Enhanced liver fibrosis test (ELF) in rheumatic arthritis and psoriatic arthritis patients.

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ABSTRACT

Background: Recently the enhanced liver fibrosis (ELF) test was introduced as noninvasive biomarker for liver fibrosis in patients with liver diseases and healthy controls, but it has not been tested in inflammatory joint diseases. Liver fibrosis is prevalent in arthritis, especially in methotrexate users.

Objective: To evaluate the applicability of ELF test among an outpatient group with psoriatic arthritis (PsA) and rheumatoid arthritis (RA), and to test whether ELF is related to disease activity.

Methods: In this daily practice cross sectional study ELF test was performed in 281 patients. Furthermore, demographic and disease-specific data were collected.

Results: Increased ELF (>9.8 and >11) was found in 36.2% and 7.7% of RA patients and in 13.2% and 0.7% of PsA patients. In the multivariate linear model for PsA and RA patients, ELF was minimal associated with disease activity.

Conclusions: ELF may be a promising non-invasive screening tool for PsA and RA to monitor liver fibrosis. ELF is minimal related to disease activity. However further research is needed to find clinically meaningful cut—off values for inflammatory diseases and to explore the increased risk of liver fibrosis.

INTRODUCTION

The incidence of liver fibrosis in patients with rheumatic arthritis (RA) has been described to range between 15.3% for mild fibrosis and 1.3% for severe fibrosis. In RA, 0,5% of patients has liver cirrhosis.[1] In psoriatic arthritis (PsA) this ranges between 9.9% and 1.4% resp., while 1.4% actually has cirrhosis.[1] In these two diseases the use of hepatotoxic drugs, especially methotrexate (MTX), as well as presence of comorbidities and/or systemic inflammation may result in decreased liver function. [2]

Liver fibrosis is often asymptomatic for years until cirrhosis develops and therefore it is difficult to detect with standard noninvasive techniques. It can develop despite normal liver function tests and normal images from ultrasound and radionuclide scans.[3] Although liver biopsy is the golden standard to detect liver fibrosis and cirrhosis, the potential risk of complications restricts its use to those patients with a strong indication. Hence, there clearly is a need for an accurate, valid and pragmatic non-invasive diagnostic test to detect liver fibrosis early.[4]

The Enhanced Liver Fibrosis (ELF) test is a relatively new noninvasive test that combines an automated in-vitro immunoassay for the quantitative measurement of three serological markers being procollagen-3 N-terminal peptide (P3NP), tissue inhibitor of matrix metalloproteinase 1 (TIMP1) and hyaluronic acid (HA). The results are then combined in an algorithm to produce an ELF score.[5,6] The ELF score has been validated as a biomarker of fibrosis in healthy subjects and in patients with a wide range of chronic liver diseases.[7-10] This has resulted in validated cut off values for liver fibrosis and cirrhosis. Furthermore, it has been shown that the ELF test can predict the clinical outcome in chronic liver disease.[6]

The objective of this cross-sectional daily practice study is to evaluate the applicability of the ELF test in PsA and RA patients and whether abnormal tests outcomes are related to different markers of disease activity and increased inflammatory stage of PsA and RA. Secondly we aim to evaluate the effect of MTX use on the ELF test. This is important, because ELF can be a potentially valuable tool in the future to monitor liver fibrosis in inflammatory joint 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 have been described previously.[11] The PsA and RA patients were recruited from the rheumatology department of the Maxima Medical Center 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.[12]

Co-variables and disease characteristics,

The following data were collected in a standardized manner directly after inclusion: demographic data, disease specific information, general medical history, medication use and lifestyle.

The disease activity and course severity in psoriatic and rheumatic arthritis patients were assessed with Disease Activity Score 28 (DAS28). The DAS28 score was categorized into mild<3.2, moderate 3.2-5.1 and severe >5.1.[13]

Disease specific medication was divided into four subgroups; (1) no medication or non-steroidal anti-inflammatory drugs (NSAIDs) only; (2) disease related systemic drugs excluding MTX; (3) MTX irrespective of any other medication except biologicals; and (4) biologicals irrespective of medication from group one to three. Data on dosing regimens were not available.

Laboratory analysis

Serum was collected at the time of clinical assessment 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. The analyses were all performed on the same day to avoid measurement bias. 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 PsA and RA.[14,15]

Serum alanine aminotransferase (ALT), aspartate transaminase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), C reactive protein (CRP), blood sedimentation rate (BSE) and anti-cyclic citrullinated peptides (CCP) were measured using standard enzymatic immunoassays.

Statistics

Statistical analysis was performed using SPSS software version 20. Variables were described using standard descriptive statistics. 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. *P*-values were two-sided and values <0.05 were considered statistically significant.

In order to identify the clinical variables associated with ELF scores , stratification analysis for DAS28 score, anti-CCP, CRP levels, BSE and MTX use were conducted.

A linear univariate model was conducted for ELF on CRP, anti-CCP, BSE and DAS28. In the linear multivariate regression model on the ELF, Age, Sex, MTX, BMI, smoking,

Table 1. General characteristics of the patients

	Psoriatic arthritis	**Rheumatoid arthritis**
	(n=151)	**(n=130)**
Covariables		
Age (years)	52,8 ± 11,7	62,0 ± 11,7
Female (%)	45.7	64,6
Alcohol intake (drinks/day)		
None (%)	30.9	39.2
≤ 3 (%)	66.2	58.4
> 3 (%)	2,9	2.4
BMI	26.5 ± 4.2	25.9 ± 4.5
Smoking		
Never (%)	40,5	32.3
Former (%)	42,5	52.6
Current (%)	17,0	15.0
Disease		
Duration of disease, years	9.9± 9.3	10.7 ± 8.4
Activity of disease DAS28	2.16 ± 0.91	2.66 ± 1.00
Current medication use, n (%)		
None	8 (5.2)	4 (2.9)
Prostagladinesynthetase inhibors	60 (39)	75 (55.1)
MTX	81 (52.6)	91 (66.9)
prednison	15 (9.7)	21 (15.4)
Other systemic medication	50 (32.5)	35 (25.7)
Biologicals	21 (13.6)	17 (12.5)
Laboratory data (non fasting)		
AST (U/L)	28.3±9.7	27.5±16.3
ALT (U/L)	31.2±22.2	26.2±16.7
GGT (U/L)	34.5±33.3	33.2±22.8
ALP (U/L)	76.6±19.7	82.3±31.9
CRP	5.7 ±10.5	9.9 ±19.0

Abbreviations: 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.

alcoholic use, CRP, anti-CCP, BSE and DAS28 were included. In the logistic multivariate analyses enter model for RA the following variables were included: Age, Sex, DAS28, CRP, BSE, and anti-CCP.

The study was approved by the Medical Ethics Committee of the Erasmus Medical Center in Rotterdam (MEC- 2007-181). Written informed consent was obtained from all participants.

RESULTS

In total, 281 patients with eligible ELF values were included for further analyses, of whom 151 PsA patients (mean age 52.8±11.7; 45.7% female) and 130 RA patients (mean age 62.0±11.7; 64.6% female). For details regarding demographics and baseline data, see Table 1.

Disease characteristics and medication

The mean DAS28 score was 2.16±SD0.91 for PsA patients and 2.66±1.00 for RA. At the moment of inclusion 5.2% of PsA and 3% of RA patients used no disease specific medication. NSAIDs were used by 60 (39%) of the PsA and 75 (55%) of the RA patients. MTX was the most frequently used systemic drug in PsA and RA patients (81 (52.3%) vs 91(66.9%)), followed by hydroxychloroquine. Biologicals were use by 17(12,5%) of the RA patients and 21(13.6%) of the PsA patients. There were no known other causes of chronic liver disease. There were no subjects on MTX who had excessive alcoholic use.

ELF test: distribution and categorization

The mean ELF score was 8.96±0.76 within PsA and 9.55±1.04 in RA patients. In RA 10 (7.7%) and PsA 1 (0.7%) of the subjects had an abnormal ELF test, based on the higher cutoff level for chronic liver diseases (ELF≥11), compared to 47 (36.2%) vs 20 (13.2%) based on the cutoff value for healthy blood donors (ELF>9.8). Table 2

Table 2. Different cut-off values of ELF and P3NP

% (n)		**PSA** (151)	**RA** (130)	TOTAL
ELF (>9,8)	healthy blood donors	13.2% (20)	36.2% (47)	23.8% (67)
ELF (>11)	chronic liver disease	0.7% (1)	7.7% (10)	3.9% (11)

Elf >11(chronic liver disease), ELF >9.8(healthy blood donors)
Abbreviations: PSA: psoriatic arthritis; RA rheumatic arthritis.

After comparing the outcomes of subanalyses in PsA, DAS28 scores, CRP, anti-CCP, BSE and MTX use, showed no significant differences in the proportion with an ELF cut-off value of 9.8 and ≥11. By RA patients using the 9.8 cut-off value, significant differences were only seen for DAS and CRP (p=0.04 and 0.01) and on the cut-off value of 11 for BSE (Figure 1a-e). There was no significant difference between the different medication subgroups.

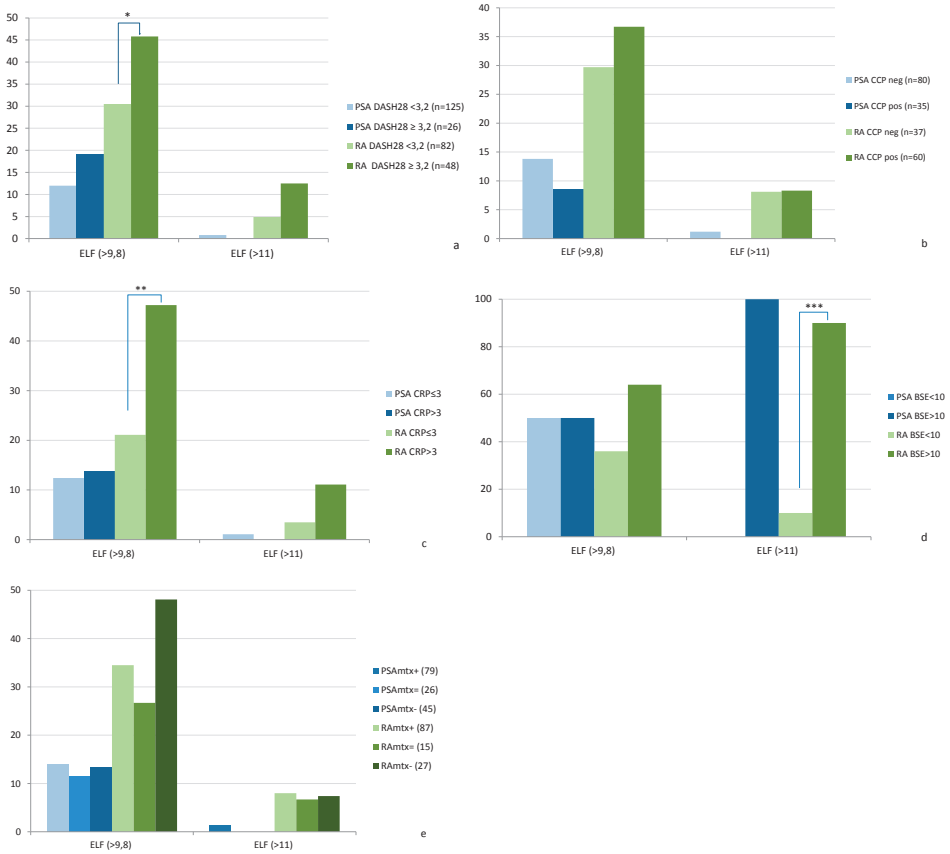

Figure 1 a to e. Proportion of patients with elevated ELF values stratified on disease activity, disease severity, systemic inflammation and MTX use.

a: disease activity based on DAS score; b: disease severity based on anti-CCP levels; c: systemic inflammation based on CRP level; d: systemic inflammation based on BSE level; e: current, former and never MTX use.

Abbreviations: PSA: psoriatic arthritis; RA rheumatic arthritis; MTX+ current methotrexate use; MTX= ever MTX use, but not current; MTX- never MTX use; ELF, Enhanced liver fibrosis test . * p=0.04; ** p=0.01; ***p=0.02

Vertical border are % of patients with a positive value.

Cut-off values: ELF (>9,8) healthy blood donors, ELF (>11) chronic liver disease

Table 3. Linear regression univariate model on the ELF

ELF	RA	PSA
CRP	0.12 (-0.003-0.16)	0.10 (-0.004-0.19)
Anti-CCP	0.15 (0.00-0.00)*	0.41 (0.002-0.007)
BSE	**0.28 (0.007-0.03)**	**0.18 (0.001-0.026)**
DASH28	**0.38 (0.23-0.60)**	**0.20 (0.03-0.31)**

Data shown represents B-coefficients (95% CI)
*p=0.33 Abbreviations: ELF, Enhanced liver fibrosis test ; PSA: psoriatic arthritis; RA rheumatic arthritis; CCP: anti-cyclic citrullinated peptides; CRP:C reactive protein ;BSE: blood sedimentation rate; DAS: disease activity score

Predictors of elevated ELF test

The univariate linear regression, stratification showed no association between ELF and CRP or anti-CCP. In both RA and PsA a positive relation was found on BSE and DASH28, in which a higher BSE or DASH28 related with a higher ELF. (Table 3) In the multivariate linear regression model, there was no longer a positive association with BSE or DAS28 in RA or PsA patients. In RA patients only a positive association was seen with increased age. In PsA patients, ELF was associated with increased age and present MTX use and a negative association with smoking was found. (Table 4)

Table 4. Linear regression multivariate model examining the effect of ELF on known parameters of RA and PSA.

ELF	RA	PSA
sex	0,19 (-0,15-0,92)	-0.03 (-0.41-0.32)
age	**0.59 (0,03-0.08)**	**0.52 (0.02-0.05)**
MTX	0.22 (-0,04-0,59)	**0.34 (0.04-0.42)**
alcohol	-0.02 (-0,60-0,51)	0.12 (-0.06-0.14)
BMI	0.10 (-0.03-0.09)	-0.02 (-0.05-0.05)
Smoking	-0,01 (-0,40-0,37)	**-0.47 (-0.65—0.18)**
Anti-CCP	0.12 (0,00-0,00)*	0.01 (-0.03-0.03)
CRP	0,23 (-0,01-0,03)	0.35 (-0.001-0.13)
BSE	0.08 (-0,02-0,03)	-0.33 (-0.06-0.01)
DAS28	0.19 (-0,22-0,57)	0.27 (-0.08-0.44)
R square adjusted	0.48	0.34
R square	0.60	0.48

Results are expressed in B-coefficients with (95% CI)
*p=0.34 Abbreviations: ELF, Enhanced liver fibrosis test; PSA: psoriatic arthritis; RA rheumatic arthritis; MTX: methotrexate; BMI: body mass index; CCP: anti-cyclic citrullinated peptides; CRP:C reactive protein ;BSE: blood sedimentation rate; DAS: disease activity score.

In the multivariable adjusted logistic regression model, using the cut of value of 9.8 of the ELF test, in RA patients, age was no longer associated with a positive ELF test (adjusted OR 1.05 (0.92-1.19)), also no relation was found on the different factors of disease activity.

DISCUSSION

This cross sectional study explores the levels of ELF in PsA and RA in relation to inflammatory status and disease activity. RA patients had a higher value of ELF score on both cut off values compared to PsA in our study. Higher ELF in RA/PsA might suggest that a higher prevalence of (preclinical) liver fibrosis, however ELF is not yet validated for rheumatic diseases. Thus, further validation studies are needed.[16,17]

These higher values of ELF in RA and PsA patients can have a couple of possible explanations. First, the fact of measuring inflammation instead of fibrosis. It has been described previously that arthritis activity may affect the value of P3NP (part of the ELF test).[18] However, this was described in active or severe subgroup analyses of RA patients not in the PsA subgroup and this correlation does not yet prove a causal relation.[19] Another study of 100 patients showed no relation between P3NP and disease activity, but the level of P3NP was correlated to early stage joint destruction.[20] Joint erosions were not investigated in our study. In an article were ELF test was tested as an outcome measure in systemic sclerosis, no relation was found with arthritis or specific auto-antibodies, in line with our study results. They did find a relation with male gender, age and BSE.[21] In the articles of Kikuchi and Toubi, no relation was found between rheumatic arthritis and TIMP-1.[22,23] In patients with RA but not PsA, increased levels of circulating HA can be found, which may originate as a spillover from either the synovium, the synovial fluid and/or the cartilage. There is no close relation between HA levels and biomarker of the acute fase response in RA, which may reflect differFent processes of the inflammatory reaction.[24] In this study, systemic inflammation measured by CRP was weakly related to the ELF value (ELF >9.8) for RA, but this was not confirmed in the linear regression models. No associations were found with disease activity or other inflammatory markers. This makes it unlikely that ELF can be seen as an inflammatory marker in arthritis patients. Furthermore ELF test has a correlation with liver inflammation (ALT), this inflammation does not influence the clinical reliability of the test and ELF is seen as a 'good' diagnostic tool in clinical practice for the staging of advanced liver fibrosis and cirrhosis in the hepatology.[10,25]
Another reason of this increased ELF score in RA patients can be though selection bias for the inclusion criterion to conduct a liver biopsy (i.e. long term MTX use). This may

have led to underestimating the true prevalence of liver fibrosis in RA patients in the literature.[26,27] Among the limited number of studies with noninvasive imaging of liver fibrosis (e.g. transient or shear wave elastography) in RA patients, a higher prevalence (3.4% to 8%) of severe liver fibrosis was found compared to liver fibrosis detected by liver biopsy.[26,27] In a systemic review, even a prevalence up to 15% of mild liver fibroses was found in RA patients who use MTX and in up to 9.1% of patients undergoing pre-methotrexate biopsy.[28]

Strengths & Limitations

This cross-sectional study provides a useful comparison of the test outcomes for liver fibrosis in arthritis diseases, which makes extrapolation of the results to the clinical practice more possible. However due to the heterogeneity of the data, it is hard to find significant associations. Furthermore, we have tried to investigate the association between the potentially important confounders using multivariable analyses and stratification of the data. However, the cross-sectional study design does not allow drawing conclusions about temporal relationships and causality.

Advantages of using the ELF test are that it is non-invasive, simple test; it is well validated and readily available for clinicians. However the 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 large groups of patients including healthy controls. Finally, the cut-off points for the ELF test have not been validated for PsA or RA, and were hence 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

ELF test has an increased prevalence in RA and PsA patients. Next step is to evaluate whether ELF test may be a promising noninvasive screening and monitoring tool for liver fibrosis by rheumatologists for PsA and RA patients. But further research is needed to validate the ELF-test by using another noninvasive test e.g. ultrasound transient elastography (FibroScan®) and determine the appropriate cut-off values in PsA and RA patients. Nevertheless (mild) liver fibrosis may more frequent in patients with inflammatory arthritis than expected.

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