Meta-analysis. We followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement for reporting methods. 10

Search and sources of information

We searched PubMed and Web of Science from 2011 to present with search terms **liver fibrosis/cirrhosis related to methotrexate use**. No restrictions were applied. 138 articles were included in the initial review. Retrieved citations were exported to citation manager and duplicates were removed.

Eligibility criteria and selection process

Initial vetting was done by reviewer (HIC) based on study titles and abstract information. We excluded studies with pediatric data, basic science focus, case reports, studies with multiple different therapies, meeting abstracts, posters, letters, common narrative reviews and opinion articles. Included articles (n=20) were then acquired in full text form. A consensus was established at each step, when not reached, by a second reviewer (JAD). We included 20 (11-31) studies in the systematic review and out of these three were included in the final meta-analysis (11,12,13) Step-wise selection process is shown in Figure1.

Data collection and processing

Due to considerable heterogeneity in patient population, study design and methods of reporting results, we pooled similar patient populations with chronic inflammatory diseases that were

treated with methotrexate. All these studies excluded patients with chronic liver disease, alcoholic liver disease, autoimmune hepatitis, chronic hepatitis B&C, liver cirrhosis, chronic kidney disease, chronic heart disease and pregnancy. As a result of this, 5 studies (11, 12, 13, 14, 15) were shortlisted. Out of these one was a retrospective cohort (15) and the remaining four (11, 12, 13, 14) were cross sectional studies. This included 505 subjects. Varying criteria for excluding obese patients were reported with each study. Average body mass index (BMI) was 25 +/- 5. Indications for MTX therapy were rheumatoid arthritis and/or psoriasis. Range of MTX dose was between 1.5 gram to 22 grams. Duration of MTX therapy ranged from 3 to 11 years.

All of these studies used fibroscan to assess liver stiffness and used 7.1 kilopascals as the cutoff for any fibrosis. The average cut off for severe fibrosis was >9 kilopascals and cirrhosis was defined as more than 12.5 kilopascals. Two of these studies (11,15) performed biopsies on selected patients. Meta-analysis could be performed on only three studies (11,12,13) because of variability in reported results. Study characteristics of all 20 studies are shown in tables 2,3 and 4.

Pooled means and standard deviations (SD) were computed as below using excel (see (a) and (b) below).

To include information from Bafna et al. 2021 (11), the median was assumed to equal the mean and the SD was calculated from the interquartile range assuming normality as below (see (c) below). Confidence intervals were calculated as follows (d).

Results shown in Table 1.

(a) Pooled mean calculation

$m_T=(n_1m_1 +n_2m_2 + n_3m_3)/(n_T)$,

where m=mean, n=patients in study, #=study of interest, T=all studies being pooled in sample

(b) Pooled SD calculation

$SD_P = [(((n_1-1)SD_1^2)+ ((n_2-1)SD_2^2))/(n_1+n_2-2)]^{0.5}$

where SD_P=pooled standard deviation, $SD_\#$ = standard deviation for study of interest

(c) Given that the Z score for the 75th percentile = +0.67 and the Z score for the 25th percentile = - 0.67. Assuming normality, the following can be used to approximate the SD.

$SD =IQR/(Z_{IQR}) = IQR/(Z_{75} -Z_{25}) = IQR/(0.67- -0.67) = IQR/1.34$

where IQR= interquartile range from 75th percentile minus the 25th percentile and Z_{IQW} = difference in Z scores between the 75th and 25th percentiles.

(d) Pooled confidence interval calculations

95% Confidence interval = mean +/- 1.96 $(SD/(n_T^{0.5}))$

Outcomes

Our primary endpoint was association of cumulative MTX dose to liver fibrosis. We also evaluated any reported cirrhosis related to methotrexate dose and if it was verified by biopsy. Reported confounding factors were documented and assessed for statistical significance.