Enhanced liver fibrosis test (ELF) in psoriasis, psoriatic arthritis and rheumatoid arthritis patients: a cross-sectional comparison with procollagen-3 N-terminal peptide (P3NP).

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ABSTRACT

Background: Recently the enhanced liver fibrosis (ELF) test, a combined use of three serum biomarkers to detect liver fibroses, was introduced to screen, diagnose and/or monitor liver conditions in large groups of patients with liver diseases and healthy controls, but it has not been used in inflammatory skin or joint diseases.

Objective: To evaluate the distribution of the ELF test, apply existing cut offs for hepatic patients and healthy controls, and compare it to the corresponding procollagen-3 N-terminal peptide (P3NP) test among patients with psoriasis (PSO), psoriatic arthritis (PsA), rheumatoid arthritis (RA) and controls.

Methods: In total 531 patients were included in this cross sectional study. Demographic, lifestyle and disease-specific data were collected. ELF and P3NP test was performed.

Results: The prevalence of an increased ELF (>11) and P3NP was highest in RA patients (7.7% and 6.1%) followed by PSO patients (1.7% and 5.2%) and PsA (0.7% and 1.3%). Mean score ELF: PSO 9.09±0.86; PsA 8.96±0.76; RA 9.55±1.04. All subgroups with moderate to severe disease severity had higher ELF scores (ELF>9.8: PSO 27.0%vs 18.3%, PsA 19.2%vs12%, RA 45.8%vs30.5%) and P3NP values. The distribution of the ELF score was smaller compared to P3NP value (mean 9.15±0.92 and 8.37±4.30; range 6.53-13.05 and 0.53-63.88).

Conclusions: ELF score and P3NP values are elevated in PSO, PsA and RA. ELF may be superior to P3NP alone but further research should be done to validated ELF test susceptible for developing liver fibrosis for PSO, PsA and RA.

INTRODUCTION

Compared to the general population, patients with psoriasis (PSO) have approximately twice the risk of developing nonalcoholic fatty liver disease (NAFLD) and liver fibrosis.[1-4] This is partly due to the use of hepatotoxic drugs and shared comorbidities, but possibly also through other independent mechanisms.[2,3] Furthermore, PSO patients using methotrexate (MTX) have a higher likelihood of developing liver fibrosis as compared to those with psoriatic arthritis (PsA) and rheumatic arthritis (RA) using MTX.[5] A recent systematic review reported that the prevalence of methotrexate-induced liver fibrosis and cirrhosis (Roenigk stage ≥3a) in PSO patients varies from 5,7%-71,8% [6], depending on underlying risk factors and comorbidities. The incidence of MTX-induced liver fibrosis in patients with RA and PsA seems to be much lower, with 15.3% for mild, 1.3% for severe liver fibrosis and 0.5% for cirrhosis in RA and 9.9%, 1.4% and 1.4% in PsA, respectively.[5]

Cirrhosis is the main cause of morbidity and mortality in chronic liver disease, but is often asymptomatic until the synthetic and filtering functions of the liver are finally compromised and/or portal hypertension develops. Hepatic fibrosis is also difficult to detect with standard noninvasive techniques: it can develop despite normal liver function tests and normal images from ultrasound and radionuclide scans.[7] Although liver biopsy remains the golden standard, it carries a risk for serious complications in > 1% of patients. Hence, there clearly is a need for an accurate, valid and reliable non-invasive diagnostic test to detect early liver fibrosis.[8]

The European psoriasis EDF guidelines recommend to determine procollagen-3 N-terminal peptide (P3NP) as a marker for liver fibroses prior to starting methotrexate as well as serially every 2–3 months throughout treatment, for patients at risk and where available.[9-11] P3NP has however not been accepted as the standard by other specialties, including hepatology, requires serial measurements, is quite expensive, is not specific to liver fibrosis and may be falsely elevated in patients with inflammatory diseases such as an active arthritis.

A relatively new noninvasive test is the Enhanced Liver Fibrosis (ELF) test. The ELF test employs a combined automated in-vitro immunoassay for the quantitative measurement of three serological markers; P3NP, tissue inhibitor of matrix metalloproteinase 1 (TIMP1) and hyaluronic acid (HA). The individual results of these markers are combined in an algorithm to produce an ELF score,[12,13] which has been validated as a biomarker of fibrosis in healthy subjects and in patients with a wide range of chronic liver diseases, including nonalcoholic fatty liver disease, hepatitis C and primary biliary cirrhosis.[14-17] This has resulted in proposed cut off values for liver fibrosis and cirrhosis. Furthermore, it has been shown that the ELF test is superior to liver biopsy in predicting the clinical outcome in chronic liver disease.[13] A recent pilot study suggested that a single ELF

measurement may be at least equivalent or possibly superior to single as well as serial P3NP in the detection of liver fibrosis in 27 patients with PSO treated with MTX as part of routine clinical practice.[18]

The objective of this cross-sectional study is to evaluate and compare the distribution, cut off scores and values and predictors of the elevated noninvasive liver fibrosis tests ELF and P3NP in three different patient populations being PSO, PsA and RA. Secondary to explore if the ELF test can be a potentially valuable tool to monitor liver fibrosis in inflammatory diseases especially for those treated by hepatotoxic medication.

METHODS

Study design and population

The study subjects were included from March 2009 until August 2012, which has been described previously.[19] The patients with PSO had chronic plaque psoriasis and were diagnosed and recruited by dermatologists from the department of dermatology Erasmus Medical Center in Rotterdam. At the same center, the control group consisting of individuals with varicose veins or benign moles without PSO, PsA and/or RA were recruited. The PsA and RA patients were recruited from the rheumatology department of the Maxima Medisch Centrum in Eindhoven. An expert rheumatologist confirmed PsA and RA diagnosis based on the Classification Criteria for Psoriatic Arthritis (CASPAR) and 2010 ACR/EULAR RA Classification Criteria.[20] All PSO subjects had no history or signs of inflammatory arthritis.

Co-variables and disease characteristics

The following data were collected in a standardized manner, at the same day the patient was included: demographic data (age, gender, weight, height), disease onset, disease duration, general medical history including comorbidities, concomitant medication, current and previous disease specific medication and lifestyle (including alcohol intake and smoking).

Body mass index (BMI) was calculated as weight (kg)/height (m2). Patients were defined as having diabetes if they used diabetic medication including all insulin preparations and oral agents, had diabetes mentioned in their medical history or had an elevated serum glucose level (> 6.1 mmol/L) or HbA1c(Glycohemoglobine) (>42 mmol/mol Hb). Hypercholesterolemia was defined as serum total cholesterol >6.5 mmol/L, serum triglycerides >2.0 mmol/L; serum high-density lipoprotein cholesterol < 0.9 mmol/L, serum low-density lipoprotein cholesterol > 2.59 mmol/L or drug treatment for low high-density lipoprotein cholesterol, elevated triglycerides or elevated high low-cholesterol. Hypertension was determined based on a medical history of hypertension or the use of blood pressure lowering drugs. Excessive alcohol consumption was defined as more

than 3 drinks per day for men and women. Pack-years of smoking were calculated as years of smoking (excluding years of nonsmoking) multiplied by the average number of packs (containing 20 cigarettes) smoked per day.

For psoriasis and PsA patients, Psoriasis Severity Index Score (PASI)<7 was defined as mild; PASI 7–12 as moderate and a PASI>12 as severe disease.[21] The disease activity and course severity in psoriatic and rheumatic arthritis patients were assessed with Disease Activity Score 28 (DAS28) and a DAS<3.2 was defined as mild, 3.2-5.1 as moderate and >5.1 as severe disease activity. [22, 23] In case of a discrepancy in disease severity score between skin and joints in PsA, the most severe stage was taken. This occurred only in 4 patients with skin severity higher than joint severity.

Disease specific medication was divided into four subgroups; (1) patients without medication or who only used topicals, UV and/or non-steroidal anti-inflammatory drugs (NSAIDs); (2) patients who used disease related systemic drugs excluding MTX; (3) MTX use irrespective of any other medication except biologicals; and (4) patients who used a biologicals irrespective of medication from group one to three. Data on dosing regimens were not available.

Laboratory analysis

Serum samples were collected at the same visit and stored at –80°C until assayed. Serum samples were analyzed for levels of HA, TIMP-1 and P3NP using the proprietary assays developed for the ELF test by Siemens Healthcare Diagnostics Inc. These assays are magnetic particle separation immunoassays, and samples were analyzed on an ADVIA®CentaurXP immunoassay system (Siemens Medical Solutions Diagnostics Inc., Tarrytown, NY, USA). Results were entered into the manufacturer's published algorithm to derive an ELF score. These samples were analyzed by an independent reference laboratory (Star-MDC, Rotterdam, NL).The analyses were all performed on the same day to avoid measurement bias.

The ELF (ELF) score was calculated using the algorithm: DS = 6.38 – (ln (age) 9 0.14) + (ln (HA) 9 0.616 + (ln (P3NP) 9 0.586) + (ln (TIMP1) 90.472). Validated ELF test cut off values to high specificity identification of fibrosis, have been determined for healthy blood donors (>9.8) and patients with chronic liver diseases (>11), but this has not yet been validated in PSO, PsA and RA.[10,24] The cut off values for P3NP in PSO patients with MTX are >12.2 for a liver biopsy indication and >15.3 for withdrawal MTX.[11]

Serum alanine aminotransferase (ALT), aspartate transaminase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) and C reactive protein (CRP) were measured using standard enzymatic immunoassays.

Statistics

Statistical analysis was performed using SPSS software version 20. Variables were described using standard descriptive statistics. Continuous variables were expressed as mean ± SD or as median ± interquartile range; and categorical data as number and percentage. The unpaired t test and analysis of variance (ANOVA) test and, when indicated, two-tailed Mann– Whitney and Kruskall–Wallis tests were used to perform comparison between two or more groups, respectively. Bonferroni and Dunn's tests were used for multiple comparisons. $\chi 2$ and Fisher's exact tests were used to compare categorical variables. Parametric and non-parametric correlations were calculated using Pearson's and Spearman's rank correlation tests, respectively.

The distribution of the general characteristics were compared between the different groups using the Chi-square tests and one way ANOVA or Kruskal Wallis tests for statistical significance of categorical data and continuous data, respectively.

In order to identify the clinical variables independently associated with P3NP and ELF scores in the whole cohort, multivariate logistic regression analyses were performed. The first multivariable model adjusted for age and gender. In the fully adjusted model, multivariable logistic backward regression model was selected to determine which confounder substantially affected the test outcome considering the other variables in the model. Based on the literature we selected age, gender, alcohol consumption, smoking, ALT, CRP, BMI, disease type and activity and liver toxic medication as potential relevant confounders. The variables ALT, CRP and BMI were however excluded from the fully adjusted multivariable model because of too much missing data. The Nagelkerke R square was used to calculate the proportion of explained variation in the final adjusted backward model. Furthermore, missing data on the ELF test (n=70; 8,5%) were due to technical problems or insufficient stored samples, and hence considered to have occurred at random. These cases were therefore excluded in further analyses.

The study was approved by the Medical Ethics Committee of the Erasmus Medical Center in Rotterdam. Written informed consent was obtained from all participants.

RESULTS

In total, 531 subjects with ELF scores and P3NP values were included for further analyses. Of these 119 had PSO, 151 PsA, 130 RA and 131 were control subjects. On average the RA population was the eldest (mean age 62.0+/-11.7) and the PSO population the youngest (mean age 49.8+/-14.3; table 1). Furthermore, the populations differed significantly in the proportion of females, which was lowest in the PSO (37.8%) and highest in the RA group (64.6%; P=<0.001).

Table 1. General characteristics of the study population

	psoriasis (n=119)	Psoriatic arthritis (n=151)	Rheumatoid arthritis (n=130)	Controls (n=131)	Total (n=531)	*P*-value*
Covariables						
Age (years)	49.8 ± 14.3	52.8 ± 11.7	62.0 ± 11.7	54.4 ± 14.4	54.3 ± 13.7	<0.001
Female, n (%)	45 (38%)	70 (46%)	85 (65%)	76 (58%)	276 (52%)	<0.001
BMI	27.2 ± 5.8	26.5 ± 4.2	25.9 ± 4.5	27.5 ± 6.1	26.8 ± 5.3	0.054
Alcohol intake (drinks/day)						0.07
None (%)	43,2	30.9	39.2	36,3	37,1	
≤ 3 (%)	47.7	66.2	58.4	60,5	58,7	
> 3 (%)	9.0	2,9	2.4	3.2	0.8	
Smoking						<0,001
Never (%)	27.7	40,5	32.3	52,3	37,4	
Former (%)	29.4	42,5	52.6	32,3	38,6	
Current (%)	42,9	17,0	15.0	15,4	23,9	
Personal medication use, n(%)						
Diabetes drugs*	16 (12.1%)	8 (7.3%)	11 (9.8%)	4 (5.7%)	39 (9.2%)	0.41
Antihypertensives	40 (22.2%)	38 (24.7%)	50 (36.8%)	38 (29%)	166 (27.6%)	0.08
Lipid lowering agents	80 (44.7%)	38 (24.7%)	25 (18.4%)	16 (12.4%)	159 (26.6%)	<0.001
Disease activity, n(%)				-		<0.001
mild	80 (67.2%)	124 (82.1%)	84 (65.6%)	-	288 (72.0%)	
moderate	25 (20.6%)	25 (16.6%)	42 (32.8%)	-	92 (23.0%)	
severe	15(12.2%)	2 (1.3%)	2 (1.6%)	-	19 (4.8%)	
PASI	5.9 ± 5.8	1.5±2.4	-	-	-	<0.001
DAS28	-	2.16±0.91	2.66±1.00	-	-	<0.001
Current medication use, n (%)[§]						<0.001
None/ cutaneous	35 (29.4%)	13 (8.6%)	4 (3.1%)	-	52 (9.8%)	
Other systemic medication	42 (35.3%)	49 (32.5%)	34 (26.2%)	-	125 (23.5%)	
Methotrexate	18 (15.1%)	69 (45.7%)	77 (59.2%)	-	164 (30.9%)	
Biologicals	24 (20.2%)	20 (13.2%)	15 (11.5%)	-	59 (11.1%)	
Laboratory data (non fasting)						
AST (U/L)*	29.9±10.6	28.3±9.7	27.5±16.3	-	28.6±10.8	0.61
ALT (U/L)*	34.6±25.1	31.2±22.2	26.2±16.7	-	30.1±21.4	0.017
GGT (U/L)*	40.4±34.6	34.5±33.3	33.2±22.8	-	39.5±61.2	<0.0001
ALP (U/L)*	75.0± 15.9	76.6±19.7	82.3±31.9	-	79.0±25.9	<0.0001
CRP (U/L)*	2.7 ±2.9	5.7 ±10.5	9.9 ±19.0	-	6.8 ± 13.9	0.004

Data are represented as mean (± standard deviation) or percentages.

Abbreviations: PASI, Psoriasis Severity Index Score; DAS28, Disease Activity Score 28; BMI, Body Mass Index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transferase; ALP, alkaline phosphatase; CRP, C reactive protein;

Normal values: AST 0-34 (U/L), ALT 0-44 (U/L), GGT 0-54 (U/L), ALP 0-114(U/L)

[§] Disease specific medication: subdivided into four subgroups; (1) without medication or only cutaneous medication, UV and/or non-steroidal anti-inflammatory drugs; (2) systemic drugs excluding methotrexate (MTX), (3) MTX use irrespective of other medication except biologic therapy.; (4) biologic therapy irrespective of medication from group one to three.

* missing data if >7%: diabetic total = 29.6%; AST control= 93.1%; AST Pso=34.5%; AST RA=78.5%; ALT control = 93.1%; ALT PSO=29.4%; ALT RA 13.8%; GGT control=93.1%; GGT PSO=29.4%; GGT RA=80%; ALP control=93.1%; ALP Pso=59.7%; ALP RA=13.8%; CRP control=96.2%; CRP PSO=84.0%

Disease characteristics and medication

Psoriasis patients had the longest mean disease duration (20.1±14.5 years) compared to PsA and RA (9.9±9.3 and 10.7±8.4 years respectively, p<0.001) with a mean PASI of 5.9 ± 5.8 in PSO compared to 1.5±2.4 in PsA patients. (Table 1) The mean DAS28 score for PsA patients was 2.16±0.91 and 2.66±1.00 for RA.

Table 2. Demographic and clinical details of patients with and without elevated ELF and/or P3NP test

Variables	ELF Score		p-value	P3NP		p-value
	<9.8 %(n)	≥9.8 %(n)		≤ 12.2 %(n)	>12.2 %(n)	
Age mean age						
Sex	75.9% (195)	24.1% (62)	0.25	91.4% (234)	8.6% (22)	0.72
men						
female	80.3% (220)	19.7% (54)		90.5% (248)	9.5% (26)	
Body Mass Index						0.25
Healthy	81% (170)	19% (40)	0.58	92.9% (195)	7.1% (15)	
Overweight	77.9% (162)	22.1% (46)		91.3% (189)	8.7% (18)	
Obese	76.2% (77)	23.8% (24)		87.1% (88)	12.9% (13)	
Disease etiology			<0.001			0.08
PSO	79% (94)	21.0% (25)		94.0% (109)	6.0% (7)	
PsA	86.8% (131)	13.2% (20)		94.0% (142)	6.0% (9)	
Ra	63.8% (83)	36.2% (47)		86.4% (114)	13.6% (18)	
Controls	81.7% (107)	;8.3% 24 ()		89.3% (117)	10.7% (14)	
Disease severity			0.02			0.01
mild	81.0% (234)	19.0% (55)		94.4% (270)	5.6% (16)	
Moderate	67.1% (55)	32.9% (27)		84.5% (71)	15.5% (13)	
severe	66.7% (14)	33.3% (7)		81.0% (17)	19.0% (4)	
Diabetes	54.8% (17)	45.2% (14)	0.007	83.9% (26)	16.1% (5)	0.28
Dyslipidemia	68.1% (81)	31.9% (38)	0.002	86.4% (102)	13.6% (16)	0.056
Hypertension	64% (96)	36% (54)	<0.001	84.9% (129)	15.1% (23)	0.007
Smoking			0.11			0.25
Never	80.8% (160)	19.2% (38)		88.4% (176)	11.6% (23)	
Former	73.9% (153)	26.1% (54)		92.3% (192)	7.7% (16)	
Current	82.6% (100)	17.4% (21)		93.2% (110)	6.8% (8)	
Excess alcohol intake	85.7% (18)	14.3% (3)	0.39	95.2% (20)	4.8% (1)	0.51
Medication			0.74			0.74
cutaneous	76.9% (40)	23.1% (12)		90.2% (46)	9.8% (5)	
systemic	77.6% (97)	22.4% (28)		93.5% (115)	6.5% (8)	
MTX	78% (128)	22.0% (36)		91.5% (151)	8.5% 14 ()	
biological	72.9% (43)	27.1% (16)		88.3% (53)	11.7% (7)	
Hepatotoxic medication*	74.3% (153)	25.7% (53)	0.09	91.3% (190)	8.7% (18)	0.88

Abbreviations: PSO, psoriasis; RA, rheumatoid arthritis; PSA, psoriatic arthritis; P3NP, procollagen-3 N-terminal peptide; ELF, Enhanced liver fibrosis test; MTX, methotrexate

* hepatotoxic medication is defined as: amiodarone , corticosteroids, MTX and tamoxifen .

At the moment of inclusion 3.3% of the PSO, 5.2% of PsA and 3% of RA patients used no disease specific medication. Topical medication was used 76.1% in PSO and 50% in PsA patients, and 5% (all PSO) had UVB phototherapy. NSAIDs were used by 39% of the PsA and 55% of the RA patients.

MTX was the most frequently used systemic drug in PsA and RA patients (52.3%vs66.9%), followed by hydroxychloroquine. In PSO patients, fumaric acid was most frequently used (31%), while 16.8% used MTX.

In total 222 (42%) subjects used potentially hepatotoxic medications, of whom 186 used MTX, 36 systemic corticosteroids and one patient received isoniazid. Furthermore, there were no know other causes of chronic liver disease (e.g. autoimmune liver diseases, alpha-1 antitrypsin deficiency, cholestatic liver diseases or Wilson's disease).

Lifestyle and comorbidities

In the group of patients with an increased ELF (>9.8) score, diabetes (6.2%vs15.4%, p=0.007), dyslipidemia (19.6%vs33.0%, p=0.002)and hypertension (23.1%vs46.6%, p<0.001) were more prevalent.

In contrast, BMI, smoking and excessive alcohol intake was not more prevalent in this group. (table 2)

ELF test vs P3NP: distribution and categorization

In the total population the ELF score ranged between 6.53 and 13.05 with an overall mean score of 9.2±0.92 and median of 9.06 interquartile range (IQR) (7.86- 10.26). This range was much smaller compared to P3NP, which varied between 0.53 and 63.88 with an overall mean value of 8.37±4.30 and a median of 8.50 IQR (5.62-11.38). For the disease groups separately a comparable narrow spread of the ELF test was seen compared to the P3NP outcomes as shown in Figure 1 and Table 3. The controls showed a similar distribution compared to those with inflammatory diseases.

Table 3. Median values of P3NP and ELF stratified by disease

	Psoriasis	Psoriatic arthritis	Rheumatoid arthritis	Controls	**Total**	P-value
P3NP	7.56 (2.92)	7.23 (2.8)	7.87 (4.08)	7.49 (2.54)	7.50 (2.88)	0.26
ELF	8.96 (1.20)	8.93 (0.98)	9.48 (1.17)	9.05 (1.33)	9.06 (1.2)	<0.001

Data are presented as median with IQR

Abbreviations: P3NP, procollagen-3 N-terminal peptide; ELF, Enhanced liver fibrosis test

Elf >11(chronic liver disease), ELF >9.8(healthy blood donors), P3NP> 12.2 (biopsy indication for MTX users with psoriasis), P3NP >15.3 (indication on psoriasis patients to withdrawal of MTX)

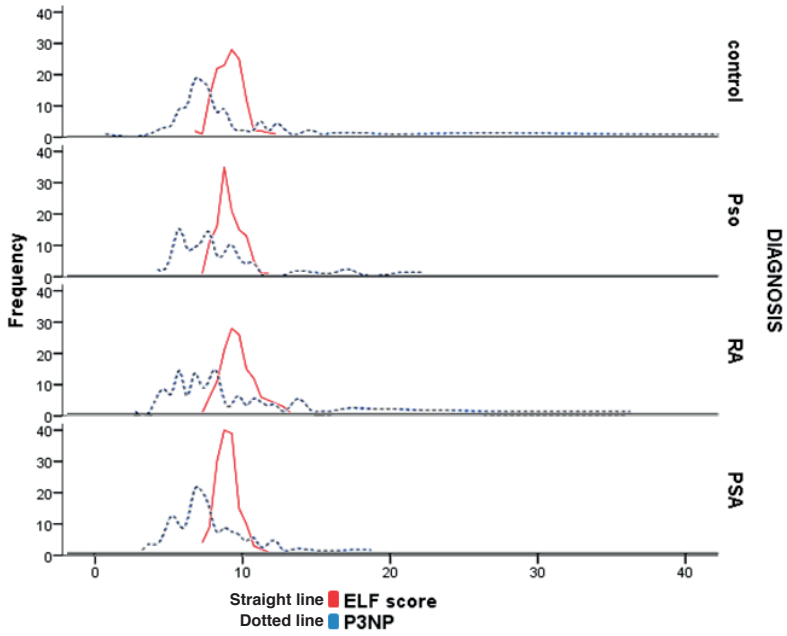

Figure 1. Distribution of the values of P3NP and ELF stratified on diagnoses
Abbreviations: P3NP, procollagen-3 N-terminal peptide; ELF, Enhanced liver fibrosis test; PSO, psoriasis; RA, rheumatoid arthritis; PSA, psoriatic arthritis
Distribution of the values of P3NP and ELF stratified on diagnoses.
Cut-off values: P3NP (>12.2) biopsy indication for MTX users with PSO, P3NP (>15.3) indication on PSO to withdrawal of MTX; ELF (>9,8) healthy blood donors, ELF (>11) chronic liver disease

Sixteen of the 531 (3.0%) subjects had an abnormal ELF test, based on the higher cut off value for chronic liver diseases (ELF≥11), compared to 21.8% (n=116) based on the cut off value for healthy blood donors (ELF>9.8). In total 9.1% of the study population had a P3NP value of >12.2 (i.e. indication for liver biopsy), and 4% had a P3NP value that would require withdrawal of MTX(>15.3; see table 4) None of the PSO patients, however had a liver biopsy.

Subgroup analyses

The highest proportion of increased ELF scores and P3NP values were seen in RA patients (7.7% and 6.1% respectively, using the high cut off values), followed by PSO (1.7% and 5.2%) and finally PsA (0.7% and 1.3%; table 4). After stratifying for disease activity scores, as shown in Figure 2, higher proportions of elevated ELF scores and P3NP values were seen for those with more active disease.

A quarter of the patients who used hepatotoxic medication had an elevated ELF score (>9.8).

Table 4. Different cut-off values of ELF and P3NP test

Values % (n)	Reference group	**PSO**(119)	**PSA**(151)	**RA** (130)	**CO** (131)	**Total (531)**
P3NP >12.2	biopsy indication for MTX users with PSO	6.0% (7)	6.0% (9)	13.6% (18)	10.7% (14)	9.1% (48)
P3NP >15.3	indication on PSO to withdrawal of MTX	5.2% (6)	1.3% (2)	6.1% (8)	3.8% (5)	4.0% (21)
ELF >9,8	healthy blood donors	21.0% (25)	13.2% (20)	36.2% (47)	18.3% (24)	21.8% (116)
ELF >11	chronic liver disease	1.7% (2)	0.7% (1)	7.7% (10)	2.3% (3)	3.0 (16)

Elf >11(chronic liver disease), ELF >9.8(healthy blood donors), P3NP> 12.2 (biopsy indication for MTX users with psoriasis), P3NP >15.3 (indication on psoriasis patients to withdrawal of MTX)
Abbreviations: PSO, psoriasis; RA, rheumatoid arthritis; PSA, psoriatic arthritis; P3NP, procollagen-3 N-terminal peptide; ELF, Enhanced liver fibrosis test

In the group of patient with an increased ELF (>9.8) score or P3NP (>12.2) values, there was no significant difference between the different medication subgroups. For ELF, these proportions were 10.3% for those using none or topical treatments, 24.1% among those on systemic not MTX, 31.0% for MTX and 13.8% for those using biologicals.

Figure 3 showed data based on stratification on current, past and never MTX use. In PSO patients, those who used MTX seemed to have increased ELF scores and P3NP values compared to ever and never MTX users. For PsA and RA, this is less clear.

Table 5. multivariate logistic regression unadjusted and age/gender adjusted model

		ELF >9.8	***P3NP >12.2***
		Crude / Adjusted OR (95% CI)	**Crude / Adjusted OR (95% CI)**
Crude univariate model			
Disease	control	1.0 (ref)	1.0 (ref)
	PSO	1.186 (0.635-2.215)	0.537 (0.209-1.380)
	PSA	0.681 (0.357-1.299)	0.530 (0.221-1.267)
	RA	**2.525 (1.429-4.461)**	1.320 (0.627-2.778)
Age and gender adjusted			
Age, years		**1.081 (1.058-1.104)**	**1.027 (1.004-1.051)**
Gender	women	1.0 (ref)	1.0 (ref)
	men	**1.635 (1.028-2.601)**	1.042 (0.566-1.922)
Disease	control	1.0 (ref)	1.0 (ref)
	PSO	1.505 (0.755-3.00)	0.578 (0.223-1.493)
	PSA	0.798 (0.400-1.594)	0.553 (0.230-1.327)
	RA	**1.877 (1.011-3.485)**	1.145 (0.534-2.453)

Cut-off values: P3NP (>12.2) biopsy indication for MTX users with PSO, ELF (>9,8) healthy blood donors.
Abbreviations: PSO, psoriasis; PSA, psoriatic arthritis; RA, rheumatoid arthritis; P3NP, procollagen-3 N-terminal peptide; ELF, Enhanced liver fibrosis test

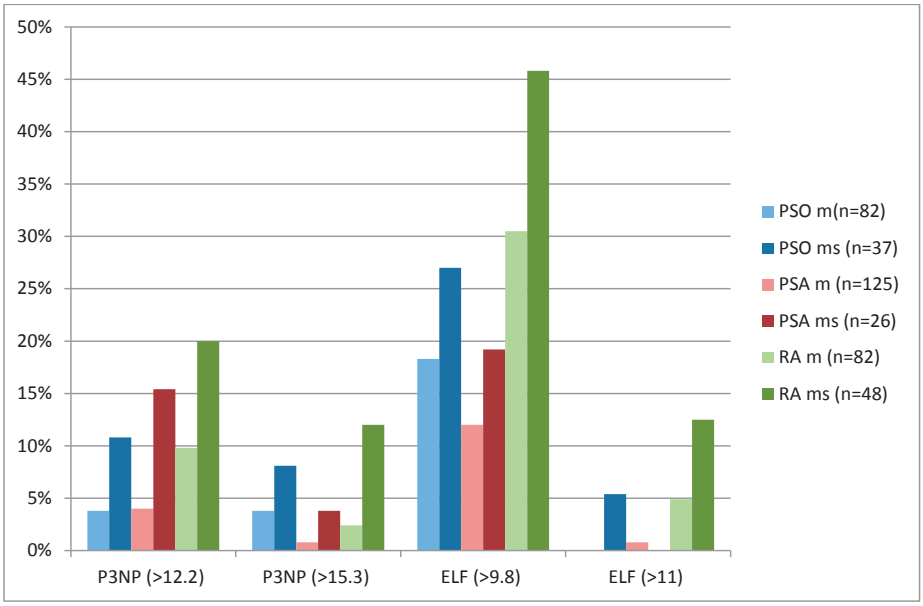

Figure 2. Proportion of patients with elevated P3NP and ELF values based on disease activity
Abbreviations: PSO, psoriasis; RA, rheumatoid arthritis; PSA, psoriatic arthritis; m= mild (PASI < 7; DAS28 <3.2) and ms = moderate / severe disease ((PASI ≤7; DAS28 ≤3.2)). P3NP, procollagen-3 N-terminal peptide; ELF, Enhanced liver fibrosis test
Vertical border are % of patients with a positive value.
Cut-off values: P3NP (>12.2) biopsy indication for MTX users with PSO, P3NP (>15.3) indication on PSO to withdrawal of MTX; ELF (>9,8) healthy blood donors, ELF (>11) chronic liver disease

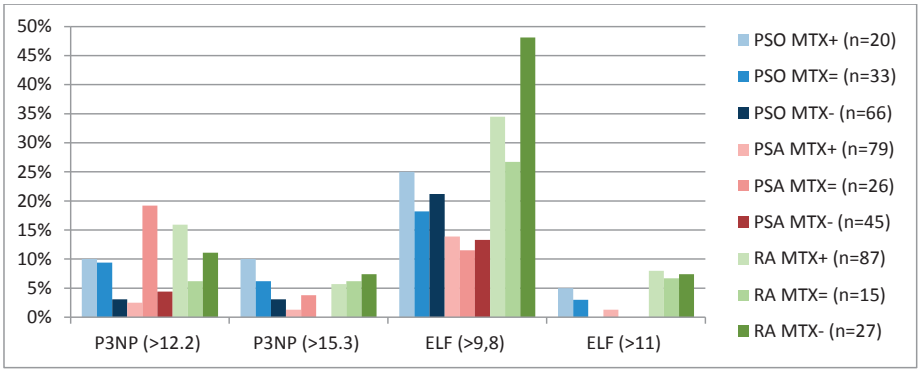

Figure 3. Proportion of patients with elevated P3NP and ELF values; based on never, ever and current methotrexate use.
Abbreviations: PSO, psoriasis; RA, rheumatoid arthritis; PSA, psoriatic arthritis; MTX+ current MTX use; MTX= ever MTX use, but not current; MTX- never MTX use; P3NP, procollagen-3 N-terminal peptide; ELF, Enhanced liver fibrosis test
Cut-off values: P3NP (>12.2) biopsy indication for MTX users with PSO, P3NP (>15.3) indication on PSO to withdrawal of MTX; ELF (>9,8) healthy blood donors, ELF (>11) chronic liver disease

Table 6. multivariate analyses

		ELF	*P3NP*
		Adjusted OR (95% CI)	**Adjusted OR (95% CI)**
Age, years		**1.095 (1.071-1.120)**	**1.026 (1.003-1.049)**
Sex	women	1.0 (ref)	
	men	**2.081 (1.252-3.459)**	
Alcoholic use	No	1.0 (ref)	1.0 (ref)
	Yes	**0.435 (0.260-0.726)**	**0.448 (0.234-0.859)**
Liver toxic medication	No	1.0 (ref)	
	Yes	**2.816 (1.142-6.944)**	
Disease activity	mild	1.0 (ref)	1.0 (ref)
	moderate	1.826 (0.971-3.434)	**2.779 (1.249-6.181)**
	severe	**5.850 (1.740-19.673)**	**5.672 (1.616-19.902)**
Medication	cutaneous	1.0 (ref)	1.0 (ref)
	systemic	1.384 (0.837-3.568)	
	MTX	0.466 (0.134-1.618)	
	biological	1.786 (0.602-5.300)	
Nagelkerke R square final model		0.307	0.102

Abbreviations: P3NP, procollagen-3 N-terminal peptide; ELF, Enhanced liver fibrosis test
Multivariate logistic regression model with backward method. The following variables were excluded in the analyses in the following order; ELF: smoking, disease; P3NP: smoking, sex, medication, liver toxic medication, disease. BMI is not in the multivariable model because there has been no relation described with the ELF test. ALT and CRP are not included in the multivariate model because of too much missing data.
linear regression: Elf dependent and P3NP independent: B=0.139, p<0.0001, adjusted R square 0.423

Predictors of elevated ELF test

In the age and gender adjusted multivariable logistic regression model, a higher age, male gender and RA were significant predictors for an increased risk of an elevated ELF score (i.e. >9.8) compared to only a higher age for P3NP (table 5). In the fully adjusted logistic regression model, disease activity and age were important confounders, but for the ELF score male gender and hepatotoxic medications were additional significant confounders (table 6). Remarkably, alcohol intake was protective for a high test score. An explanation for this may be that the selection bias for prescribing MTX. There were no subject on MTX who had excessive alcoholic use. Using Nagelkerke R square on the final adjusted backward model, 30% for the ELF test compared to 10% for the P3NP test, was explained by the included risk factors. (table 6).

DISCUSSION

This cross sectional study explores the levels of ELF scores and P3NP values in three different inflammatory diseases. The highest proportion of elevated ELF scores and P3NP values were seen in RA, followed by PSO and PsA. However, in all diseases the overall range of the ELF score was smaller than for P3NP.

European guidelines currently advise sequential measuring of serial P3NP for detecting liver fibrosis in patients using MTX.[11] However it is important to note that serum P3NP has several limitations; it is not specific for fibrosis in the liver, can only be interpreted serially and is not be properly validated.[18] These limitations advocate the search for a more reliable, non-serial test to screen for liver fibrosis especially when hepatotoxic medication is prescribed.

The ELF test has been shown to be a well validated, non-serial, non-invasive liver fibrosis test in healthy controls as well in a multiple chronic liver disease like alcoholic liver disease, NAFLD and viral hepatitis, with a sensitivity of 83% (95% CI=0.80–0.86) and a specificity of 73% (95% CI=0.69–0.77).[17,25] A recent pilot study in patients with psoriasis already suggested that single ELF score may be superiour to single P3NP value. [18] The ELF test has not yet been properly validated for inflammatory diseases and no cut off value has been described so far. In this article we have therefore selected the validated cut off values for chronic liver disease and healthy blood donors, as we expect the disease specific threshold for these inflammatory diseases will be somewhere in between these values. As P3NP is part of the ELF test, a certain effect of inflammation on the outcome of the ELF score can be expected, although this effect is less than for a single P3NP test.[7,24,26,27]

In the multivariate model, as expected, higher age, male gender, hepatotoxic medication and active disease were associated with the ELF score.[25] For P3NP comparable trend was visible, however moderate disease was also associated. Remarkably, alcohol intake was protective for a high test score. An explanation for this may be the selection bias for not prescribing MTX. In case of high alcoholic intake less hepatotoxic therapies will be prescribed.

Contradictory to the available literature, RA patients had the highest values of P3NP and ELF score on both cut off values in our study in de unadjusted model, which may suggests a higher prevalence of liver fibrosis. Alternatively, it could reflect arthritis activity, [27] instead of liver fibrosis, but this effect was not seen in the PsA subgroup.[26] On the contrary, selection bias for the inclusion criterion to conduct a liver biopsy (i.e. long term MTX use), may have led to underestimating the true prevalence of liver fibrosis in RA patients in the literature.[28,29]

For now, the position of the ELF test in clinical practice could be in the work up for systematic antipsoriatic drugs of all patients because it could direct in selecting potential hepatotoxic medication or not. This implementation in practice is especially valid for countries where P3NP monitoring is recommended in the treatment guidelines because of ELF's advantages. The ELF test could also be used to monitor patients using hepatotoxic drugs annually, but optimal frequency needs to be investigated. In the current absence of validated ELF cut-off points for inflammatory diseases yet, we suggest to use the cut off point for healthy people. Although the use of the healthy cut off values would lead to false positive cases, a negative test is sufficiently reliable to exclude those with liver conditions. Altogether if the ELF value is above the 9.8 additional investigations such as transient elastography or referral to a hepatologist is warranted.

Strengths & Limitations

This real life cross-sectional study provides a useful comparison of the test outcomes for liver fibrosis in various inflammatory diseases, which makes extrapolation of the results to the clinical practice more possible. However due to the heterogeneity of the data, it harder to find significant associations. Furthermore, we have tried to investigate the association between the potentially important confounders and abnormal liver tests using multivariable analyses and stratification of the data. However, the cross-sectional study design does not allow to draw conclusions about temporal relationships. Secondly, the data on liver disease were extracted from the general medical history, without specific question on liver disease. However, by asking about the general medical history we assume that we did not miss major liver diseases and additionally we do know that there we no liver biopsies taken in our population. Despite this, it could have been possible than patients with mild or subclinical liver diseases may have been a priori unknown leading to none differential reporting bias or a minimal increased proportion of positive test results.

Another limitation of this study is the lack of validation of the ELF test by a golden standard. Although a liver biopsy is the golden standard for liver fibrosis, it is unethical to perform this on a large groups of patients including healthy controls. Neither the P3NP test can serve as a gold standard, both due to its own practical limitation, but also because P3NP is part of the ELF test which would result in circulation bias. Finally, the cut-off points for the ELF test have not been validated for PSO, PsA or RA , and were extrapolated from the hepatology literature. Given the considerable influence of disease prevalence on the predictive values of diagnostic tests, the results from liver disease hospital-based studies cannot be transferred to our own, 'low prevalence' population without resulting in an unacceptably number of false positive and negative results. This issue also probably holds true for healthy blood donors, which a priori have a lower prevalence of liver fibrosis than those patients with an inflammatory disease.

Conclusion & Future prospective

This study suggests that the ELF test may be a promising noninvasive screening and monitoring tool for liver fibrosis by dermatologists and rheumatologists, but further research is needed to validate the ELF-test, through dermatologist, rheumatologists and hepatologists together, by using another noninvasive test e.g. ultrasound transient elastography (FibroScan®) and determine the appropriate cut-off values in PSO, PsA and RA patients.

Secondly, increased ELF scores are found by PSO, PsA and especially by RA patients and were associated with increased age, male, use of liver toxic medication and severe disease. A challenge in interpreting these results clinically is the lack of validated cut-off points to diagnose hepatic fibrosis in population-based cohorts. Despite this, our results suggest that liver fibrosis may more frequent in patients with inflammatory arthritis than would be expected based on the available literature.

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