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Observational Study

HEPATIC STEATOSIS IN PATIENTS WITH RHEUMATOID ARTHRITIS TREATED WITH METHOTREXATE IS ASSOCIATED WITH BODY MASS INDEX.

Liver steatosis in rheumatoid arthritis.

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Abstract

BACKGROUND

Methotrexate (MTX) is the usual first-line treatment for rheumatoid arthritis (RA). Long-term use of MTX has been associated with liver steatosis (LS) and liver fibrosis (LF).

AIM

The aim of our study was to determine if LS in patients treated with MTX for **RA** is associated with MTX cumulative dose (MTX-CD), metabolic syndrome (MtS), body mass index (BMI), male gender or LF.

METHODS

A single-centre, prospective study of patients receiving MTX for **RA** was performed from February 2019 to February 2020. The inclusion criteria were patients aged 18 years or older, diagnosed with RA by a rheumatologist and on treatment with MTX (without limitation on the duration of treatment). The exclusion criteria were previous diagnosis of liver disease (hepatitis B or C virus infection; known non-alcoholic fatty liver disease), alcohol consumption greater than 60 g/day in men or 40 g/day in women, HIV infection on antiretroviral therapy, diabetes mellitus, chronic renal failure, congestive heart failure or body mass index (BMI) greater than 30 kg/m². Patients receiving leflunomide in the 3 years prior to the study were also excluded.

Transient elastography (Fibroscan, echosens®) was used for fibrosis determination (LF >7 KpA) and computer attenuation parameter (CAP) for liver steatosis (CAP >248 dB/m).

Demographic variables, laboratory data, MTX-CD (>4000 mg), MtS criteria, BMI (>25), TE and CAP scores were collected from all patients.

RESULTS

Fifty-nine patients were included. Forty-three were women (72.88%), and the mean age was 61.52 years (SD 11.73).

When we compared MTX-CD ≤4000 mg (26 patients; 14 with LS and 12 without) with >4000 mg (33 patients; 12 with LS and 21 without), no statistical differences were found (P = 0.179).

We compared CAP scores stratified by MtS, BMI, gender and LF. There were no significant differences in CAP scores based on the presence or not of MtS, male sex or LF. CAP/MtS: 50 no MtS (84.75%), 9 MtS (15.25%), P = 0.138. CAP-gender: 8 man/ 18 women LS; 8 man/ 25 women no LS, P = 0.576. CAP-Fibrosis: 53 No LF (89.83%); 6 LF (10.17%), P = 0.239. LS determined by CAP was significantly associated with BMI >25: CAP/BMI: 22 BMI ≤25 (37.29%); 37 BMI >25 (62.71%), P = 0.002.

CONCLUSION

Hepatic steatosis in patients with **RA** treated with MTX is not associated with MTX-CD, LF, male sex or MtS. However, BMI is significantly related to LS in these patients.

Key words: Methotrexate; rheumatoid arthritis; liver steatosis; liver fibrosis; transient elastography; computed attenuation parameter

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Core Tip: Methotrexate is the cornerstone of treatment for rheumatoid arthritis (RA) and has been associated with the development of hepatic fibrosis and liver steatosis. The objective of this work was to study if hepatic steatosis in patients with RA treated with MTX is associated with body mass index (BMI), MTX cumulative dose (MTX-CD), gender, hepatic fibrosis (HF) and metabolic syndrome (MtS). We conclude that liver

steatosis in patients with RA on MTX treatment is not realated to MTX-CD, HF, male sex or MtS. In our study, BMI is significantly associated to hepatic steatosis in these patients.

INTRODUCTION

Methotrexate (MTX) has been used in the treatment of oncological and chronic inflammatory diseases. It is also the cornerstone of treatment for rheumatoid arthritis (RA). The most concerning long-term adverse effect of this treatment is the development of hepatic fibrosis (HF) (1-5). Liver steatosis (LS) has been associated with RA and with MTX treatment (6). Liver biopsy has been the gold standard for the study of HF and LS, but it has several limitations (3): there is a disparity of fibrosis values between biopsy samples, and it is an invasive technique accompanied by risks (3).

Recent studies have been carried out on non-invasive measurements of fibrosis in the liver. Transient elastography (TE) is a non-invasive method without side effects that also allows the sequential determination of liver fibrosis measurements over time, which makes it of great interest for the follow-up of these patients (2-4).

MTX, as a risk factor for secondary hepatic steatosis, has been studied recently. RA has been associated with moderate to severe hepatic steatosis; predisposing factors as higher body mass index, male sex, and MTX cumulative dose (MTX-CD) have been published (7). However, there have been conflicting results, and the impact of MTX on NAFLD is still unclear (6- 11).

The computed attenuation parameter (CAP) measures carried out at the time of TE correlates with the histological hepatic steatosis (12). The CAP algorithm calculates the ultrasound signal attenuation (12). LS have been evaluated recently using CAP in chronic MTX users and was common with moderate and severe LS predicting moderate to severe LF (13).

The objective of our work is to determine if LS in patients with **RA** treated with MTX is associated with body mass index (BMI), MTX-CD, gender, hepatic fibrosis and metabolic syndrome (MtS).

MATERIALS AND METHODS

We performed a single-centre, prospective study of patients receiving MTX for RA. The principle objective of this work was to study the presence of HF by TE and APRI (14) as well as the detection of LS by ultrasonography and CAP.TE, Fibroscan® (FS) (Fibroscan®402, Echosens, France, www.echosens.com) was used for fibrosis determination (LF >7 KpA). CAP for LS (CAP >248 dB/m) (11). Demographic variables, laboratory data, MTX-CD (>4000 mg), MtS criteria, BMI (>25), TE and CAP scores were collected from all patients.

Patients were recruited between 1 February 2019 and 31 January 2020 from the Gastroenterology-Rheumatology clinics of our Hospital. The inclusion criteria were patients aged 18 years or older, diagnosed with RA by a rheumatologist and on treatment with MTX (without limitation on the duration of treatment). The exclusion criteria were previous diagnosis of liver disease (hepatitis B or C virus infection; known non-alcoholic fatty liver disease), alcohol consumption greater than 60 g/day in men or 40 g/day in women, HIV infection on antiretroviral therapy, diabetes mellitus, chronic renal failure, congestive heart failure or body mass index (BMI) greater than 30 kg/m². Patients receiving leflunomide in the 3 years prior to the study were also excluded.

Demographic data analysis, treatment history and cumulative MTX dose were collected through computerised medical records. LF was defined by FS (measurement greater than 7 Kpa) and by APRI score (result greater than 0.7). The FS assessment was performed by a trained nurse. At the time of inclusion in the study, a blood test was performed to calculate the **APRI score (APRI: aspartate aminotransferase to platelet ratio index. AST level: AST(ULN)/ platelet level x 100).** High transaminase levels were defined as results above 33 U/L. Finally, disease activity was defined by a rheumatologist using the DAS28-CRP score. Data were collected by means of a

questionnaire, a review of the computerised clinical history and a visit to the gastroenterology clinic.

Statistical analysis

Initially, a descriptive analysis was performed by calculating the mean and standard deviation (or median and interquartile range) for quantitative variables. For qualitative variables, absolute and relative frequencies were calculated as percentages. To compare the distribution of qualitative variables, the chi-square test or Fisher's exact test was used. Similarly, the Student's *t*-test or the Mann-Whitney U test was used to compare quantitative variables. STATA 16.1 software was used for all the analyses. Statistical review of the study was performed by a biomedical statistician (IU).

Ethics

The clinical research ethics committee of the Gipuzkoa health area (Código de Protocolo: ACLFSC-2018-01; Acta 01/2019) approved this study, and participants signed an informed consent form prior to inclusion.

RESULTS

We included 59 patients in the study. There were 43 women (72.88%), and 61.52 years (SD 11.73) was the mean age. Clinical characteristics are presented in Table 1 and laboratory data in Table 2 **(Supplementary materials).** The mean duration of the MTX treatment was 82.4 months (SD 65.1). The mean MTX-CD of the patients was 5214.5 mg (SD 4031.9). Twenty six patients presented an MTX-CD ≤ to 4000 mg. Thirty three had an MTX-CD > than 4000 mg.

Treatment duration and times of disease progression were longer in the MTX-CD > 4000 mg group. MTX monotherapy was used in 46 patients (77.9%). Only 7 patients (11.8%) were on **NSAIDs therapy in association with MTX.**

Ultrasonography was performed in 56 patients, of whom 39 presented no steatosis (69.64%), and 17 (30.36%) had LS. CAP was determined in all 59 patients, categorising 33 patients without LS and 26 patients with LS.

We then compared both methods (56 patients in total): ultrasonography presented a PPV of 88.2% (CI 95%: 63.6%–98.5%) and an NPV of 76.9% (CI 95%: 60.7%–88.9%), with a sensitivity of 62.5% (CI 95%: 40.6%–81.2%) and a specificity of 93.8% (CI 95%: 79.2%–99.2%), compared to CAP. When comparing MTX-CD ≤4000 mg (26 patients; 14 with LS and 12 without) with >4000 mg (33 patients; 12 with LS and 21 without), we found no statistical differences in LS between low and high MTX-CD (P = 0.179) (**Figure 1).** CAP scores were compared stratified by BMI, gender, LF or MtS: no significant differences were obtained based on the presence or absence of male sex, LF or MtS. CAP/MtS: 50 no MtS (84.75%), 9 MtS (15.25%), P = 0.138. **CAP-gender: 8 man/ 18 women LS; 8 man/ 25 women no LS**, P = 0.576. CAP-Fibrosis: 53 No LF **(89.83%);** 6 LF (10.17%), P = 0.239.

Nonetheless, hepatic steatosis measured by CAP was significantly related with BMI >25. CAP/BMI: 22 BMI ≤25 (37.29%); 37 BMI >25 (62.71%), *P* = 0.002 (**Figure 2).**

DISCUSSION

MTX is the gold standard of RA treatment, both in monotherapy or associated with biological therapies (15). Hepatic fibrosis has been associated with the chronic MTX use in this disease. There is increasing evidence that LF is broadly affected by other factors: alcohol, other associated drugs and **MtS** are directly related with the development of LF (16-19).

Drugs can affect LS development. The possible effect of MTX in the presence of LS in patients with RA is currently being studied. According to laboratory research folate deficiency produced by chronic MTX treatment could promote liver fat accumulation (20), but folic acid supplementation has been recommended and is currently being used in treatment regimens. Studies have shown conflicting results, and the impact of MTX on LS is still unclear (6).

Choi *et al* (6) investigated whether MTX-CD in 368 RA patients led to LS, determined by ultrasound, but they did not detect a significant association between LS development and MTX administration, suggesting that to adjust for individualized risk factors for NAFLD may be more efficient than MTX discontinuation in LS detection/management. Hypertriglyceridemia and higher BMI were associated with an increased risk of LS.

Erre *et al* (7) have recently studied the independent association of LS and RA. In 223 patients with RA, they found that RA is independently associated with LS (moderate to severe), scored by ultrasound, and male gender, higher BMI and MTX-CD are independent risk factors for the development of LS (7).

Mori *et al* (8) studied the association between NAFLD and liver injury during MTX treatment in 846 patients with RA. They did not observe a significant impact of MTX dose and duration on histological severity. On the other hand, Sakthiswary *et al* (9) concluded, in a retrospective study, that the MTX-CD was the only independent predictor of MTX-associated LS with transaminitis in a cohort of 978 patients with RA.

Recently, detection of LS by CAP in chronic MTX users was published for the first time. Tomaszewski *et al* (14) studied 172 patients on MTX (45 with RA). DM, hypertension and BMI ≥30 were predictors of LS. LS determined by CAP was frequent. Moderate and severe LS in this study predicted moderate to severe fibrosis of the liver.

Our prospective study was designed to determine in patients with RA treated with MTX if LS, as measured by CAP, is associated with BMI, gender, hepatic fibrosis or MTX-CD. When we compared MTX-CD ≤4000 mg with >4000 mg, no statistical differences were found. There were no significant differences between the presence and absence of MtS, male sex or LF, but LS determined by CAP was significatively associated with BMI >25 ($P = 0.002$).

Our study has limitations. The sample size is relatively small, and we included all the patients with RA on MTX treatment, without a treatment duration limitation. **More women than men were included in this study, and given the limited sample size, it is difficult to conclude that there is no relationship between gender and LS.** The strengths of the study are that it is a prospective study and that liver steatosis was determined as measured by the CAP.

CONCLUSION

We can conclude that**, in our series** of patients treated with MTX for **RA,** LS is not associated with MTX-CD, HF, male sex or MtS.

In our study, BMI is significatively associated with hepatic steatosis. It seems that other factors, apart from MTX-CD or treatment duration, are more important for the development of LS in these patients.

ARTICLE HIGHLIGHTS

Research background

Methotrexate (MTX) remains the cornerstone of treatment for rheumatoid arthritis (RA), both in monotherapy and in association with other treatments.

The most concerning adverse effect of this treatment, in the long term, is liver fibrosis. Liver steatosis (LS) has been associated with RA and with MTX.

Research motivation

MTX, as a risk factor for secondary LS, has been studied recently. RA has been independently associated with moderate to severe LS; gender, higher body mass index and cumulative dose of MTX (CD-MTX) are predisposing factors. However, the studies have shown conflicting results, and the impact of MTX on LS is still unclear.

Research objectives

The objective of our work is to study if hepatic steatosis in RA patients treated with MTX is related to body mass index (BMI), MTX-CD, metabolic syndrome (MtS), gender or hepatic fibrosis.

Research methods

We performed a prospective study of RA patients treated with MTX. The principal objective of this work was to study the presence of HF by transient elastography and

aspartate aminotransferase to platelet ratio index (APRI) as well as the detection of LS by ultrasonography and CAP.

Research results

Fifty-nine patients were included in the study. When comparing MTX-CD ≤4000 mg with >4000 mg, we found no statistical differences in LS between low and high MTX-CD. We compared CAP scores with MtS, BMI, gender and LF: there were no significant differences based on the presence or absence of MtS, male sex or LF. Nonetheless, LS determined by CAP was significantly associated with BMI >25.

Research conclusions

We conclude that, in our series, LS in RA patients treated with MTX is not related to gender, MTX-CD, MtS or hepatic fibrosis. BMI >25 is significatively associated to LS in our study. Other factors, apart from MTX-CD or time in treatment, are more important for the development of LS in these patients.

Research perspectives

The routine incorporation of FS for the study of LF and LS in RA patients with MTX treatment is critical and will aid in understanding the real impact of MTX on LS. More studies (larger and multicentric) are recommended to validate these results.

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