Early View

Original article

Methotrexate and rheumatoid arthritis associated interstitial lung disease


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METHOTREXATE AND RHEUMATOID ARTHRITIS ASSOCIATED INTERSTITIAL LUNG DISEASE

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Take home message
This multi-ethnic case-control study showed that methotrexate use is not associated with an increased risk of interstitial lung disease in patients with rheumatoid arthritis.
ABSTRACT

Question addressed by the study
Methotrexate (MTX) is a key anchor drug for rheumatoid arthritis (RA) management. Fibrotic interstitial lung disease (ILD) is a common complication of RA. Whether MTX exposure increases the risk of ILD in patients with RA is disputed. We aimed to evaluate the association of prior MTX use with development of RA-ILD.

Methods
Through a case-control study design with discovery and international replication samples, we examined the association of MTX exposure with ILD in 410 patients with chronic fibrotic ILD associated with RA (RA-ILD) and 673 patients with RA without ILD. Estimates were pooled over the different samples using meta-analysis techniques.

Results
Analysis of the discovery sample revealed an inverse relationship between MTX exposure and RA-ILD (adjusted odds ratio [OR], 0.46; 95% confidence interval [CI], 0.24-0.90; P=0.022), which was confirmed in the replication samples (pooled adjusted OR, 0.39; 95% CI, 0.19-0.79; P=0.009). The combined estimate using both the derivation and validation samples revealed an adjusted OR of 0.43 (95% CI, 0.26-0.69; P=0.0006). MTX ever users were less frequent among patients with RA-ILD compared to those without ILD, irrespective of chest high resolution computed tomography pattern. In patients with RA-ILD, ILD detection was significantly delayed in MTX ever users compared to never users (11.4±10.4 years and 4.0±7.4 years, respectively; P<0.001).

Answer to the Question
Our results suggest that MTX use is not associated with an increased risk of RA-ILD in patients with RA, and that ILD was detected later in MTX treated patients.
BACKGROUND

Interstitial lung disease (ILD) is a severe manifestation of rheumatoid arthritis (RA) that affects 2.2 to 30% of RA patients based on high-resolution computed tomography (HRCT) chest scan findings [1, 2]. RA-ILD is the second leading cause of mortality in RA and contributes to death in 6.8 – 9.8% of RA patients [3, 4].

Methotrexate (MTX) is recommended as the first-line treatment of RA as it effectively reduces disease activity, morbidity and mortality [5, 6]. MTX has long been suspected as a causative agent in lung disease, including fibrotic ILD [7-9] and many rheumatologists and pneumologists are reluctant at introducing or maintaining MTX in patient with RA-ILD. However, other than acute or sub-acute hypersensitivity pneumonitis, which is a rare complication of MTX [10], the evidence for a cause and effect relationship in modern populations between MTX and chronic fibrotic ILD in a patient with RA (i.e. RA-ILD) is unsettled. Recent studies of the incidence of ILD among RA and non-RA populations have cast doubt on the causal role of MTX and some data have even suggested a possible protective effect of MTX against RA-ILD [11-15]. However, most of these studies had several potential biases: i) the ILD status was not systematically assessed by HRCT chest scan in cases (RA-ILD) and controls (RA-noILD) resulting in potential misclassification (i.e., classification of patients with pre-clinical RA-ILD in the RA-noILD group) and precluding sub-analyses according to the HRCT pattern; ii) identification of ILD based on data recorded on case report forms and death certificates without independent validation leading to an underestimation of the RA-ILD incidence; iii) the design of the study did not take into account exposure to MTX before the diagnosis of ILD, thus leading to a potential bias of MTX use (i.e. non-initiation or discontinuation of MTX in patients with RA-ILD); and iv) the year of RA onset and the corresponding guidelines for the management of RA, thus influencing MTX use, were not considered. The aim of this study was to evaluate whether MTX exposure is associated with an increased risk of RA-ILD.
METHODS

Study populations

This case-control association study included a discovery and a replication step. The discovery sample included patients with chronic fibrotic ILD associated with RA (RA-ILD) (cases) and patients with RA who did not have ILD (RA-noILD) (controls), from the French RA-ILD network [16]. The replication step included patients from multi-ethnic case series from five countries (Italy, United Kingdom, Mexico, Brazil and United States). All the patients included in the study were investigated by a chest HRCT scan, data were collected through a systematic chart review. The date of inclusion in the study was defined as follows: date of ILD diagnosis for patients with RA-ILD and date of the chest HRCT scan excluding the ILD diagnosis in patients with RA-noILD. Patients with RA in whom ILD onset preceded RA onset were not included in the study. All cases fulfilled the 2010 European League Against Rheumatism-American College of Rheumatology (EULAR-ACR) and/or 1987 ACR revised criteria for RA and were included consecutively in each participating center [17, 18]. Because of the known relationship between the risk of occurrence of ILD and RA duration [19], cases and controls (i.e. RA-ILD and RA-noILD) were matched according to the RA duration at the date of inclusion. The ILD status of patients with RA was established by chest HRCT images that were centrally reviewed by experienced radiologist and pulmonologist readers at each participating center. The chest HRCT ILD pattern was classified as the usual interstitial pneumonia (UIP) pattern, possible UIP or inconsistent with UIP according to international criteria [20] and all readers were blinded to the clinical data. In each participating center, patients classified by a pulmonologist senior as having a diagnosis of MTX related hypersensitivity pneumonitis according to previously published criteria were not included in the study [21]. The institutional review boards at each institution approved all protocols.

Methotrexate exposure assessment

MTX exposure was assessed through a systematic chart review of all the patients included in the study. To avoid any bias resulting in MTX withdrawal secondary to ILD detection in patients with RA, MTX exposure was systematically assessed during the period encompassing the date of RA onset (year or year and month, when available) to the date of ILD diagnosis.
(year or year and month, when available) for patients with RA-ILD and to the date of ILD negative HRCT scan (year or year and month, when available) for the patients with RA-noILD. This period was termed MTX exposure duration. MTX exposure was evaluated by including the MTX ever/never use status for the period defined above (i.e. MTX exposure duration). Due to the retrospective design of the study, the accuracy of both MTX doses and MTX duration exposure were considered as low. Consequently, the cumulative dose of MTX (exposure duration x mean MTX dose) information was not considered sufficiently robust, therefore, corresponding P-values were considered as descriptive.

**Statistical analysis**

The association between MTX ever use and the occurrence of ILD in patients with RA was expressed in terms of odds ratio and analyzed in the discovery and in all replication samples. Following the same principles as the two-stage approach to individual patients’ meta-analysis, the association in all replication samples was then pooled by a random effects meta-analysis model with inverse variance weighting to obtain a single estimate of the replication odds ratio. Discovery and replication odds ratios were then pooled similarly to obtain an overall (combined) odds ratio. Given the risk for sparse data bias in cohorts with small number of unexposed subjects, Firth’s penalized logistic regression was used [22]. Analyses were adjusted for age at RA onset, sex, ever smoking, biologics ever use, study site, MTX exposure duration and periods of RA onset.

To avoid bias secondary to MTX practice patterns at the year of RA onset of each included patient, we defined and considered 4 distinct periods of MTX use at RA onset. According to major publications and available guidelines these 4 periods were defined as follows: unlikely (before 1985) [23], less often (1985 to 1995) [24, 25], often (1996-2007) [26] and standard of care (since 2008). These 4 periods were considered as covariates when adjusting for periods of MTX use at year of RA onset. Missing data were handled by multiple imputations using the chained equations method, with 30 independent imputed datasets generated and analyzed separately. All adjustment factors were considered in the imputation model, as well as MTX ever use and RA-ILD status. Results were then pooled over imputed datasets using Rubin’s rule. Sensitivity analyses were carried out by 1) using a logistic model with random sample effects (one-stage IPD meta-analysis), 2) removing the replication samples from Italy and
Mexico in which very few patients were unexposed to MTX, and 3) by repeating the analysis on complete cases, and by adding MTX doses and durations in the imputation model. Of note, in one of the replication samples (UK population), all patients were exposed to MTX. This sample was therefore omitted from the statistical analyses assessing the association of MTX ever use and ILD. Other analyses also involved mixed-effects multivariable models, with sample as a random effect. All analyses were carried out using R 3.6.1 statistical software (The R Foundation of Statistical Computing, Vienna, Austria).
RESULTS

This case-control study included 1083 patients with RA, 410 with ILD and 673 without ILD. We computed that, given the correlation between MTX use and adjustment variables and the proportions of patients exposed to MTX, the study would have 62% power to detect an odds ratio of 0.5 in the discovery sample, 78% in the replication sample and 96% in the combined analysis.

Characteristics of the rheumatoid arthritis population

The discovery case series included 100 RA-ILD cases and 165 RA-noILD controls. The replication step comprised a multi-ethnic case series and included 310 RA-ILD and 508 RA-noILD patients. Characteristics of the overall population of 1083 patients with RA are summarized in Table 1. Characteristics of patients with RA-ILD and patients with RA-noILD in each case series are summarized in Table S1. As compared with RA-noILD, patients with RA-ILD were more frequently male, older, older at RA onset, ever smokers and had a shorter MTX exposure duration (Table 1 and 2). RA-ILD and RA-noILD patients did not differ in rheumatoid factor (RF) and/or anti-citrullinated protein antibody (ACP) positivity, RA duration, the periods of MTX use at year of RA onset and the biologic DMARDs use (Table 1 and 2, Figure S1). The frequency of UIP or possible UIP pattern on HRCT ranged from 36.4% to 55.6% (Table S1). Overall, 45.1% of patients with RA-ILD had UIP or possible UIP pattern (Table 1).

Methotrexate ever use and risk of RA-ILD

In the discovery sample, the frequency of MTX ever user was 60.0% in RA-ILD patients and 83.0% in RA-noILD patients. After controlling for age at RA onset, sex, ever smoking, MTX exposure duration, periods of MTX use at RA onset and biologic use, a negative association was found between the ever use of MTX and RA-ILD when compared to RA-noILD (ORadj=0.46; 95% CI, 0.24 – 0.90; Padj=0.022) (Figure 1, Table 2). A similar association was found in the pooled replication population, where fewer MTX ever users were significantly observed among the patients with RA-ILD compared to those with RA-noILD (79.7% and 95.5%, respectively, ORadj=0.39; 95% CI, 0.19 – 0.79; Padj=0.009). The inverse relationship between
the MTX ever use and occurrence of ILD among RA patients was confirmed in the combined population (OR_{adj}=0.43; 95% CI, 0.26 – 0.69; \( P_{adj}=0.0006 \)) (Figure 1, Table 2). Of note, no heterogeneity was found among the replication samples (\( I^2 = 0\% \)), and no difference was found between the discovery and replication samples (\( I^2 = 0\% \), test for between-group differences: \( P=0.73 \)). Sensitivity analyses yielded similar results (Table S2).

**Methotrexate ever use and risk of RA-ILD according to the chest HRCT scan patterns**

In the combined population the frequency of MTX ever use was 70.0% and 79.9% in cases of RA-UlIP and RA-nonUIP, respectively, compared to 92.4% in cases of RA-noILD (RA-UlIP: OR_{adj}=0.34; 95% CI, 0.19 – 0.61, \( P=0.0003 \); RA-nonUIP: OR_{adj}=0.44; 95% CI 0.24 – 0.81, \( P=0.008 \)). Details are given in Table 3. In the combined population, the comparison of adjusted ORs for RA-UlIP vs. RA-noILD to RA-nonUIP vs. RA-noILD did not reach statistical significance (\( P=0.54 \)).

**Effect of methotrexate ever use on delay of detection of RA-ILD**

In the discovery population and after adjusting for covariates, ILD was detected later in MTX ever users as compared to never users (\( P_{adj}=0.001 \)). These findings were replicated in the replication multi-ethnic case series sample (\( P_{adj}<0.001 \)), leading in the combined analysis to a mean delay of detection of 11.4 ± 10.4 years in MTX ever users compared to 4.0 ± 7.4 years in MTX never users in the combined analysis (\( P_{adj}<0.001 \)) (see Supplementary Appendix Table S3).
DISCUSSION

MTX is currently recommended as the first-line disease-modifying treatment for RA [5, 6]. Even though fibrotic ILD is a well-recognized extra-articular complication of RA (i.e. RA-ILD), the effect of MTX on the development of RA-ILD remains unsettled as conflicting results have been published [11-15]. Interpretation of previous studies is complicated by a number of biases affecting the association of MTX ever use and development of ILD, one of the main bias being the absence of systematic evaluation of the lung phenotype with chest HRCT. Both MTX-related hypersensitivity pneumonitis and the putative association between MTX and RA-ILD [15, 27] have led rheumatologists and pulmonologists to withdraw MTX therapy not only in patients with established prior history of MTX-related hypersensitivity pneumonitis, but also in patients with RA who developed ILD, and to limit the use of MTX in patients with RA-ILD.

In this study, we found a lower frequency of MTX ever use in RA-ILD patients compared to RA-noILD patients within the French discovery population and within the international replication population. One of the major strengths of our study is that the inverse relationship was consistent in all studied populations, which is reassuring (Figure 1 and Supplementary Appendix, Tables S1) and, sensitivity analyses did not change the direction or magnitude of the association signal of MTX exposure on risk of ILD among patients with RA (see Supplementary Appendix, Tables S2). We also observed that the HRCT ILD detection was delayed by 3.6 years in MTX ever users compared to never users, supporting the hypothesis of a possible inverse relationship between MTX ever use and ILD development in RA patients [13].
Most importantly, the lung phenotype (ILD or noILD) was systematically assessed with chest HRCT scan, avoiding the misclassification of patients with pre-clinical RA-ILD at the time of inclusion, and allowing us to demonstrate that the inverse relationship was found whatever the pattern of ILD (UIP or non-UIP). Even if the temporality of the chest HRCT scan (median RA duration time of 13(7-21)) is relevant according to the reported mean time of ILD occurrence in RA [28], a late occurrence of ILD in some individuals classified as RA-noILD cannot be fully excluded, which is one limitation of the study. In addition, there is a possibility that patients with ILD or respiratory symptoms would be less likely to be prescribed MTX. However, to take this into account the MTX exposure was systematically assessed before the detection of ILD. Nonetheless, these findings are consistent with three previously reported meta-analyses of randomized controlled trials (RCT) in RA and non-RA inflammatory diseases in which MTX was not associated with non-infectious respiratory adverse events and with two British cohorts in which MTX was not associated with an increased risk of incident RA-ILD [11, 13, 29, 30].

Beside the practical implications for RA-ILD management, these results may also have important implications for the interpretation of ongoing and future clinical trials addressing RA-ILD or other connective tissue diseases related ILD. Putative beneficial effects of other agents studied for their effect on RA-ILD in the context of MTX therapy may be misattributed to the study drug rather than MTX.

Although an association between anti-CCP positivity and RA-ILD has been previously suggested [31, 32], concordant with previously reported large studies [33, 34]. we did not detect such association. Several arguments would explain this apparent discrepancy of our findings: i) a multi-ethnic and larger population of patients investigated, ii) the ILD status defined with chest HRCT scan, avoiding misclassification and iii) the use of the ACR/EULAR 2010 classification criteria which include the seropositivity for anti-CCP [35]. In our multi-ethnic case-control study the lack of association of anti-CCP positivity with RA-ILD was constantly observed in each investigated population, strengthening our finding.

Because of the retrospective design of this study, the inverse relationship between MTX exposure and the risk of RA-ILD should be interpreted with caution. First, age, sex and smoking history was statistically different between the patients with and without ILD and may
have influenced MTX prescription. Second, information about biological treatment (drugs, dosage, treatment duration...) could not be assessed in this retrospective study. Third, due to missing data, the cumulative dose of MTX could only be considered as descriptive. Fourth, the ratio of patients with and without ILD varies between countries resulting in a potential bias of confounding by center. Fifth, some physicians may perform PFTs before MTX initiation in order to avoid MTX prescription in patients with abnormal lung function. Such evaluation was not assessed in our study inducing a possible prescription bias. In order to avoid such bias, confounders such as age at RA onset, sex, ever smoking, biologics ever use, participating center, MTX exposure duration and periods of RA onset were taken into account using a regression adjustment. However, it is possible that all the confounding factors that affect the observed inverse relationship between MTX ever use and RA-ILD could not be taken into account.

Altogether, these results suggest that MTX could be considered as having a disease modifying effect on RA-ILD that could result from distinct anti-inflammatory mechanisms, involving i) a direct immune suppressive effect of MTX specifically targeting the lung, similar to that observed in some other immune-mediated pulmonary diseases such as sarcoidosis[36], and ii) an indirect effect related to MTX-driven decrease of RA-related systemic inflammation. Indeed, previous studies have reported that higher RA disease activity is associated with an increased risk of extra-articular manifestations including ILD [37-39] and a recent study confirmed that active RA was associated with an increased risk for developing RA-ILD [40]. It is worth noting that we did not observe a difference in biological agents ever use between RA-ILD and RA-noILD patients, suggesting that the inverse relationship detected in this study may be specific to MTX as compared to other DMARDs. However, even if the strength of the inverse relationship and the reproducibility in the different populations investigated are in favor of a causal relationship, our study only describes a statistical relationship and did not provide definitive evidence for causality. Indeed, the observed effect may be due to an unexpected confounder that was not taken into account in our statistical analysis. Although we acknowledge that a prospective study would be required to further establish that MTX ever use is delaying the onset of RA-ILD, such a trial is unlikely to be performed due to the central role of MTX in the management of RA.
Our results suggest that MTX use is not associated with an increased risk of RA-ILD in patients with RA, and that ILD was detected later in MTX treated patients.

**Acknowledgment**

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REFERENCES


Figure 1. Methotrexate ever use and risk of rheumatoid arthritis associated interstitial lung disease

Forest plot of odds ratios (OR) for interstitial lung disease among patients with rheumatoid arthritis (RA) according to methotrexate (MTX) ever use. The square boxes indicate ORs, and the horizontal lines indicate 95% CIs for each sample. Diamonds display the pooled estimates. The black dotted line represents a mean OR value of 1. ORs were adjusted for age at RA onset, sex, ever smoking, case series origin, biologic ever use, MTX exposure duration and periods of MTX use at year of RA onset.
Table 1. Characteristics of patients with rheumatoid arthritis at inclusion

<table>
<thead>
<tr>
<th></th>
<th>Discovery</th>
<th>Pooled replication</th>
<th>Combined</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>RA-ILD</td>
<td>RA-noILD</td>
<td>P*</td>
</tr>
<tr>
<td>No. patients</td>
<td>100</td>
<td>165</td>
<td></td>
</tr>
<tr>
<td>Female — no. (%)</td>
<td>56 (56.0)</td>
<td>131 (79.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age at inclusion — yr</td>
<td>60 (53-68)</td>
<td>55 (46-65)</td>
<td>0.002</td>
</tr>
<tr>
<td>Age at RA onset — yr</td>
<td>54 (44-62)</td>
<td>42 (30-52)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RA duration — yr</td>
<td>10 (4-18)</td>
<td>10 (4-16)</td>
<td>0.53</td>
</tr>
<tr>
<td>Ever smoker — no. (%)</td>
<td>59 (59.0)</td>
<td>70 (45.2)</td>
<td>0.040</td>
</tr>
<tr>
<td>Tobacco exposure, pack per year — mean (SD)</td>
<td>13.8 ± 17.0</td>
<td>10.0 ± 21.2</td>
<td>0.002</td>
</tr>
<tr>
<td>Biologic ever use — no. (%)</td>
<td>37 (57.8)</td>
<td>86 (55.5)</td>
<td>0.77</td>
</tr>
<tr>
<td>RA autoimmunity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACPA-positive — no. (%)</td>
<td>83 (85.6)</td>
<td>140 (88.1)</td>
<td>0.57</td>
</tr>
<tr>
<td>RF-positive — no. (%)</td>
<td>71 (77.2)</td>
<td>120 (77.4)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>UIP or possible UIP HRCT pattern — no. (%)</td>
<td>47 (52.8)</td>
<td>133 (42.9)</td>
<td></td>
</tr>
<tr>
<td>Pulmonary function testing</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>FVC— % predicted</td>
<td>88 (66-102)</td>
<td>76 (61-90)</td>
<td></td>
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<tr>
<td>DLCO— % predicted</td>
<td>59 (50-70)</td>
<td>55 (41-68)</td>
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<tr>
<td>TLC— % predicted</td>
<td>81 (69-94)</td>
<td>77 (67-87)</td>
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* Unadjusted P-values obtained by Wilcoxon rank-sum tests (continuous variables) and Fisher’s exact tests (categorical variables).
Table 2. Methotrexate use in patients with rheumatoid arthritis with or without interstitial lung disease

<table>
<thead>
<tr>
<th></th>
<th>Discovery</th>
<th>Pooled replication</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RA-ILD</td>
<td>RA-noILD</td>
<td>RA-ILD</td>
</tr>
<tr>
<td>MTX exposure duration t yr</td>
<td>4 (1-13)</td>
<td>10 (4-16)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>6 (2-15)</td>
<td>15 (8-22)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>6 (1-14)</td>
<td>13 (7-21)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Periods of MTX use at year of RA onset — no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unlikely</td>
<td>6 (6.0)</td>
<td>19 (11.5)</td>
<td>25 (8.1)</td>
</tr>
<tr>
<td>Less often</td>
<td>18 (18.0)</td>
<td>24 (14.5)</td>
<td>51 (16.5)</td>
</tr>
<tr>
<td>Often</td>
<td>42 (42.0)</td>
<td>81 (49.1)</td>
<td>126 (40.6)</td>
</tr>
<tr>
<td>Standard of care</td>
<td>34 (34.0)</td>
<td>41 (24.8)</td>
<td>108 (34.8)</td>
</tr>
<tr>
<td>MTX ever use — no. (%)*</td>
<td>60 (60.0)</td>
<td>137 (83.0)</td>
<td>247 (79.7)</td>
</tr>
<tr>
<td>MTX cumulative dose — g</td>
<td>0.1 (0.0-3.1)</td>
<td>2.2 (0.2-5.5)</td>
<td>1.5 (0.0-4.7)</td>
</tr>
</tbody>
</table>

RA: rheumatoid arthritis, ILD: interstitial lung disease, RA-ILD: patients with rheumatoid arthritis associated interstitial lung disease, RA-noILD: rheumatoid arthritis patients without interstitial lung disease, MTX: methotrexate. Values are median (interquartile range) or else if indicated.

For MTX ever use, odds ratio (OR adj) P values are adjusted for age at RA onset, sex, ever smoking, biologic ever use, MTX exposure duration and periods of MTX use at year of RA onset, and obtained by random-effects meta-analysis of pooled estimates over multiply imputed datasets.

For MTX exposure duration, periods of MTX use at year of RA onset and MTX cumulative dose, P values are unadjusted.

* To avoid any bias resulting in MTX withdrawal secondary to ILD co-occurrence in patients with RA, the MTX exposure for patients with RA-ILD was established during the period before the diagnosis of ILD.

* UK excluded from the Pooled replication and Combined
Table 3. Association of the methotrexate ever use with rheumatoid arthritis related interstitial lung disease, according to chest high resolution computed tomography scan patterns

<table>
<thead>
<tr>
<th></th>
<th>Discovery</th>
<th>Pooled replication*</th>
<th>Combined*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RA-UIP</td>
<td>RA-nonUIP</td>
<td>RA-noILD</td>
</tr>
<tr>
<td>No. patients</td>
<td>47</td>
<td>42</td>
<td>165</td>
</tr>
<tr>
<td>MTX ever use — no. (%)</td>
<td>26 (55.3)</td>
<td>28 (66.7)</td>
<td>137 (83.0)</td>
</tr>
<tr>
<td>OR adj (95% CI)</td>
<td>0.44 (0.20–0.98)</td>
<td>0.41 (0.18–0.95)</td>
<td>0.25 (0.11–0.59)</td>
</tr>
<tr>
<td>P adj</td>
<td>0.044</td>
<td>0.038</td>
<td>0.002</td>
</tr>
</tbody>
</table>

RA: rheumatoid arthritis, UIP: usual interstitial pneumonia, ILD: interstitial lung disease, RA-noILD: rheumatoid arthritis patients without interstitial lung disease, MTX: methotrexate. The RA-UIP subset includes RA-ILD patients with the following HRCT scan patterns: usual interstitial pneumonia and possible usual interstitial pneumonia. The RA non-UIP subset includes RA-ILD patients having the following HRCT scan patterns: Non-specific interstitial pneumonia, organizing pneumonia, unclassifiable ILD. Odds ratios (OR adj) and their corresponding P values are adjusted for age at RA onset, sex, ever smoking, biologic use ever, MTX exposure duration and periods of MTX use at year of RA onset, respectively, and obtained by random-effects meta-analysis of pooled estimates over multiply imputed datasets. OR adj are adjusted odds ratios for RA-UIP or RA-nonUIP vs. RA-noILD.

* UK excluded from the Pooled replication and Combined analyses.
### Table S1. Baseline characteristics of patients in each case series

<table>
<thead>
<tr>
<th></th>
<th>France</th>
<th>Brazil</th>
<th>Italy</th>
<th>Mexico</th>
<th>United Kingdom</th>
<th>United States of America</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>100</td>
<td>165</td>
<td>61</td>
<td>201</td>
<td>46</td>
<td>47</td>
</tr>
<tr>
<td>Female — no. (%)</td>
<td>56 (56.0)</td>
<td>131 (79.4)</td>
<td>43 (70.5)</td>
<td>201 (100.0)</td>
<td>27 (58.7)</td>
<td>35 (74.5)</td>
</tr>
<tr>
<td>Age at inclusion — yr</td>
<td>60 (52.6)</td>
<td>55 (46.6)</td>
<td>58 (52.6)</td>
<td>62 (55.6)</td>
<td>71 (63.7)</td>
<td>72 (62.7)</td>
</tr>
<tr>
<td>RA duration — yr</td>
<td>10 (4.18)</td>
<td>10 (4.16)</td>
<td>14 (9.25)</td>
<td>17 (11.27)</td>
<td>16 (12.22)</td>
<td>17 (12.22)</td>
</tr>
<tr>
<td>MTX exposure duration — yr</td>
<td>4 (1.13)</td>
<td>10 (4.16)</td>
<td>8 (3.17)</td>
<td>17 (11.27)</td>
<td>9 (5.17)</td>
<td>17 (12.22)</td>
</tr>
<tr>
<td>Age at RA onset — yr</td>
<td>54 (44.62)</td>
<td>42 (30.52)</td>
<td>50 (41.57)</td>
<td>42 (32.50)</td>
<td>54 (46.66)</td>
<td>53 (46.61)</td>
</tr>
<tr>
<td>No. missing</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

#### Periods of MTX use at year of RA onset — no. (\%)

<table>
<thead>
<tr>
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<th>Brazil</th>
<th>Italy</th>
<th>Mexico</th>
<th>United Kingdom</th>
<th>United States of America</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. missing</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

#### Methotrexate exposure

<table>
<thead>
<tr>
<th></th>
<th>France</th>
<th>Brazil</th>
<th>Italy</th>
<th>Mexico</th>
<th>United Kingdom</th>
<th>United States of America</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTX ever use — no. (%)</td>
<td>60 (60.0)</td>
<td>137 (83.0)</td>
<td>44 (72.1)</td>
<td>194 (96.5)</td>
<td>40 (80.7)</td>
<td>47 (100.0)</td>
</tr>
<tr>
<td>No. missing</td>
<td>36</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</table>

#### Pulmonary function testing

<table>
<thead>
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<th>Brazil</th>
<th>Italy</th>
<th>Mexico</th>
<th>United Kingdom</th>
<th>United States of America</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC — % predicted</td>
<td>88 (66.102)</td>
<td>NA</td>
<td>84 (74.91)</td>
<td>60 (50.85)</td>
<td>93 (84.113)</td>
<td>74 (61.87)</td>
</tr>
<tr>
<td>No. missing</td>
<td>35</td>
<td>22</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>DLCO — % predicted</td>
<td>59 (50.70)</td>
<td>NA</td>
<td>65 (54.68)</td>
<td>38 (21.72)</td>
<td>56 (46.68)</td>
<td>53 (40.68)</td>
</tr>
<tr>
<td>No. missing</td>
<td>40</td>
<td>19</td>
<td>19</td>
<td>2</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>TLC — % predicted</td>
<td>81 (69.94)</td>
<td>NA</td>
<td>77 (65.89)</td>
<td>73 (62.83)</td>
<td>83 (76.87)</td>
<td>76 (67.86)</td>
</tr>
<tr>
<td>No. missing</td>
<td>52</td>
<td>39</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>46</td>
</tr>
</tbody>
</table>

**RA**: rheumatoid arthritis, **ILD**: interstitial lung disease, **RA-ILD**: patients with rheumatoid arthritis associated interstitial lung disease, **RA-nolILD**: rheumatoid arthritis patients without interstitial lung disease, **MTX**: methotrexate, **ACPA**: anti-citrullinated protein antibody, **RF**: rheumatoid factor, **HRCT**: high-resolution computed tomography, **UIP**: usual interstitial pneumonia, **FVC**: forced vital capacity, **DLCO**: diffusion capacity of carbon monoxide, **TLC**: total lung capacity, **NA**: not assessed. Values are median (interquartile range) or else if indicated.
Table S2. Sensitivity analyses of the association between methotrexate ever use and interstitial lung disease in patients with rheumatoid arthritis.

<table>
<thead>
<tr>
<th>Sensitivity analysis</th>
<th>Discovery OR&lt;sub&gt;adj&lt;/sub&gt; (95% CI)</th>
<th>Pooled replication OR&lt;sub&gt;adj&lt;/sub&gt; (95% CI)</th>
<th>Combined OR&lt;sub&gt;adj&lt;/sub&gt; (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One-stage analysis with random case series effects</td>
<td>0.46 (0.24 to 0.90)</td>
<td>0.43 (0.21 to 0.84)</td>
<td>0.44 (0.28 to 0.70)</td>
</tr>
<tr>
<td>Removing replication samples with too few unexposed</td>
<td>0.46 (0.24 to 0.90)</td>
<td>0.39 (0.19 to 0.81)</td>
<td>0.43 (0.26 to 0.70)</td>
</tr>
<tr>
<td>Complete cases analysis</td>
<td>0.41 (0.19 to 0.88)</td>
<td>0.41 (0.20 to 0.81)</td>
<td>0.41 (0.24 to 0.68)</td>
</tr>
</tbody>
</table>

Odds ratios (OR<sub>adj</sub>) are adjusted for age at RA onset, sex, ever smoking, biologic ever use, MTX exposure duration and periods of MTX use at year of RA onset.

To add further sensitivity analyses, we considered that all missing biologic use data would be either « no » or « yes » in the discovery sample, which are rather extreme assumptions. This corresponded to proportions of biologic use of 37% or 73% for RA-ILD, respectively, and 52.1% or 58.2% for RA-noILD, respectively. In the first case, the odds ratio in the discovery sample was increased to 0.52 (95%CI 0.27 to 1.00), and in the second case it was 0.43 (0.22 to 0.84). The combined odds ratios were 0.45 (0.28 to 0.74) and 0.41 (0.25 to 0.67), respectively.
Table S3. Effect of methotrexate ever use on the delay of onset of interstitial lung disease among patients with rheumatoid arthritis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>MTX never use Mean (SD)</th>
<th>MTX ever use Mean (SD)</th>
<th>Difference (95% CI)</th>
<th>( P_{\text{adj}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discovery</td>
<td>3.7 (7.1)</td>
<td>10.7 (9.3)</td>
<td>3.5 (1.4–5.6)</td>
<td>0.001</td>
</tr>
<tr>
<td>Pooled replication</td>
<td>4.2 (7.6)</td>
<td>11.5 (10.7)</td>
<td>3.5 (2.2–4.8)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Combined</td>
<td>4.0 (7.4)</td>
<td>11.4 (10.4)</td>
<td>3.6 (2.6–4.7)*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

MTX: methotrexate. Mean differences and \( P \)-values are adjusted for age at RA onset, sex, ever smoking, biologic ever use, MTX exposure duration and periods of MTX use at year of RA onset, and obtained by a single-stage model with a random effect for case series origin (*), pooled over multiply imputed datasets. * UK excluded from the Pooled validation and Combined analyses.
REFERENCES