For this outcome, patients with ICD-9-CM diagnosis code for other acute and subacute forms of ischemic heart disease (411.xx), angina (413.xx), heart failure (428.9x), other forms of chronic ischemic heart disease (414.xx) or ICD-9-CM procedure code or common practice terminology code (CPT) for CABG and PCI before the initiation date were excluded.

Primary Exposure Definitions for Methotrexate Use: Time-varying methotrexate use was defined as current exposure (i.e. days of supply, without extension), and updated for each person-day. In a sensitivity analysis, a 90-day extension was added to days of supply of methotrexate. A second sensitivity analysis defined "concomitant methotrexate" in a non-time-varying ("fixed") fashion as any prescription for methotrexate within 120 days after initiation of bDMARDS. This sensitivity analysis required patients be event free for those 120 days, and therefore, follow-up started at day 121 to avoid immortal person-time bias.

Demographic characteristics, co-morbidities, and health behaviors: Age was updated at each bDMARD initiation. We identified history of CVD other than MI or stroke using all available data and other covariates using data in 365 days prior to each bDMARD initiation date, which was also defined as baseline. Both co-morbidities and healthcare utilization were dichotomous and identified using ICD-9-CM diagnosis codes. Using NDC and HCPCS codes, we also identified patients' concurrent medications. Glucocorticoids dose was calculated as a 183 days average before initiation and classified as none, low dose (<7.5 mg/day) or higher dose (≥7.5 mg/day). Statin use was identified using NDC codes and classified as none, low, medium and high potency [33] based on the last prescription in baseline. Folic acid use was identified using NDC codes and defined dichotomously and time varying. Non-alcoholic fatty liver disease and metabolic syndrome were defined using ICD-9 diagnosis codes using all available data before index date as fixed covariate for

concomitant methotrexate analysis, and using all available data before and after index date as time varying covariate for time-varying methotrexate analysis.

CVD Outcome assessment: The primary outcome was a composite of incident MI, stroke, and fatal CVD. Incident MI was defined as at least one ICD-9-CM diagnosis code for MI (410.x1) from a hospital discharge with at least one night stay in a hospital unless the patient died (PPV≥90%).[34] Incident stroke was defined as at least one ICD-9-CM diagnosis code for stroke (430.xx, 431.xx, 433.x1, 434.x1 and 436.xx) from a hospital discharge (PPV ≥90%).[35] Fatal CVD was identified by a validated claims based algorithm with PPV ≥80%.[36] The secondary outcomes were individual components of the primary outcome. A broader CVD outcome definition included angina identified using ICD-9 diagnosis codes, CABG and PCI using both ICD-9 procedure codes and CPT codes.

Follow-up: Follow-up began at the initiation of each specific bDMARD medication and ended at the earliest of 1) end of exposure to each specific agent (i.e. days of supply for injection bDMARDS or the standard dosing interval for infusion bDMARDS) plus a 90 day extension); 2) a switch to another bDMARD or tofacitinib; 3) CVD outcome; 4) death date (inclusive for fatal CVD); 5) loss of Medicare coverage or 6) end of study.

Statistical methods

Descriptive statistics were calculated for covariates of interest, comparing patients with and without methotrexate use on their biologic initiation date, and to characterize the distribution of those risk factors during follow-up according to methotrexate exposed versus unexposed person-time. Mean and standard deviation were calculated for continuous variables and proportions were calculated for categorical variables. Standard mean differences (SMD) were calculated for both continuous and categorical variables, SMD greater than 0.1 was considered as indication for non-balance. Incidence rates (IRs) of CVD were calculated by dividing number of events by person years (PY) exposed; 95% confidence intervals (CIs) were estimated

with Poisson regression. Unadjusted and multivariable adjusted hazard ratios (aHR) and 95% CI were estimated using Cox regression. Age, sex, race, heart failure, atrial fibrillation, abdominal aortic aneurism, peripheral arterial disease, diabetes, hyperlipidemia, hypertension, obesity, chronic kidney disease, chronic obstructive pulmonary disease, fibromyalgia, any hospitalized infection, any hospitalization, number of physician visits, methotrexate, hydroxychloroquine, leflunomide, sulfasalazine, NSAIDS, statin use and potency, other lipid lowering drug use in baseline, number of other bDMARDS used prior to initiation, oral glucocorticoid dose in the six months before initiation, smoking, low income (i.e. enrolled in state buy-in program), and reason for Medicare eligibility other than age (e.g. disability) were included in the multivariable adjusted model. Robust sandwich covariance matrix estimate was used to account for the clustered nature of the data, since each patient could initiate multiple bDMARDs over time and thus contribute more than one treatment episode (with the time axis reset and covariates updated). The proportional hazard assumption was assessed in the fixed concomitant methotrexate analysis and was not violated.

A subgroup analysis was performed that included patients previously exposed to methotrexate to assure that the comparator group not exposed to methotrexate was at some point in the past deemed well enough to have received it. This subgroup would expect to therefore exclude individuals with strong (but potentially unmeasured) confounding related to factors for which clinicians would not give patients methotrexate (e.g. alcoholism, non-compliance with methotrexate-related monitoring, chronic liver disease) that might not be measured well in health plan data. A second subgroup analysis evaluated risk according to specific bDMARD used. One variable with 16 levels (e.g. abatacept with methotrexate, abatacept without methotrexate) was created and included in a multivariable adjusted Cox model; the effect of methotrexate on the risk for CVD for each bDMARD was calculated with a contrast statement in SAS.

Using external adjustment methods, potential confounding for RA disease activity was examined using the Multi-Biomarker Disease Activity (MBDA) test results,[37] assessed in the subgroup of patients who had their data linked to MBDA test results (within six months before bDMARD initiation) according to the method described by Schneeweiss et al.[38] The effect size of MBDA (RR_{CD}) on CVD risk was 1.5-fold elevated for patients in moderate or high RA disease activity compared to people who had low RA disease activity, measured by the MBDA[37].

All analyses were conducted using SAS 9.4. Use of the Medicare data was governed by a Data Use Agreement with the Centre for Medicare and Medicaid Services. The Institutional Review Board of the University of Alabama at Birmingham approved the study (IRB-121029003).

RESULTS

A total of 475,651 bDMARDS initiations from 334,092 patients were identified. After applying the inclusion and exclusion criteria, 88,255 initiations (64,218 patients) were included in the main analysis, and 71,328 initiations (55,543 patients) in the fixed concomitant methotrexate sensitivity analysis (Figure 1). The average age at initiation was 64.6 (12.3) years, 84.0% were female, 68.2% were non-Hispanic white.

Patients with methotrexate use (38.8%) concomitant to bDMARD initiation date were similar to patients without methotrexate use in age, sex, and race (Table 1). Methotrexate users were more likely to initiate infliximab, be bDMARD naïve, and less likely to use leflunomide in baseline. They had a lower prevalence of chronic kidney disease and chronic obstructive pulmonary disease. They had a somewhat lower likelihood to have liver disease or a hospitalized infection, had fewer physician visits, and were less likely to be considered disabled by Medicare. Median (IQR) follow up time was 288 (148,602) days.

Association of methotrexate use with CVD risk

A total of 1,324 composite CVD events were identified over 74,127 methotrexate unexposed PY, resulting in an IR of 17.9 (95%CI:16.9–18.8) (Table 2, left panel) per 1,000 PY. A total of 537 composite CVD events were identified during 44,312 methotrexate exposed PY, resulting in an IR of 12.1 (95%CI:11.1–13.2) events per 1,000-PY. Given this difference of approximately 5/1000 PY, the number of RA patients needed to treat (NNT) with methotrexate for one year to prevent one additional composite CVD event would be about 200. The unadjusted HR (Table 2, right panel) was 0.68 (95%CI:0.61,0.75) and the multi-variable adjusted HR (Figure 2, top panel) for exposure was 0.76 (95%CI:0.68,0.85). Multi-variable adjusted HRs consistently showed a protective effect for methotrexate for the four CVD outcomes studied: MI: HR 0.78 (0.66,0.91); stroke: 0.74 (0.62,0.88); composite of (MI or stroke): 0.77 (0.68,0.86); and a broader CVD event definition including the above events plus angina, CABG and PCI: 0.82 (0.73,0.93). There was a suggestion of a protective effect for hydroxychloroquine (0.92, 95% CI 0.82,1.03 for MI outcome) that was not significant.

The sensitivity analysis for time-varying methotrexate exposure with a 90 days extension resulted in similar IRs and aHRs compared with the main analysis (Figure 2, middle panel) and found a multi-variable adjusted HRs for the composite CVD outcome of 0.85 (0.75,0.96). The sensitivity analysis for concomitant methotrexate as a fixed exposure resulted in similar IRs (Figure 2, bottom panel); the multivariable adjusted HRs were all less than 1.0, although some confidence intervals included 1.0.

Subgroup analyses

The subgroup analysis limited to RA patients who used methotrexate prior to bDMARDS initiation resulted in similar HR to the main analysis (Table 3). The overall multi-variable adjusted HR for composite CVD was 0.76 (0.67,0.85) for methotrexate use. The IRs for composite CVD for bDMARDS without methotrexate exposure updated in a time-varying fashion ranged from a low of 14.6 (95% CI: 11.8–18.0) for tocilizumab initiators to high of 22.4 (95%CI: 20.1–25.0) for infliximab initiators (Supplementary Table 1, top panel).The IRs for composite CVD events for bDMARDs with concomitant methotrexate exposure updated in a time-varying

fashion ranged from low of 8.6 (95%CI: 5.5–13.5) for tocilizumab initiators to high of 13.6 (95%CI: 10.2–18.3) for rituximab initiators (Supplementary Table 1, top panel, middle). Contrasts for comparing CVD risk with and without methotrexate exposure ranged from low of 0.59 (95%CI: 0.36–0.98) for certolizumab initiators to high of 0.93 (95%CI: 0.70–1.23) for etanercept initiators. All remaining contrasts evaluating risk for the composite CVD outcome were within these boundaries and all were < 1. Contrasts for each component of the CVD outcome (MI, stroke, or either) generally were similar to that for composite CVD (Supplementary Table 1, lower three quarters).

External adjustment for disease activity

The proportion of moderate or high MBDA scores was 87.0% among all MBDA tests (n=123,921) analyzed. Measured at the start of biologic therapy, the proportion of RA patients with moderate or high MBDA scores were nearly identical, comparing bDMARD initiators with and without methotrexate (94% vs. 93%). Assuming an HR of 1.5 for the association between moderate/high MBDA score and CVD based on published data, external adjustment for the MBDA by exposure group therefore led to no change in the overall estimated HR for composite CVD associated with time varying methotrexate use (aHR=0.76).

DISCUSSION

In this retrospective cohort study of Medicare-insured RA patients who initiated bDMARDs, we observed a 24% reduction in the risk for CVD events associated with concomitant methotrexate use. The observed results were robust in two sensitivity analyses and a subgroup analysis of patients who had previously been on methotrexate but who had discontinued it. These findings lend additional support to methotrexate yielding an incremental benefit to reducing CVD risk, even among RA patients treated with biologics. We note that the absolute rate difference of the composite CVD event of approximately 5/1000 patient-years and the associated NNT of about 200 is relatively small. By itself, a benefit of this magnitude is unlikely to be a strong motivator for patients or clinicians. Nevertheless, for those taking methotrexate mainly for its

clinical effects to treat RA, its potential benefits to further reduce CVD risk may be compelling for some patients.

Several previous studies have demonstrated that methotrexate is associated with a reduced risk for CVD events among RA patients using conventional synthetic DMARD.[18-21,28] In a prospective, single-center study including 1,240 RA patients between Jan 1, 1981 and Dec 31, 1999, Choi et al. found the HR associated with methotrexate use for CVD death was 0.3 (95%CI:0.2,0.7). In a study including veterans with RA, Prodanowich *et al*. found the risk ratio for CVD for methotrexate use was 0.83 (95%CI: 0.71,0.96). Suissa *et al*. conducted a cohort study using the PharMetrics claims database and found the risk ratio for MI for methotrexate use to be 0.81 (95%CI: 0.60,1.08). These studies were conducted among RA patients never exposed to bDMARDS and are compatible with the aHR we observed 0.76 (95%CI: 0.68,0.86). Of importance from a study design perspective, because methotrexate is now considered an 'anchor drug' in the treatment of RA, it is challenging to construct a reasonable comparator therapy for methotrexate in any contemporary analysis given how it is used in RA. Thus, our study design where all patients were initiating bDMARDs, with some receiving background methotrexate and others not, is particularly relevant in terms of being able to study its effects in the modern era.

The clinical implications of our study are notable. While combination therapy with methotrexate may only marginally increase the clinical efficacy of some bDMARDS (e.g. tocilizumab), combination therapy may further reduce the risk for CVD. The recently completed CIRT trial showed no benefit of methotrexate for CVD risk reduction among patients with stable coronary artery disease and either type 2 diabetes or metabolic syndrome who were not selected on the basis of having systemic inflammation.[31] In contrast, the canakinumab anti-inflammatory thrombosis outcome study (CANTOS) showed that when given to patients with prior MI with elevated inflammatory markers (hsCRP ≥2mg/L), canakinumab led to a 17% reduction in the risk of heart attack, stroke, urgent need for revascularization, or cardiovascular death. The effect size of

methotrexate for CVD risk reduction that we observed in RA patients treated with bDMARDS is comparable to the effect size associated with canakinumab. Our study suggests that when tolerability is not a problem, concomitant methotrexate to bDMARDS not only improves symptoms and reduces the risk for radiographic progression but may offer the possibility of further reduction of CVD risk.

Our study has several strengths. We included fatal CVD as a part of a CVD composite outcome. Fatal CVD was identified through a validated claims based algorithm,[36] and not including fatal CVD events in such an analysis will underestimate the IR for CVD events and may result in biased estimates of its exposure-outcome associations. We used a validated algorithm[32] to identify infusion represented with non-specific HCPCS codes 3490 and 3590 before a permanent HCPCS code were available; failure to identify these infusion drugs will reduce the sample size and more importantly, will misclassify some prevalent users as new users by misclassifying their initiation date. Our large sample size made it possible to assess effect of methotrexate on CVD risk according to specific bDMARD exposure and to estimate IR and HRs with high precision. Recognizing the potential for residual confounding due to unmeasured factors such as RA disease activity, and notable given that large claims databases typically lack lab test results, we used the MBDA to assess the distribution of disease activity. There was no difference in the proportion of patients with moderate or high MBDA biomarker scores between patients initiating bDMARDs with or without methotrexate. Recognizing that a one-time measurement of the MBDA is not a gold standard to control for the influence of RA disease activity on the cardiovascular system and noting that other clinical measures of disease activity were not available in this data source, this approach nevertheless may help address the concern that RA disease activity, previously shown to be associated with CVD risk,[39] might confound our observed associations. This concern may be further attenuated given that all patients were initiating biologic therapy, making baseline disease activity more likely to be in the moderate-to-high end of the range and thus more comparable between the methotrexate and the non-methotrexate exposure groups.

Our study has limitations common to other studies using administrative data. Despite adjusting for a multitude of covariates, some may be unascertained (e.g. obesity), misclassified (e.g. smoking, over-the-counter folate), or unavailable (e.g. family history). Claims data contains only information on whether a prescription was filled, and patients filling a prescription do not necessarily take the medication as directed, and thus we could have misclassified exposure. For this reason, we did not study methotrexate dose, given that patients could change dose (particularly down-titration) without requiring a new prescription. We also recognize that patients discontinue methotrexate for a variety of reasons, only some of which could be controlled for in this type of data source. CVD outcome events were not adjudicated individually, although we relied on claims-based algorithms that have a positive predictive value in excess of 80–90% in their accuracy, and there is little reason to expect that performance of these would be differential by specific biologic exposure or methotrexate use. Finally, a minority of RA patients had MBDA test results available for external adjustment, patients may have been tested with the MBDA up to 6 months prior to bDMARD initiation, and tested patients may be systematically different than those not tested.

CONCLUSIONS

In conclusion, our observational study suggests an overall 18-25% reduction of CVD risk associated with methotrexate use, incremental to the inflammatory disease control conferred by concomitant biologic use. These findings provide additional information to support the importance of optimal disease activity control and reduction of inflammation attainable by combination RA therapy with methotrexate.

Figure legends

Figure 1: Flow chart for RA cohort selection

Footnote for figure 1:

CABG: coronary artery bypass grafting; CHD: Coronary heart disease; HF: heart failure; HIV: human immunodeficiency virus; MI: myocardial infarction; MTX: methotrexate; PCI: percutaneous coronary intervention; RA: rheumatoid arthritis.

Note that an 'episode' refers to initiation of a specific biologic medication for RA. Patients could have initiated more than 1 biologic over the study time period and therefore contributed more than 1 episode to the analysis.

Figure 2: Forest plot showing the adjusted* hazard ratio for CVD risk associated with methotrexate in the primary analysis and sensitivity analyses referent to methotrexate non-use, varying the definition used for methotrexate exposure

Footnote for figure 2:

CI: confidence interval; CVD: Cardiovascular disease; HR: hazard ratio; IR: incidence rate per 1,000 patient years;

Primary outcome including myocardial infarction, stroke and fatal CVD identified with algorithm with cut-points for positive predictive value ≥0.80. CVD plus including myocardial infarction, stroke and fatal CVD, angina, coronary artery bypass grafting, percutaneous coronary intervention.

Concomitant methotrexate was defined as any prescription within 120 days after initiation of biologic DMARD.

*Adjusted for age, sex, race, Charlson comorbidity index, biologic DMARDS initiated, baseline MTX, hydroxychloroquine, leflunomide, sulfasalazine, heart failure, atrial fibrillation, abdominal aortic aneurism, peripheral arterial disease, diabetes, hyperlipidemia, hypertension, obesity, chronic kidney disease, chronic obstructive pulmonary disease, fibromyalgia, any hospitalized infection, any hospitalization, number of physician visit, nonsteroidal anti-inflammatory drug, statin potency, other lipid lowering drug use in baseline, number of biologic used prior initiation, oral steroid dose in six month before initiation, smoking, state buy-in (i.e. low income), and reasons for Medicare eligibility other than age (e.g. disability). For time varying MTX analysis also adjusted for time varying folic acid, non-alcoholic fatty liver disease, and metabolic syndrome. For concomitant MTX analysis, also adjusted for folic acid, non-alcoholic fatty liver disease, other forms of liver disease, and metabolic syndrome assessed using baseline data.

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Figure 1: Flow chart for RA cohort selection

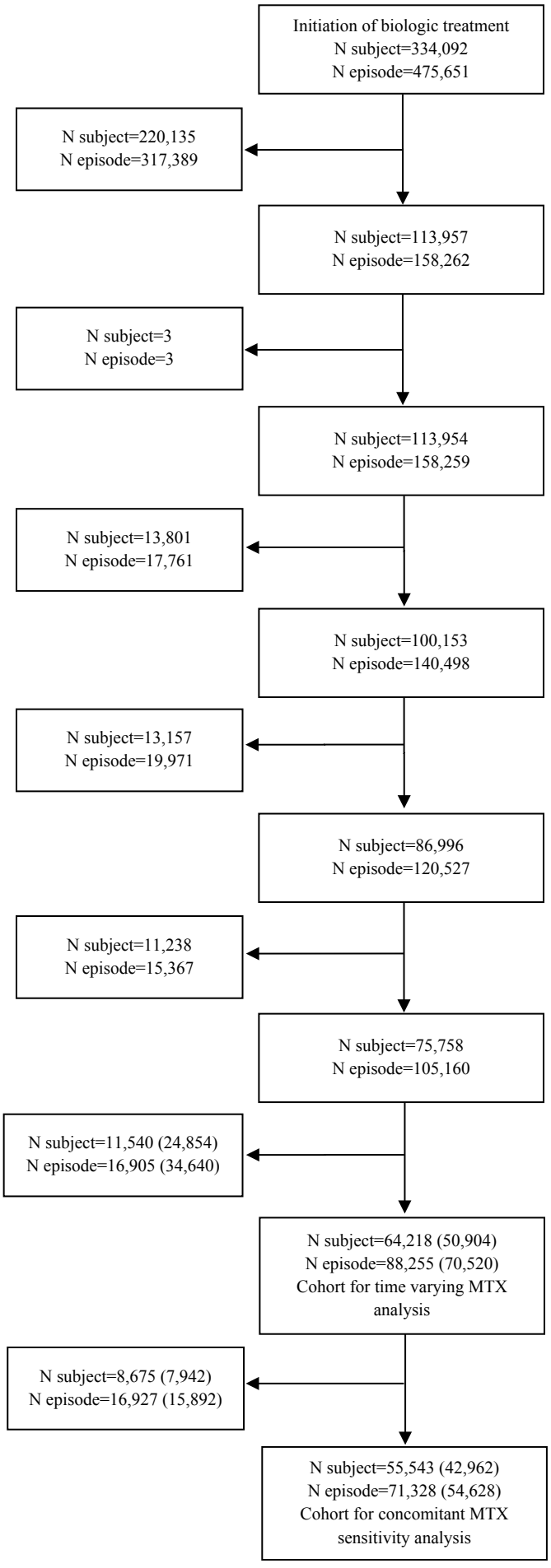
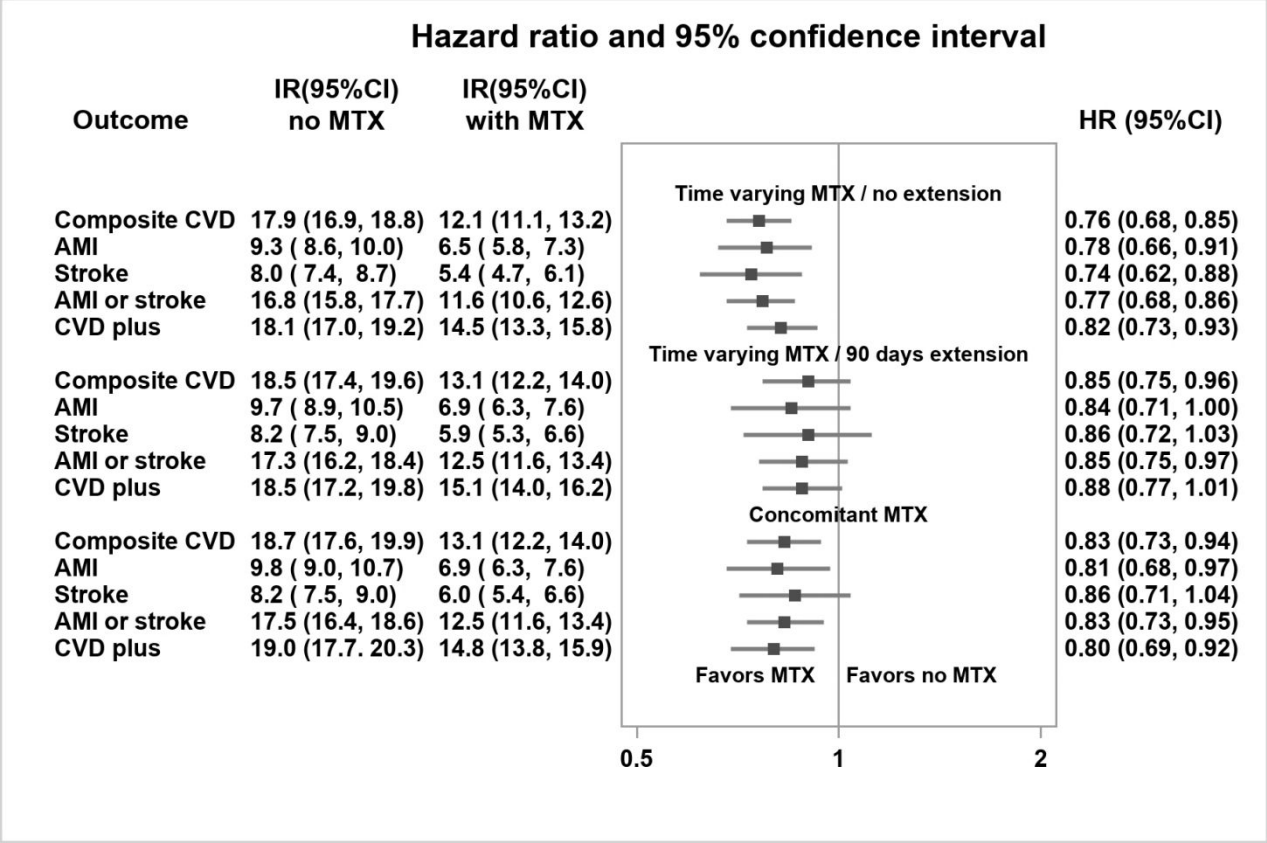


Figure 2: Forest plot showing the adjusted* hazard ratio for CVD risk associated with methotrexate in the primary analysis and sensitivity analyses referent to methotrexate non-use, varying the definition used for methotrexate exposure



CI: confidence interval; CVD: Cardiovascular disease; HR: hazard ratio; IR: incidence rate per 1,000 patient years; Primary outcome including myocardial infarction, stroke and fatal CVD identified with algorithm with cut-points for positive predictive value ≥0.80. CVD plus including myocardial infarction, stroke and fatal CVD, angina, coronary artery bypass grafting, percutaneous coronary intervention. Concomitant methotrexate was defined as any prescription within 120 days after initiation of biologic DMARD. *Adjusted for age, sex, race, Charlson comorbidity index, biologic DMARDS initiated, baseline MTX, hydroxychloroquine, leflunomide, sulfasalazine, heart failure, atrial fibrillation, abdominal aortic aneurism, peripheral arterial disease, diabetes, hyperlipidemia, hypertension, obesity, chronic kidney disease, chronic obstructive pulmonary disease, fibromyalgia, any hospitalized infection, any hospitalization, number of physician visit, folic acid, nonsteroidal anti-inflammatory drug, statin potency, other lipid lowering drug use in baseline, number of biologic used prior initiation, oral steroid dose in six month before initiation, smoking, state buy-in (i.e. low income), and reasons for Medicare eligibility other than age (e.g. disability). For time varying MTX analysis also adjusted for time varying folic acid, non-alcoholic fatty liver disease, and metabolic syndrome. For concomitant MTX analysis, also adjusted for folic acid, non-alcoholic fatty liver disease, other forms of liver disease, and metabolic syndrome assessed using baseline data.

Table 1: Distribution of baseline characteristics by methotrexate use at the time of biologic initiation

	MTX[†] unexposed episodes N=54,055	MTX[†] exposed episodes N=34,200	SMD[‡]
Age in years, mean (STD)	64.1 (12.6)	65.2 (11.8)	0.09
Female, %	84.7	82.9	0.05
Race, %			0.04
White	67.4	69.4	
Black	9.8	9.8	
Other	22.7	20.8	
Observable time before index date in years median (IQR)	2.8 (1.7- 4.6)	2.6 (1.6- 4.4)	
Biologic initiated, %			0.26
Abatacept	21.8	18.3	
Adalimumab	14.8	16.3	
Certolizumab	7.4	6.6	
Etanercept	14.7	15.4	
Golimumab	5.7	5.5	
Infliximab	15.6	22.9	
Rituximab	10.7	8.1	
Tocilizumab	9.3	6.8	
Comorbidity, %			
Charlson comorbidity index			0.12
0	59.2	64.0	
1	30.9	28.2	
2	8.1	6.3	
3 and more	1.8	1.2	
History of cardiovascular disease[†]	21.9	19.8	0.05
Heart failure	6.3	4.7	0.07
Atrial fibrillation	5.4	4.8	0.03
Abdominal aortic aneurism	0.6	0.5	0.01
Peripheral arterial disease	2.2	2.0	0.02
Diabetes	23.7	21.8	0.05
Hyperlipidemia	42.9	43.5	0.01
Hypertension	61.1	60.3	0.02
~~Obesity~~	~~5.0~~	~~4.4~~	~~0.03~~
Chronic kidney disease	10.6	7.0	0.13
Chronic obstructive pulmonary disease	28.6	24.0	0.10
Fibromyalgia	20.8	17.8	0.08
Hospitalized infection	10.8	7.4	0.12
Non-alcoholic fatty liver	1.7	0.8	0.08
Other forms of liver disease	1.5	0.3	0.12
Metabolic syndrome	1.7	1.7	0.00
Medication use, %			
Any methotrexate during 1 year baseline[§]	36.9	100.0	
Hydroxychloroquine	24.3	23.8	0.01
Leflunomide	25.9	7.9	0.50
Sulfasalazine	11.4	9.2	0.07

Nonsteroidal anti-inflammatory drug	45.3	48.3	0.06
Prior biologic DMARDs			0.38
None (i.e. biologic naïve)	39.3	49.4	
1	36.7	32.8	
2	15.7	12.4	
3 and more	8.3	5.4	
Statin			0.15
None	67.1	64.8	
Low potency	4.6	5.2	
Moderate potency	23.2	24.9	
High potency	5.1	5.1	
Other lipid lowering drug	10.9	9.7	0.04
Prednisone equivalent, mg/day			0.06
None	35.6	34.8	
≤7.5	47.9	49.7	
>7.5	16.5	15.4	
Health behavior and healthcare utilization			
Smoking	14.2	12.2	0.06
Prostate specific antigen (male only)	42.0	44.5	0.05
PAP smear (female only)	13.6	14.0	0.01
Mammography (female only)	38.9	42.3	0.07
Any hospitalization	23.4	19.2	0.10
Number physician visit, mean (STD)	18.0 (10.5)	16.6 (9.6)	0.14
State buy in program (proxy for low income)	40.1	37.1	0.06
Reason for eligibility for Medicare other than age (e.g. disability)	54.9	48.8	0.12
MBDA score category*			0.06
Low (1-29)	6.7	6.0	
Medium, or high (30-100)	93.3	94.0	

DMARDS: Disease-modifying anti-rheumatic drug; MTX: methotrexate; SMD: Standardized mean difference.

† MTX was defined as MTX exposure on the date that biologic DMARDs were initiated.

‡SMDs contrasted columns 2 and 3. SMDs >0.1 was considered as potentially important (i.e., a large difference in the distributions between MTX exposed and unexposed groups).

§Patients in the methotrexate unexposed group may have previously received methotrexate in the one-year baseline period but discontinued it at the time that follow-up began at the time of biologic initiation.

*MBDA score was assessed for a subgroup of 6,034 patients with MBDA score data linked to Medicare data through patients' birth date, sex, test date, NPI for rheumatologist ordered the test; tests for MBDA in Medicare were identified through a set of common practice codes submitted by Crescendo Bioscience

Table 2: Incidence rate and unadjusted hazard ratio of composite CVD events, myocardial infarction and stroke associated with time varying methotrexate[†] use, referent to MTX unexposed

	MTX[†] exposed		MTX[†] unexposed		Crude Hazard Ratio (95% CI)	Adjusted[‡] Hazard Ratio (95% CI)
	Event Person years	IR (95% CI)	Event Person years	IR (95% CI)		
Myocardial infarction, stroke or fatal CVD	537 44,312	12.1 (11.1, 13.2)	1324 74,127	17.9 (16.9, 18.8)	0.68 (0.61, 0.75)	0.76 (0.68–0.85).
Myocardial infarction	289 44,497	6.5 (5.8, 7.3)	694 74,526	9.3 (8.6, 10.0)	0.70 (0.61, 0.80)	0.78 (0.66, 0.91),
Stroke	239 44,555	5.4 (4.7, 6.1)	599 74,732	8.0 (7.4, 8.7)	0.67 (0.58, 0.78)	0.74 (0.62, 0.88)
Myocardial infarction or stroke	513 44,312	11.6 (10.6, 12.6)	1242 74,127	16.8 (15.8, 17.7)	0.69 (0.62, 0.76)	0.77 (0.68, 0.86)
Myocardial infarction, stroke , angina, PCI, CABG ,fatal CVD	1036 57,259	18.1 (17.0, 19.2)	513 35,499	14.5 (13.3, 15.8)	0.68 (0.61, 0.75)	0.82 (0.73, 0.93)

CI: Confidence interval; CVD: Cardiovascular disease; MTX: Methotrexate; HR: Hazard ratio; IR: Incidence rate per 1,000 person years.

[†] Time varying MTX was defined as days of supply with no extension

[‡] Adjusted for age, sex, race, Charlson comorbidity index, biologic DMARDS initiated, baseline MTX, hydroxychloroquine, leflunomide, sulfasalazine, heart failure, atrial fibrillation, abdominal aortic aneurism, peripheral arterial disease, diabetes, hyperlipidemia, hypertension, obesity, chronic kidney disease, chronic obstructive pulmonary disease, fibromyalgia, any hospitalized infection, any hospitalization, number of physician visit, , nonsteroidal anti-inflammatory drug, statin potency, other lipid lowering drug use in baseline, number of biologic used prior initiation, oral steroid dose in six month before initiation, smoking, State Buy-In (poor), reasons other than age for eligible for Medicare, time varying folic acid, non-alcoholic fatty liver disease, and metabolic syndrome. Time varying folic acid was defined as days of supply with no extension. Time varying non-alcoholic fatty liver disease, and metabolic syndrome were defined using diagnosis code in both baseline and follow-up period.

Table 3: Subgroup analysis evaluating the incidence rate and hazard ratio of composite CVD events and each component associated with methotrexate use, restricted to patients exposed to methotrexate at any time before initiation of biologic DMARDs

	MTX[†] exposed		MTX[†] unexposed		Unadjusted HR (95% CI)	Adjusted HR[‡] (95% CI)
	Event Person Year	IR (95% CI)	Event Person Year	IR (95% CI)		
Myocardial infarction, stroke, or fatal CVD	518 42,981	12.1 (11.0, 13.1)	791 47,132	16.8 (15.6, 18.0)	0.72 (0.64, 0.80)	0.76 (0.67, 0.85)
Myocardial infarction	276 43,163	6.4 (5.7, 7.2)	416 47,387	8.8 (8.0, 9.7)	0.73 (0.62, 0.85)	0.75 (0.64, 0.89)
Stroke	233 43,213	5.4 (4.7, 6.1)	364 47,526	7.7 (6.9, 8.5)	0.71 (0.60, 0.83)	0.75 (0.63, 0.90)
Myocardial infarction or stroke	494 42,981	11.5 (10.5, 12.6)	750 47,132	15.9 (14.8, 17.1)	0.72 (0.65, 0.81)	0.76 (0.67, 0.86)
Myocardial infarction, stroke , angina, PCI, CABG ,fatal CVD	494 34,430	14.3 (13.1, 15.7)	627 36,305	17.3 (16.0, 18.7)	0.83 (0.74, 0.94)	0.81 (0.71, 0.92)

CI: Confidence interval; CVD: Cardiovascular disease; DMARDS: Disease-modifying anti-rheumatic drug; MTX: Methotrexate; HR: Hazard ratio; IR: Incidence rate.

[†] Time varying MTX was defined as days of supply with no extension.

[‡] Adjusted for age, sex, race, Charlson comorbidity index, biologic DMARDS initiated, baseline MTX, hydroxychloroquine, leflunomide, sulfasalazine, heart failure, atrial fibrillation, abdominal aortic aneurism, peripheral arterial disease, diabetes, hyperlipidemia, hypertension, obesity, chronic kidney disease, chronic obstructive pulmonary disease, fibromyalgia, any hospitalized infection, any hospitalization, number of physician visit, folic acid, nonsteroidal anti-inflammatory drug, statin potency, other lipid lowering drug use in baseline, number of biologic used prior initiation, oral steroid dose in six month before initiation, smoking, State Buy-In (poor), reasons other than age for eligible for Medicare, time varying folic acid, non-alcoholic fatty liver disease, and metabolic syndrome. Time varying folic acid was defined as days of supply with no extension. Time varying non-alcoholic fatty liver disease, and metabolic syndrome were defined using diagnosis codes in both baseline and follow-up periods.