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## **A pragmatic non-invasive assessment of liver fibrosis in patients with psoriasis, rheumatoid arthritis or Crohn's disease receiving methotrexate therapy**

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**Data Availability Statement:** the data that support the findings of this study are available from the corresponding author upon request.

### **Highlights:**

#### **What is already known about this subject**

- The fear of liver fibrosis development limits the use of methotrexate
- Until recently, only liver biopsy was recommended for sequential assessment of liver fibrosis.

#### **What this study adds**

- Our study suggests that methotrexate has negligible effect regarding the risk of liver fibrosis in comparison with the metabolic syndrome.
- Liver biopsy should be limited as much as possible to render methotrexate cost-effective and widely acceptable.
- Non-invasive markers should be recommended for monitoring patients before and during therapy

**Abstract:**

**Background and aims:** The reported hepatotoxicity of methotrexate underlines the need for a repeated non-invasive and reliable evaluation of liver fibrosis. We estimated, using a non-invasive strategy, the prevalence of significant liver fibrosis in patients treated by methotrexate and the predictors of significant fibrosis (fibrosis  $\geq$ F2).

**Methods:** Fibrosis was prospectively evaluated using 9 non-invasive tests in consecutive patients with psoriasis, rheumatoid arthritis, or Crohn's disease. Significant fibrosis was assessed without liver biopsy by defining a 'specific method' (result given by the majority of the tests) and a 'sensitive method' (at least one test indicating a stage  $\geq$ F2).

**Results:** 131 patients (66 Psoriasis, 40 rheumatoid arthritis, and 25 Crohn's disease) were enrolled, including 83 receiving methotrexate. Seven tests were performed on average per patient, with a complete concordance in 75% of cases. Fibroscan<sup>®</sup> was interpretable in only 61% of patients. The best performances (AUROC $>$ 0.9) for predicting significant fibrosis were obtained by tests dedicated to steatohepatitis (FibroMeter NAFLD, NFS and FPI). The prevalence of fibrosis  $\geq$ F2 according to the 'specific' or the 'sensitive' assessment of fibrosis was 10% and 28%, respectively. Methotrexate exposure did not influence the fibrosis stage. Factors independently associated with significant fibrosis according our 'sensitive method' were age, male gender, and metabolic syndrome.

**Conclusion:** We provided a non-invasive approach for identifying liver fibrosis  $\geq$ F2 by using 8 biochemical tests and Fibroscan<sup>®</sup>. In this population, the risk of significant fibrosis was related to age, male gender, and presence of metabolic syndrome, but was not influenced by methotrexate.

Methotrexate (MTX) is an anti-folate chemotherapeutic agent and immunosuppressant used since the 50's. It is prescribed as a first-line therapy in various inflammatory disorders such as psoriasis (Pso) [1], rheumatoid arthritis (RA) [2] and inflammatory bowel diseases [3] and can induce sustained remissions in more than two-third of patients [3-5]. Its efficiency, low cost and good acceptance have been however balanced by concerns about its liver toxicity, resulting in limiting its use. Current guidelines to monitor liver fibrosis established in last decades by dermatologists [1,6] are no longer appropriate because they required too many unnecessary liver biopsies, an invasive procedure. The American College of Rheumatology suggests monitoring only transaminases levels, but it is established that persistently normal liver biochemistry cannot exclude cirrhosis [7,8]. The development of non-invasive liver fibrosis markers in chronic viral hepatitis C and subsequently in a variety of chronic liver diseases renders liver biopsy unsuitable for prognostic purposes. In patients receiving MTX the most common histological feature is steatohepatitis [9], a condition that can be evaluated by non-invasive fibrosis tests [10-12].

The more recent studies have focused on non-invasive screening of significant liver fibrosis in patients with Crohn's disease (CD) [13-16], psoriasis [17-22] and rheumatoid arthritis [23-25] treated by MTX. These studies indicated that significant liver fibrosis is a rare event in patient under MTX without comorbidities, underlying the futility of serial liver biopsy samples in this context. However, these studies were limited to one or two non-invasive tests and did not provide comparison between different strategies of non-invasive evaluation of fibrosis.

The purposes of the present study were therefore 1) to define an appropriate strategy (both specific and sensitive) to assess significant liver fibrosis exclusively based on non-invasive methods in three distinct inflammatory diseases (Pso, RA and CD); 2) to evaluate the prevalence of liver fibrosis in patients treated with MTX when compared with patients with the same inflammatory diseases without MTX (control group); and 3) to evaluate in this population the impact of risks factors

(alcohol consumption, metabolic syndrome and other causes of liver disease) on liver fibrosis to investigate whether MTX remains an independent risk factors of liver fibrosis over the long term.

## **Patients and methods**

### *Study design*

We conducted a prospective observational study including consecutive patients with Pso, CD or RA seen between February 2008 and October 2010, in our University hospital. The recruitment of patients was performed by two hepatologists in collaboration with the dermatology, rheumatology and gastroenterology departments. The study protocol was approved by the ANSM (Agence Nationale de Sécurité du Médicament et des produits de santé) N°Eudact2008-001149-24 and by our local ethics committee (Comité de Protection des Personnes Est II) N°08/474, providing it did not require liver biopsy and did not change patients care. Every enrolled patient gave written informed consent.

### *Patients*

Pso, RA or CD patients already treated by MTX (treated group) or not (control group) were either referred by their dermatologist, rheumatologist, and gastroenterologist, or recruited during a planned hospitalization for TNF $\alpha$  antagonist treatment. The only exclusion criterion was an age<18.

### *Collected data*

During a medical visit in the hepatology department, 189 variables were collected for each patient using a questionnaire focused on chronic liver disease's risk factors and metabolic syndrome, MTX treatment's modalities, other associated therapies, and coffee drinking supposed to have anti-fibrosing effects [26,27]. Clinical data included age, weight, body mass index and waist circumference. Biochemical parameters of metabolic syndrome and liver disease risk factors were

determined on blood samples and doppler ultrasonography and assessed for each patient. Metabolic syndrome was defined according to the National Cholesterol Education Program Adult Treatment III (NCEP-ATP III) [28].

#### *Non-invasive fibrosis scoring system and Definition of significant fibrosis*

Liver stiffness was measured by Fibroscan<sup>®</sup> [10,29,30] performed by an experienced hepatologist (BA or JPC) with the M probe. Eight biochemical liver fibrosis scores: FibroTest<sup>®</sup> [11,31], Forns index [32], ASAT to Platelet Ratio Index (APRI) [33], Fibrosis Probability Index (FPI) [34], Hepascore [35], Fibrosis-4 (FIB-4) [36], NAFLD fibrosis score (NFS) [37] and FibroMeter NAFLD [38,39] were also used in our study by combining appropriately the available biochemical variables.

We developed a pragmatic algorithm to assert a high probability of significant fibrosis without liver biopsy by using all the fibrosis tests available for each patient. We used a three-step approach summarized in figure 1. First, we excluded 'invalid' tests and tests with results ranged within the 'grey zone', i.e., unable to predict with sufficient accuracy if the fibrosis stage was lower or greater than F2. The relevant values for each score for predicting fibrosis stage F2 or higher were then: Fibroscan<sup>®</sup>>7.1kPa [17], Fibrotest<sup>®</sup>>0.49 [31], Forns index>6.9 [32], APRI score>1.5 [33], FPI>0.8 [34], Hepascore>0.5 [35], FIB-4 score>3.25 [36], NFS>0.676 [37] and FibroMeter NAFLD>0.49 [39]. The liver stiffness values which did not meet the quality criteria (i.e., inter-quartile range <30% of the median elasticity and success rate >50% [29]) were also excluded from our final assessment. Second, we identified, for each patient, the number of discordant results between each test. By combining all valid results, we then defined two detection methods of significant fibrosis: a "specific" method corresponding to the result given by the majority of the tests (this algorithm is called 'Fspec') and a "sensitive" method, when at least one score concluded to significant fibrosis (this algorithm is called 'Fsens'). If more than two conflicting results were identified for a patient, we controlled the patient's record and each score was recalculated.

### *Statistical analysis*

The statistical analyzes were used to 1) compare the 3 populations (Pso, RA and CD), 2) compare the patients treated or not with methotrexate, 3) identify the factors associated with liver fibrosis as defined by our algorithm and 4) evaluate the diagnostic performance of each noninvasive score for the prediction of significant fibrosis. The distribution of patient demographic, clinical, biochemical and radiological characteristics at baseline was analyzed in the three patient populations (Pso, RA and CD) and then in the "treated" and "control" groups. Univariate analyzes used the chi-2 test for comparing qualitative variables, Student's t-test, ANOVA, or Mann-Whitney test for comparing quantitative variables, and linear regression to evaluate correlations between quantitative variables, as appropriate. Multivariate analyzes used the logistic regression model for the analysis of factors associated with significant fibrosis. The explanatory variables used in the different models were those whose degree of significance was considered sufficient in univariate analysis ( $p < 0.10$ ). The cumulative dose of methotrexate was systematically incorporated in each model.

Finally, the performance of the different non-invasive tests for the diagnosis of significant fibrosis was assessed by the area under the ROC curve (AUROC). For this specific analysis, Fspec and Fsens algorithms were revised to exclude the fibrosis test evaluated.

## **RESULTS**

### *Study population*

A total of 131 consecutive patients were included: 66 Pso, 40 RA and 25 CD. Eighty patients (63%) had received MTX for a median duration of 34 months (range 3-235 months) and with a median cumulative dose of 1575 mg (range: 280-15000mg). Demographics and clinical characteristics of the study population are summarized in table 1: median age was 52 years; 61% of patients had BMI $\geq$ 25, 26% had BMI $\geq$ 30 and 27% patients met the NCEP-ATP III criteria of metabolic syndrome [28]; the



mean homeostasis model assessment estimate of insulin resistance (HOMA-IR) index was 2.55. Forty-four percent of patients were alcohol consumers, with a reported consumption of 35g per day. Eight patients were anti-HBc antibody positive and one was anti-HCV positive but HBVDNA or HCVRNA were not detectable. One additional patient had a primary sclerosing cholangitis.

The Comparison between the 3 groups of patients is summarized in table 1. Patients with CD were younger ( $p<0.0001$ ), had a lower BMI ( $p=0.02$ ) and HOMA-IR index ( $p=0.01$ ). Patients with RA received higher cumulated dose of MTX (mean dose of 3022mg vs. 971mg and 1047mg in the Pso group and the CD group, respectively).

**Table 1: Main characteristics of the population: comparison of psoriasis, rheumatoid arthritis and Crohn disease population.**

	Population N=131	Psoriasis N=66	Rheumatoid arthritis N=40	Crohn's disease N=25	p-value
age, years (mean±SE)	52±1	54±2	58±2	41±3	<0.0001
Male gender (%)	48%	61%	35%	36%	0.015
MTX treatment <sup>(a)</sup>	63%	71%	62%	44%	0.05
median Cumulated dose >1500mg, %	37%	30%	55%	28%	0.02
Alcohol consumption (mean±SE)	15±3	19±5	7±6	17±8	NS
Anti-HBc antibodies, %	6.3%	7.6%	7.7%	0%	NS
HCV <sup>(b)</sup> antibodies	0.8%	1.5%	0	0	NS
Number of cups of coffee per day (mean±SE)	2.6±0.2	2.8±0.3	2.8±4	1.7±0.5	0.07
ALT > ULN <sup>(c)</sup>	27%	27%	25%	28%	NS
Hyaluronic acid (µg/L) (mean±SE)	36±4	29±6	58±7	18±9	0.004
Presence of steatosis (ultrasound)	47%	56%	47%	19%	0.01
Weight (kg) (mean±SE)	76±2	78±2	79±3	66±4	0.003
BMI <sup>(d)</sup> (kg/m <sup>2</sup> ) (mean±SE)	26.6±0.5	27.2±0.7	27.2±0.9	23.9±1.1	0.02
<b>NCEP-ATP III <sup>(e)</sup> criteria:</b>					
- Waist circumference	48%	50%	60%	24%	0.02
- Dyslipidemia	43%	39%	55%	32%	NS
- High blood pressure	64%	70%	75%	32%	<0.001
- Hyperglycemia	17%	23%	15%	4%	NS
- Number of criteria (mean±SE)	1.8±0.1	1.9±0.1	2.6±0.2	1.6±0.2	0.002
HOMA-IR <sup>(f)</sup> (mean±SE)	2.55±0.3	2.86±0.4	2.75±0.5	1.29±0.7	0.01

<sup>(a)</sup> MTX: methotrexate; <sup>(b)</sup> HCV: hepatitis C virus ; <sup>(c)</sup> ALT: alanine transaminase; <sup>(d)</sup> BMI: Body mass index; <sup>(e)</sup> NCEP-ATP III: National Cholesterol Education Program Adult Treatment III; <sup>(f)</sup> HOMA-IR: Homeostasis model assessment of insulin resistance; SE: standard error.

The comparison between patients receiving or not MTX is summarized in table 2. Males were more often treated with MTX whereas patients with CD were not. There was no difference in terms of prevalence of metabolic syndrome or fatty liver detected by ultrasonography.

**Table 2: Comparison of patients treated and untreated with methotrexate.**

	<b>Untreated patients n=48</b>	<b>Treated patients n=83</b>	<b>p-value</b>
<b>Age (years) (mean±SE)</b>	50 ±3	54 ±1	NS
<b>Male gender, n (%)</b>	17 (35%)	46 (55%)	0.03
<b>SRAC<sup>(a)</sup> (g/day) (mean±SE)</b>	17 ±5	14 ±5	NS
<b>Psoriasis</b>	19 (40%)	47 (57%)	0.06
<b>Rheumatoid arthritis</b>	15 (31%)	25 (30%)	NS
<b>Crohn's disease</b>	14 (29%)	11 (13%)	0.03
<b>Metabolic syndrome</b>	15 (31%)	20 (24%)	NS
<b>High blood pressure</b>	30 (63%)	54 (65%)	NS
<b>Waist circumference (cm) (mean±SE)</b>	93 ±2	96 ±2	NS
<b>Weight (kg) (mean±SE)</b>	74 ±3	77 ±2	NS
<b>Insuline resistance <sup>(b)</sup></b>	8 (17%)	14 (17%)	NS
<b>Dyslipidemia <sup>(c)</sup></b>	23 (48%)	33 (40%)	NS
<b>Uricemia (mean±SE)</b>	307 ±13	345 ±12	0.04
<b>Abnormal liver tests</b>	19 (40%)	29 (35%)	NS
<b>Ultrasound steatosis</b>	19 (40%)	40 (52%)	NS
<b>Fspec <sup>(d)</sup></b>	4 (8%)	9 (11%)	NS
<b>Fsens<sup>(e)</sup></b>	9 (19%)	28 (34%)	NS

<sup>(a)</sup> **SRAC**: Self-Reported Alcohol Consumption; <sup>(b)</sup> **Insulin resistance** defined by history of diabetes, fasting blood glucose >6,1mmol/L; <sup>(c)</sup> **Dyslipidemia** defined by triglycerides levels >1,5g/L and/or HDL-cholesterol <0,4g/L in men and 0,5g/L in women; <sup>(d)</sup> **Fspec**: Significant fibrosis >F2 defined by majority of tests; <sup>(e)</sup> **Fsens**: Significant fibrosis >F2 defined by at least one test.

*Factors influencing the crude values of liver fibrosis tests*

Old age was correlated with high hyaluronic acid ( $R^2=0.12$ ;  $p<0.0001$ ), and FibroMeter NAFLD values ( $R^2=0.23$ ;  $p<0.001$ ). Male gender was associated with a higher Forns index (4.61 vs. 3.38,  $p<0.001$ ), APRI (0.33 vs. 0.23,  $p<0.001$ ), FPI (0.25 vs. 0.06,  $p<0.001$ ), FIB-4 (1.31 vs. 0.98,  $p=0.043$ ), FibroMeter NAFLD (0.13 vs. 0.02,  $p<0.001$ ), and Fibroscan<sup>®</sup> (5.6 vs. 4.4;  $p=0.003$ ). CD was associated with lower hyaluronic acid (12 vs. 21,  $p=0.03$ ), Forns index (2.65 vs. 4.31,  $p=0.001$ ), APRI (0.23 vs. 0.29,  $p=0.02$ ), FPI (0.02 vs. 0.20,  $p<0.001$ ), FIB-4 (0.70 vs. 1.15,  $p<0.001$ ), NFS (-3.23 vs. -1.95,  $p<0.001$ ), Fibrotest<sup>®</sup> (0.11 vs. 0.16,  $p=0.02$ ) and FibroMeter NAFLD (0.01 vs. 0.11,  $p<0.001$ ). Conversely, Pso was associated with a higher FibroMeter NAFLD (0.11 vs. 0.06,  $p=0.02$ ) and RA was associated with higher Hepascore (0.17 vs. 0.11,  $p=0.028$ ), FIB-4 (1.31 vs. 0.97,  $p=0.003$ ), and NFS (-1.75 vs. -2.51,  $p=0.006$ ). The Cumulative dose of MTX significantly correlated with the levels of hyaluronic acid ( $R^2=0.048$ ;  $p=0.01$ ) and of Hepascore ( $R^2=0.53$ ;  $p=0.01$ ), but not with the other fibrosis scores. Alcohol consumption was associated with higher Forns index (4.49 vs. 3.44,  $p=0.003$ ), APRI (0.31 vs. 0.24,  $p=0.03$ ), FPI (0.27 vs. 0.06,  $p<0.001$ ) and FibroMeter NAFLD (0.11 vs. 0.03,  $p=0.03$ ). Coffee drinking did not influence the result of the fibrosis tests. Patients with metabolic syndrome had higher Forns index (4.97 vs. 3.69,  $p<0.001$ ), APRI (0.34 vs. 0.25,  $p=0.009$ ), FPI (0.29 vs. 0.08,  $p<0.001$ ), FIB-4 (1.32 vs. 1.00,  $p=0.01$ ), NFS (-1.08 vs. -2.52,  $p<0.001$ ), Fibrotest (0.18 vs. 0.13,  $p=0.006$ ), FibroMeter (0.27 vs. 0.02,  $p<0.001$ ) and Fibroscan<sup>®</sup> (6.95 vs. 4.65 KPa;  $p<0.001$ ).

*Non-invasive Assessment of liver fibrosis*

All patients underwent a Fibroscan® but liver stiffness measurements failed for 29 patients and were not interpretable in 22 additional patients. A total of 80 Fibroscan® results were thus contributing. Only 40% of overweight patients had interpretable Fibroscan® measures. Seven biochemical tests could be interpreted for each patient on average, with extremes ranging from three (for three patients) to nine (for 28 patients) tests. At least five tests could be interpreted for 92% of the patients (figure 1). All the patented tests, i.e; FibroMeter®, Fibrotest®, and Hepascore could be interpreted. Regarding the non-patented fibrosis tests, because a substantial proportion of NFS (n=36), APRI (n=13), FIB-4 (n=35), FPI (n=44) and Forns (n=49) provided inconclusive results (i.e., within the 'grey zone'), we evaluated the interest of combining two, three, four, or five tests (table 3). The best combinations of two, three, or four non-patented fibrosis tests always included the Forns index and the APRI. By combining APRI, Forns and NFS or FPI, the proportion of failures was restricted to the minimum value of 2.4%.

In 98 patients (75%), there was a complete concordance between all the available tests. Conflicting values concerned one single test for 20 patients, two tests for eight patients, and three tests for five patients. Of the 33 patients with conflicting estimates of fibrosis stage, the Hepascore was involved 14 times, the Fibrotest 10 times, the Fibroscan® and the FibroMeter NAFLD 9 times, the APRI test 5 times, the FIB-4, the NFS and the FPI thrice, and the Forns index once.

**Table 3: contribution of the non-patented fibrosis tests for the diagnosis of significant fibrosis**

Combinations of available non patented fibrosis tests	nb patients tested	nb patients with tests within the grey zone	Nb with less than 2 concordant tests*	% patients with non-contributive testing**
<b>5 tests</b>				
APRI + Forns + FPI + NFS + Fib4	124	3	NA	2.4
<b>4 tests</b>				
APRI + Forns + FPI + NFS	124	3	NA	2.4
APRI + Forns + FPI + Fib4	124	3	NA	2.4
APRI + Forns + NFS + Fib 4	127	4	NA	3.1
APRI + FPI + NFS + Fib 4	124	4	NA	3.2
Forns + FPI + NFS + Fib 4	124	9	NA	7.3
<b>3 tests</b>				
APRI + Forns + FPI	124	3	NA	2.4
APRI + Forns + NFS	127	3	NA	2.4
APRI + Forns + Fib 4	127	4	NA	3.1
APRI + FPI + NFS	124	4	NA	3.2
APRI + FPI + Fib 4	124	5	NA	4.0
APRI + NFS + Fib 4	128	7	NA	5.5
Forns + FPI + NFS	124	16	NA	12.9
Forns + FPI + Fib 4	124	15	NA	12.1
Forns + NFS + Fib 4	127	12	NA	9.4
FPI + NFS + Fib 4	124	10	NA	8.1
<b>2 tests</b>				
APRI + Forns	127	5	2	5.5
APRI + FPI	124	7	5	9.7
APRI + NFS	128	7	5	9.4
APRI + Fib 4	130	11	2	10.0
Forns + FPI	124	28	4	25.8
Forns + NFS	127	25	1	20.5
Forns + Fib 4	127	22	0	17.3
FPI + NFS	124	10	4	11.3
FPI + Fib 4	124	18	0	14.5
NFS + Fib 4	128	17	0	13.3

This table explores the ability of obtaining a majority of (and two or more) concordant fibrosis tests for predicting significant fibrosis. By combining all the available non patented fibrosis tests, the proportion of patients with non-contributive tests changed regarding the tests used. The best contribution was obtained by combining 3 tests: APRI, Forns, and either NFS or FPI, with no additional contribution obtained by the addition of the remaining two tests. By combining only two tests, the best contribution was given by APRI and Forns. NA: not applicable.

**APRI:** ASAT to Platelet Ratio Index; **FPI:** Fibrosis Probability Index; **NFS:** NAFLD fibrosis score; **Fib-4:** Fibrosis-4

\* after excluding test results within the 'grey zone'

\*\* no answer given due to discrepancies and/or test results within the 'grey zone'

The prevalence of significant fibrosis as defined by the Fspec and by the Fsens algorithms was 10% (13 patients) and 28% (37 patients), respectively (table 4).



**Table 4: Non-invasive significant liver fibrosis assessment according to the inflammatory disease.**

	Population (+/- SD) <sup>a</sup>	Psoriasis (+/-SD)	Rheumatoid arthritis (+/- SD)	Crohn's disease (+/- SD)	p- value
	n=131	n=66	n=40	n=25	
<b>Fibroscan<sup>®</sup></b>	<b>102</b>	<b>49</b>	<b>30</b>	<b>23</b>	
Fibroscan <sup>®</sup> (mean in kPa)	6.3	7.4 ±0.7	5.4 ±0.9	4.9 ±1.1	NS
Fibroscan <sup>®</sup> Failure rate	22%	26%	25%	8%	
Fibroscan <sup>®</sup> NI <sup>b</sup>	38.6%	39.7%	45%	25%	
Fibroscan <sup>®</sup> ≥F2 (%)	7.9%	9.5%	10%	0%	0.06
<b>Forns index (n)</b>	<b>127</b>	<b>63</b>	<b>40</b>	<b>24</b>	
Mean Forns index	4.0 ±0.3	4.2 ±0.2	4.5 ±0.3	2.8 ±0.4	0.004
Forns NI	38.6%	39.7%	45%	25%	
Forns ≥F2 (%)	9.5%	9.5%	10%	0%	NS
<b>APRI score (n)</b>	<b>130</b>	<b>65</b>	<b>40</b>	<b>25</b>	
Mean APRI <sup>c</sup> score	0.38 ±0.04	0.41 ±0.06	0.41 ±0.07	0.26 ±0.09	0.06
APRI NI	10%	14.1%	7.5%	4%	
APRI ≥F2 (%)	3.1%	3.1%	5%	0%	NS
<b>FPI (n)</b>	<b>124</b>	<b>64</b>	<b>40</b>	<b>20</b>	
Mean FPI <sup>d</sup>	0.26 ±0.03	0.29 ±0.04	0.28 ±0.04	0.09 ±0.06	<0.001
FPI NI	35.5%	39.1%	40%	15%	
FPI ≥F2 (%)	9.7%	12.5%	10%	0%	0.05
<b>Hepascore (n)</b>	<b>124</b>	<b>64</b>	<b>38</b>	<b>22</b>	
Mean Hepascore	0.22 ±0.02	0.18 ±0.03	0.32 ±0.04	0.15 ±0.05	0.08
Hepascore ≥F2 (%)	10.5%	7.8%	21%	0%	0.02
<b>FIB-4 (n)</b>	<b>130</b>	<b>65</b>	<b>40</b>	<b>25</b>	
Mean FIB-4 <sup>e</sup>	1.37 ±0.11	1.44 ±0.15	1.60 ±0.19	0.83 ±0.24	<0.001
FIB-4 NI	26.9%	26.2%	37.5%	12%	
FIB-4 ≥F3	3.8%	4.6%	5%	0%	NS
<b>NFS<sup>f</sup> (n)</b>	<b>128</b>	<b>64</b>	<b>39</b>	<b>25</b>	
Mean NFS	-2.05 ±0.15	-1.98 ±0.21	-1.53 ±0.26	-3.02 ±0.33	<0.001
NFS NI	28.1%	28.1%	35.9%	16%	
NFS ≥F2 (%)	5.5%	6.3%	7.7%	0%	NS
<b>Fibrotest<sup>®</sup>(n)</b>	<b>119</b>	<b>61</b>	<b>37</b>	<b>21</b>	
Mean Fibrotest	0.21 ±0.02	0.22 ±0.02	0.24 ±0.03	0.14 ±0.04	0.06
Fibrotest <sup>®</sup> ≥F2 (%)	10.1%	11.4%	13.3%	0%	NS
<b>Fibrometer-NAFLD (n)</b>	<b>125</b>	<b>64</b>	<b>38</b>	<b>23</b>	
Mean Fibrometer-NAFLD	0.19 ±0.02	0.24 ±0.03	0.21 ±0.04	0.05 ±0.05	<0.001
Fibrometer-NAFLD ≥F2	15.5%	21.9%	13.2%	0%	0.04
<b>Number of tests</b>	<b>8.5 ±0.1</b>	<b>8.5 ±0.1</b>	<b>8.6 ±0.1</b>	<b>8.3 ±0.2</b>	<b>NS</b>
Uninterpretable tests	7.1 ±0.1	7.0 ±0.2	6.9 ±0.3	7.5 ±0.3	NS
Significant fibrosis ≥F2	0.7 ±0.1	0.9 ±0.2	0.9 ±0.3	0 ±0.3	0.003
Complete concordance	74%	71.2%	62.5%	100%	0.01
<b>Number of conflicting results</b>	<b>0.4 ±0.1</b>	<b>0.4 ±0.1</b>	<b>0.6 ±0.1</b>	<b>0 ±0.1</b>	<b>0.03</b>
Fspec	9.9%	12.1%	12.5%	0%	NS
Fsens	28.2%	33.3%	37.5%	0%	0.002

(<sup>a</sup>)SD: Standard deviation; (<sup>b</sup>) NI : Non interpretable ; (<sup>c</sup>) APRI: ASAT to Platelet Ratio Index; (<sup>d</sup>) FPI: Fibrosis

Probability Index; (<sup>e</sup>) FIB-4: Fibrosis-4; (<sup>f</sup>) NFS : non-alcoholic fatty liver disease fibrosis score

*Risks factors associated with significant fibrosis*

In univariate analyses, significant fibrosis according to Fspec, was associated with age ( $p < 0.001$ ), metabolic syndrome ( $p < 0.001$ ) and its different components, and high GGT levels ( $p < 0.001$ ). Significant fibrosis defined by Fsens was still associated with age ( $p < 0.001$ ), metabolic syndrome ( $p = 0.004$ ), and also with the absence of Crohn's disease ( $p < 0.001$ ). The use of MTX (any dose) even with a cumulative dose  $> 1500$ mg, did not impact on liver fibrosis as assessed by the Fspec or Fsens algorithms. A logistic regression analysis identified three predictors of a significant fibrosis defined by the Fsens method (table 5): old age (OR=1.06,  $p < 0.001$ ), male gender (OR=2.87,  $p = 0.026$ ) and presence of metabolic syndrome (OR=3.64,  $p = 0.007$ ). The cumulative dose of MTX had no effect. The small number of patients diagnosed with significant fibrosis through the Fspec algorithm did not allow multivariate analysis.

**Table 5: Multivariate analysis of factor associated with significant liver fibrosis according to the 'Fsens' algorithm**

	Logistic regression N=131 ; R <sup>2</sup> =0,20		
	OR	IC 95%	p
<b>Age (year)</b>	1.06	(1.02-1.10)	<0.001
<b>Male gender</b>	2.87	(1.13-7.29)	0.026
<b>Cumulated dose of methotrexate</b>	1.00	(0.99-1.00)	0.15
<b>Metabolic syndrome</b>	3.64	(1.41-9.35)	0.007

*Performance in liver fibrosis assessment*

The diagnostic performances of the fibrosis tests are summarized in table 6 and 7. Six tests showed excellent diagnostic performances ( $AUROC \geq 0.9$ ) for predicting significant fibrosis according to the Fspec algorithm: FibroMeter NAFLD, FPI, FIB-4 score, Fibrotest<sup>®</sup>, NFS, and Forns test. FibroMeter NAFLD obtained the best performance with a proportion of well categorized patients PPV and NPV of 89.3%, 92.3% and 89.0%, respectively. The FibroMeter NAFLD was also the best test for predicting significant fibrosis through the Fsens algorithm ( $AUROC=0.88$ ).

**Table 6: Positive and negative predictive value of significant liver fibrosis according to the 'Fspec' algorithm.**

	PPV <sup>a</sup> (%)	NPV <sup>b</sup> (%)	Well ranked tests (%)
<b>Fibroscan® (kPa)</b>	46.2	56.8	55.7
<b>Forns index</b>	69.2	57.6	58.8
<b>APRI<sup>c</sup></b>	30.8	91.5	85.5
<b>FPI<sup>d</sup></b>	76.9	57.6	59.5
<b>Hepascore</b>	38.5	88.1	83.2
<b>FIB-4<sup>e</sup></b>	30.8	74.6	70.2
<b>NAFLD<sup>f</sup> fibrosis score</b>	46.2	71.2	68.7
<b>Fibrotest®</b>	69.2	88.1	86.3
<b>Fibrometer-NAFLD</b>	92.3	89.0	89.3

<sup>a</sup>PPV: Positive Predictive Value; <sup>b</sup>NPV: Negative Predictive Value; <sup>c</sup>APRI: ASAT to Platelet Ratio Index; <sup>d</sup>FPI: Fibrosis

Probability Index; <sup>e</sup>FIB-4: Fibrosis-4; <sup>f</sup>NAFLD: Non alcoholic fatty liver disease

Table 7: Non-invasive tests performance for the diagnosis of significant liver fibrosis.

	AUROC <sup>(a)</sup> value			
	« Fspec »		« Fsens »	
	AUROC	IC 95%	AUROC	IC 95%
Age	0.83	0.56-0.94	0.77	0.61-0.87
Cumulated dose of MTX <sup>(b)</sup>	0.34	0.11-0.52	0.52	0.35-0.66
Waist circumference	0.65	0.28-0.85	0.69	0.51-0.81
BMI <sup>(c)</sup>	0.64	0.30-0.83	0.73	0.56-0.84
GGT	0.87	0.57-0.97	0.66	0.50-0.78
Fibroscan® (kPa)	0.65	0.27-0.85	0.67	0.48-0.80
Hyaluronic acid (µg/l)	0.64	0.32-0.83	0.62	0.45-0.75
PIIIP (kU/l) <sup>(d)</sup>	0.59	0.23-0.81	0.63	0.46-0.76
Forns index	0.95	0.77-0.99	0.76	0.60-0.86
APRI score <sup>(e)</sup>	0.90	0.76-0.96	0.72	0.54-0.84
FPI <sup>(f)</sup>	0.97	0.84-1	0.81	0.65-0.90
HEPASCORE	0.78	0.47-0.92	0.62	0.43-0.76
FIB-4 <sup>(g)</sup>	0.95	0.67-0.99	0.78	0.61-0.88
NFS <sup>(h)</sup>	0.90	0.72-0.97	0.84	0.68-0.92
Fibrotest®	0.92	0.68-0.98	0.75	0.59-0.86
Fibrometer-NAFLD	0.93	0.79-0.98	0.88	0.73-0.95

<sup>a</sup>AUROC: Area Under the Receiver Operating Characteristic Curve; <sup>b</sup>MTX: Methotrexate;

<sup>c</sup>BMI: Body Mass Index; <sup>d</sup>PIIIP: Procollagen type 3; <sup>e</sup>APRI: ASAT to Platelet Ratio Index;

<sup>f</sup>FPI: Fibrosis Probability Index; <sup>g</sup>FIB-4: Fibrosis-4; <sup>h</sup>NAFLD: Non-alcoholic fatty liver disease

### *Sensitivity analyses*

Because hyaluronic acid may increase in the event of inflammatory rheumatism regardless of liver fibrosis, we conducted several sensitivity analyses by excluding 1) the 40 patients with RA and the 19 patients with identified psoriatic arthritis; 2) the six cases of liver fibrosis identified on the basis of Fibrometer (which includes hyaluronic acid) results only. Through multivariate analyses, independent predictors of significant liver fibrosis according to the 'Fsens' algorithm were age and metabolic syndrome (ORs varying from 2.55 to 5.02). By comparing the new test performances for predicting fibrosis according to the Fsens algorithm with those given in table 7, three parameters decreased their AUROC by 0.03 or more: age (-0.03), Fibrometer (-0.07), and NFS (-0.04); six parameters increased their AUROC by 0.03 or more: waist circumference (+0.06), PIIIP (+0.10), Hepascore (+0.12), Forns' index (+0.04), Fibrotest (+0.03) and Fibroscan (+0.07), and for the remaining four parameters, the difference of AUROC values was less than 0.02. The best predictors of liver fibrosis according to Fsens were thus: FPI (AUROC=0.82; 95%CI:0.68-0.90), Fibrometer (AUROC=0.81; 95%CI:0.70-0.89), NFS (AUROC=0.80; 95%CI:0.68-0.88), and Forns (AUROC=0.80; 95%CI:0.67-0.88).

## DISCUSSION

The fear of liver fibrosis development limits the use of MTX and highlights the need of a reliable, non-invasive, routinely available and inexpensive sequential assessment of liver fibrosis. Until recently, only liver biopsy was recommended for this purpose, thus considerably hampering the use of MTX over the long term, because its acceptability is low. It was therefore appropriate to evaluate all the non-invasive alternatives to liver biopsy available that could detect significant fibrosis. We evaluated them in an original fashion, i.e., without imposing liver biopsy as a diagnostic reference tool, but by analyzing the non-invasive tests altogether and considering their concordance as the guarantee of true assessment. The non-use of liver biopsy was imposed by our local ethic committee and allowed a good adhesion of patients and their referring physicians to our study. It also permitted to prospectively include all consecutive patients without biasing the results by selecting patients with presumed severe liver disease, a well-known situation which encourages liver biopsy. Our approach allowed us to define algorithms evaluating risks of fibrosis according to a 'specific' method (Fspec) and a 'sensitive' method (Fsens). Using this original diagnostic strategy, we were able to compare patients with Pso, RA and CD and evaluate the real impact of MTX on liver fibrosis taking into account the confounding factors as much as possible. Moreover, we investigated from all the combination of tests available, the ability of obtaining at least two concordant fibrosis tests for predicting significant fibrosis (table 3). We found that by combining at least three non-patented tests allowing a 'grey zone', only a minority of patients (i.e., 2.4%) could not be classified according to the presence of significant liver fibrosis. Such a finding may be useful for all patients with fatty liver disease, a condition largely represented in our population, and justify that our approach will be further investigated in the setting of NAFLD.

The main results of our work were the following: 1) In patients with Pso, RA or CD, MTX treatment was not associated with more frequent significant liver fibrosis (no cumulative dose effect); 2) significant fibrosis was associated with a metabolic syndrome, which was common in patients with



RA or Pso, conversely to that observed in patients with CD; 3) some non-invasive fibrosis markers among the most popular ones, such as the PIIIP assay widely used by dermatologists, and the Fibroscan<sup>®</sup>, which enjoys significant promotional communication in the hepatology field, appeared to be disappointing and less contributive than the biochemical tests. In particular, the FibroMeter NAFLD, the Fibrotest, and the combination of the non-patented tests, even despite their grey zone, were contributive in more than 97% of patients, a proportion much higher than the proportion of Fibroscan<sup>®</sup> successes with the M probe.

The originality of the present study was the pragmatic use and integrated analysis of numerous non-invasive fibrosis tests in patients receiving MTX. In previous studies on the topic, only a limited number of non-invasive tests (i.e., PIIIP [40,41], and Fibroscan<sup>®</sup>, alone or combined with Fibrotest<sup>®</sup>[13,15-17,20,21,23-25]) were studied. Comparisons with liver biopsy examination were performed when available. In the study by Chalmers et al., the PIIIP monitoring reduced the number of liver biopsies by 7 fold and increased its cost-effectiveness [40]. In our work, this marker seemed not effective for the diagnosis of significant fibrosis, since its AUROC did not significantly differ from 0.5 whatever the algorithm chosen for defining significant fibrosis (table 7). In our work, Fibroscan<sup>®</sup> was performed by two experimented operators with the standard M probe and provided disappointing results, conversely to that enthusiastically reported by Laharie et al. with the same probe [15]. We collected only 61% interpretable liver stiffness measures. Most failures or inaccurate measures were observed in overweight subjects, who represented a high proportion of the study population (76%). Our failure rate was consistent with that published by Lynch et al. [22]. Moreover, even when considering interpretable results of Fibroscan<sup>®</sup>, we observed low predictive values for the diagnosis of significant fibrosis (Table 6). In the present study, the most efficient test was the FibroMeter NAFLD, which was always interpretable and provided the highest performances for predicting significant fibrosis (table 6). Interestingly, APRI provided excellent NPV.

According to our more specific algorithm (Fspec), the estimated prevalence of fibrosis, was 10 % in our population: 12% in Pso, 12% in RA and 0% in CD. This result, consistent with the literature [8,15,42], may be explained in part by the inclusion of a population unselected by the availability of liver biopsy, which is rarely systematic and rather encouraged by the presumption of severe liver disease. The non-influence of MTX treatment on liver fibrosis progression reported in our study was also previously reported by Laharie et al. [15] in similar populations of consecutive patients. One may hypothesize that such reassuring data may be explained by lower cumulative doses of MTX than those used in the historical series that incriminated MTX in liver fibrosis development [43-45], but our results do not support this hypothesis. Indeed, in the present study, only 49 patients had received a cumulative dose of MTX >1500 mg, but even with this dose considered to be risky, no trend towards advanced fibrosis was observed. Our results emphasize the preponderant role of confounding factors in patients receiving MTX treatment to explain the variability of liver fibrosis. Those factors, especially the metabolic syndrome, were not studied in historical reports but were reported to have a detrimental impact in the more recent ones [46-48]. In the study by Langman et al, which included patients with repeated liver biopsies, the 13 patients with metabolic syndrome had histologically proven non-alcoholic steatohepatitis and subsequently developed liver fibrosis [47]. In our study, the prevalence of the metabolic syndrome, defined by the most stringent criteria (NCEP- ATPIII) was twice as high as that described in European populations [49], and higher than the prevalence of excessive alcohol consumption or viral hepatitis. In patients with RA, an increased risk of cardiovascular disease has been described recently [50], which is clearly influenced by the metabolic syndrome [51]. In patients with psoriasis, the high prevalence of metabolic syndrome (up to 30%) reported in the literature [52] was similar to that observed in our patients (35%). It favors non-alcoholic steatohepatitis, often reported in this population [53,54]. Moreover, steatohepatitis was reported to be associated with severe forms of psoriasis which require MTX therapy [54]. The low prevalence of significant fibrosis in patients with Crohn's disease was explained by the low

prevalence of metabolic syndrome (12%), the predominance of females and the young age of the majority of patients, consistently to that observed in other liver diseases [55,56]. The question whether MTX may contribute to the development of metabolic syndrome and NAFLD remains unresolved but is not supported by our data. In our series, the prevalence of metabolic syndrome was not higher in treated patients (Table 2), and liver steatosis was less common in patients with CD, despite a similar proportion of CD and Pso patients receiving a cumulative dose of MTX>1500mg (table 1).

Our study acknowledges several limitations. First, liver biopsy was not performed although it is considered as the gold standard for identifying liver fibrosis in patients receiving MTX. We dared conduct this study without liver biopsy, consistently with what is done in the more recent studies in patients with chronic hepatitis C and with the recommendations of our ethics committee, who was aware of the risks and sampling biases associated with this invasive procedure [57]. As mentioned above, it allowed us to reduce selection biases and increase acceptability of the study. Second, we used an original strategy to combine non-invasive tests results and identify patients with significant fibrosis, that had not been validated before. Similar strategies, however, are encouraged by several guidelines devoted to the management of various chronic liver diseases, in order to restrict the use of liver biopsy to patients with discordant results according to non-invasive evaluations [58]. They are particularly important for the detection of fibrosing non-alcoholic steatohepatitis in the large population potentially concerned [59]. We improved this approach by using the largest number of scores ever done and defining both a specific algorithm based on the majority of concordant tests, and a sensitive algorithm that did not omit any patient with significant fibrosis predicted by only one positive test. The excellent agreement between the tests used (Figure 1) for predicting significant fibrosis was a convincing finding and a pragmatic validation of our diagnostic approach.

In conclusion, our study suggests the safety of MTX regarding the risk of liver fibrosis or, in any case, its negligible effect in comparison with risk factors such as the metabolic syndrome. In this context,

liver biopsy should be reduced as much as possible to render MTX, a very cost-effective treatment, widely acceptable. The use of non-invasive markers should be recommended, providing it integrates the analysis of discordant results. The use of a single direct marker such as PIIIP or hyaluronic acid should no longer be recommended, particularly hyaluronic acid in the context of rheumatoid arthritis. Our preference goes to biochemical tests dedicated to steatohepatitis such as the FibroMeter NAFLD or to a combination of at least three tests including APRI, Forns, and FPI or NFS. Our experience of Fibroscan® with M probe in this population was disappointing. Further studies using XL probe would be useful in patients receiving MTX.

## References

- [1] Roenigk HH, Jr., Auerbach R, Maibach H, Weinstein G, Lebwohl M. Methotrexate in psoriasis: consensus conference. *J Am Acad Dermatol* 1998;38:478-85.
- [2] Mikuls TR, O'Dell J. The changing face of rheumatoid arthritis therapy: results of serial surveys. *Arthritis Rheum* 2000;43:464-5.
- [3] Mate-Jimenez J, Hermida C, Cantero-Perona J, Moreno-Otero R. 6-mercaptopurine or methotrexate added to prednisone induces and maintains remission in steroid-dependent inflammatory bowel disease. *Eur J Gastroenterol Hepatol* 2000;12:1227-33.
- [4] Griffiths RI, Bar-Din M, MacLean CH, Sullivan EM, Herbert RJ, Yelin EH. Medical resource use and costs among rheumatoid arthritis patients receiving disease-modifying antirheumatic drug therapy. *Arthritis Care Res* 2000;13:213-26.
- [5] Kinder AJ, Hassell AB, Brand J, Brownfield A, Grove M, Shadforth MF. The treatment of inflammatory arthritis with methotrexate in clinical practice: treatment duration and incidence of adverse drug reactions. *Rheumatology* 2005;44:61-6.
- [6] Menter A, Korman NJ, Elmets CA, Feldman SR, Gelfand JM, Gordon KB, et al. Guidelines of care for the management of psoriasis and psoriatic arthritis. Section 3. Guidelines of care for the management and treatment of psoriasis with topical therapies. *J Am Acad Dermatol* 2009;60:643-59.
- [7] Shergy WJ, Polisson RP, Caldwell DS, Rice JR, Pisetsky DS, Allen NB. Methotrexate-associated hepatotoxicity: retrospective analysis of 210 patients with rheumatoid arthritis. *Am J Med* 1988;85:771-4.
- [8] Te HS, Schiano TD, Kuan SF, Hanauer SB, Conjeevaram HS, Baker AL. Hepatic effects of long-term methotrexate use in the treatment of inflammatory bowel disease. *Am J Gastroenterol* 2000;95:3150-6.
- [9] Roenigk HH, Jr., Auerbach R, Maibach HI, Weinstein GD. Methotrexate in psoriasis: revised guidelines. *J Am Acad Dermatol* 1988;19:145-56.
- [10] de Ledinghen V, Wong VW, Vergniol J, Wong GL, Foucher J, Chu SH, et al. Diagnosis of liver fibrosis and cirrhosis using liver stiffness measurement: comparison between M and XL probe of FibroScan(R). *J Hepatol* 2012;56:833-9.
- [11] Ratziu V, Massard J, Charlotte F, Messous D, Imbert-Bismut F, Bonyhay L, et al. Diagnostic value of biochemical markers (FibroTest-FibroSURE) for the prediction of liver fibrosis in patients with non-alcoholic fatty liver disease. *BMC Gastroenterol* 2006;6:6.

- [12] Wong VW, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, et al. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. *Hepatology* 2010;51:454-62.
- [13] Barbero-Villares A, Mendoza Jimenez-Ridruejo J, Taxonera C, Lopez-Sanroman A, Pajares R, Bermejo F, et al. Evaluation of liver fibrosis by transient elastography (Fibroscan(R)) in patients with inflammatory bowel disease treated with methotrexate: a multicentric trial. *Scand J Gastroenterol* 2012;47:575-9.
- [14] Gonzalez-Lama Y, Taxonera C, Lopez-Sanroman A, Perez-Calle JL, Bermejo F, Pajares R, et al. Methotrexate in inflammatory bowel disease: a multicenter retrospective study focused on long-term efficacy and safety. The Madrid experience. *Eur J Gastroenterol Hepatol* 2012;24:1086-91.
- [15] Laharie D, Seneschal J, Schaefferbeke T, Doutre MS, Longy-Boursier M, Pellegrin JL, et al. Assessment of liver fibrosis with transient elastography and FibroTest in patients treated with methotrexate for chronic inflammatory diseases: a case-control study. *J Hepatol* 2010;53:1035-40.
- [16] Laharie D, Zerbib F, Adhoute X, Boue-Lahorgue X, Foucher J, Castera L, et al. Diagnosis of liver fibrosis by transient elastography (FibroScan) and non-invasive methods in Crohn's disease patients treated with methotrexate. *Aliment Pharmacol Ther* 2006;23:1621-8.
- [17] Berends MA, Snoek J, de Jong EM, Van Krieken JH, de Knegt RJ, van Oijen MG, et al. Biochemical and biophysical assessment of MTX-induced liver fibrosis in psoriasis patients: Fibrotest predicts the presence and Fibroscan predicts the absence of significant liver fibrosis. *Liver Int* 2007;27:639-45.
- [18] Bray AP, Barnova I, Przemioslo R, Kennedy CT. Liver fibrosis screening for patients with psoriasis taking methotrexate: a cross-sectional study comparing transient elastography and liver biopsy. *Br J Dermatol* 2012;166:1125-7.
- [19] Chladek J, Simkova M, Vaneckova J, Hroch M, Vavrova J, Hulek P. Assessment of methotrexate hepatotoxicity in psoriasis patients: a prospective evaluation of four serum fibrosis markers. *J Eur Acad Dermatol Venereol* 2013;27:1007-14.
- [20] Rongngern P, Chularojanamontri L, Wongpraparut C, Silpa-Archa N, Chotiyaputta W, Pongpaibul A, et al. Diagnostic performance of transient elastography for detection of methotrexate-induced liver injury using Roenigk classification in Asian patients with psoriasis: a retrospective study. *Arch Dermatol Res* 2017;309:403-8.

- [21] Talme T, Nikamo P, Rosenberg P, Stahle M. Transient Elastography May Improve Detection of Liver Fibrosis in Psoriasis Patients Treated with Methotrexate. *Acta Derm Venereol* 2017;97:952-4.
- [22] Lynch M, Higgins E, McCormick PA, Kirby B, Nolan N, Rogers S, et al. The use of transient elastography and FibroTest for monitoring hepatotoxicity in patients receiving methotrexate for psoriasis. *JAMA Dermatol* 2014;150:856-62.
- [23] Arena U, Stasi C, Mannoni A, Benucci M, Maddali-Bongi S, Cammelli D, et al. Liver stiffness correlates with methotrexate cumulative dose in patients with rheumatoid arthritis. *Dig Liver Dis* 2012;44:149-53.
- [24] Park SH, Choe JY, Kim SK. Assessment of liver fibrosis by transient elastography in rheumatoid arthritis patients treated with methotrexate. *Joint Bone Spine* 2010;77:588-92.
- [25] Rouhi A, Hazlewood G, Shaheen AA, Swain MG, Barber CEH. Prevalence and risk factors for liver fibrosis detected by transient elastography or shear wave elastography in inflammatory arthritis: a systematic review. *Clin Exp Rheumatol* 2017;35:1029-36.
- [26] Freedman ND, Everhart JE, Lindsay KL, Ghany MG, Curto TM, Shiffman ML, et al. Coffee intake is associated with lower rates of liver disease progression in chronic hepatitis C. *Hepatology* 2009;50:1360-9.
- [27] Ruhl CE, Everhart JE. Coffee and tea consumption are associated with a lower incidence of chronic liver disease in the United States. *Gastroenterology* 2005;129:1928-36.
- [28] Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001;285:2486-97.
- [29] Sandrin L, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, et al. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003;29:1705-13.
- [30] Yoneda M, Mawatari H, Fujita K, Endo H, Iida H, Nozaki Y, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with nonalcoholic fatty liver disease (NAFLD). *Dig Liver Dis* 2008;40:371-8.
- [31] Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001;357:1069-75.
- [32] Forns X, Ampurdanes S, Llovet JM, Aponte J, Quinto L, Martinez-Bauer E, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002;36:986-92.

- [33] Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003;38:518-26.
- [34] Sud A, Hui JM, Farrell GC, Bandara P, Kench JG, Fung C, et al. Improved prediction of fibrosis in chronic hepatitis C using measures of insulin resistance in a probability index. *Hepatology* 2004;39:1239-47.
- [35] Adams LA, Bulsara M, Rossi E, DeBoer B, Speers D, George J, et al. Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. *Clin Chem* 2005;51:1867-73.
- [36] Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006;43:1317-25.
- [37] Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007;45:846-54.
- [38] Cales P, Laine F, Boursier J, Deugnier Y, Moal V, Oberti F, et al. Comparison of blood tests for liver fibrosis specific or not to NAFLD. *J Hepatol* 2009;50:165-73.
- [39] Cales P, Oberti F, Michalak S, Hubert-Fouchard I, Rousselet MC, Konate A, et al. A novel panel of blood markers to assess the degree of liver fibrosis. *Hepatology* 2005;42:1373-81.
- [40] Chalmers RJ, Kirby B, Smith A, Burrows P, Little R, Horan M, et al. Replacement of routine liver biopsy by procollagen III aminopeptide for monitoring patients with psoriasis receiving long-term methotrexate: a multicentre audit and health economic analysis. *Br J Dermatol* 2005;152:444-50.
- [41] Maurice PD, Maddox AJ, Green CA, Tatnall F, Schofield JK, Stott DJ. Monitoring patients on methotrexate: hepatic fibrosis not seen in patients with normal serum assays of aminoterminal peptide of type III procollagen. *Br J Dermatol* 2005;152:451-8.
- [42] Whiting-O'Keefe QE, Fye KH, Sack KD. Methotrexate and histologic hepatic abnormalities: a meta-analysis. *Am J Med* 1991;90:711-6.
- [43] Boffa MJ, Chalmers RJ, Haboubi NY, Shomaf M, Mitchell DM. Sequential liver biopsies during long-term methotrexate treatment for psoriasis: a reappraisal. *Br J Dermatol* 1995;133:774-8.
- [44] Zachariae H, Sogaard H. Liver biopsy in psoriasis. A controlled study. *Dermatologica* 1973;146:149-55.
- [45] Zachariae H, Sogaard H. Methotrexate-induced liver cirrhosis. A follow-up. *Dermatologica* 1987;175:178-82.



- [46] Belinsky GS, Parke AL, Huang Q, Blanchard K, Jayadev S, Stoll R, et al. The contribution of methotrexate exposure and host factors on transcriptional variance in human liver. *Toxicol Sci* 2007;97:582-94.
- [47] Langman G, Hall PM, Todd G. Role of non-alcoholic steatohepatitis in methotrexate-induced liver injury. *J Gastroenterol Hepatol* 2001;16:1395-401.
- [48] Shetty A, Cho W, Alazawi W, Syn WK. Methotrexate Hepatotoxicity and the Impact of Nonalcoholic Fatty Liver Disease. *Am J Med Sci* 2017;354:172-81.
- [49] Balkau B, Charles MA, Drivsholm T, Borch-Johnsen K, Wareham N, Yudkin JS, et al. Frequency of the WHO metabolic syndrome in European cohorts, and an alternative definition of an insulin resistance syndrome. *Diabetes Metab* 2002;28:364-76.
- [50] Meune C, Touze E, Trinquart L, Allanore Y. Trends in cardiovascular mortality in patients with rheumatoid arthritis over 50 years: a systematic review and meta-analysis of cohort studies. *Rheumatology* 2009;48:1309-13.
- [51] Zonana-Nacach A, Santana-Sahagun E, Jimenez-Balderas FJ, Camargo-Coronel A. Prevalence and factors associated with metabolic syndrome in patients with rheumatoid arthritis and systemic lupus erythematosus. *J Clin Rheumatol* 2008;14:74-7.
- [52] Gisondi P, Tessari G, Conti A, Piaserico S, Schianchi S, Peserico A, et al. Prevalence of metabolic syndrome in patients with psoriasis: a hospital-based case-control study. *Br J Dermatol* 2007;157:68-73.
- [53] Gisondi P, Targher G, Zoppini G, Girolomoni G. Non-alcoholic fatty liver disease in patients with chronic plaque psoriasis. *J Hepatol* 2009;51:758-64.
- [54] Miele L, Vallone S, Cefalo C, Torre GL, Stasi CD, Vecchio FM, et al. Prevalence, characteristics and severity of non-alcoholic fatty liver disease in patients with chronic plaque psoriasis. *J Hepatol* 2009;51:778-86.
- [55] Di Martino V, Lebray P, Myers RP, Pannier E, Paradis V, Charlotte F, et al. Progression of liver fibrosis in women infected with hepatitis C: long-term benefit of estrogen exposure. *Hepatology* 2004;40:1426-33.
- [56] Poynard T, Munteanu M, Deckmyn O, Ngo Y, Drane F, Castille JM, et al. Validation of liver fibrosis biomarker (FibroTest) for assessing liver fibrosis progression: proof of concept and first application in a large population. *J Hepatol* 2012;57:541-8.
- [57] Poynard T, Benhamou Y, Thabut D, Ratziu V. Liver biopsy: the best standard...when everything else fails. *J Hepatol* 2009;50:1267-8.

- [58] Castera L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, et al. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005;128:343-50.
- [59] Xiao G, Zhu S, Xiao X, Yan L, Yang J, Wu G. Comparison of laboratory tests, ultrasound, or magnetic resonance elastography to detect fibrosis in patients with nonalcoholic fatty liver disease: A meta-analysis. *Hepatology* 2017;66:1486-501.

**Figures legend:**

Figure 1: Non-invasive liver fibrosis diagnosis strategy: results from used tests and significant fibrosis definition.

**Non invasive significant liver fibrosis assessment**  
(n=131)

**Fibroscan®**  
(n=131)

**8 biological tests:**  
Fibrometer NAFLD (n=125)  
NFS (n=128)  
Fibrotest (n=119)  
Forns (n=127)  
APRI (n=130)  
FPI (n=124)  
Hepascore (n=124)  
FIB-4 (n=130)

-Failures (n=29)  
-Insufficient reliability (n=22)

**Tests within the grey area**  
FibroMeter NAFLD (n=0)  
Fibrotest (n=0)  
Hepascore (n=0)  
APRI (n=13)  
Fib4 (n=35)  
NAFLD fibrosis (n=36)  
FPI (n=44)  
Forns (n=49)

**7 interpretable tests [range 3 to 9]:**  
125 FibroMeter NAFLD  
124 Hepascore  
119 Fibrotest  
117 APRI  
95 FIB-4  
92 NFS  
80 FPI  
78 Forns  
80 Fibroscan®

*3 tests were interpretable in 3 patients  
At least 5 tests were interpretable in 121 patients  
At least 8 tests were interpretable in 62 patients*

**Liver fibrosis classification according to each test**

**Discrepancies identification**  
20 patients with 1 discrepant test  
8 patients with 2 discrepant tests  
5 patients with 3 discrepant tests

**Specific strategy « Fspec »**  
(Significant fibrosis found by most tests)  
  
N=13 patients

**Sensitive strategy « Fsens »**  
(At least 1 test showing significant fibrosis)  
  
N=37 patients