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Review Title	A review of the clinical utility of the Enhanced Liver Fibrosis test in multiple aetiologies of Chronic Liver Disease
Running Title	Clinical Utility of the ELF Test
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Abstract (250 words max)

The rising incidence of chronic liver disease (CLD) continues to be an increasing health burden. The morbidity and mortality associated with CLD typically occurs in patients with advanced fibrosis. Hence early identification of those at-risk is of vital importance to ensure appropriate ongoing management. Currently, tools for appropriate risk stratification remain limited. Increasing awareness of the limitations of liver biopsy has driven research into alternative non-invasive methods of fibrosis assessment including serological markers assessing functional changes. One such biomarker, the Enhanced Liver Fibrosis test, was initially validated in a cohort of 1,021 patients with mixed aetiology chronic liver disease and shown to perform well. Since this pathfinder study, it has been independently validated in cohorts of Hepatitis C, non-alcoholic fatty liver disease, alcoholic liver disease, primary biliary cirrhosis and primary sclerosing cholangitis. In addition to performing well as a diagnostic tool, the Enhanced Liver Fibrosis test has been shown to out-perform liver biopsy in prognostic studies and is the only non-invasive marker to do so. However, questions remain regarding the use of this test, particularly regarding the possible effect age and alcohol may have on test scores. This review examines the current literature published in relation to the enhanced liver fibrosis test and its clinical utility and highlights areas requiring further study.

INTRODUCTION

The rising incidence of chronic liver disease (CLD) continues to be an increasing health burden.[1] The morbidity and mortality associated with CLD typically occurs in patients with advanced fibrosis and cirrhosis. Concerningly, these patients often remain asymptomatic until they develop decompensated disease. Hence early identification of those at-risk is of vital importance to ensure appropriate ongoing management.

Currently, tools for appropriate risk stratification remain limited. Although liver biopsy remains the gold standard for identifying advanced fibrosis, it requires access to hospital services, is invasive, costly and not without risk. Increasing awareness of the limitations of liver biopsy has driven research into alternative non-invasive methods of fibrosis assessment (NIT), including either novel imaging techniques assessing liver stiffness or serological markers assessing functional changes. Whilst transient elastography offers a user-friendly bedside test, it still requires access to a dedicated machine with a trained operator and may be inaccurate in patients with severe obesity, narrow intercostal spaces and mild steatosis.[2-5] Conversely, serum biomarkers provide a standardised automated test with good reproducibility,[6-9] and do not require specialist equipment or personnel. The wider availability of serum biomarkers facilitates their utility in clinical practice, especially for screening of patients with compensated disease or in settings where liver biopsy is not pragmatic. Biomarkers can be direct (reflecting underlying fibrosis) or indirect markers (using markers of liver function). Table 1 illustrates a comparison between these biomarkers. Although indirect markers are inexpensive as they rely upon routine blood tests and are not subject to patents, they are less accurate, less precise and not as reproducible.[10,11]

The Enhanced Liver fibrosis (ELF) is one such direct serum biomarker. It is the result of a logarithmic algorithm combining quantitative serum measurements of three markers of hepatic extracellular matrix metabolism – hyaluronic acid (HA), tissue inhibitor of metalloproteinase-1 (TIMP-1) and N-terminal peptide of procollagen III (PIIINP),[6] whereby a greater concentration of measured analytes generates a higher ELF score and reflects more significant fibrosis.[6,12-14] ELF performs best when two thresholds are used; an upper threshold to confirm advanced fibrosis (high specificity and positive predictive value [PPV]) and a lower threshold to exclude fibrosis (high sensitivity and negative predictive value [NPV]).[15] According to the manufacturer, ELF should be interpreted as follows: <7.7 – no to mild fibrosis; ≧7.7 to <9.8 – moderate fibrosis (F≧2); ≧9.8 – significant fibrosis (F≧3).[6] Recent evidence has suggested additional thresholds of <8.3 to exclude moderate fibrosis, ≧10.5 to indicate advanced fibrosis (F≧3) and ≧11.3 for cirrhosis.[16,17]

In the primary care setting, use of serum biomarkers such as ELF enables testing for fibrosis severity in patients unable to access or unsuited to liver biopsy or transient elastography (TE). Patients at risk may be screened for fibrosis, possibly in combination with simple tests, to reduce the need for biopsy and facilitate more appropriate referrals to tertiary care, with consequent health economic benefits.[18,19] A significant role for ELF has also been outlined in secondary and tertiary care, where it has been shown to not only accurately stratify fibrosis severity but also provide greater prognostic insight than liver biopsy. The prognostic utility of ELF has been shown to be graded, whereby a one unit increase in ELF is associated with a doubling in the risk of a liver related event.[20] ELF is one of only two serum biomarker panels designed for all aetiologies. Whilst originally validated in a mixed cohort of 1021 CLD patients,[6] it has since been validated in cohorts of hepatitis C virus (HCV),[14,15] primary biliary cirrhosis (PBC), [21] primary sclerosing cholangitis

(PSC) [22], alcoholic[23], and non-alcoholic fatty liver disease (NAFLD), [11] however has been shown to perform poorly in autoimmune hepatitis. [24]

In comparison to alternative patented serum biomarkers, ELF does not have an upper threshold, meaning it can be used to evaluate fibrotic liver damage in cirrhotic patients to provide a quantitative measure that correlates with portal hypertension and prognosis.[25-27] Further, its linear relationship to fibrosis severity and non-invasive nature renders it an excellent tool to monitor disease course and response to anti-fibrotic therapies. [28,29]

CURRENT CLINICAL USES OF ELF

Viral Hepatitis C

Globally approximately 71 million people are infected with the HCV. Untreated HCV may progress to cirrhosis and hepatocellular carcinoma, causing an estimated 399,000 deaths annually.[30] Recent developments in antiviral medications have facilitated clearance of the virus in many patients, however the significant global prevalence necessitates ongoing progress be made in both treatment and monitoring of this disease. ELF has shown good efficacy in the stratification of fibrosis severity in both chronic HCV infection and HIV/ HCV co-infection,[6,14,15,31,32] is able to predict evolution of fibrosis in response to antiviral treatment,[33-35] and is cost effective.[36] EASL-APASAL guidelines on the use of NIT have recommended the use of ELF in viral hepatitis and other CLD. ELF may prove particularly valuable in assessing fibrosis severity in prison populations, where HCV prevalence is high but liver biopsy is not routinely performed due to practical restrictions.[37]

The original validating ELF cohort included a substantial HCV population (49%), whereby subgroup analysis found an area under receiver operator characteristic curve (AUROC) of 0.773 (95% CI: 0.697-0.848) for the diagnosis

of significant fibrosis.[6] Perhaps of greater clinical utility, a NPV of 94.9% and PPV of 90.0% were found. Recruitment of this cohort was completed in tertiary centers, therefore generalization of PPV and NPV to primary care should be undertaken with caution. Further investigation of ELF in HCV in populations where the spectrum of disease reflects that seen in primary care is required. The heterogenous aetiology of this cohort has also been criticized[38] and subsequently, further validation in HCV exclusive cohorts has been undertaken. One independent validation study recruited patients in three different cohorts.[15] They identified similar AUROCs to the original ELF cohort and determined that if sensitivity and specificity of 85% are accepted, biopsy could be avoided in 81% of patients. As the study did not use a centralized pathologist to review all histology, inter-observer variation may compromise the validity of these findings. Further investigation into the avoidance of biopsy found that 63% and 74.7% respectively of patients could avoid biopsy by using ELF as a surrogate fibrosis marker.[35,39] Each of these studies utilized different acceptable error rates when calculating the proportion of biopsies which might have been avoided in their respective cohorts, with sensitivities and specificities ranging from 76% to 90%. This highlights the variability in acceptable error rates between clinicians, which impacts the number of biopsies potentially avoided. The largest independent study to date assessed 512 patients with HCV and confirmed ELF's utility in predicting fibrosis or cirrhosis in HCV.[32] Their findings were similar to those of the original validating cohort whereby significant and severe fibrosis and cirrhosis could be detected with AUROCs of 0.78 (95% CI: 0.74-0.82), 0.82 (0.78-0.86) and 0.85 (0.81-0.90) respectively. These results were not significantly different to FibroTest or Hepascore when statistical comparisons were completed. However the greater stability of the ELF analytes means that the ELF test is more widely applicable to clinical settings in which there may be delays in sample processing or analysis.[40-42] Although it is difficult to compare AUROCs from different studies

given the potential influence of spectrum bias, generally the utility of ELF in fibrosis assessment has been shown to be relatively consistent. Significant fibrosis can be identified with AUROCs ranging from 0.74-0.87, advanced fibrosis 0.82-0.89 and cirrhosis 0.82-0.90.[6,15,32,33,35,39,43]

Nonalcoholic Fatty Liver disease

NAFLD is estimated to affect approximately 25% of the adult population worldwide,[44] **it is the commonest cause of CLD seen in primary care and is now the second leading cause of liver disease**[45]**, primary liver cancer and decompensation amongst adults awaiting liver transplantation**[46] **in the USA.**[47,48] **Therefore identifying either those patients who require ongoing management or those who are suitable for either existing and emerging remedies remains essential. Although NAFLD screening in the community is not currently recommended, European clinical practice guidelines recommend the use of serum biomarkers (NAFLD fibrosis score [NFS], fibrosis 4 [FIB-4] or ELF test) as first line risk stratification to help identify at-risk patients with NAFLD and advanced fibrosis due to its prognostic implications. In contrast to the European Associations, the United Kingdom National Institute for Health and Care Excellence (NICE) guidelines on the assessment and management of NAFLD recommend using the ELF test as the first-line test for advanced fibrosis in all patients who have been diagnosed with NAFLD.**[49] The ELF test was validated for diagnosing moderate and severe fibrosis in an independent cohort of patients with NAFLD with AUROC of 0.90 (95%CI 0.84-0.96) and 0.93 (95%CI 0.88-0.98) respectively.[11]

To date the utility of ELF as a screening tool for NAFLD has been tested in two international prospective studies. The UK based study used a stepwise biomarker protocol with ELF as a secondary direct biomarker to triage primary

care referrals requiring secondary assessment. Although the addition of the secondary "direct biomarker" ELF test was only required in 26.7% of cases, its use enabled the primary care practice to allocate 59.9% of the remaining cases of NAFLD for referral to secondary care leaving 40.1% to be managed in primary care. Use of the biomarker pathway resulted in 20% cost saving compared to standard care.[18] The Australian based study used ELF in combination with TE and demonstrated that together they have an negative predictive value of 91.7% for excluding advanced fibrosis and a positive predictive value of 95.8% for identifying advanced fibrosis.[50] Interestingly the sub-analysis highlighted that older age was significantly associated with higher odds of having an ELF>9.8 independent of fibrosis status (p<0.001).[50] Although both studies have highlighted the potential value of the use of ELF in a pragmatic way, due to comorbidities in the cohorts investigated, histological confirmation of fibrosis status was only available in a subset of patients.

Alcoholic liver disease

The spectrum of ALD ranges from simple steatosis in almost all heavy drinkers through to steatohepatitis and ultimately fibrosis and cirrhosis, which develops in 20% of chronic alcoholics.[13,51] Whilst the performance of serum biomarkers in ALD is comparable to other aetiologies in that they better diagnose more severe stages of fibrosis, supporting literature is relatively sparse compared to viral hepatitis and NAFLD populations. The original ELF cohort included 64 patients with ALD and found an AUROC of 0.944 (95% CI:0.836-1.000) for diagnosis of significant fibrosis (F≧2).[6] Although a small cohort, the perfect specificity and sensitivity (100% when using two different thresholds; no CIs published) indicated good marker performance. More recent validation of ELF in a population of 289 ALD patients completed in 2018 [16] found the utility of ELF in detecting significant and advanced fibrosis and cirrhosis was excellent, with AUROCs of 0.84 (95% CI:0.80-0.89), 0.92 (0.89-

0.96) and 0.94 (0.91–0.97) respectively. Sensitivity (89%) and specificity (91%) were only published for advanced fibrosis. A comparison of ELF with FibroTest, TE, APRI, FIB-4 and Forns found no significant difference between FibroTest and ELF, however they both outperformed indirect markers (p<0.001-0.008). This corroborates findings whereby indirect markers have been shown to perform poorly in ALD populations relative to other aetiological cohorts.[16,52-59] TE performed significantly better than ELF (P<0.002) in the per protocol analysis, although this difference was not observed in the intention to treat analysis due to the 5-15% failure rate of TE.[5]

Concerns regarding the effect of alcohol on the constituents of ELF have been expressed, however a comparison of histologically matched cases and controls to properly investigate this is yet to be completed. Studies which have attributed differences in analyte levels to alcohol have not matched patients according to fibrosis severity, thus making the results difficult to interpret accurately as the differences in ELF scores may be due to early fibrotic changes in alcoholics rather than alcohol consumption.[60] A small study (n=10) assessing the impact of acute intoxication on the markers used in the ELF algorithm found no significant differences in analyte levels measured in blood taken whilst patients were intoxicated compared to bloods taken following two weeks of sobriety (p>0.01).[61] [61]

Primary biliary cirrhosis

ELF is particularly useful in PBC as in typical presentations, liver biopsy is not required to confirm diagnosis[62] and a single biopsy may well not be representative of the whole liver. A non-invasive assessment of fibrosis severity with ELF in these situations aids clinicians in making the most appropriate treatment decisions and predicts clinical outcomes. Whilst the original ELF cohort included 53 PBC/ PSC patients, there is no published sub-

group analysis.[6] A prospective cohort study of 161 PBC patients followed up for 7.3 years demonstrated ELF scores correlate well with histology, albeit with significant overlap between ELF scores in the intermediate stages of fibrosis (AUROC = 0.75 for significant fibrosis and 0.76 for cirrhosis).[21] This good performance has been corroborated by subgroup analysis of 28 PBC patients in a comparison of ELF, biopsy, FibroTest and TE.[63] ELF may also be used to monitor fibrosis progression, as it has been shown to increase on average by 0.032 per year in those with fibrosis and has also performed as well as histology as a prognostic marker, whereby each unit increase in ELF was associated with a threefold increase in future complications.[21]

Primary Sclerosing Cholangitis

Patients with PSC have an unpredictable disease course with a median transplant free survival of 12-21 years.[64,65] To date studies investigating potential predictors of poor outcome in PSC patients have either relied upon biochemical variables[65-71] or clinical evidence of decompensated disease.[65,72,73] Currently there remain no reliable prognostic tools for use in patients with PSC.[74] However recent literature has demonstrated promising results utilising the ELF test. A Norwegian study using ELF to stratify PSC patients demonstrated higher ELF scores at baseline were associated with shorter survival. The ELF test reliably distinguished between mild and severe disease with an AUROC of 0.81 (95% CI 0.73-0.87). They also identified that ELF score was associated with transplant free survival independent of the mayo risk score (hazard ratio 1.9, 95% CI 1.4-2.5, and 1.5, 95% CI 1.1-2.1, respectively).[22] The multivariate Cox regression analysis identified that both ELF score and Mayo risk score were independently associated with transplant-free survival. Interestingly, amongst the other variables in the regression model only PSC duration and age at diagnosis were also independently associated with survival time.

A subsequent international multicenter retrospective PSC population study estimated rates of transplant free survival. They identified that ELF levels were higher in patients with the combined outcome of liver transplantation or death (median 10.9 [Interquartile range (IQR): 9.8-12.1]; n=24 deaths, 79 liver transplantations, p < 0.001). They also identified that ELF independently predicted clinical outcomes with an AUROC of 0.79.[75] Unfortunately in this study they were unable to calculate the Mayo risk score, the most commonly used prognostic tool in PSC due to unreliable clinical data. Therefore the authors could not validate whether ELF added incremental value to the Mayo risk score as identified by the Norwegian study[22] As liver biopsies are not indicated in the management of PSC other than to diagnosis concomitant autoimmune hepatitis, neither study had histological confirmation of fibrosis status and instead used endpoints of either a composite of all-cause mortality and liver transplantation, or transplant free survival.

COST-EFFECTIVENESS

Cost-effectiveness analyses have demonstrated the utility of ELF in populations of HCV, ALD and NAFLD. [18] [19] Theoretical cohorts of ALD and HCV, have compared the use of annual ELF, annual sequential TE/ ELF and single biopsy. ELF test alone was the most effective strategy in both HCV and ALD however was also the most expensive (€11,484 per quality adjusted life year [QALY] in HCV and €189 per QALY in ALD). The significantly greater cost associated with HCV is due to the cost of antiviral therapies. At a willingness-to-pay threshold of €30,000 per QALY, sequential ELF/ TE was found to be a cost-effective strategy in 90.1% of HCV cases and >99.9% of ALD cases.

A previous cost-effectiveness investigation into the use of NIT in HCV found that treatment with antiviral agents without liver testing was the most cost-

effective strategy to reduce liver fibrosis occurrence and progression, however this study was completed during the age of interferon-only therapy. Since the development of direct acting antivirals (DAAs), which are substantially more costly, NITs are able to identify those in early stages of fibrosis requiring access to treatment in countries where not everyone with a positive test can receive treatment.

As previously discussed, the sequential use of ELF and an indirect fibrosis marker in NAFLD in a UK primary care setting is associated with a 5-fold increase in the identification of advanced fibrosis and cirrhosis and a 20% cost saving compared to standard care.[18] There are yet to be cost effectiveness analyses for ELF performed in other aetiologies of CLD. All studies of cost-effectiveness are dependent on determining the cost of the ELF test. As with most diagnostic tests the cost has fallen since first introduction and the cost of the test is likely to be inversely proportional to the number of tests performed up to a certain value. Few of the cost-effectiveness studies performed to date have incorporated the current NHS price of ELF of £42.

AREAS REQUIRING FURTHER STUDY

Although the promising utility of ELF has been highlighted in a variety of pathologies of CLD there are still areas that require further clarification. Age has been highlighted as an independent factor influencing the ELF score in some studies in HCV,[76] NAFLD[50] and PSC. [22] This may, in part, be explained by the higher incidence of cardiovascular and connective tissue disease seen in elderly populations, which are known to impact on ELF. The original ELF algorithm did take age in account however subsequent studies designed to specifically address the necessity for incorporating age in the ELF algorithm found that age could be omitted with no loss in diagnostic performance.[77] One potential reason for this may have been the relatively young age of the cohorts

that were used, compared to the higher age seen in the subsequent studies.[50] Clearly further analysis is required as to clarify the impact of age on the ELF score. Assessment of ELF in ALD has shown promising results, however there is a need for studies assessing ELF's performance in the setting of severe alcoholic hepatitis, decompensated liver disease and HCV co-infection. The impact of alcohol on ELF scores must be more thoroughly investigated as it is unclear if this will affect ELF performance as a diagnostic or prognostic test and if so, for better or worse. Further studies assessing its longitudinal utility, either alone or in combination with other risk scores or non-invasive tests are required to help us understand the full potential of ELF in clinical practice. To this point, no statistically significant differences have been shown between ELF and other patented panels used to assess fibrosis stage (including Hepascore, FibroMeter, FibroSure, FibroTest), however generally, ELF has been shown to consistently out-perform simple panels (including FIB-4, Forn's Index and APRI).

CONCLUSION

The ELF test is a combination of serological biomarkers that has been developed and validated in multiple underlying aetiologies of CLD. Its utility both in risk stratification and prognostication has been subsequently determined and validated by multiple international studies in several different populations and settings.[11,20,35,78,79] The analytical stability and excellent reproducibility of the test differentiate it from other non-invasive tests for liver fibrosis. Its value appears particularly promising where access to imaging modalities is limited, such as in prisons or rural areas, however further studies to clarify its uses with regards to longitudinal data and the concerns related to older age, and concomitant heavy alcohol use are required.

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