

## BACKGROUND

Methotrexate (MTX) is an immunosuppressant drug used in the management of severe psoriasis. Its use is associated with hepatotoxicity, requiring patients to be continuously monitored for the development of liver fibrosis. Currently the gold standard for the assessment of liver fibrosis is biopsy. However, due to its invasive, distressing nature and expense to the health service, this has brought about the emergence of non-invasive methods of assessment.

Siemens Healthcare has developed the Enhanced Liver Fibrosis (ELF) test for the measurement of liver fibrosis. The ELF test combines the measurement of three direct serum biomarkers; amino terminal propeptide of type III procollagen (P3NP), hyaluronic acid (HA) and tissue inhibitor metalloproteinase 1 (TIMP-1). Results are then combined in an algorithm to report a unit-less numeric score, with an increasing score related to fibrosis severity. The ELF assay is a validated biomarker of liver fibrosis in a number of chronic liver diseases, however is yet to be validated in patients with liver fibrosis as a result of MTX therapy.

Currently NHS Greater Glasgow & Clyde (GG&C) dermatology patients are monitored for methotrexate-induced liver damage using the single measurement of P3NP. If a patient has two consecutive results of >13ug/L or three results of >7ug/L within a 12 month period, then ultimately biopsy is recommended to determine the extent of liver fibrosis.

This study aims to evaluate the clinical utility of the ELF test in a cohort of patients with psoriasis treated with MTX, by assessing the influence of age and sex on ELF results.

## MATERIALS AND METHODS

### Sample Collection & Preparation

Serum samples are collected at routine dermatology clinics, measurement of P3NP is performed and then samples are stored at -20°C. From this database, 38 samples fulfilling the following criteria were collected: ≥ 18 years old, on MTX therapy for psoriasis and a P3NP result of >16ng/mL. Excess serum was aliquoted into 2mL tubes, labelled only with age and a unique ELF barcode, and then stored at -20°C until required. Prior to analysis, samples were thawed, thoroughly mixed and centrifuged at 1000g for 10 minutes.

### Assay verification

The P3NP assay was already verified for patient analysis at Royal Alexandra Hospital (RAH) biochemistry laboratory, therefore only verification of HA and TIMP-1 was carried out. Assays were all implemented as per the manufacturer's recommendation, specific to the Siemens Centaur XP analyser. The assay verification protocol was carried out in line with the Clyde Biochemistry standard operating procedure, and the performance of both HA and TIMP-1 assays were deemed acceptable for use for the purpose of this study.

### Measurement

Quantitative measurement of each direct marker of the ELF test were performed on every sample and then combined to produce an ELF score. Results were then separated into four categories of severity (Figure 1) (Siemens Healthcare Diagnostics Ltd).



Figure 1: Severity of ELF Score

## RESULTS

### Patient Evaluation Results

Following analysis, ELF scores were calculated and results found that the majority of patients had severe fibrosis (25/38 patients), while 9 patients had moderate fibrosis and 5 patients livers were cirrhotic (Table 1). The ELF cohort was divided into three age groups: 18–35 years old, 36-59 years old and >60 years old. The ratio of male to female patients varied amongst each age group, 18-35 was 71% female and 29% male, 36-59 was 44% female and 56% male, and the >60 group was 39% female and 61% male.

### Gender

The mean ELF score for female patients (n=18) was 10.6 ± 0.5, which was slightly higher than the mean ELF score for male patients (n=20) of 10.4 ± 1.3. Figure 2 shows females have a larger variation of ELF scores than males (IQR<sub>F</sub> = 4.7, IQR<sub>M</sub> = 2.2), however t-test analysis shows there is no significant difference between both groups (P >0.05).

Male	20
Female	18
Age Range	20 - 86
<b>Age Groups</b>	
18-35	7
36-59	18
>60	13
<b>ELF Score</b>	
<7.7 None to Mild	0
≥7.7 - <9.8 Moderate	9
≥9.8 Severe	25
≥11.3 Cirrhosis	5

Table 1: ELF Cohort Demographics

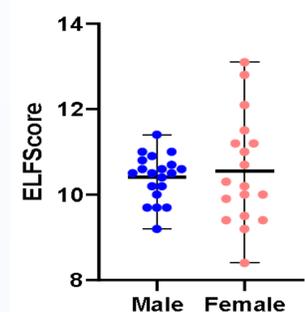


Figure 2: Distribution of ELF Scores by gender

### Age

Linear regression analysis (figure 3) was used to determine the correlation between ELF score and age showed a very weak positive correlation, with a R<sup>2</sup> value of 0.0348. Regression analysis generated a P-value of 0.26, therefore it cannot be said that age has no effect on ELF score, however correlation is very small (r=0.1866). The mean ELF score for each age group was, 9.8 (18-35), 10.6 (36-59) and 10.7 (≥60). A box-plot graph was generated (figure 4) to give a visual representation of the distribution of results of each age group. There was no significant variation between the mean value of each group, ANOVA analysis generated a P-value of 0.12 (>0.05).

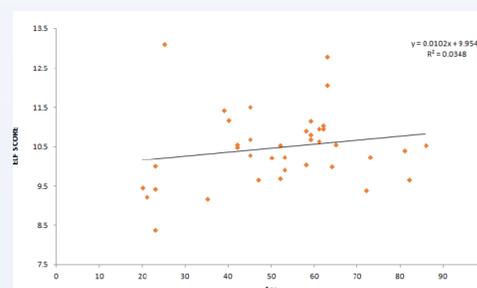


Figure 3: Correlation of ELF score against age

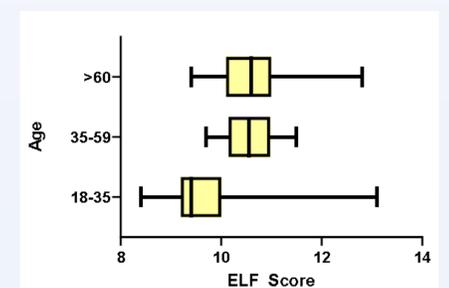


Figure 4: Box & whisker plot of ELF vs Age

## CONCLUSION

This small study was conducted to evaluate the ELF test as a non-invasive marker of methotrexate-induced liver fibrosis, and in particular, assess the influence of age and sex on ELF results. The study found that overall, in patients with P3NP levels of >16 ng/mL, age and sex had no significant impact on ELF scores, supporting the implementation of the ELF test as a screening and monitoring tool for assessing liver fibrosis in this patient group. However, as the patient cohort in this study was very small, and did not consider influence of other factors that may cause liver fibrosis, larger prospective studies are required.

### References

Siemens Healthcare Diagnostics (2021), The Enhanced Liver Fibrosis (ELF) Blood Test, Literature Compendium (Volume 1) [Online] Available: [Siemens Healthineers · brochure S4 portrait · Template \(scrvt.com\)](#) [Accessed 05/02/2022]