Ther Adv Musculoskel Dis
2020, Vol. 12: 1–12
DOI: 10.1177/1759720X20981221

Methotrexate at middle and high accumulative doses might be associated with lower risk of new-onset cancers in patients with rheumatoid arthritis: a nationwide population-based cohort study

Wuu-Tsun Perng*, Yao-Min Hung*, Renin Chang, Cheng-Li Lin, Jeng-Yuan Chiou, Huang-Hsi Chen, Chia-Hung Kao and James Cheng-Chung Wei

Abstract

Background: We investigated whether taking methotrexate (MTX) is associated with a lower risk of new-onset cancers in patients with rheumatoid arthritis (RA).

Methods: We conducted a 12-year retrospective cohort study from a population-based National Health Insurance Research Database in Taiwan. A total of 21,699 patients with newly diagnosed RA were enrolled during 2000–2009. The overall cancer rate was compared between 10,352 new users of MTX and 11,347 non-users. We used the WHO Defined Daily Dose (DDD) as a tool to assess drug exposure. Cox proportional hazard regression models were used to estimate the hazard ratio (HR) of disease after controlling for demographics and other comorbidities.

Results: After adjusting for age, sex, cancer-related comorbidities, and RA-combined medication, the HR of cancer risk was 0.87 (95% CI = 0.74–1.02) for the MTX user group compared with the MTX non-user group. The cumulative incidence of cancer in the MTX non-user group was significantly higher than that of the MTX user group (log-rank test $p < 0.001$). In the low accumulative dose group [cumulative dose < 1125 mg, cumulative defined daily dose (cDDD) < 450], the HR of cancer risk for MTX users was 1.20 (95% CI = 1.01–1.42) compared with the MTX-non-user group. However, the adjusted HR of cancer risk was reduced to 0.66 (95% CI = 0.49–0.87) in MTX middle-dose users (cumulative dose 1125–2250 mg, cDDD: 450–899) and 0.33 (95% CI = 0.23–0.48) for the MTX high-dose group (cumulative dose ⩾ 2250 mg, cDDD ⩾ 900), respectively (p for trend < 0.0001).

Conclusion: MTX at middle and high accumulative doses might be associated with lower risk of new-onset cancers in patients with RA.

Keywords: cancer, cohort study, methotrexate, rheumatoid arthritis

Received: 16 September 2019; revised manuscript accepted: 21 November 2020.

Correspondence to:
James Cheng-Chung Wei
Division of Allergy, Immunology and Rheumatology, Chung Shan Medical University Hospital, Taichung, Taiwan
Institute of Medicine, Chung Shan Medical University
Graduate Institute of Integrated Medicine, China Medical University, Taichung
No. 110, Sec. 1, Jianguo N. Rd., South District, Taichung City 40201, Taiwan.
wei3228@gmail.com

Chia-Hung Kao
Graduate Institute of Biomedical Sciences, College of Medicine, China Medical University, Taichung
Department of Nuclear Medicine and PET Center, China Medical University Hospital, Taichung, Taiwan
Department of Bioinformatics and Medical Engineering, Asia University, Taichung
Center of Augmented Intelligence in Healthcare, China Medical University Hospital, Taichung
d10040@mail.cmuh.org.tw

Wuu-Tsun Perng
Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan
Department of Recreational Sport & Health Promotion, National Pingtung University of Science and Technology

Yao-Min Hung
Department of Internal Medicine, Kaohsiung Municipal United Hospital, Kaohsiung, Taiwan
Institute of Medicine, Chung Shan Medical University, Taichung

Introduction

Rheumatoid arthritis (RA) is a chronic life-long inflammatory disease characterized by destructive polyarthritis. Comorbidities of RA are common, including Sjogren's syndrome, cardiovascular diseases, osteoporosis, lymphoproliferative diseases, and cancers.[1] Several epidemiologic studies[2–5] and two meta-analyses[6,7] have reported a higher risk of cancer in patients with RA relative to the general population, although three recently published studies focusing on Asian populations showed that the overall malignancy risk was not higher in the RA population.[8–10]

Among all malignancies in RA patients, lymphoproliferative cancers and lung cancers have consistently

Yuhing Junior College of Health Care and Management, Kaohsiung, Taiwan;

School of Medicine, National Yang Ming University, Taipei 11221, Taiwan

Renin Chang
Department of Emergency Medicine, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan

Institute of Biotechnology and Chemical Engineering, I-Shou University, Kaohsiung 84001, Taiwan

Cheng-Li Lin
Management Office for Health Data, China Medical University Hospital, Taichung, Taiwan

College of Medicine, China Medical University, Taichung

Jeng-Yuan Chiou
School of Health Policy and Management, Chung Shan Medical University, Taichung, Taiwan

Huang-Hsi Chen
Division of Allergy, Immunology and Rheumatology, Chung Shan Medical University Hospital, Taichung, Taiwan

*WT Perng and YM Hung contributed equally.

been found to be more common;[6,7] however, the incidence of breast and colon cancers is lower.[4,6,7,11] Indeed, cancer risk may be related to disease severity or treatment regimens. Baecklund *et al.* first proposed that the increased risk of lymphoma in patients with RA can be mainly attributed to underlying chronic inflammation rather than the treatment for RA.[12] However, it is still uncertain whether RA treatment with disease-modifying antirheumatic drugs (DMARDs) may affect the cancer risk in RA. Methotrexate (MTX), the "anchor drug" in the treatment of RA,[13] might have a dual effect on cancer; it probably has an anticancer effect due to its anti-metabolite property, but may also promote cancer due to its immunosuppressive effects. An eight-case series reported that MTX was not responsible for generating cancers.[14] However, discontinuation of MTX has been followed by the disappearance of lymphoma in some patients.[15] The relationship between MTX and cancer risk has been explored in patients with RA, but the results have been inconsistent.[16–19] Buchbinder *et al.* reported RA patients who had been exposed to MTX had a 50 percent greater risk of developing cancers of any type. However, their study design was questioned due to its small sample size (only 309 women and 150 men) and few confounding adjustments, and whether MTX or the disease itself was the culprit.[19]

Despite this debate, the literature presents little evidence currently regarding MTX's long-term effect on cancers in large cohorts of RA patients. Therefore, we conducted a large nationwide population-based cohort study in order to investigate MTX's effect on cancer development in patients with RA.

Methods

Study design

This study is a 12 year, retrospective cohort study based on a nationwide, population-based database in Taiwan. The study was approved by the Ethics Review Board of China Medical University (CMUH104-REC2-115).

Data source

The study was constructed using data from the National Health Insurance Research Database (NHIRD), including claims data from Taiwan's National Health Insurance (NHI), which is a nationwide, single-payer health insurance program and compulsorily covered over 99% of Taiwan's 23 million citizens in 2005. The NHIRD contains all claims data, including the registry for beneficiaries, inpatient and outpatient records, and the registry for drug prescriptions and other medical services; in addition, the database is renewed every year. The NHI records diseases based on the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM). Before releasing the database for research, the Taiwanese government replaced the original identification numbers with anonymized numbers to safeguard patients' privacy.

Study population

We undertook a retrospective, population-based cohort study to investigate the association between MTX use and the development of cancer risks in RA patients. We selected patients with new-onset RA (ICD-9-CM 714 with a catastrophic illness card) between 2000 and 2009. The RA history was collected from the registry for catastrophic illness, and the disease history information for insured people was collected from inpatient and outpatient files. In Taiwan, insured patients with major diseases can apply for a catastrophic illness certificate that grants exemption from co-payment. The issuance of catastrophic illness certificates for RA is reviewed by an expert rheumatologist, based on the latest ACR-EULAR criteria. This dataset had very good validity and positive predictive value.[2]

The criteria for the MTX user group were RA patients who were new users of MTX treatment, and the follow-up period started at 180 days after the initial MTX use day. The MTX non-user group included RA patients not undergoing MTX treatment. We selected a random day after RA diagnosis and began counting the follow-up period 180 days after this random day. We excluded RA patients with a history of cancer before the follow-up began. The main outcome of this study was the presence or absence of cancer development (ICD-9-CM 140-208 with a catastrophic illness card). Follow-up stopped when the individual was withdrawn from the health insurance system, at the occurrence of cancer, or on 31 December 2011.

The effect of the MTX cumulative dose was calculated to evaluate the cancer risk. The cumulative defined daily dose (cDDD), the gold standard for international drug utilization research, was defined by The Anatomical Therapeutic Chemical

(ATC) classification and was used in many studies.[20–22] To standardize the MTX dosage, we used The ATC system to transfer the drug dose as the defined daily dose (DDD). The ATC code for MTX is L04AX03. The MTX exposure group included patients who were using MTX for the first time. In contrast, the non-users of MTX group included patients who never used MTX during the study period. We used the World Health Organization (WHO) DDD as a tool to assess the drug exposure. There were three groups according to the cumulative dose of MTX. That is, the MTX low accumulative dose group (cumulative dose <1125 mg, cDDD <450), MTX middle-dose users (cumulative dose 1125–2250 mg, cDDD: 450–899) and the MTX high-dose group (cumulative dose $\geqslant 2250$ mg, cDDD $\geqslant 900$). The rationale of the cut-off by 1125 and 2250 mg was based on previous literature.[23]

Comparison population

In order to eliminate the potential for confounding by indication, we attempted to minimize it with the use of propensity score 1:1 matching. The propensity score was calculated using logistic regression to estimate the probability of MTX usage, based on the baseline variables including age, gender, comorbidities, glucocorticoids, and biologics.

Outcome and relevant variables

The main outcome was diagnosis of new-onset cancer of any type during the follow-up period. The confounding factors of the study were age, sex, cancer-related comorbidities, and RA-combined medication. The history of cancer-related comorbidities was defined as the individual being diagnosed with the comorbidity before the follow-up date. Cancer-related comorbidities included hypertension (ICD-9-CM 401–405), diabetes mellitus (DM, ICD-9-CM 250), hyperlipidemia (ICD-9-CM 272), alcohol-related disorder (ALD, ICD-9-CM 291, 303, 305, 571.0, 571.1, 571.2, 571.3, 790.3, and V11.3), coronary artery disease (CAD, ICD-9-CM 410–414), *Helicobacter pylori* (HP, ICD-9-CM 041.86), hepatitis B virus (HBV, ICD-9-CM V02.61, 070.20-070.33), hepatitis C virus (HCV, ICD-9-CM V02.62, 070.41, 070.44, 070.51, 070.54, 070.70, 070.71), chronic kidney disease (CKD, ICD-9-CM 585), and chronic obstructive pulmonary disease (COPD, ICD-9-CM 491, 492, and 496). The RA-combined medication included TNF-α inhibitors (adalimumab and etanercept), rituximab, other conventional DMARDS (cDMARDs), including cyclosporine, hydroxychloroquine, leflunomide, and sulfasalazine, and corticosteroids. Other RA medication drugs, including humanized IL-6 receptor antibody (tocilizumab), selective T-cell co-stimulatory modulator (abatacept), and golimumab, were not covered by the NHI before 2012. Combined medication users were defined as individuals undergoing drug treatment during the follow-up period.

Statistical analysis

To describe the distribution of the study population, we presented the means and standard deviations for age and number, as well as percentages for sex, cancer-related comorbidities, and RA-combined medication. To compare the distribution difference between the MTX user and non-user groups, we used a *t*-test for age and chi-square test for sex, comorbidity, and medication. The incidence density for developing cancer was calculated for the MTX user and non-user groups. We also measured the cumulative incidence curves of the MTX user and non-user groups using the Kaplan–Meier method, and tested the curve differences with the log-rank test. To present the risk of cancer in RA patients with and without MTX use, the hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using single-variable and multivariable Cox proportional hazard models. The data management and statistical analyses were implemented in SAS 9.4 software (SAS Institute, Cary, NC, USA), and the incidence curve was plotted by R software (R Foundation for Statistical Computing, Vienna, Austria). The significance level was set at $p<0.05$ for two-sided testing of the *p*-value.

Results

This study enrolled 21,699 patients who had been newly diagnosed with RA in 2000–2009. Among them, 10,352 patients were classified into the MTX user group and 11,347 into the MTX non-user group (Table 1). The MTX users were generally younger than the non-users (mean age: 52.0 *versus* 56.7 years, $p<0.0001$), and the proportion of males among the MTX users was lower than among the non-users (21.6% *versus* 23.2%, $p=0.0043$). The cancer-related comorbidities in the non-user group were significantly greater than in the MTX user group ($p<0.0001$).

Table 2 shows the incidence of cancer and HR for MTX users and non-users. In the non-user group,

Table 1. Comparison of incidence densities of cancers hazard ratio between non-MTX and MTX by demographic characteristics and comorbidity.

	RA group						Crude HR (95% CI)	Adjusted HR (95% CI)
	MTX non-users *n* = 11,347		(%)	MTX users *n* = 10,352		(%)		
	Event	PY	Rate	Event	PY	Rate		
Age, years								
<50	42	11,854	35.4	64	24,531	26.1	0.71 (0.48, 1.05)	0.71 (0.47, 1.08)
50–64	130	12,254	106.1	149	20,352	73.2	0.68 (0.54, 0.87)	0.80 (0.62, 1.03)
⩾65	176	9404	187.2	139	8755	158.8	0.84 (0.67, 1.06)	0.91 (0.72, 1.15)
Sex								
Female	243	26,024	93.4	234	42,499	55.1	0.59 (0.49, 0.71)	083 (0.68, 1.00)
Male	105	7489	140.2	118	11,139	105.9	0.77 (0.59, 1.01)	0.95 (0.71, 1.26)
Comorbidity								
ALD								
No	335	32,857	102.0	347	52,952	65.5	0.65 (0.55, 0.75)***	0.87 (0.74, 1.02)
Yes	13	656	198.1	5	686	72.9	0.39 (0.14, 1.11)	1.61 (0.43, 6.02)
COPD								
No	245	26,679	91.8	273	46,085	59.2	0.65 (0.54, 0.77)***	0.85 (0.71, 1.03)
Yes	103	6834	150.7	79	7552	104.6	0.69 (0.51, 0.94)*	0.90 (0.66, 1.24)
DM								
No	280	29,049	96.4	295	48,387	61.0	0.63 (0.53, 0.75)***	0.86 (0.72, 1.03)
Yes	68	4464	152.3	57	5251	108.6	0.74 (0.51, 1.05)	0.92 (0.63, 1.35)
Hyperlipidemia								
No	227	24,384	93.1	238	41,443	57.4	0.61 (0.51, 0.74)***	0.84 (0.69, 1.03)
Yes	121	9129	132.5	114	12,194	93.5	0.72 (0.55, 0.93)*	0.91 (0.69, 1.20)
Hypertension								
No	165	20,037	82.4	178	38,012	46.8	0.56 (0.45, 0.70)***	0.71 (0.57, 0.89)**
Yes	183	13,477	135.8	174	15,626	111.4	0.82 (0.67, 1.02)	1.05 (0.84, 1.31)
CAD								
No	232	25,740	90.1	246	44,594	55.2	0.61 (0.51, 0.73)***	0.80 (0.66, 0.98)*
Yes	116	7773	149.2	106	9043	117.2	0.80 (0.61, 1.04)	0.99 (0.74, 1.32)
Helicobacter pylori								
No	348	33,397	104.2	351	53,519	65.6	0.63 (0.54, 0.74)***	0.87 (0.74, 1.03)
Yes	0	117	0.00	1	119	84.2	–	–

(Continued)

Table 1. (Continued)

	RA group						Crude HR (95% CI)	Adjusted HR (95% CI)
	MTX non-users *n* = 11,347		(%)	MTX users *n* = 10,352		(%)		
	Event	PY	Rate	Event	PY	Rate		
HBV								
No	321	31,852	100.8	335	52,105	64.3	0.64 (0.55, 0.75)***	0.88 (0.74, 1.04)
Yes	27	1662	162.5	17	1532	111.0	0.66 (0.35, 1.23)	0.79 (0.40, 1.54)
HCV								
No	316	32,132	98.3	336	52,528	64.0	0.65 (0.56, 0.76)***	0.90 (0.76, 1.06)
Yes	32	1381	231.7	16	1110	144.2	0.67 (0.36, 1.25)	0.76 (0.40, 1.44)
CKD								
No	314	31,654	99.2	321	51,473	62.4	0.63 (0.54, 0.74)***	0.86 (0.73, 1.02)
Yes	34	1859	182.9	31	2165	143.2	0.81 (0.49, 1.34)	0.97 (0.57, 1.66)
Combined medication								
TNF-α								
No	336	31,007	108.4	300	40,293	74.5	0.70 (0.60, 0.82)***	0.86 (0.73, 1.02)
Yes	12	2506	47.9	52	13,345	39.0	0.72 (0.38, 1.36)	0.80 (0.42, 1.52)
Rituximab								
No	344	33,234	103.5	345	51,942	66.4	0.65 (0.56, 0.76)***	0.88 (0.75, 1.03)
Yes	4	280	143.0	7	1695	41.3	0.22 (0.06, 0.80)*	0.08 (0.01, 0.48)**
Other nbDMARDs								
No	111	9189	120.8	39	4814	81.0	0.70 (0.49, 1.02)	0.88 (0.60, 1.31)
Yes	237	24,324	97.4	313	48,823	64.1	0.65 (0.55, 0.77)***	0.86 (0.72, 1.03)
Corticosteroid								
No	348	33,461	104.0	336	49,365	68.1	0.66 (0.57, 0.77)***	0.88 (0.75, 1.03)
Yes	0	53	0.00	16	4273	37.5	–	–

Model adjusted for age, sex, ALD, COPD, DM and hyperlipidemia, hypertension, CAD, *Helicobacter pylori*, HBV, HCV, CKD, TNF-α, Rituximab, other cDMARDs, and corticosteroid.
*$p<0.05$; **$p<0.01$; ***$p<0.001$
ALD, alcohol-related disorder; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; PYs, person-years; Rate, incidence rate, per 10,000 person-years.

the cancer incidence was 104 per 10,000 person-years, compared with only 65.6 per 10,000 person-years in the MTX user group. After adjusting for age, sex, cancer-related comorbidities, and RA-combined medication, the adjusted HR (aHR) of cancer risk was 0.88 (95% CI = 0.75–1.04) for the MTX user group compared with the non-user group. For other cDMARDs, hydroxychloroquine and leflunomide users demonstrated lower incidences of cancers than non-users. The HRs of cancer risk were 0.81 (95% CI = 0.68–0.96) and 0.62 (95% CI = 0.48–0.81), respectively. In addition, the TNF-α inhibitors (adalimumab and etanercept) users of RA patients also exhibited lower incidences of cancers than non-users. The HR of cancer risk was 0.47 (95% CI = 0.29–0.77) and 0.66 (95%

Table 2. Incidence of cancers and multivariate Cox proportional hazards regression analysis measured hazard ratio in patients with rheumatoid arthritis.

Variable	Event	PYs	Rate	Crude HR (95% CI)	Adjusted HR (95% CI)
Age group					
<50	55	24,991	22.0	ref	ref
50–64	330	44,000	75.0	3.41 (2.56–4.53)	3.17 (2.37–4.24)
⩾65	315	18,159	173	7.89 (5.92–10.5)	6.12 (4.48–8.36)
Sex					
Female	477	68,523	69.6	ref	ref
Male	223	18,628	120	1.72 (1.46–2.01)	1.60 (1.36–1.88)
ALD					
No	682	85,809	79.5	ref	Ref
Yes	18	1342	134	1.67 (1.04–2.66)	1.29 (0.80–2.08)
COPD					
No	518	72,764	71.2	ref	Ref
Yes	182	14,387	127	1.76 (1.49–2.09)	1.04 (0.87–1.25)
DM					
No	575	77,436	74.3	ref	Ref
Yes	125	9715	129	1.72 (1.42–2.09)	1.14 (0.93–1.40)
Hyperlipidemia					
No	465	65,827	70.6	ref	Ref
Yes	235	21,323	110	1.55 (1.32–1.81)	1.02 (0.86–1.21)
Hypertension					
No	343	58,049	59.1	ref	Ref
Yes	357	29,102	123	2.06 (1.78–2.39)	1.09 (0.91–1.30)
CAD					
No	478	70,334	68.0	ref	Ref
Yes	222	16,817	132	1.93 (1.65–2.26)	1.11 (0.92–1.33)
Helicobacter pylori					
No	699	86,915	80.4	ref	Ref
Yes	1	235	42.5	0.51 (0.07, 3.64)	0.37 (0.05–2.62)
HBV					
No	656	83,957	78.1	ref	Ref
Yes	44	3193	137.8	1.74 (1.28, 2.36)	1.54 (1.12–2.11)
HCV					
No	652	84,660	77.0	ref	Ref
Yes	48	2491	192.7	2.47 (1.84, 3.32)	1.79 (1.33–2.43)

(Continued)

Table 2. (Continued)

Variable	Event	PYs	Rate	Crude HR (95% CI)	Adjusted HR (95% CI)
CKD					
No	635	83,127	76.4	ref	Ref
Yes	65	4024	161.5	2.11 (1.63, 2.72)	1.25 (0.96–1.63)
MTX					
No	348	33,513	104	ref	ref
Yes	352	53,638	65.6	0.64 (0.55–0.74)	0.88 (0.75, 1.04)
Other nbDMARDs					
Cyclosporin					
No	611	72,565	84.2	ref	ref
Yes	89	14,586	61.0	0.73 (0.59–0.92)	1.15 (0.90–1.46)
Hydroxychloroquine					
No	521	60,044	86.8	ref	ref
Yes	179	27,107	66.0	0.77 (0.65–0.91)	0.81 (0.68–0.96)
Leflunomide					
No	633	72,123	87.8	ref	ref
Yes	67	15,027	44.6	0.51 (0.40–0.66)	0.62 (0.48–0.81)
Sulfasalazine					
No	339	39,904	85.0	ref	ref
Yes	361	47,247	76.4	0.91 (0.78–1.05)	1.02 (0.88, 1.19)
TNF-α					
Adalimumab					
No	683	81,434	83.9	ref	ref
Yes	17	5717	29.7	0.36 (0.22–0.58)	0.47 (0.29–0.77)
Etanercept					
No	650	75,571	86.0	ref	ref
Yes	50	11,580	43.2	0.51 (0.38–0.68)	0.66 (0.48–0.90)
Rituximab					
No	689	85,176	80.89	ref	Ref
Yes	11	1975	55.7	0.69 (0.38–1.26)	1.44 (0.76–2.71)
Corticosteroid					
No	684	82,825	82.6	ref	ref
Yes	16	4325	37.0	0.46 (0.28, 0.75)	0.62 (0.38–1.03)

Model adjusted for age, sex, ALD, COPD, DM and hyperlipidemia, hypertension, CAD, *Helicobacter pylori*, HBV, HCV, CKD, MTX, adalimumab, etanercept, rituximab, cyclosporin, hydroxychloroquine, leflunomide, sulfasalazine, and corticosteroid. ALD, alcohol-related disorder; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; PYs, person-years; Rate, incidence rate, per 10,000 person-years.

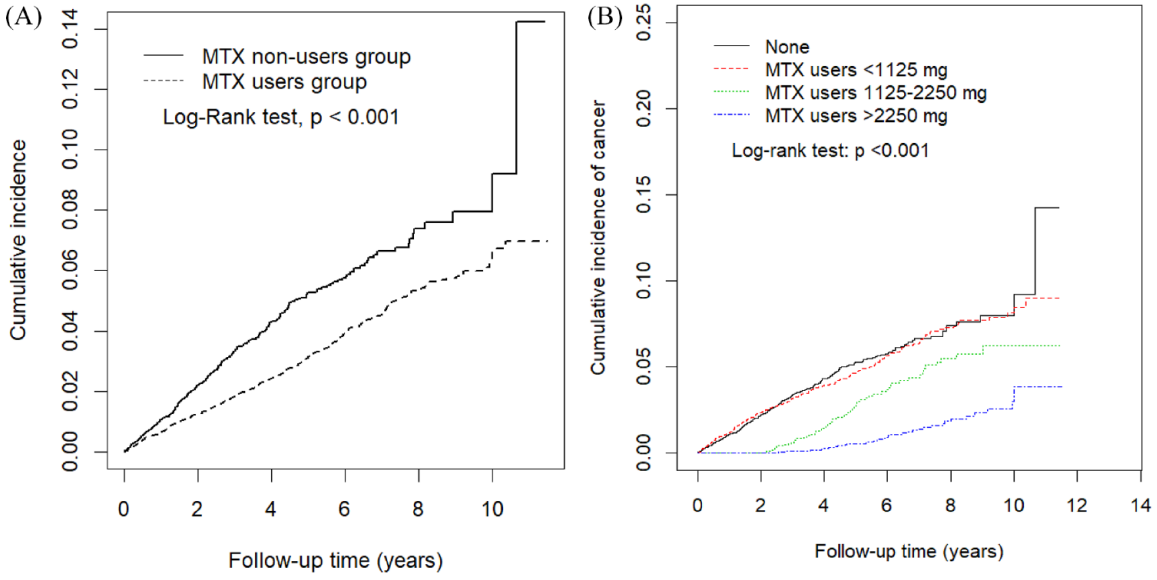

Figure 1. (A) The cumulative incidence of cancer for MTX users and non-users. (B) The cumulative incidence of cancer among different cumulative defined daily dose (cDDD) of methotrexate.

CI = 0.48–0.91) for the users compared with the non-users. Figure 1A indicates that the cancer cumulative curve for the MTX user group was significantly lower than that of the non-users group (log-rank test, $p < 0.001$). Figure 1B demonstrates the cumulative incidence of cancer among different cDDD of MTX. After 2 years' follow-up time, MTX at middle and high accumulative doses showed a significant difference between RA patients with low accumulative dose and without MTX usage.

Table 3 displays the risk of cancer for RA patients at different cumulative doses of MTX after propensity score matching. There were 7809 RA patients engaged in MTX use and 7809 RA patients belonging to the non-user group. After adjusting for age, sex, cancer-related comorbidities, and RA-combined medication, no difference existed in the cancer risk between non-users and the MTX user group (the aHR of cancer risk was 0.85 with 95% CI = 0.71–1.02). At different accumulative doses of MTX, the cancer risk in RA was different: with high accumulative doses of 1125–2250 mg the aHR of cancer risk was 0.63 with 95% CI = 0.46–0.86, and at accumulative doses ⩾2250 mg there was a lower risk than among non-users (the aHR of cancer risk was 0.28 with 95% CI = 0.18–0.44) (p for trend < 0.0001).

Supplemental Table 1 shows the subgroup analysis of the Cox proportional hazard model, comparing MTX users and non-users in patients with RA. These comparisons of incidence densities of cancers and the HR between non-MTX and MTX users were made by different subgroup demographic characteristics, comorbidities, and RA-combined medications. In the sex-subgroup analysis, both genders showed a non-significant difference in risk of developing cancers between MTX users and non-users. Data in supplemental Table 1 demonstrate that MTX has no synergistic effect with TNF blockers in cancer risk reduction in the RA population (aHR = 0.80, 95% CI = 0.42–1.52).

Supplemental Table 2 shows the comparison of the incidence and HR of sub-division cancer according to MTX status among RA patients. The aHR of lymphoma risk for MTX users was 1.22 (95% CI = 0.27–5.59) compared with the MTX-non-users group, whereas aHR for non-lymphoma cancers was 0.90 (95% CI = 0.77–1.06). Furthermore, hepatocellular carcinoma (HCC) had a lower incidence in MTX users than non-users among RA patients (aHR = 0.57, 95% CI = 0.34–0.95).

Supplemental Table 3 presented the Cox model to analyze sub-division cancer according to gender, based on MTX status among RA patients. Given a difference in cancer risks between males and females, we undertook a stratified analysis in

Table 3. Incidence of cancer and multivariate Cox proportional hazards regression analysis measured hazard ratio for study cohort by different cumulative dose and propensity scores matched.

Cumulative dose (cDDD)	*n*	Event	PYs	Rate	Crude HR (95% CI)	Adjusted HR (95% CI)
None	7809	207	23,826	86.9	ref	ref
All	7809	300	39,249	76.4	0.88 (0.73, 1.05)	0.85 (0.71, 1.02)
<1125 mg (450)	4655	230	21,236	108.3	1.24 (1.02, 1.50)	1.14 (0.94, 1.38)
1125–2250 mg (450–899)	1743	48	8394	57.2	0.66 (0.48, 0.90)	0.63 (0.46, 0.86)
⩾2250 mg (900)	1411	22	9619	22.9	0.26 (0.17, 0.40)	0.28 (0.18, 0.44)
p for trend					<0.0001	<0.0001

Model adjusted for age, sex, ALD, COPD, DM and hyperlipidemia, hypertension, CAD, *Helicobacter pylori*, HBV, HCV, CKD, TNF-α, Rituximab, other cDMARDs, and corticosteroid.
ALD, alcohol-related disorder; CAD, coronary artery disease; cDDD, cumulative defined daily dose by The Anatomical Therapeutic Chemical (ATC) classification, the gold standard for international drug utilization research; cDMARDs, non-biological disease-modifying anti-rheumatic drugs; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; PYs, person-years; Rate, incidence rate, per 10,000 person-years.

Supplemental Table 3. MTX use was associated with a lower risk of colorectal cancer than MTX non-users in females (aHR = 0.54, 95% CI = 0.30–0.96), whereas MTX use was associated with a lower risk of HCC than MTX non-use in males (aHR = 0.35, 95% CI = 0.13–0.91).

Discussion

In this study, we demonstrated cancer risk reduction effects of high and middle doses of MTX in patients with RA. MTX is neutral in terms of cancer risk in low accumulative doses, and might be protective in high accumulative doses. MTX also showed a dose-dependent effect in cancer risk reduction of the RA population. Further stratified analysis by sex revealed MTX use was associated with a lower risk of colorectal cancer than MTX non-use in females, whereas MTX use was associated with a lower risk of HCC than MTX non-use in males.

Several previous studies have mentioned the association between MTX and an increased risk of lymphoma.[23–27] Usman *et al.* describe two cases and reviewed 16 more patients who developed non-Hodgkin's lymphoma during treatment with low-dose MTX for RA. Among these 18 patients, the mean RA duration was 16 years, and lymphoma developed after a mean of 2.8 years of treatment with MTX. The mean total dose of MTX was 1224 mg.[23] Saleh *et al.* described a case report which focused on clinical-pathologic correlation and lacked recording of dose of MTX.[24] Hazleman's study showed a MXT dose of 5–20 mg per week for 3–12 months was required to achieve maximal effects, but less than 50% of patients can tolerate it for 1 year due to its frequent adverse effect.[25] Generally, the cumulative dose was about 360–960 mg a year. Rizzi *et al.* identified 26 patients in the literature who achieved spontaneous complete remission of their lymphoproliferative disorders, and eight others showing partial remission. Most were affected by RA, received low-dose MTX, and developed lymphoma. They focused on reversible lymphoproliferative disorders in MXT users.[26] In a more recent large study, Hellgren *et al.* collected data from the Swedish Rheumatology Quality Register, and this study of 12,656 RA patients found no increase in lymphoma risk among MXT users.[27] In our study, a 12-year, large-scale, population-based cohort study, Table 3 showed the dose-dependent association of MTX with cancer risk. The risk of cancer in RA patients with or without treatment with MTX demonstrated no statistically significant differences in terms of lymphoma (aHR = 1.22, 95% CI = 0.27–5.59). The major strength of our study was to demonstrate the dose-dependent effect of MTX, which had not been clearly shown in previous studies. We found that MTX at a higher dose of more than 1125 mg is protective.

Several explanations exist for the anticancer mechanisms of MTX in patients with RA. First, MTX is a structural analog of folic acid that inhibits dihydrofolate reductase, a key enzyme in cell

replication.[28] MTX was one of the earliest cancer chemotherapy agents, having been used for the treatment of leukemia and other tumors for more than 60 years. Second, cancer is an inflammation-related disease; therefore, the anti-inflammation effect of MTX probably contributes to its anticancer effect, and may also reduce cardiovascular disease in some patients with RA.[29] Recently published findings also suggest that MTX affects glyoxalase and antioxidant systems,[30] and decreases vascular endothelial growth factor levels, in breast cancer patients.[31] For lower risk of colorectal cancer than MTX non-users in female (aHR = 0.54, 95% CI = 0.30–0.96) in our preliminary real-world data, we proposed that MTX might have antimetabolite and anti-inflammatory effects to reduce colorectal cancer rate.[32,33] However, there has been no scientific or animal model research to verify our finding currently. The specific mechanism of sex difference on this effect remains unclear and still requires to be answered in future studies.

The strengths of our study are its population-based design; the generalizability of its findings; and the 12 years of follow-up in the national insurance database, which constitutes a highly representative sample of Taiwan's general population because the reimbursement policy is universal and operated solely by the Taiwanese government. This study cohort was representative of the general population, and the possible confounding factors were minimized through propensity score matching with age, gender, and medical comorbidities in both cohorts. Further, the diagnoses of RA and cancer in this database were reviewed by expert rheumatologists to confirm their accuracy. For case ascertainment, RA and cancer were obtained from the Registry of Catastrophic Illness Database in Taiwan. Therefore, the diagnosis of RA and cancer in our study population can be considered reliable. We also performed a subgroup analysis to demonstrate the effects on different subpopulations.

This study had several limitations. First, the NHIRD provides no detailed information on patients regarding their lifestyle, smoking, alcohol ingestion, RA disease severity, or family history, all of which are possible confounding factors. However, we tried to use ALDs and COPD as proxies for tobacco, alcohol, and environmental factors. To mitigate this problem, we only selected patients in the catastrophic registry to ensure accurate diagnosis and comparable severity. Besides, many studies using the NHI research databases have been published in high-impact journals such as *JAMA Oncology*, *JAMA Internal Medicine*, and *Lancet Oncology*, and so forth.[34–36] Second, the evidence derived from a cohort study is generally of lower methodological quality than that from randomized trials, because of the necessary adjustments for confounding factors. Though a population-based prospective cohort study serves best to analyze the risk factors, a retrospective population-based cohort study using insurance data is a suitable alternative. Indeed, such limitations are natural in NHIRD, but NHIRD is suitable for epidemiological and medical research,[34–36] and we conducted a sensitivity analysis by propensity score matching to check the robustness of our findings. Third, the high risk of confounding by indication is a major limitation. It is well known that MTX users are likely to be different from non-users. Hence, we used propensity score matching for possible confounders. Different sensitivity tests and subgroup analyses, including dose effects, were also used to minimize this bias. Fourth, although we matched or adjusted possible confounders, there still may have been residual confounders, especially confounding by indication, in this study.[37] For example, MTX use is associated with a lower risk of colorectal cancer than MTX non-use in females, whereas MTX use is associated with a lower risk of HCC than MTX non-use in males; this might be confounded by indication, that is, physicians might avoid MTX usage in chronic hepatitis patients due to fear of MTX liver toxicity, thus leading to unbalanced baseline comparability. To minimize this bias, we had matched the coding of chronic hepatitis. Nevertheless, some uncoded or undiagnosed patients might still exist.

In conclusion, we demonstrated that MTX at middle and high accumulative doses might be associated with lower risk of new-onset cancers in patients with RA.

Authors' contributions

Study conception and design: Perng, Hung, Wei, Kao

Acquisition of data: Kao, Lin

Analysis and interpretation of data: Perng, Hung, Chang, Lin, Chiou, Chen, Kao, Wei

Validation: Hung, Kao, Wei

Writing (original draft preparation): Perng, Hung

Writing (review and editing): Kao, Wei, Hung

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported in part by the Taiwan Ministry of Health and Welfare Clinical Trial and Research Center of Excellence (MOHW104-TDU-B-212-113002)

Conflict of interest statement

The authors declare that there is no conflict of interest.

ORCID iDs

Yao-Min Hung https://orcid.org/0000-0001-6920-8799

Chia-Hung Kao https://orcid.org/0000-0002-6368-3676

James Cheng-Chung Wei https://orcid.org/0000-0002-1235-0679

Supplemental material

Supplemental material for this article is available online.

References

1. Norton S, Koduri G, Nikiphorou E, *et al.* A study of baseline prevalence and cumulative incidence of comorbidity and extra-articular manifestations in RA and their impact on outcome. *Rheumatology (Oxford)* 2013; 52: 99–110.
2. Chen YJ, Chang YT, Wang CB, *et al.* The risk of cancer in patients with rheumatoid arthritis: a nationwide cohort study in Taiwan. *Arthritis Rheum* 2011; 63: 352–358.
3. Yamada T, Nakajima A, Inoue E, *et al.* Incidence of malignancy in Japanese patients with rheumatoid arthritis. *Rheumatol Int* 2011; 31: 1487–1492.
4. Kim SC, Glynn RJ, Giovannucci E, *et al.* Risk of high-grade cervical dysplasia and cervical cancer in women with systemic inflammatory diseases: a population-based cohort study. *Ann Rheum Dis* 2015; 74: 1360–1367.
5. Lim XR, Xiang W, Tan JWL, *et al.*; TTSH Rheumatoid Arthritis Study Group. Incidence and patterns of malignancies in a multi-ethnic cohort of rheumatoid arthritis patients. *Int J Rheum Dis*. Epub ahead of print 11 July 2019. DOI: 10.1111/1756-185X.13655.
6. Smitten AL, Simon TA, Hochberg MC, *et al.* A meta-analysis of the incidence of malignancy in adult patients with rheumatoid arthritis. *Arthritis Res Ther* 2008; 10: R45.
7. Simon TA, Thompson A, Gandhi KK, *et al.* Incidence of malignancy in adult patients with rheumatoid arthritis: a meta-analysis. *Arthritis Res Ther* 2015; 17: 212.
8. Kim YJ, Shim JS, Choi CB, *et al.* Mortality and incidence of malignancy in Korean patients with rheumatoid arthritis. *J Rheumatol* 2012; 39: 226–232.
9. Jin S, Li M, Fang Y, *et al.*; CREDIT Co-authors. Chinese registry of rheumatoid arthritis (CREDIT): II. Prevalence and risk factors of major comorbidities in Chinese patients with rheumatoid arthritis. *Arthritis Res Ther* 2017; 19: 251.
10. Wang H-L, Zhou Y-M, Zhu G-Z, *et al.* Malignancy as a comorbidity in rheumatic diseases: a retrospective hospital-based study. *Clin Rheumatol* 2018; 37: 81–85.
11. Damjanov N, Nurmohamed MT and Szekanecz Z. Biologics, cardiovascular effects and cancer. *BMC Med* 2014; 12: 48.
12. Baecklund E, Iliadou A, Askling J, *et al.* Association of chronic inflammation, not its treatment, with increased lymphoma risk in rheumatoid arthritis. *Arthritis Rheum* 2006; 54: 692–701.
13. Favalli EG, Biggioggero M and Meroni PL. Methotrexate for the treatment of rheumatoid arthritis in the biologic era: still an "anchor" drug? *Autoimmun Rev* 2014; 13: 1102–1108.
14. Bologna C, Picot MC, Jorgensen C, *et al.* Study of eight cases of cancer in 426 rheumatoid arthritis patients treated with methotrexate. *Ann Rheum Dis* 1997; 56: 97–102.
15. Salloum E, Cooper DL, Howe G, *et al.* Spontaneous regression of lymphoproliferative disorders in patients treated with methotrexate for rheumatoid arthritis and other rheumatic diseases. *J Clin Oncol* 1996; 14: 1943–1949.
16. Georgescu L, Quinn GC, Schwartzman S, *et al.* Lymphoma in patients with rheumatoid arthritis: association with the disease state or methotrexate treatment. *Semin Arthritis Rheum* 1997; 26: 794–804.
17. Bachman TR, Sawitzke AD, Perkins SL, *et al.* Methotrexate-associated lymphoma in patients with rheumatoid arthritis: report of two cases. *Arthritis Rheum* 1996; 39: 325–329.

18. Mariette X, Cazals-Hatem D, Warszawki J, *et al.* Lymphomas in rheumatoid arthritis patients treated with methotrexate: a 3-year prospective study in France. *Blood* 2002; 99: 3909–3915.

19. Buchbinder R, Barber M, Heuzenroeder L, *et al.* Incidence of melanoma and other malignancies among rheumatoid arthritis patients treated with methotrexate. *Arthritis Rheum* 2008; 59: 794–799.

20. Chan V and Tett SE. Changes in use of disease-modifying anti-rheumatic drugs in Australia over the period 1992-2004. *Pharmacoepidemiol Drug Saf* 2006; 15: 462–468.

21. Blomqvist P, Feltelius N, Ekbom A, *et al.* Rheumatoid arthritis in Sweden. Drug prescriptions, costs, and adverse drug reactions. *J Rheumatol* 2000; 27: 1171–1177.

22. Donges E, Staatz CE, Benham H, *et al.* Patterns in use and costs of conventional and biologic disease-modifying anti-rheumatic drugs in Australia. *Clin Exp Rheumatol* 2017; 35: 907–912.

23. Usman AR and Yunus MB. Non-Hodgkin's lymphoma in patients with rheumatoid arthritis treated with low dose methotrexate. *J Rheumatol* 1996; 23: 1095–1097.

24. Saleh JZ, Lee LH, Schieke SM, *et al.* Methotrexate-induced CD30^{+} T-cell lymphoproliferative disorder of the oral cavity. *JAAD Case Rep* 2016; 2: 354–356.

25. Hazleman B. Incidence of neoplasms in patients with rheumatoid arthritis exposed to different treatment regimens. *Am J Med* 1985; 78: 39–43.

26. Rizzi R, Curci P, Delia M, *et al.* Spontaneous remission of "methotrexate-associated lymphoproliferative disorders" after discontinuation of immunosuppressive treatment for autoimmune disease. Review of the literature. *Med Oncol* 2009; 26: 1–9.

27. Hellgren K, Baecklund E, Backlin C, *et al.* Rheumatoid arthritis and risk of malignant lymphoma: is the risk still increased? *Arthritis Rheumatol* 2017; 69: 700–708.

28. Huennekens FM. The methotrexate story: a paradigm for development of cancer chemotherapeutic agents. *Adv Enzyme Regul* 1994; 34: 397–419.

29. Landewe RB, van den Borne BE, Breedveld FC, *et al.* Methotrexate effects in patients with rheumatoid arthritis with cardiovascular comorbidity. *Lancet* 2000; 355: 1616–1617.

30. Neradil J, Pavlasova G and Veselska R. New mechanisms for an old drug; DHFR- and non-DHFR-mediated effects of methotrexate in cancer cells. *Klin Onkol* 2012; 25(Suppl. 2): 2S87–2S92.

31. Colleoni M, Rocca A, Sandri MT, *et al.* Low-dose oral methotrexate and cyclophosphamide in metastatic breast cancer: antitumor activity and correlation with vascular endothelial growth factor levels. *Ann Oncol* 2002; 13: 73–80.

32. Rubin DT, Huo D, Kinnucan JA, *et al.* Inflammation is an independent risk factor for colonic neoplasia in patients with ulcerative colitis: a case-control study. *Clin Gastroenterol Hepatol* 2013; 11: 1601–1608.e1-e4.

33. Gridley G, McLaughlin JK, Ekbom A, *et al.* Incidence of cancer among patients with rheumatoid arthritis. *J Natl Cancer Inst* 1993; 85: 307–311.

34. Chien HC, Kao YH and Bai JP. Trastuzumab-related cardiotoxic effects in Taiwanese women: a nationwide cohort study. *JAMA Oncol* 2016; 2: 1317–1325.

35. Lee CC, Lee MT, Chen YS, *et al.* Risk of aortic dissection and aortic aneurysm in patients taking oral fluoroquinolone. *JAMA Intern Med* 2015; 175: 1839–1847.

36. Lin JN, Lin CL, Lin MC, *et al.* Risk of leukaemia in children infected with enterovirus: a nationwide, retrospective, population-based, Taiwanese-registry, cohort study. *Lancet Oncol* 2015; 16: 1335–1343.

37. Benedetto U, Head SJ, Angelini GD, *et al.* Statistical primer: propensity score matching and its alternatives. *Eur J Cardiothorac Sur* 2018; 53: 1112–1117.