# Risk of liver fibrosis induced by methotrexate and other rheumatoid arthritis medications according to the Fibrosis-4 Index

J. Avouac, R. Degrave, H. Vergneault, A. Combier, S. Wanono, M. Boisson, C. Frantz, Y. Allanore

*Université de Paris, Service de Rhumatologie, Hôpital Cochin, AP-HP.CUP, Paris, France.*

## Abstract

### Objective

*We aimed to estimate the amount of scarring in the liver with the fibrosis-4 (FIB-4) index in patients with rheumatoid arthritis (RA) with special interest in methotrexate (MTX) influence.*

### Methods

*This was a cross-sectional monocentric study including successive RA patients recruited for a 12-month period. Data on liver function, disease activity, hepatotoxic and cardiovascular risk factors were systematically collected. The FIB-4 index was calculated according the following formula: (age(years)× AST(U/L)/platelet (PLT) (109/L)×√ALT(U/L)).*

### Results

*We included 170 patients with established RA: 141 (83%) were women with a mean age of 59±12 years and mean disease duration of 15±11 years.The FIB-4 was low and not significantly different between patients receiving MTX (n=102), patients previously treated with MTX (n=39) and patients never treated with MTX (n=29). No correlation was observed between FIB-4 values and cumulative MTX dose (r=0.09, p=0.271). No relationship was observed between FIB-4 and MTX treatment duration. The FIB-4 index was found significantly increased in patients receiving leflunomide (n=24), (median (range) 1.58 (0.46–3.16) vs. 1.18 (0.54–3.40), p=0.019) and tocilizumab (n=14), (median (range) 1.82 (0.75–3.73) vs. 1.18 (0.54–3.40), p=0.005) compared to patients not receiving DMARDs (n=29). Multivariate logistic regression analyses revealed an independent association between increased FIB-4 (>1.45) and male gender, low disease activity, and treatment with leflunomide and tocilizumab.*

### Conclusion

*RA patients with long-term maintenance MTX therapy have low FIB-4 values suggesting that MTX is not associated with an increased risk of advanced liver fibrosis. Increased FIB-4 values have been detected in leflunomide- and tocilizumab-treated patients, which will deserve dedicated further investigations.*

**Key words**

rheumatoid arthritis, methotrexate, liver, fibrosis

*Jérôme Avouac, MD, PhD*
*Raphaël Degrave, MD*
*Hélène Vergneault, MD*
*Alice Combier, MD*
*Sarah Wanono, MD*
*Margaux Boisson, MD*
*Camélia Frantz, MD*
*Yannick Allanore, MD, PhD*

*Please address correspondence to:*
*Jérôme Avouac,*
*Service de Rhumatologie,*
*Hôpital Cochin,*
*27 rue du Faubourg Saint Jacques,*
*75014 Paris, France.*
*E-mail: jerome.avouac@aphp.fr*

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*Competing interests: none declared.*

## Introduction

Methotrexate (MTX) holds a unique place in the management of rheumatoid arthritis (RA) with a role at every stage in the evolution of this chronic condition, given its favourable balance between efficacy and safety (1). However, conflicting data still suggest a potential risk of MTX-induced long-term liver fibrosis.

Hepatotoxicity is the most frequently seen side effect in MTX-treated RA patients. Elevations of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) occur in 27–69% of RA patients (2). The enzyme elevations are usually mild and self-limiting, or they resolve spontaneously after MTX dose reduction and/or the addition of folic acid. However, even if transaminase values are almost within normal range, prolonged MTX exposition may result in fatty liver, steatohepatitis, and fibrosis leading to cirrhosis (3, 4). Taking into account the limitation of troublesome and expensive liver biopsies, there is a need to introduce, relevant, frequently assessable and non-invasive (serum) markers for fibrosis that would be able to replace or to be used as surrogate for liver imaging/biopsy.

Several non-invasive physical or biochemical markers for liver fibrosis have been suggested for patients with chronic liver diseases (5). Although physical examinations such as transient elastography or shear wave elastography have demonstrated a higher accuracy in assessing the extent of liver fibrosis than biochemical markers (6), physical examinations are usually expensive and frequently restricted to tertiary academic centres. In contrast, biochemical markers, including the fibrosis-4 (FIB-4) index have important clinical applicability, as they are easily available with routine laboratory tests (7). The FIB-4 index was originally proposed as a simple and non-invasive marker of liver fibrosis in HIV/HCV co-infection. This index is calculated based on age, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, and platelet count. It has shown powerful diagnostic performance in assessing the degree of liver fibrosis (8). FIB-4 has been proven to be a significant predictor of poor outcomes in chronic liver diseases, including hepatic fibrotic changes in Non-Alcoholic Steato-Hepatitis (NASH) (6, 9, 10). In patients with RA, a preliminary Japanese study reported that FIB-4 values were correlated to the amount of histologic liver lesions, suggesting that this index may be a valuable marker to diagnose liver disease in RA patients (11).

Our primary objective was to estimate the amount of scarring in the liver with the FIB-4 index in RA patients on maintenance therapy with MTX. Our secondary objectives were to evaluate the impact of other treatments on the FIB-4 index and identify potential associations between this score and RA disease characteristics.

## Patients and methods

*Inclusion and exclusion criteria*

We included patients with RA, >18 years of age, fulfilling the 2010 ACR/European League Against Rheumatism (EULAR) classification for RA (12, 13), who attended the one day hospitalization program of the department of Rheumatology, Cochin Hospital, over a 12-month period, for the evaluation and/or the treatment of their disease, as previously described (14). All included patients agreed to participate in the study after informed consent, which was recorded in the medical source file. The protocol and the informed consent document have received Institutional Review Board/Independent Ethics Committee (IRB/IEC) approval before initiation of the study ("Comité de Protection des Personnes" Paris Ile de France I, n° CPPIDF1-2016-Juin-DAP13).

*Data collection from RA patients*

History taking, physical examination, laboratory tests, and review of medical files were systematically performed to collect data from RA patients. CV risk factors (high blood pressure, tobacco, diabetes, fasting glycaemia, body mass index (BMI), metabolic syndrome), hepatotoxic factors (medication such as analgesics, non-steroidal anti-inflammatory drugs, NSAIDs, alcohol consumption) and current/past medication use were obtained from information provided by patients, and based on the

review of medical records. RA disease activity was assessed using the Disease Activity Score based on evaluation of 28 joints (DAS28) (15), using erythrocyte sedimentation rate (ESR) (16). Health status was measured by the self-administered Stanford Health Assessment Questionnaire (HAQ). Systematic hand and foot x-rays were performed to measure joint destruction, defined by the presence of erosions.

*Laboratory tests*

Routine laboratory study tests were obtained in RA patients on the morning of hospital visit. They included complete blood cell count, Westergren ESR) CRP concentration, serum creatinine concentration, and liver function tests (serum-glutamyl-oxaloacetate-transferase SGOT, serum glutamate-pyruvate transaminase SGPT, γGT, alkaline phosphatase, APL and bilirubin). Rheumatoid factor (RF) and second-generation anti-cyclic citrullinated peptide (anti-CCP2) antibodies were detected by enzyme-linked immunosorbent assay (ELISA).

*Definitions*

The FIB-4 index was calculated according the following formula:
(age(years) × AST(U/L)/platelet (PLT) (109/L) × √ALT(U/L)).

Using a lower cut-off value of 1.45, a FIB-4 score <1.45 had a negative predictive value of 90% for advanced fibrosis in the patient cohort in which this formula was first validated. In contrast, a FIB-4 >3.25 had 97% specificity and a positive predictive value of 65% for advanced fibrosis (8).

Metabolic syndrome was defined according to the NCEP-ATP III classification criteria. Metabolic syndrome was considered as present when patients had 3 of the 5 following criteria: fasting glycaemia ≥6.1 mmol/L, triglycerides ≥1.7 mmol/L, high density lipoproteins (HDL) <1.04 mmol/L in men/1.29 mmol/L in women, high blood pressure with systolic arterial pressure, (SAP)/ diastolic arterial pressure (DAP) ≥135/85 mmHg, waist circumference ≥ 102 cm in men/88 cm in women. If patients received anti-hypertensive agent, there were considered as suffering from high blood pressure. When waist circumference was not available, it was replaced by BMI ≥25 kg/m$^2$ according to the AACE criteria (17). ESR and CRP were considered as elevated above 28mm hour-1 and 10 mg/L, respectively.

**Table I.** Study population.

| | Patients with rheumatoid arthritis and FIB-4 ≤1.45 n=121 | Patients with rheumatoid arthritis and FIB-4>1.45 n=49 | *p*-value |
|---|---|---|---|
| **Demographics** | | | |
| Age (years), mean ± SD | 57 ± 12 | 69 ± 7 | <0.001 |
| Females, n (%) | 105 (87) | 36 (73) | 0.028 |
| **Disease characteristics** | | | |
| Disease duration (years), mean ± SD | 14 ± 11 | 18 ± 12 | 0.038 |
| Positive rheumatoid factor, n (%) | 99 (82) | 35 (71) | 0.112 |
| Positive anti-CCP2 antibodies, n (%) | 95 (79) | 39 (80) | 0.884 |
| Erosions on hand/foot x-rays, n (%) | 70 (58) | 31 (63) | 0.548 |
| **Disease activity:** | | | |
| DAS28, mean ±SD | 3.65 ± 1.39 | 3.14 ± 1.37 | 0.031 |
| DAS28 >3.2, n (%) | 55 (45) | 13 (27) | 0.030 |
| ESR (mmH1), mean ±SD | 22 ± 21 | 20 ± 14 | 0.541 |
| ESR >28 mmH1, n (%) | 34 (28) | 15 (31) | 0.696 |
| CRP (mg/L), mean ±SD | 8.9 ± 22.1 | 6.6 ±9.5 | 0.484 |
| CRP >10 mg/L, n (%) | 29 (24) | 8 (16) | 0.253 |
| **Function** | | | |
| HAQ, mean ±SD | 0.92 ± 0.77 | 1.18 ± 1.03 | 0.073 |
| **Treatment received** | | | |
| Current analgesic use | 17 (14) | 6 (12) | 0.730 |
| Current corticosteroid use, n (%) | 81 (67) | 31 (63) | 0.619 |
| Current corticosteroid use, >10 mg/day, n (%) | 10 (8) | 3 (6) | 0.653 |
| Current use of NSAIDs | 22 (18) | 10 (20) | 0.762 |
| Current conventional DMARD use, n (%) | 87 (72) | 38 (78) | 0.422 |
| Current MTX use, n (%) | 75 (62) | 27 (55) | 0.400 |
| Current MTX dose, mg/week, mean±SD | 10.02 ± 8.43 | 9.30 ± 8.48 | 0.615 |
| MTX treatment duration, years, mean±SD | 8.7 ± 9.5 | 9.1 ± 9.7 | 0.805 |
| MTX cumulative dose, g, mean±SD | 4.9 ± 5.1 | 6.2 ± 5.2 | 0.136 |
| Current leflunomide use | 12 (10) | 12 (25) | 0.012 |
| Current targeted biologic therapies, n (%) | 63 (52) | 30 (61) | 0.238 |
| Current anti-TNF-α use, n (%) | 23 (19) | 8 (16) | 0.645 |
| Current rituximab use, n (%) | 23 (19) | 13 (26) | 0.181 |
| Current tocilizumab use, n (%) | 4 (3) | 10 (20) | 0.001 |
| Current abatacept use, n (%) | 13 (11) | 1 (2) | 0.081 |
| **Modifiable cardiovascular risk factors** | | | |
| Smokers, n (%) | 35 (29) | 18 (37) | 0.309 |
| High blood pressure, n (%) | 43 (36) | 21 (43) | 0.396 |
| Diabetes mellitus, n (%) | 37 (31) | 18 (37) | 0.451 |
| Dyslipidaemia, n (%) | 48 (40) | 17 (35) | 0.545 |
| BMI, kg/m$^2$, mean ±SD | 25.7 ± 5.5 | 24.8 ± 4.08 | 0.302 |
| BMI >30 kg/m$^2$, n (%) | 20 (17) | 9 (18) | 0.876 |
| **Hepatic disorders** | 15 (12) | 5 (10) | 0.898 |
| **Regular alcohol intake** | 13 ( 11) | 4 (8) | 0.558 |
| **Metabolic syndrome** | 12 (10) | 7 (14) | 0.454 |
| **ALT, U/L,** mean ±SD | 23 ± 13 | 25 ± 13 | 0.365 |
| **AST, U/L,** mean ±SD | 22 ± 7 | 31 ± 15 | <0.001 |
| **Platelets /mm$^3$,** mean ±SD | 285 ± 68 | 226 ± 64 | <0.001 |

SD: standard deviation; DAS: Disease Activity Score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; HAQ: Health Assessment Questionnaire; DMARD: disease-modifying anti-rheumatic drug; NSAIDs: non-steroidal anti-inflammatory drugs; MTX: methotrexate; TNF-α: tumour necrosis factor-α; BMI: Body Mass Index; CV: cardiovascular.

*Statistical analysis*

All data were expressed as mean values ± standard deviation (SD) or median (range), unless stated otherwise. Statistical analysis was performed using using Medcalc (v. 18.9.1) and Prism 8 (v. 8.4.3).

Given its non-parametric distribution (Kolmogorov-Smirnov distance of 0.091, $p<0.001$), the value of FIB-4 according to binary variables was tested using Kruskal-Wallis test with Dunn

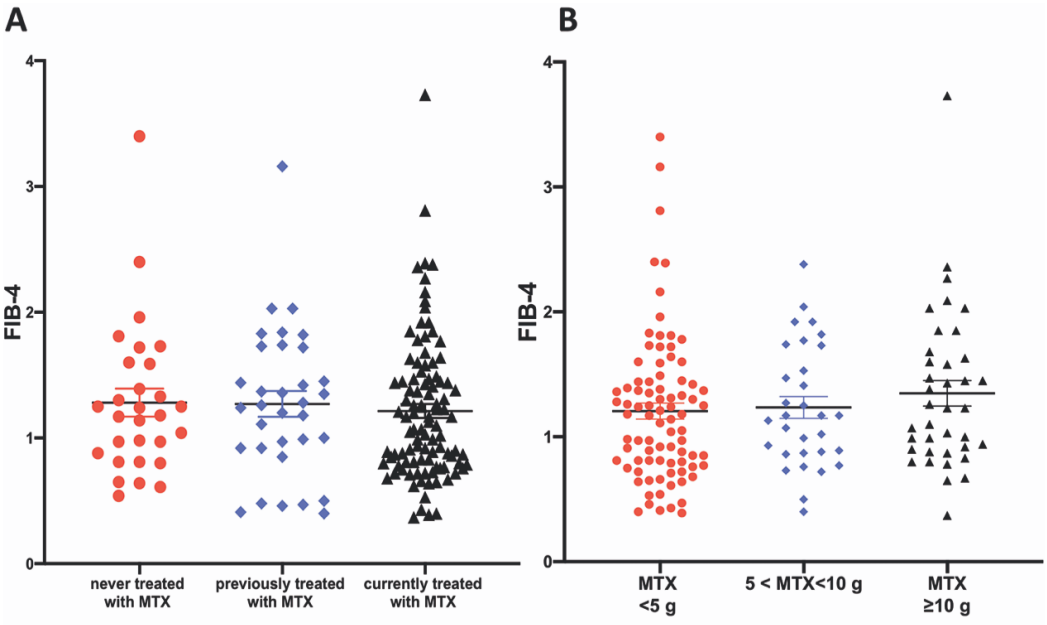


**Fig. 1. A-B.** Fibrosis-4 index according to never, past, or current treatment with methotrexate (MTX) (**A**), and to the cumulative dose of methotrexate (MTX) (**B**) in patients with rheumatoid arthritis.

correction. Comparisons of mean values were assessed by the Mann Whitney U-test, and the chi-square test was used to seek for differences in frequency. Multivariate analyses by logistic regression were also performed to determine the factors independently associated with increased FIB-4 index. These analyses included the FIB-4 index (>1.45) as the dependent variables. All relevant identified covariates with a *p*-value <0.1 in the single variable analysis were then entered in one single step in each model. Odds ratio (OR) and 95% confidence intervals (CI) were then calculated. In this model, a *p*-value <0.05 was considered statistically significant.

## Results

*Study population*

A total of 170 patients (141 females, 83%) with established RA were included, with a mean age of 59±12 years and a mean disease duration of 15±11 years. Positive rheumatoid factors and anti-CCP antibodies were detected in 134 (79%) patients. Erosions were present in 101 (59%) patients. Detailed characteristics of our study sample are provided in Table I.

No patient had unstable hepatic disease associated with biologic signs of liver dysfunction (decreased albumin and procoagulant synthesis, altered bilirubin metabolism) or liver failure. Twenty patients had known hepatic disorders: 3 RA patients had associated-primary biliary cirrhosis with positive anti-mitochondrial antibodies, all treated with ursodeoxycholic acid; 14 had occult hepatitis B infection (undetectable HBV DNA, HBsAg-negative, anti-HBc and anti-HBs positive antibodies) requiring no specific treatment; and 3 had non-alcoholic fatty liver disease detected by liver ultrasound, associated with metabolic syndrome.

*Distribution of the FIB-4 in our population*

The mean FIB-4 value was 1.24±0.57, with 121 patients (71%) with value <1.45, 47 (29%) with values ranging from 1.45 to 3.25 and 2 patients with FIB-4 >3.25 (Supplementary Fig. S1).

*Primary outcome: analysis of the FIB4 index in RA patients receiving MTX maintenance therapy*

The FIB-4 was low and not significantly different between patients receiving MTX, patients previously treated with MTX and patients never treated with MTX (Fig. 1A, Table II). The proportion of patients with increased FIB-4 was not influenced by MTX: 27/102 patients receiving MTX (26%) had a FIB-4 >1.45 compared to 22/68 patients (32%) not treated by MTX ($p$=0.397). Moreover, only a single patient had a score >3.25 in each group. Interestingly, The FIB-4 index did not markedly change when MTX was associated with targeted biological therapies (Fig. 2A), except for tocilizumab. Indeed, the combination of tocilizumab with MTX was associated with a median FIB-4 index of 2.36 (range: 0.75–3.73) *vs*. 1.03 (range: 0.79–2.39) in patients receiving MTX in monotherapy ($p$<0.001).

No correlation was observed between FIB-4 values and the cumulative dose of MTX (r=0.09, $p$=0.271). The FIB-4 index was low and similar between patients receiving cumulative MTX doses <5g, between 5 and 10g and >10g (Fig. 1B). In addition, the cumulative MTX dose was not significantly higher in patients with a FIB-4 index >1.45 (median cumulative MTX dose 5.5g *vs*. 3.5g, $p$=0.302). No relationship was observed between FIB-4 and MTX treatment duration (Table I).

*Secondary outcomes*

*– Analysis of the FIB-4 index in RA patients receiving other treatments*

The FIB-4 index was significantly lower in untreated patients compared to patients receiving conventional synthetic and/or biologic disease-modifying anti-rheumatic drugs (csDMARDs and bDMARDs, respectively) ((median (range) 0.97 (0.40–3.40) *vs*. 1.23 (0.37–3.73), $p$=0.045).

The FIB-4 index was found significantly increased in patients receiving leflunomide compared to patients not

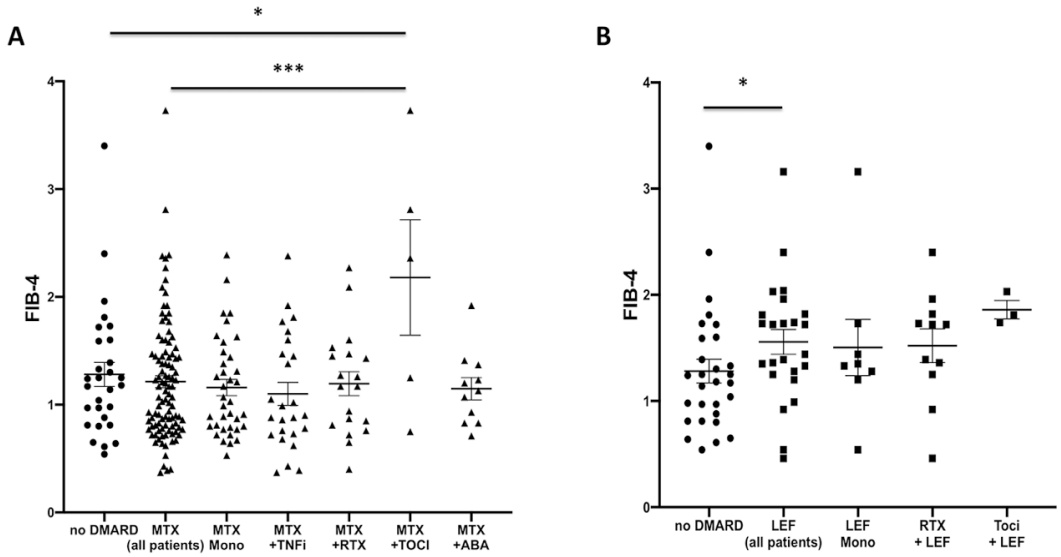


**Fig. 2. A-B.** Fibrosis-4 index according to current treatment with methotrexate (MTX) (**A**) and leflunomide (LEF) (**B**) in monotherapy (Mono) or in combination with targeted biologic disease-modifying antirheumatic drugs (DMARDs). TNFi: TNF-α inhibitors; RTX: rituximab; TOCI: tocilizumab; ABA: abatacept. Statistical test: Mann Whitney U-test, *$p$<0.05, ***$p$<0.001.

receiving DMARDs (median (range) 1.58 (0.46–3.16) *vs*. 1.18 (0.54–3.40), $p$=0.019). In addition, patients treated with this drug were more likely to present a FIB-4 index >1.45 (12/24, 50% *vs*. 37/146, 25%, $p$=0.006). No patient with leflunomide had a FIB-4 >3.25. As observed for MTX, the combination of leflunomide with tocilizumab increased the median value of the FIB-4 index from 1.34 (range: 0.54–3.16) to 1.81 (range: 1.74–2.03) (Fig. 2B).

Patients treated with tocilizumab presented a markedly increased FIB-4 compared to patients not receiving DMARDs (median (range) 1.82 (0.75–3.73) *vs*. 1.18 (0.54–3.40), $p$=0.005). The percentage of patients with a FIB-4 >1.45 was significantly higher in tocilizumab-treated patients (10/14, 71% *vs*. 39/156, 25%, $p$=0.008). The combination of tocilizumab with MTX or leflunomide did not amplify the FIB-4 increase. One patient had an index >3.25 (3.73).

A trend for decreased FIB-4 values was observed in patients treated with TNF-α inhibitors compared to patients not receiving this drug class (median (range) 0.89 (0.37–2.38) *vs*. 1.21 (0.40–3.73), $p$=0.055), but the number of patients with a FIB-4 >1.45 was similar between patients receiving or not this drug class (8/31, 26% *vs*. 41/139, 29%, $p$=0.739).

The FIB-4 index was not modified by other therapies including rituximab, abatacept, corticosteroids, NSAIDs or analgesics (Table II).

**Table II.** FIB-4 index according to rheumatoid arthritis therapies.

| | Median | Range | $p$-value |
|---|---|---|---|
| Analgesics | | | |
| Treated | 1.13 | 0.46-1.85 | 0.865 |
| Not treated | 1.18 | 0.37-3.73 | |
| NSAIDs | | | |
| Treated | 1.18 | 0.40-2.27 | 0.954 |
| Not treated | 1.17 | 0.37-3.73 | |
| Corticosteroids | | | |
| Treated | 1.18 | 0.37-3.40 | 0.872 |
| Not treated | 1.12 | 0.39-3.73 | |
| MTX | | | |
| Treated | 1.10 | 0.37-3.73 | 0.383 |
| Not treated | 1.24 | 0.40-3.40 | |
| Leflunomide | | | |
| Treated | 1.58 | 0.46-3.16 | <0.001 |
| Not treated | 1.07 | 0.37-3.73 | |
| TNF-α inhibitors | | | |
| Treated | 0.89 | 0.37-2.38 | 0.055 |
| Not treated | 1.21 | 0.40-3.73 | |
| Rituximab | | | |
| Treated | 1.35 | 0.40-2.40 | 0.104 |
| Not treated | 1.10 | 0.37-3.73 | |
| Tocilizumab | | | |
| Treated | 1.82 | 0.75-3.73 | <0.001 |
| Not treated | 1.13 | 0.37-3.40 | |
| Abatacept | | | |
| Treated | 1.10 | 0.71-1.92 | 0.669 |
| Not treated | 1.18 | 0.37-3.73 | |

NSAIDs: non-steroidal anti-inflammatory drugs; MTX: methotrexate; TNF-α: tumour necrosis factor-α.

*– Relationship between FIB-4 and other disease characteristics*

No correlation was observed between the DAS28, ESR and CRP levels with the FIB-4 index (r=-0.04, $p$=0.538; r=-0.01, $p$=0.989 and r=-0.01, $p$=0.862, respectively), but patients with a FIB-4 >1.45 were more likely to have lower disease activity (DAS28: 3.14±1.37 *vs*. 3.65±1.39, $p$=0.031). The FIB-4 index was not modified by the presence of bone erosions (median (range)

1.17 (0.37–3.73) *vs*. 1.18 (0.40–3.40), $p$=0.693) and a trend for a correlation with the HAQ was observed (r=0.14, $p$=0.088).

The FIB-4 index did not significantly change according to the body mass index, traditional cardiovascular risk factors and metabolic syndrome, except for elevated blood pressure (Table III).

*Multivariate analysis*

We next conducted a multivariate logistic regression analysis to identify variables independently associated with a FIB-4 index >1.45. A first analysis was conducted with covariates with a $p$-value <0.1, and confirmed the independent association between increased FIB-4 and male gender, disease activity, current treatment with leflunomide and current treatment with tocilizumab (Table IV). A second analysis added known risk factors of liver fibrosis (hepatic disorders, alcohol consumption, metabolic syndrome) and did not change results obtained in the first model. A third model included as covariates conventional and targeted biologic DMARDs and confirmed the independent association between increased FIB-4 with current treatment with leflunomide and current treatment with tocilizumab, as well as the absence of significant influence of MTX in FIB-4 elevation (Table IV).

**Table III.** FIB-4 index according to hepatic disorders, cardiovascular risk factors and metabolic syndrome.

| | Median | Range | $p$-value |
|---|---|---|---|
| Hepatic disorders | | | |
| Yes | 1.17 | 0.40-2.38 | 0.338 |
| No | 1.24 | 0.37-3.73 | |
| Smokers | | | |
| Yes | 1.15 | 0.37-3.40 | 0.926 |
| No | 1.22 | 0.46-3.73 | |
| High blood pressure | | | |
| Yes | 1.31 | 0.37-3.73 | 0.033 |
| No | 1.07 | 0.39-3.40 | |
| Diabetes | | | |
| Yes | 1.17 | 0.37-3.73 | 0.873 |
| No | 1.20 | 0.39-3.40 | |
| Dyslipidaemia | | | |
| Yes | 1.22 | 0.37-3.73 | 0.204 |
| No | 1.14 | 0.39-2.81 | |
| BMI >30 kg/m$^2$ | | | |
| Yes | 1.24 | 0.50-1.85 | 0.607 |
| No | 1.15 | 0.37-3.73 | |
| Regular alcohol intake | | | |
| Yes | 1.17 | 0.67-2.38 | 0.627 |
| No | 1.18 | 0.37-3.73 | |
| Metabolic syndrome | | | |
| Yes | 1.43 | 0.65-3.73 | 0.065 |
| No | 1.18 | 0.37-3.40 | |

BMI: Body Mass Index

## Discussion

FIB-4 is a simple and inexpensive but reproducible method that may help identify patients at risk of liver outcomes. This index is now used beyond HIV/HCV co-infection and hepatitis B virus-related liver fibrosis in different diseases including diabetes mellitus or non-alcoholic fatty liver disease (18). In addition, FIB-4 was reported to predict all-cause mortality even in non-liver disease like heart failure (19). The use of FIB-4 is particularly suitable for RA in clinical practice. Indeed, the reliability and sensitivity to change of this marker to diagnose liver disease in RA patients treated with long-term MTX administration has been reported in a preliminary study of 14 patients (11). In addition, a retrospective monocentric study has shown that FIB-4 at diagnosis can predict all-cause mortality in patients with RA, suggesting that, besides conventional risk factors, FIB-4 might be valuable as a complementary prognostic index for risk stratification of newly diagnosed RA patients (20). Other markers of fibrosis (*e.g*. hyaluronic acid (HA), type IV collagen) may be useful, but are too expensive to monitor frequently. The Fibrotest, FibrometerA, and Hepascore use expensive laboratory tests, such as alpha-2-microglobulin, apolipoprotein, or HA, which are not measured in rheumatology daily practice (11). Regarding other simple tests, APRI (AST to Platelet Ratio Index) has limited diagnostic performance and FORNS (combination of age, platelets, gamma-GT and cholesterol) may be biased by dyslipidaemia and/or cholesterol-modifying treatments that are regularly applied in RA (21, 22).

Despite the beneficial effects of methotrexate, hepatotoxicity remains an important concern when methotrexate is prescribed to RA patients. RA patients with long-term maintenance MTX therapy presented low FIB-4 values, similar to patients who never received MTX and those who received MTX in the past, suggesting that MTX is not associated with an increased risk of advanced liver fibrosis. These results are interesting given the high cumulative dose of MTX in our population (5.34±5.12 g). Indeed, a previous study showed that a cumulative dose of approximately 5 g was associated with NASH-like hepatotoxicity in psoriasis patients with risk factors (3, 23), and in the past, the dermatology community recommended monitoring for liver disease by liver biopsy performed after a total cumulative dose of 1.5 g of MTX and again after each additional 1 g cumulative dose (24). However, the type of disease may confer differing risk for MTX hepatotoxicity, as the prevalence of cytolysis appears to be greater in patients treated for psoriasis than in patients treated for RA (25). In addition, no clear evidence has demonstrated that cumulative MTX dose correlates to the development of hepatic fibrosis (26). A previous study investigating DMARDs

**Table IV.** Multivariate logistic regression analysis.

| Variables | Univariate *p*-value | Odds ratio (95% CI) | *p*-value |
|---|---|---|---|
| **First model** | | | |
| Male gender | 0.028 | 4.04 (1.33-12.21) | 0.013 |
| Disease duration | 0.038 | 1.02 (0.98-1.06) | 0.247 |
| DAS28 >3.2 | 0.030 | 0.23 (0.08-0.66) | 0.006 |
| HAQ | 0.073 | 1.65 (0.93-2.92) | 0.087 |
| Current leflunomide use | 0.012 | 3.25 (1.16-9.12) | 0.024 |
| Current tocilizumab use | 0.001 | 4.14 (1.08-15.80) | 0.037 |
| Current abatacept use | 0.081 | 0.27 (0.93-13.83) | 0.246 |
| **Second model** | | | |
| Male gender | 0.028 | 13.37 (2.80-63.86) | 0.001 |
| Disease duration | 0.038 | 1.04 (0.98-1.09) | 0.164 |
| DAS28 >3.2 | 0.030 | 0.25 (0.06-0.94) | 0.041 |
| HAQ | 0.073 | 2.59 (0.98-5.66) | 0.066 |
| Current leflunomide use | 0.012 | 4.21 (1.11-1.83) | 0.034 |
| Current tocilizumab use | 0.001 | 4.80 (1.03-22.22) | 0.044 |
| Current abatacept use | 0.081 | 0.28 (0.91-14.29) | 0.332 |
| Hepatic disorders | 0.898 | 1.06 (0.23-4.92) | 0.935 |
| Regular alcohol intake | 0.558 | 0.879 (0.20-3.86) | 0.126 |
| Metabolic syndrome | 0.454 | 0.879 (0.20-3.86) | 0.865 |
| **Third model** | | | |
| Current MTX | 0.400 | 2.06 (0.80-5.32) | 0.134 |
| Current leflunomide use | 0.012 | 4.42 (1.39-14.08) | 0.012 |
| Current anti-TNF-α use | 0.645 | 1.49 (0.53-4.21) | 0.445 |
| Current rituximab use | 0.181 | 1.59 (0.61-4.11) | 0.338 |
| Current tocilizumab use | 0.001 | 7.11 (1.92-26.34) | 0.003 |
| Current abatacept use | 0.081 | 0.29 (0.03-2.41) | 0.250 |

DAS: Disease Activity Score; HAQ: Health Assessment Questionnaire; MTX: methotrexate; TNF-α: tumour necrosis factor-α.

associated with abnormal liver stiffness using liver transient elastography, in which the cumulative dose of methotrexate was not significantly correlated with abnormal liver stiffness measurement (27). Another recent study assessing MTX-related liver injury in patients with RA and psoriatic arthritis had considered liver biopsy when the total cumulative dosage of MTX was ≥3.5g. In the 5 patients who consented to this procedure, no significant fibrosis or steatosis was evident on histology. MTX treatment duration was also not associated with increased FIB-4 values, in line with the conclusion of most of previous studies (28-30).

It has been recently suggested that liver fibrosis associated with methotrexate could be due to associated factors, including alcohol consumption, diabetes and obesity instead of methotrexate itself (31). Herein, all these factors have been taken into account and were not associated with increased FIB-4 values in uni- or multivariate analyses.

We also report for the first time an independent association between increased FIB-4 values and treatment with leflunomide and tocilizumab, especially when this latter was combined to MTX. It has to be noticed that no patient treated with leflunomide and only a single tocilizumab-treated patient had a FIB-4 index >3.25, supporting the absence of advanced fibrosis. Although the potential hepatic toxicity of leflunomide and tocilizumab is known and has extensively been described in trials and real-life studies (32, 33), the development of silent liver fibrosis related to these drugs is debated or not known. Leflunomide was not clearly reported as an independent predictor of liver fibrosis (34). However, the cumulative dose of leflunomide has been identified as an independent predictor of silent liver fibrosis in patients with RA who had received MTX for more than six months (27). Tocilizumab has been linked to clinically apparent liver injury, which arose after several months of treatment and was mainly hepatocellular with no immunoallergic or autoimmune features. While liver injury was severe, it was usually self-limited with complete recovery in 2 to 3 months. In these acute situations, liver biopsies have revealed findings in line with drug injury including focal necrosis of hepatocytes, steatosis and early fibrosis. However, the impact of maintenance therapy with tocilizumab on liver function and fibrosis is not known. The mechanism by which tocilizumab causes liver injury is unknown, but may be the result of its effects on the immune system and/or on the IL-6 pathway, which is important in liver regeneration (35).

Considering our findings and these data, increased FIB-4 values upon leflunomide and tocilizumab may justify further evaluation using liver stiffness measurement like transient elastography and shear wave elastography.

FIB-4 has been reported to reflect disease activity, with increased values being a consequence of joint and systemic inflammation (20). This is not reflected by our findings, as increased FIB-4 was independently associated with low disease activity in our study sample. Given the significantly lower DAS28 (3.38±1.39 *vs*. 4.23±1.19, $p$=0.004) and significantly higher FIB-4 in patients treated with csDMARDs and/or bDMARDs compared to untreated patients, increased FIB-4 values may relate more to a direct hepatic drug effect than a consequence of inflammation.

Our study included consecutive long-standing patients who were carefully assessed and phenotyped in a tertiary centre with a long-lasting experience in RA evaluation and care. However, our study is limited by its observational design, the relatively small number of patients included in some analyses and the use of surrogates for liver fibrosis.

Survivorship bias may also apply and some patients may have had in the past issue with methotrexate not anymore measurable at the time of inclusion. The inclusion of RA patients followed in hospital may have resulted in a selection bias. Since this study is cross-sectional, any pathogenic link should be taken very cautiously, with possibility of confounders and lack of evidence for causal associations.

In conclusion, the FIB-4 is a simple and inexpensive tool to evaluate underlying hepatic fibrosis in RA clinical setting. RA patients with long-term maintenance MTX therapy have low FIB-4

values suggesting that MTX is not associated with an increased risk of advanced liver fibrosis. Increased FIB-4 values have been detected in leflunomide- and tocilizumab-treated patients, which will deserve dedicated further investigations.

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