INVESTIGATIVE REPORT

Methotrexate Exposure and Risk of Cutaneous Malignant Melanoma: No Evidence of a Dose-response Relationship

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Methotrexate treatment has been linked with an increased risk of melanoma. However, a possible dose-response relationship with respect to methotrexate exposure and melanoma has not been addressed. The aim of the present study was to investigate whether higher accumulated doses of methotrexate correlate with an increased risk of melanoma, which would further support a possible association. A nationwide retrospective cohort study was conducted. All Swedish patients over 18 years of age who were dispensed methotrexate in the period 2005 to 2014 were registered ($n = 101,966$) and matched to the cancer registry. A Cox proportional hazards model, testing risk of melanoma vs. total accumulated methotrexate dose, controlled for sex, age group, and time from first to last dispensed prescription of methotrexate, yielded no significant risk dependence on dose, and a hazard ratio of 1.02 (95% CI 0.97–1.08). Overall, no conclusive dose-response relationship was observed between methotrexate exposure and risk of melanoma.

Key words: methotrexate; cutaneous malignant melanoma; dose-response; incidence; risk; exposure.

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SIGNIFICANCE

Methotrexate is a commonly prescribed drug used in autoimmune and inflammatory diseases, such as rheumatoid arthritis and psoriasis. However, it has been linked with an increased risk of melanoma. This retrospective registry-based nationwide cohort study, including all Swedish patients over 18 years of age in the period 2005 to 2014, found no increase in risk of melanoma related to higher accumulated doses of methotrexate. The absence of a dose-response relationship casts doubt on a possible association between methotrexate and the risk of melanoma, which is reassuring to physicians in everyday clinical practice.

Methotrexate (MTX) was first developed in the 1940s (1). It is an antimetabolite and antineoplastic drug with immunosuppressive properties (2) that acts as an antagonist of folic acid. MTX is used in a range of inflammatory diseases, such as psoriasis (Pso), psoriatic arthritis (PsoA) and rheumatoid arthritis (RA).

Unsurprisingly, an immunosuppressive drug such as MTX, which has been used clinically for decades, has been under scrutiny regarding risk of development of cancer. Several studies have been performed regarding exposure to MTX and risk of malignancies in patients with Pso; however, no increase in risk of cutaneous malignant melanoma (CMM) has been reported (3–5). Moreover, patients with gestational trophoblastic tumours treated with MTX and folic acid as a single chemotherapeutic regimen were not found to have an increased risk of CMM (6). In an Australian cohort consisting of patients with RA treated with MTX, Buchbinder et al. (7) demonstrated a 3-fold increase in risk of CMM; however, the accumulated dose was not taken into consideration.

In a previous study, we observed a small, but significant, increase in risk of CMM in patients treated with MTX compared with MTX-unexposed, sex- and age-matched subjects (8). However, the model considered only whether patients had ever been exposed to MTX (including trivial exposure) and did not take into account the accumulated dose. To further investigate a potential association between MTX exposure and CMM, the aim of the present study was to determine whether there was a dose-response relationship between accumulated MTX dose and CMM.

METHODS

Design overview

The raw data analysed and methods in this study used have been described previously (8). A nationwide retrospective registry-based cohort study was conducted. Data collection was approved by the regional ethics board (approval number 461-15).

Databases, study participants and exposure

Data were obtained from the Swedish prescribed drug register (9) for all patients in Sweden over 18 years of age who were dispensed a prescription of MTX (ATC codes: L04AX03 and/or L01BA01) from Swedish pharmacies in the period 1 August 2005 to 31 December 2014 (MTX-exposed group). Detailed information on all dispensed prescriptions of MTX was available, including route of administration (oral/parenteral) and dose. For the respective MTX-exposed patients, all dispensed MTX prescriptions were calculated, adding up to a total accumulated dose (in g) during the time period studied. Patients with a missing accumulated dose were excluded from the analysis. Only outpatient prescriptions were obtained, since inpatient administration is not included in the

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registry. For each MTX-exposed patient, 5 age- and sex-matched patients who had been dispensed any pharmaceutical drug other than MTX were randomly selected (MTX-unexposed group). The unexposed patients were dispensed their drugs within a period of ±1 month from the date of the first MTX prescription dispensed to the patients in the MTX-exposed group. Data generated were matched to the Swedish cancer registry (10), which has a virtually complete capture rate (11). All history of CMM (invasive and *in situ* melanomas) was obtained from the start of the registry in 1958 until 2014. Data from the Swedish cause of death register (12) were obtained, providing the date and cause of death for diseased individuals in the study time period.

Primary outcome

- To investigate whether, within the MTX-exposed group, there is an increased hazard ratio (HR) for CMM with respect to an increase in the total accumulated MTX dose, controlling for the time from first to last exposure to MTX.

Secondary outcomes

- To compare the CMM incidence rate for the MTX-exposed group, divided into subgroups according to total accumulated dose intervals, with the incidence rate of the entire Swedish population.
- To compare the MTX-exposed patients who had their first MTX prescription in 2005, and therefore had the longest follow-up time, with their MTX-unexposed counterparts with respect to time to CMM in a survival analysis. The analyses were also stratified with respect to total accumulated MTX dose.
- To compare the risk of CMM between the MTX-unexposed group and the MTX-exposed group only dispensed parenteral prescriptions of MTX.
- To compare overall mortality, including all causes of death, between the MTX-exposed and MTX-unexposed groups.

Observation period and censoring

The start dates for the observation period were taken as the dates of the first MTX prescription dispensed in the observation period and the dispensed prescription of the random drug. Patients were censored due to death or end of study period. Data on emigration was not obtained. Patients with a previous history of CMM before the first dispensed prescription were excluded.

Statistical analyses

All data were analysed using R version 3.0.3 (The R Foundation for Statistical Computing, Vienna, Austria).

Primary analysis

- A Cox proportional hazards regression model was used with the time from first observed dispensed MTX prescription to the first CMM as the dependent variable. Only patients in the MTX-exposed group were included in the primary analysis. The independent variables used were: sex, age group at treatment start, total accumulated MTX dose (g), and time from first to last dispensed prescription of MTX during the period 2005 to 2014. This last variable was divided into the following groups: 0 (MTX only dispensed at one time i.e. single day dispensation), >0 to ≤2 years, >2 to ≤4 years, >4 to ≤6 years, >6 to ≤8 years and >8 to ≤10 years. The age groups at treatment start were divided into the following intervals: ≤40, >40 to ≤50, >50 to ≤60, >60 to ≤70 and >70 years). The same analysis was repeated within each subgroup of the above 6 periods between the first and last dispensed prescription of MTX. The HRs and confidence intervals corresponding to a total MTX exposure of 1 g were calculated for each model. Finally, the above analyses were also performed in 2 subanalyses for the MTX-exposed patients who had prescriptions provided exclusively by a rheumatologist or a dermatologist, respectively.

Secondary analyses

- The overall incidence rates of CMM during the period 2005 to 2014 and the corresponding standardized incidence ratios (SIR) (MTX observed/MTX expected) were calculated and Poisson tests were performed. The expected incidences were computed, keeping the sex and age distribution from the MTX-exposed fixed, but assuming the same underlying incidence of CMM as in the Swedish general population. The above analysis was performed within subgroups divided according to total accumulated MTX dose into the following groups: ≤2, >2 to ≤4, >4 to ≤6, >6 to ≤8 and >8 g.

Table I. Demographic characteristics for methotrexate (MTX)-exposed patients

		MTX-exposed, *n* = 101,144 Mean (95% CI)			MTX-unexposed, *n* = 505,090 Mean (95% CI)	
Age at first dispensed prescription (years)						
Men		56.8 (56.7–57.0), (*n* = 37,672, 37%)			56.8 (56.7–56.9), (*n* = 188,080, 37%)	
Women		57.8 (57.6–57.9), (*n* = 63,472, 63%)			57.8 (57.7–57.8), (*n* = 317,010, 63%)	
All		57.4 (57.3–57.5)			57.4 (57.4–57.4)	
	MTX-exposed group only; Time from first to last observed dispensed prescription of MTX					
	Single dispensed prescription *n* (%)	>0–≤2 years *n* (%)	>2–≤4 years *n* (%)	>4–≤6 years *n* (%)	>6–≤8 years *n* (%)	>8–≤10 years *n* (%)
Men						
≤40 years	786 (7.2)	2,361 (7.7)	1,000 (6.0)	662 (5.3)	545 (5.0)	706 (3.6)
>40–≤50 years	677 (6.2)	1,991 (6.5)	1,027 (6.2)	729 (5.8)	646 (5.9)	1,028 (5.2)
>50–≤60 years	790 (7.2)	2,248 (7.3)	1,256 (7.6)	1,013 (8.1)	933 (8.5)	1,929 (9.8)
>60–≤70 years	950 (8.7)	2,736 (8.9)	1,588 (9.6)	1,201 (9.6)	1,052 (9.6)	1,938 (9.8)
>70	1,042 (9.5)	2,810 (9.2)	1,521 (9.2)	1,035 (8.3)	666 (6.1)	806 (4.1)
Women						
≤40 years	1,180 (10.8)	3,157 (10.3)	1,569 (9.5)	1,164 (9.3)	1,017 (9.3)	1,438 (7.3)
>40–≤50 years	945 (8.7)	2,720 (8.9)	1,466 (8.9)	1,106 (8.9)	1,067 (9.8)	2,044 (10.4)
>50–≤60 years	1,317 (12.1)	3,666 (12.0)	2,086 (12.6)	1,645 (13.2)	1,728 (15.8)	3,754 (19.1)
>60–≤70 years	1,459 (13.4)	4,117 (13.5)	2,382 (14.4)	1,891 (15.1)	1,743 (15.9)	3,876 (19.7)
>70 years	1,765 (16.2)	4,796 (15.7)	2,634 (15.9)	2,041 (16.3)	1,541 (14.1)	2,158 (11.0)

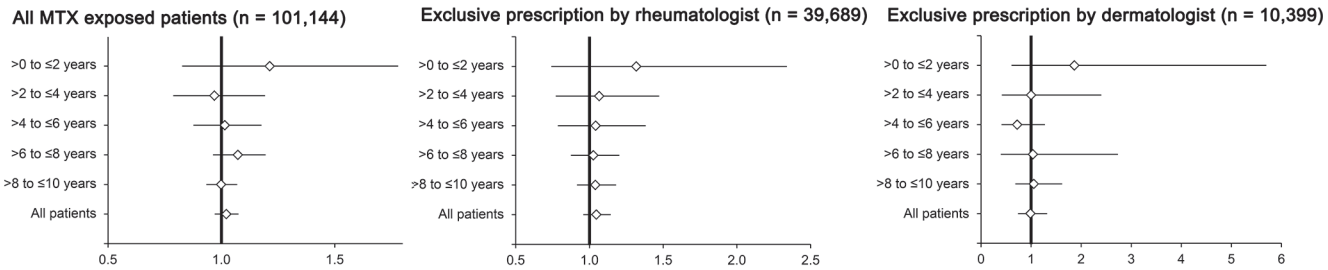

Fig. 1. Hazard ratios corresponding to a total methotrexate (MTX) exposure of 1 g and 95% confidence intervals (CI) from Cox proportional hazards regression models for subgroups dividing time from first to last MTX exposure into intervals, and a model including all patients (controlling for the above-mentioned time intervals). Hazard ratios > 1 indicate an increased risk of cutaneous malignant melanoma (CMM) with increased dose. Single day dispensed prescriptions were not included in the figure due to a large CI.

- Cox proportional hazards regression models were used to compare the time to CMM between the MTX-exposed patients who received their first dispensed prescription of MTX in 2005 and their corresponding MTX-unexposed counterparts with sex and age group as independent variables. The analysis was separated into 5 models corresponding to the above-mentioned dose intervals. In each model, the MTX-exposed individuals were compared with their respective MTX-unexposed counterparts.
- A Cox proportional hazards model was used where the MTX-exposed patients with exclusively parenteral MTX administration were compared with their corresponding MTX-unexposed subjects with respect to CMM risk, with sex and age group as independent variables.
- A Cox proportional hazards model was used to compare overall mortality (i.e. overall survival analysis), with respect to all causes of death, between the MTX-exposed and MTX-unexposed groups, stratifying with respect to sex and age group.

All tests were 2-sided and $p < 0.05$ was considered statistically significant.

RESULTS

In total, 101,144 of the 101,169 MTX-exposed patients without a prior history of CMM before initiation of MTX,

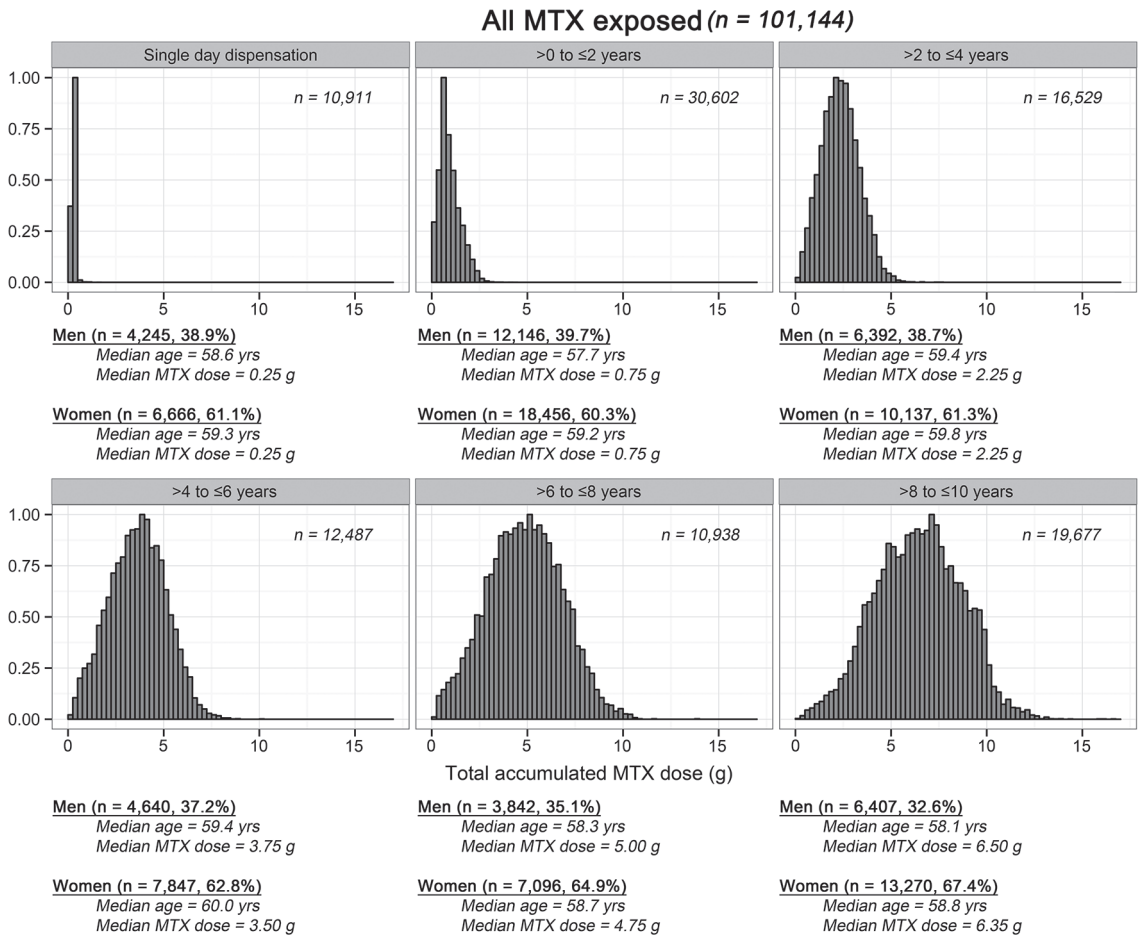

Fig. 2. Histograms of total accumulated methotrexate (MTX) dose (g) stratified into subgroups defined by dividing time from first to last MTX exposure into intervals.

Table II. Aggregated incidences for cutaneous malignant melanoma (CMM) over the period 2005 to 2014

	Total accumulated MTX dose, g				
	≤ 2 years	> 2–≤ 4 years	> 4–≤ 6 years	> 6–≤ 8 years	> 8 years
Number of CMM among MTX-exposed	389	224	169	112	58
Person-years among MTX-exposed, *n*	465,536	203,093	143,932	91,900	52,153
Crude (observed) incidence-rate[a], mean (95% CI)	84 (75–92)	110 (96–125)	117 (100–135)	122 (99–144)	111 (83–140)
Expected incidence rate for MTX-exposed[b] , mean (95% CI)	80.5 (79.8–81.3)	88.0 (87.3–88.8)	94.3 (93.5–95.2)	95.8 (94.9–96.6)	94.7 (93.9–95.6)
SIR (MTX observed/MTX expected) (95% CI)	1.0 (0.9–1.2)	1.3** (1.1–1.4)	1.2** (1.0–1.4)	1.3* (1.0–1.5)	1.2 (0.9–1.5)
Number of CMM among the Swedish population (2005 to 2014)[c]	47,910				
Person-years among MTX-exposed, *n*	71,972,798				
Crude incidence for the Swedish population, mean (95% CI)	66.6 (66.0–67.2)				

[a]per 100,000 person years among the MTX-exposed. [b]The expected incidence rate in the methotrexate (MTX)-exposed group with sex and age distribution maintained, but assuming that the underlying incidence was equal to the general population. [c]The total number of CMM (including *in situ* melanomas) among individuals > 20 years in the entire Swedish population in the period 2005 to 2014.
Standardized incidence ratios (SIR) differing significantly from 1 are denoted by *($p < 0.05$) and **($p < 0.01$). CI: confidence interval.

had an accumulated dose value and were included in the analyses (**Table I**).

Primary analysis

- The risk of CMM did not significantly depend on dose ($p = 0.41$). The model yielded a HR of 1.02 (95% CI 0.97–1.08) for 1 g of total MTX exposure. The patients were divided into groups with respect to time from the first to the last MTX exposure and the same analysis was performed within each group. No significant association with respect to dose was found for the risk of CMM in any subgroup: single day dispensed prescription, $p = 0.57$; HR 2.71 (95% CI 0.09–83.9); > 0 to ≤ 2 years, $p = 0.32$; HR 1.21 (95% CI 0.83–1.78); > 2 to ≤ 4 years, $p = 0.77$; HR 0.97 (95% CI 0.79–1.19); > 4 to ≤ 6 years, $p = 0.84$; HR 1.02 (95% CI 0.88–1.18); > 6 to ≤ 8 years, $p = 0.20$; HR 1.07 (95% CI 0.96–1.19) and > 8 to ≤ 10 years, $p = 0.98$; HR 1.00 (95% CI 0.93–1.07). When the above analysis was repeated for subgroups of patients with an exclusive prescription from a rheumatologist or a dermatologist, respectively, no significant dependence between the risk of CMM and the accumulated dose was observed in either subgroup (**Fig. 1**). The distribution of the total accumulated doses within each subgroup is shown in **Fig. 2** and Figs S1–S2[1].

Secondary analyses

- The observed and expected incidence rates of CMM within different intervals of the total accumulated MTX dose were compared. A significant risk increase was seen for MTX-exposed individuals compared with the Swedish population for the dose intervals > 2 to ≤ 4 g (standardized incidence ratio (SIR) 1.3, 95% CI 1.1–1.4; $p = 0.001$), > 4 to ≤ 6 g (SIR 1.2, 95% CI 1.0–1.4 $p = 0.006$) and > 6 to ≤ 8 g (SIR 1.3, 95% CI 1.0–1.5; $p = 0.014$). However, no risk increase was observed for the groups ≤ 2 g (SIR 1.0, 95% CI 0.9–1.2; $p = 0.47$) and > 8 g (SIR 1.2, 95% CI 0.9–1.5; $p = 0.20$) (**Table II**).
- Patients who had a first prescription of MTX in 2005 ($n = 29,235$) were compared with their MTX-unexposed counterparts with respect to risk of CMM (Table SI[1] and Fig. S3[1]). A significant difference in the risk of CMM between the MTX-exposed and unexposed individuals was observed in the subanalyses in which MTX-exposed patients had a total accumulated dose of > 4 to ≤ 6 g, $p = 0.006$; HR 1.45 (95% CI 1.11–1.88); and > 6 to ≤ 8 g, $p = 0.044$; HR 1.36 (95% CI 1.01–1.82). However, no significant differences between MTX-exposed and unexposed individuals were observed in the subanalyses corresponding to ≤ 2 g, $p = 0.49$; HR 1.12 (95% CI 0.82–1.52); > 2 to ≤ 4 g, $p = 0.18$; HR 1.23 (95% CI 0.91–1.65) and > 8 g, $p = 0.58$; HR 1.11 (95% CI 0.77–1.58).
- Patients with an exclusively parenteral MTX exposure ($n = 3,774$) were compared with their respective

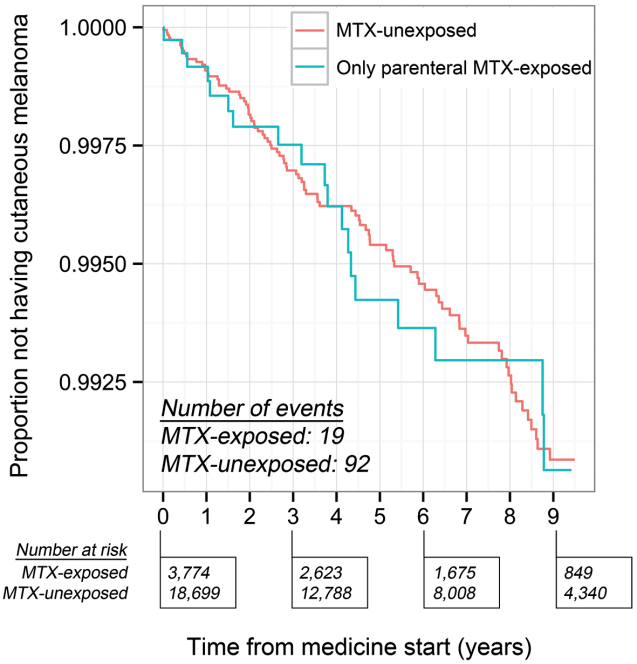

Fig. 3. Kaplan–Meier plots for patients exclusively dispensed parenteral methotrexate (MTX) and their corresponding MTX-unexposed patients. The figure depicts the proportion of patients not having cutaneous malignant melanoma vs. time from medicine start.

[1]https://doi.org/10.2340/00015555-2987

MTX-unexposed patients ($n = 18{,}699$) for a difference in risk of CMM. No significant difference was found between parenteral MTX-exposed and unexposed patients, $p = 0.85$ and a HR of 1.05 (95% CI 0.64–1.72) (**Fig. 3**). In 2 subanalyses, patients with exclusively parenteral MTX exposure only having prescriptions from a dermatologist or a rheumatologist, respectively, were compared with their respective MTX-unexposed patients and no significant differences in risk of CMM were found (data not shown).

- Finally, comparing the overall mortality after first dispensed prescription, including all causes of death between the MTX-exposed and MTX-unexposed, yielded an increased mortality for the MTX-unexposed among men > 40 years and an increased mortality for MTX-exposed among women aged ≤ 50 and > 70 years (**Table III** and **Fig. 4**).

DISCUSSION

This nationwide, retrospective and registry-based cohort study, found no conclusive or convincing evidence for a dose-response association between exposure to MTX and the risk of CMM. Since a dose-response relationship might prove difficult to assess, we believe it is essential to address a potential association using different models.

The primary analysis aimed to determine whether there was a correlation between the accumulated MTX dose and the risk of CMM controlling for sex and age group at treatment start. This analysis was repeated within groups that had a total exposure time divided into predefined intervals; in effect comparing patients who had approximately the same overall exposure time, but different doses. The purpose of performing subgroup analyses was to avoid a bias resulting from a correlation between higher accumulated doses and not being censored. In order to address indication bias, subanalyses with patients whose prescriptions were exclusively from either a dermatologist or a rheumatologist were performed. Overall, stratifying with respect to exposure time, and within each subgroup, no significant correlation between accumulated dose and the risk of CMM was found.

In a second analysis, the expected incidence rates for the time period in the MTX-exposed group were com-

Table III. Kaplan–Meier analyses with respect to time to cutaneous malignant melanoma (CMM) and time to death, respectively

	Total observation time, years[a]		Total observation time for censored patients, years[b]		Number of observed deaths[c]		Cox proportional hazards model for overall mortality		
	Median	Mean (95% CI)	Median	Mean (95% CI)	*n* (%)	95% CI	Hazard ratio[d]	95% CI	*p*-value
All patients									
MTX-unexposed	5.78	5.54 (5.53–5.55)	5.79	5.55 (5.54–5.55)	62,677 (12.4)	12.3–12.5	1.00	0.98–1.02	0.89
MTX-exposed	5.79	5.56 (5.54–5.58)	5.80	5.57 (5.55–5.59)	12,583 (12.4)	12.2–12.6			
Men									
≤ 40 years									
MTX-unexposed	5.46	5.35 (5.32–5.39)	5.47	5.35 (5.32–5.39)	362 (1.2)	1.1–1.3	1.02	0.80–1.32	0.85
MTX-exposed	5.47	5.34 (5.27–5.42)	5.48	5.35 (5.27–5.42)	74 (1.2)	1.0–1.5			
> 40–≤ 50 years									
MTX-unexposed	5.75	5.53 (5.50–5.57)	5.75	5.54 (5.50–5.57)	817 (2.7)	2.5–2.9	0.75	0.62–0.91	0.003
MTX-exposed	5.79	5.56 (5.49–5.64)	5.79	5.57 (5.49–5.64)	124 (2.0)	1.7–2.4			
> 50–≤ 60 years									
MTX-unexposed	6.28	5.82 (5.79–5.85)	6.29	5.82 (5.79–5.85)	2,804 (6.9)	6.6–7.1	0.74	0.67–0.82	< 0.00001
MTX-exposed	6.38	5.89 (5.83–5.96)	6.40	5.90 (5.84–5.97)	420 (5.1)	4.7–5.6			
> 60–≤ 70 years									
MTX-unexposed	5.44	5.35 (5.33–5.38)	5.46	5.36 (5.34–5.39)	6,826 (14.5)	14.1–14.8	0.85	0.80–0.91	< 0.00001
MTX-exposed	5.62	5.47 (5.41–5.53)	5.63	5.48 (5.41–5.54)	1,194 (12.6)	11.9–13.3			
> 70 years									
MTX-unexposed	3.79	4.31 (4.28–4.34)	3.80	4.32 (4.29–4.35)	15,485 (39.4)	38.9–39.9	0.90	0.87–0.94	< 0.00001
MTX-exposed	4.11	4.49 (4.42–4.55)	4.13	4.50 (4.43–4.56)	2,901 (36.8)	35.7–37.9			
Women									
≤40 years									
MTX-unexposed	6.04	5.69 (5.66–5.72)	6.04	5.69 (5.67–5.72)	294 (0.6)	0.5–0.7	1.31	1.02–1.68	0.034
MTX-exposed	6.04	5.67 (5.61–5.73)	6.04	5.67 (5.61–5.73)	77 (0.8)	0.6–1.0			
> 40–≤ 50 years									
MTX-unexposed	6.27	5.81 (5.78–5.84)	6.28	5.82 (5.79–5.85)	898 (1.9)	1.8–2.0	1.21	1.04–1.40	0.013
MTX-exposed	6.25	5.79 (5.73–5.85)	6.26	5.80 (5.73–5.86)	216 (2.3)	2.0–2.6			
> 50–≤ 60 years									
MTX-unexposed	6.89	6.09 (6.07–6.11)	6.91	6.10 (6.08–6.12)	3,224 (4.6)	4.4–4.7	1.01	0.93–1.09	0.88
MTX-exposed	6.90	6.10 (6.05–6.15	6.92	6.11 (6.06–6.16)	651 (4.6)	4.2–4.9			
> 60–≤ 70 years									
MTX-unexposed	6.37	5.87 (5.85–5.89)	6.41	5.88 (5.86–5.90)	7,639 (9.9)	9.7–10.1	1.02	0.97–1.08	0.40
MTX-exposed	6.40	5.90 (5.85–5.95)	6.45	5.91 (5.86–5.96)	1,572 (10.2)	9.7–10.6			
> 70 years									
MTX-unexposed	4.96	5.09 (5.06–5.11)	4.97	5.10 (5.07–5.12)	24,328 (32.6)	32.3–33.0	1.12	1.09–1.15	< 0.00001
MTX-exposed	4.85	5.02 (4.97–5.07)	4.87	5.03 (4.98–5.08)	5,354 (35.8)	35.1–36.6			

[a]Observation times are for the Kaplan–Meier analysis for time to CMM. [b]Patients are censored if dead or if end of observation period is reached. [c]The number of observed deaths, including all causes of death, in the period August 2005 to December 2014. Percentages are the proportion of patients who died. [d]Cox proportional hazards model with respect to overall mortality, including all causes of death. The hazard ratio is $\text{hazard}_{\text{MTX-exposed}}/\text{hazard}_{\text{MTX-unexposed}}$.
MTX: methotrexate.

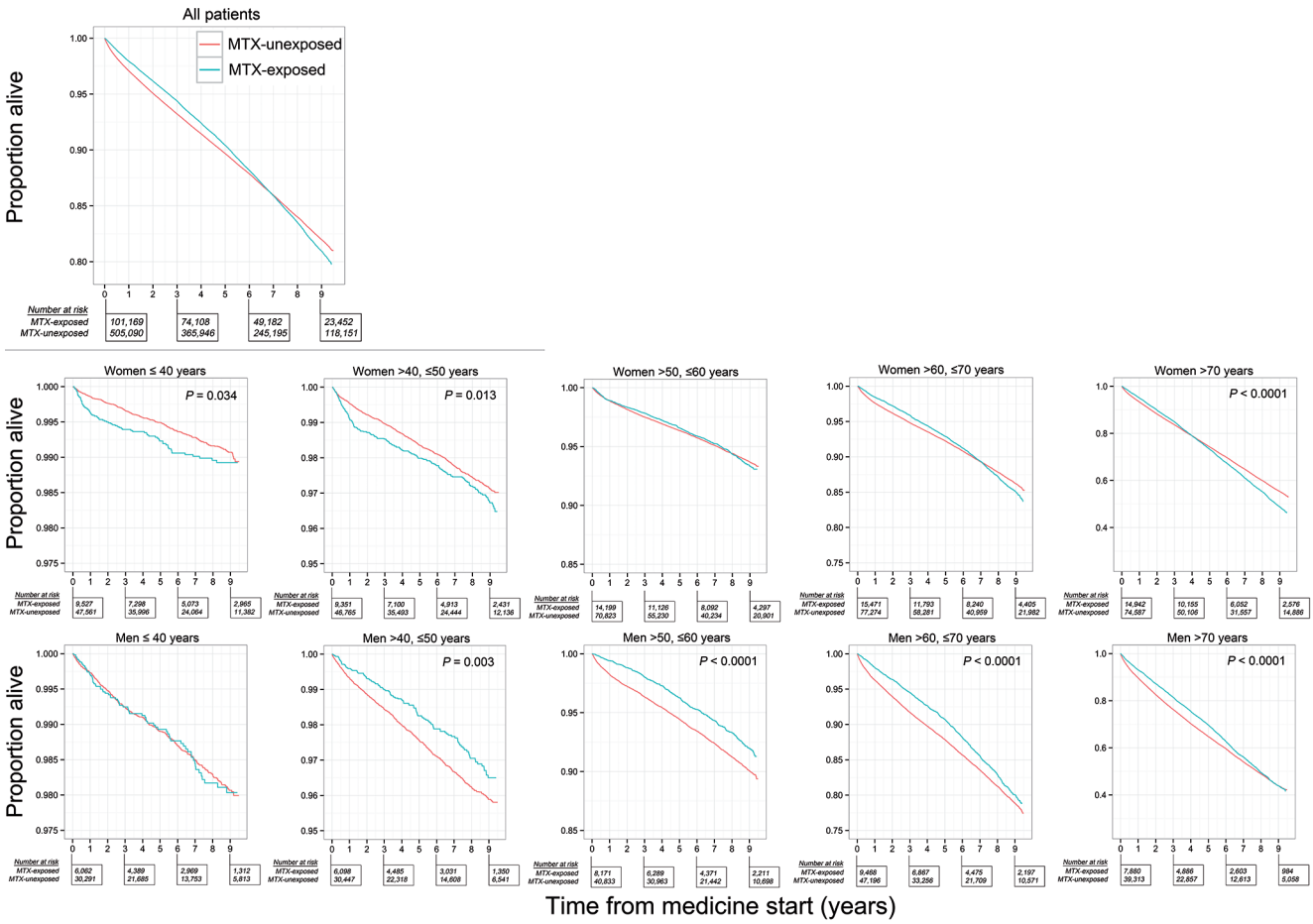

Fig. 4. Kaplan–Meier plots of overall mortality, including all causes of death, divided by sex and age groups.

pared with the observed incidence rates, using incidence for the Swedish population. A significant risk increase was found in 3 of the 5 subgroups: >2 to ≤4 g, >4 to ≤6 g, and >6 to ≤8 g.

Thirdly, the risk of CMM in the MTX-exposed individuals was compared with their corresponding unexposed counterparts, dividing them into subgroups defined by intervals of total accumulated dose of MTX. Only patients with their first dispensed prescription in 2005, and hence having the longest potential follow-up time, were included in this model. A significant increase in risk was found for the MTX-exposed among the patients, with an accumulated dose of >4 to ≤6 g and >6 to ≤8 g, but not in the other 3 groups. However, when interpreting the above results from subanalyses 2 and 3, it is important to take into account the intrinsic censoring bias for the MTX-exposed patients with higher accumulated doses, as they are less likely to be censored compared with the Swedish population and the MTX-unexposed group, respectively. On the other hand, patients with an accumulated dose of more than 8 g would be the least likely to be censored, but did not differ significantly from the MTX-unexposed or the Swedish population regarding CMM risk or CMM incidence, respectively.

Parenteral MTX administration has a higher bioavailability compared with oral intake (13, 14). Thus, it could be expected that patients with exclusive dispensed prescriptions of MTX would have an even higher increase in risk of CMM compared with their respective unexposed counterparts than in our previous article comparing all MTX-exposed and MTX-unexposed patients (8). No difference in the risk of CMM in this subset and their respective MTX-unexposed patients was seen.

Interestingly, the overall mortality, including all causes of death, differed between MTX-exposed and MTX-unexposed individuals within certain sex and age groups. Particularly for women younger than 50 years, a significantly increased mortality was observed for MTX-exposed individuals. It is unlikely that this observation is due to MTX exposure; it might rather be explained by disease-associated mortality (confounding by indication). On the other hand, for men older than 40 years, a lower mortality rate was observed among MTX-exposed compared with the MTX-unexposed group. Indeed this observation is interesting and opens up to speculation about a potential protective effect of MTX among men. However, due to residual confounding, this association is premature and further research is needed.

The results of the present study are conflicting, as they do not reproduce entirely within our models. If there is a dose-response relationship between accumulated doses of MTX and risk of CMM, the association might be too small to discern. Moreover, the risk of CMM might increase after a specific threshold value rather than being linear. Finally, the observation period used in the present study might be too short to discern a clear dose-response association. As an example, psoralen plus UVA (PUVA) treatments enhanced the risk of CMM 15 years after the first treatment, and the increase in risk was more pronounced in patients with more than 250 treatments (15, 16).

A publication investigating 6 human melanoma cell lines *in vitro*, demonstrated that MTX upregulates Fas/FasL and enhances melanoma apoptosis, which might suggest an anti-neoplastic effect. The findings indicate preclinical evidence that MTX might be used for combination therapy regimens directed against melanoma (17). Another publication suggested a lower risk of metastasis in a human melanoma cell line in mice treated with MTX (18). Moreover, some of the cytokines that may affect melanogenesis and melanocytic growth (interleukin (IL)-1a, IL-6 and tumour necrosis factor alpha (TNF-α)) have also been reported to be involved in the pathogenesis of Pso (19). MTX reduces the production of proinflammatory cytokines IL-4, IL-6, IL-13, TNF-α and interferon gamma (20). IL-6 plays an important role in the development and progression of melanoma (21) and it might be hypothesized that MTX treatment could reduce the risk of development of a CMM by reducing the production of pro-inflammatory cytokines.

Investigating and reporting adverse and unexpected effects from pharmaceutical drugs is key to the introduction of novel drugs. Moreover, side-effects might shed light on possible disease-causing mechanisms. As an example, sildenafil was recently linked to an increased risk of melanoma (22). However, subsequent publications have not been able to reproduce an evident increased risk in patients with a dispensed prescription of phosphodiesterase inhibitors. Moreover, a clear dose-response association was not seen (23, 24).

To the best of our knowledge, the present study is the first to address whether there is a dose-response relationship between risk of CMM and MTX exposure. The cohort also includes a large number of patients, which is necessary in order to discern even a weak association. Recently, an Australian publication investigated the risk of non-melanoma skin cancer (NMSC) in an Australian cohort of patients with rheumatic disease ($n=405$) (25). The authors concluded that exposure to MTX increased the risk of NMSC, and there is some suggestion of a dose-dependent trend in risk of NMSC with increasing dose. However, patients with accumulated doses over 8 g had a SIR 4.81 (95% CI 3.60–6.29). In our cohort, patients with MTX exposure over 8 g did not have an increased risk of CMM.

When analysing our results it is critical to mention confounding by indication. Even though the exact diagnosis that prompted MTX prescription was not included in our database, most patients with a prescription exclusively from a dermatologist or rheumatologist were likely to have Pso or RA, respectively. Pso as an independent risk factor for development of CMM is hard to assess due to confounding phototherapy treatment. In a large meta-analysis investigation, 14 studies were included. No increased risk of CMM was observed (SIR 1.07, 95% CI 0.85–1.35), whereas the risk of NMSC was enhanced (26). In a Danish nationwide cohort study, patients with mild Pso had an increased risk of CMM, whereas patients with severe disease did not (27). Similar results were presented in a British cohort (28). In a Swedish nationwide, population-based, prospective, cohort study, patients with RA who were not treated with TNF inhibitors did not have an enhanced risk of *in situ* or invasive CMM compared with the general population. On the other hand, patients with RA exposed to TNF inhibitors had a 50% increased relative risk of development of invasive melanoma compared with unexposed patients with RA. No corresponding risk increase was observed for *in situ* melanoma or invasive cancer at all sites (29). Interestingly, in a European collaboration project including 11 registers, no increase in risk of invasive CMM was seen for patients with RA treated with TNF inhibitors (SIR 1.2, 95% CI 0.99–1.6).

Importantly, the present study has some limitations. First, only data on dispensed prescriptions of MTX were obtained, omitting concomitant medication, which could have confounded the results. Moreover, it is likely that patients with only a trivial exposure or short duration of MTX treatment either had a more severe disease or experienced side-effects and were moved to another drug. Due to the retrospective design, data for relevant risk factors, such as a family history of CMM, skin type or detailed ultraviolet (UV) exposure history, could not be obtained. A higher level of UV exposure is expected in the group pf patients prescribed MTX by a dermatologist where no significant increase in risk of CMM was seen. Thus, it is not excluded that, albeit not being significant, the risk in this group may be exaggerated. The present study was conducted in a Swedish population, which might not be comparable to other countries in terms of the population skin types. Significantly, as mentioned above, the diagnosis that prompted MTX prescription was unknown, as this information was not part of the database analysed. Subanalyses for a cohort with Pso and patients with RA would have contributed substantially. Finally, the present study lacks data about relevant comorbidities and hospitalizations.

To summarize, these results do not prove a conclusive reproducible dose-response relationship between CMM risk and MTX dose, and thus cast doubt on such an association.

REFERENCES

1. Cronstein BN, Bertino JR. Methotrexate. Basel: Birkhäuser, 2012.
2. Kanik KS, Cash JM. Does methotrexate increase the risk of infection or malignancy? Rheum Dis Clin North Am 1997; 23: 955–967.
3. Bailin PL, Tindall JP, Roenigk HH, Jr, Hogan MD. Is methotrexate therapy for psoriasis carcinogenic? A modified retrospective-prospective analysis. JAMA 1975; 232: 359–362.
4. Nyfors A, Jensen H. Frequency of malignant neoplasms in 248 long-term methotrexate-treated psoriatics. A preliminary study. Dermatologica 1983; 167: 260–261.
5. Stern RS, Zierler S, Parrish JA. Methotrexate used for psoriasis and the risk of noncutaneous or cutaneous malignancy. Cancer 1982; 50: 869–872.
6. Savage P, Cooke R, O'Nions J, Krell J, Kwan A, Camarata M, et al. Effects of single-agent and combination chemotherapy for gestational trophoblastic tumors on risks of second malignancy and early menopause. J Clinical Oncol 2015; 33: 472–478.
7. Buchbinder R, Barber M, Heuzenroeder L, Wluka AE, Giles G, Hall S, et al. Incidence of melanoma and other malignancies among rheumatoid arthritis patients treated with methotrexate. Arthritis Rheum 2008; 59: 794–799.
8. Polesie S, Gillstedt M, Sönnergren HH, Osmancevic A, Paoli J. Methotrexate treatment and risk for cutaneous malignant melanoma: a retrospective comparative registry-based cohort study. Br J Dermatol 2017; 176: 1492–1499.
9. Socialstyrelsen. National Board of Health and Welfare: the Prescribed Drug Register 2018 [accessed 2018 May 7]. Available from: www.nepi.net/Socialstyrelsens-laekemedelsregister.htm (in Swedish).
10. Socialstyrelsen. National Board of Health and Welfare: The Swedish Cancer Register 2018 [accessed 2018 May 7]. Available from: www.socialstyrelsen.se/register/halsodataregister/cancerregistret (in Swedish).
11. Barlow L, Westergren K, Holmberg L, Talback M. The completeness of the Swedish Cancer Register: a sample survey for year 1998. Acta Oncologica 2009; 48: 27–33.
12. Socialstyrelsen. National Board of Health and Welfare: the Causes of Death Register 2018 [accessed 2018 May 7]. Available from http://www.socialstyrelsen.se/register/dodsorsaksregistret (in Swedish).
13. Visser K, van der Heijde D. Optimal dosage and route of administration of methotrexate in rheumatoid arthritis: a systematic review of the literature. Ann Rheum Dis 2009; 68: 1094–1099.
14. Hoekstra M, Haagsma C, Neef C, Proost J, Knuif A, van de Laar M. Bioavailability of higher dose methotrexate comparing oral and subcutaneous administration in patients with rheumatoid arthritis. J Rheumatol 2004; 31: 645–648.
15. Stern RS, Laird N. The carcinogenic risk of treatments for severe psoriasis. Photochemotherapy follow-up study. Cancer 1994; 73: 2759–2764.
16. Stern RS. The risk of melanoma in association with long-term exposure to PUVA. J Am Acad Dematol 2001; 44: 755–761.
17. Nihal M, Wu J, Wood GS. Methotrexate inhibits the viability of human melanoma cell lines and enhances Fas/Fas-ligand expression, apoptosis and response to interferon-alpha: rationale for its use in combination therapy. Arch Biochem Biophys 2014; 563: 101–107.
18. Piskounova E, Agathocleous M, Murphy MM, Hu Z, Huddlestun SE, Zhao Z, et al. Oxidative stress inhibits distant metastasis by human melanoma cells. Nature 2015; 527: 186–191.
19. Alwan W, Nestle FO. Pathogenesis and treatment of psoriasis: exploiting pathophysiological pathways for precision medicine. Clin Exp Rheumatol 2015; 33: S2–6.
20. Wessels JA, Huizinga TW, Guchelaar HJ. Recent insights in the pharmacological actions of methotrexate in the treatment of rheumatoid arthritis. Rheumatology 2008; 47: 249–255.
21. Hoejberg L, Bastholt L, Schmidt H. Interleukin-6 and melanoma. Melanoma Res 2012; 22: 327–333.
22. Li WQ, Qureshi AA, Robinson KC, Han J. Sildenafil use and increased risk of incident melanoma in US men: a prospective cohort study. JAMA Int Med 2014; 174: 964–970.
23. Loeb S, Folkvaljon Y, Lambe M, Robinson D, Garmo H, Ingvar C, et al. Use of Phosphodiesterase type 5 Inhibitors for erectile dysfunction and risk of malignant melanoma. JAMA 2015; 313: 2449–2455.
24. Pottegard A, Schmidt SAJ, Olesen AB, Achacoso N, Van Den Eeden SK, Hallas J, et al. Use of sildenafil or other phosphodiesterase inhibitors and risk of melanoma. Br J Cancer 2016; 115: 895–900.
25. Lange E, Blizzard L, Venn A, Francis H, Jones G. Disease-modifying anti-rheumatic drugs and non-melanoma skin cancer in inflammatory arthritis patients: a retrospective cohort study. Rheumatology 2016; 55: 1594–1600.
26. Pouplard C, Brenaut E, Horreau C, Barnetche T, Misery L, Richard MA, et al. Risk of cancer in psoriasis: a systematic review and meta-analysis of epidemiological studies. J Eur Acad Dermatol Venereol 2013; 27 Suppl 3: 36–46.
27. Egeberg A, Thyssen JP, Gislason GH, Skov L. Skin cancer in patients with psoriasis. J Eur Acad Dermatol Venereol 2016; 30: 1349–1353.
28. Chiesa Fuxench ZC, Shin DB, Ogdie Beatty A, Gelfand JM. The risk of cancer in patients with psoriasis: a population-based cohort study in the health improvement network. JAMA Dermatol 2016; 152: 282–290.
29. Raaschou P, Simard JF, Holmqvist M, Askling J. Rheumatoid arthritis, anti-tumour necrosis factor therapy, and risk of malignant melanoma: nationwide population based prospective cohort study from Sweden. BMJ 2013; 346: f1939.