DERIVATION AND PERFORMANCE OF STANDARDISED ENHANCED LIVER FIBROSIS (ELF) TEST THRESHOLDS FOR THE DETECTION AND PROGNOSIS OF LIVER FIBROSIS.

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Abstract

Introduction: Non-invasive tests are increasingly used to assess liver fibrosis and determine prognosis but suggested test thresholds vary. We describe the selection of standardised thresholds for the Enhanced Liver Fibrosis (ELF) test for the detection of liver fibrosis and for prognostication in chronic liver disease.

Methods: A Delphi method was used to identify thresholds for the ELF test to predict histological liver fibrosis stages including cirrhosis, using data derived from 921 patients in the EUROGOLF cohort. These thresholds were then used to determine the prognostic performance of ELF in a subset of 457 patients followed for a mean of 5 years.

Results: The Delphi panel selected sensitivity of 85% for the detection of fibrosis and >95% specificity for cirrhosis. The corresponding thresholds were 7.7, 9.8 and 11.3. Eighty five percent of patients with mild or worse fibrosis had an ELF score ≥7.7. The sensitivity for cirrhosis of ELF≥9.8 was 76%. ELF ≥11.3 was 97% specific for cirrhosis. ELF scores show a near linear relationship with Ishak fibrosis stages. Relative to the <7.7 group, the hazard ratios for a liver related outcome at 5 years were 21.00 (95% CI 2.68-164.65) and 71.04 (95% CI 9.4-536.7) in the 9.8 to <11.3 and ≥11.3 sub-groups respectively.

Conclusion: The selection of standard thresholds for detection and prognosis of liver fibrosis is described and their performance reported. These thresholds should prove useful both in interpreting and explaining test results and when considering the relationship of ELF score to Ishak stage in the context of monitoring.

Lay Summary

Using data derived from a large prospective study and the opinions of expert hepatologists we have identified standard thresholds for the Enhanced Liver Fibrosis test that can be used to detect liver fibrosis of different degrees of severity, and determine the prognosis of chronic liver disease.

Introduction

In most chronic liver diseases liver fibrosis is a consequence of liver injury with the accumulation of collagenous scar tissue eventually leading to cirrhosis with distortion of the hepatic vasculature. Cirrhosis, the fifth commonest cause of death in the UK in middle aged men, is still on the increase and is thus a major health problem(1). The prognosis of liver disease varies dramatically as cirrhosis advances, with the 1 year mortality rate in decompensated disease as high as 57%(2). The morbidity and mortality associated with cirrhosis can be reduced with appropriate interventions. It is therefore crucial to detect cirrhosis as early as possible in order to treat patients in the hope of reducing the incidence of the complications of decompensated disease including oesophageal varices, hepatic encephalopathy and ascites(3, 4) and to manage early hepatocellular cancers(5).

Histological analysis of liver biopsies has been the established reference standard for the assessment of liver fibrosis(6), and is usually considered the most specific test to determine the severity of liver disease(7). Clinicians readily accept the staging of a liver biopsy as indicative of mild, moderate or severe fibrosis and use numerical staging systems that assign numbers (0-6 or 0-4) to describe these stages of fibrosis. Increasing recognition of the hazards associated with biopsy and limitations in the diagnostic performance of histological staging have led to the quest for non-invasive tests for liver

fibrosis that might augment or replace the use of liver biopsies to stage disease severity and determine prognosis(8-11).

Non-invasive tests for liver fibrosis include blood tests, fibro-elastography and imaging. These tests can be standardized to yield good analytical and diagnostic performance, with reliable reproducibility, and may be repeated frequently. The information generated in the form of a numerical fibrosis score can be related to both the severity of liver fibrosis and the prognosis for clinically significant outcomes. These continuous variable scores reflect the biological process of liver fibrosis more accurately than categorical histological stages.

Both elastography and imaging have been shown to perform well in the detection of severe fibrosis and cirrhosis(12, 13) but less well in mild disease(14). Both are dependent on access to well-maintained equipment, skilled operators and interpreters.

Blood tests have the advantages that samples can be obtained easily, safely, and relatively painlessly. Analytical methods range from the calculation of scores based on the measurement of routine biochemical and haematological tests such as APRI(15), FIB4 and the Forn's index(16-19); to panels including less routine biochemical tests such as FibroTest(20); to those that incorporate the measurement of constituents of fibrous matrix such as Hepascore(21) and Fibrometer(22). These tests tend to perform better at differentiating between severe and mild fibrosis rather than precisely distinguishing between each

histological stage(23, 24), but can provide useful prognostic information(25). However, many of the studies focus on the performance of the test in one particular disease rather than taking a range of aetiologies and most are affected by liver inflammation. Combinations of biomarker panels with imaging may improve the diagnostic accuracy in distinguishing between stages of fibrosis(26-28) but carry the disadvantages associated with physical tests.

The Enhanced Liver Fibrosis (ELF) test is a serum test that measures hyaluronic acid (HA), procollagen III amino acid terminal peptide (PIIINP) and tissue inhibitor of metalloproteinase 1 (TIMP-1); three molecules directly involved in liver matrix metabolism. The ELF test was derived and validated in separate cohorts selected at random from over 1,000 patients recruited in the EUROGOLF study reported in 2004(29). Subsequently the ELF test has been validated in a wide range of chronic liver diseases for the assessment of liver fibrosis(29, 30), including the paediatric population(31), and has been shown to be an accurate prognostic marker for all-cause mortality and the complications of cirrhosis(32) and may be more accurate than liver histology in determining prognosis(33).

The ELF test is marketed by Siemens Healthineers. The instructions for use provided by the manufacturer with the ELF reagents recommend test thresholds that can be used to interpret the results. However the data underpinning these recommended have not previously been placed in the public domain. Here we describe the process that was undertaken to identify these thresholds for the ELF test and their relationship to histologically

defined stages of fibrosis through analysis of test performance in samples from the cohorts of patients in which the test was derived and validated. These data were shared with the manufacturer and used to establish the thresholds described in their instructions for use.

In addition to the manufacturer's thresholds we describe a new threshold for the detection of cirrhosis with high specificity.

Furthermore, in order to exploit the additional information captured by a continuous variable such as ELF, we have investigated the change in ELF score within each histological stage to describe the relationship between the ELF score and liver fibrosis staging.

Although the prognostic performance of ELF has been described in specific and diverse chronic liver diseases(32-34), the thresholds identified in these studies were derived from study specific data rather than the manufacturer's thresholds. In order for clinicians to interpret both fibrosis severity and prognosis using the same thresholds we have determined the prognostic performance of ELF in predicting liver-related morbidity and liver-related deaths in a mixed cohort of patients with chronic liver disease over 2, 5 and 6 years follow-up at the manufacturer's thresholds, and our new threshold specific for cirrhosis.

Materials and Methods

Clinically significant sensitivities and specificities for the detection of liver fibrosis were determined through a Delphi process(35) in which four expert

hepatologists were interviewed by WR or JP and asked to select levels of diagnostic performance that they would deem acceptable when assessing patients with chronic liver disease. Specifically they were asked what proportion of patients with mild or worse liver fibrosis they would be willing to miss-assign as having “no fibrosis”; and the proportion of patients with severe fibrosis or cirrhosis that they would be willing to miss-assign as having “moderate or milder fibrosis” to the nearest 5%. After identifying these sensitivities and specificities, the data generated in the original EUROGOLF cohort(29) were interrogated to identify the ELF test thresholds. After reviewing the performance of these thresholds, the hepatologists then requested a further threshold that was even more specific for liver cirrhosis.

We have previously reported the event-free survival, and probability of liver related morbidity and mortality, for the 501 English patients recruited in the EUROGOLF cohort(33). These data were used *a priori* to determine thresholds that correlated with categorical risks for liver related outcomes. The thresholds generated using this approach differed from those derived from the cross-sectional analysis of histological fibrosis severity as described above. In order to avoid confusion arising from the use of different thresholds for assessment and prognosis, we recalculated the prognostic performance of the ELF test at the thresholds identified for histological assessment, using the data obtained for the English participants in the EUROGOLF cohort.

The ELF prognostic performance study is based on follow up of patients recruited to the original Bayer “EUROGOLF” study in 1998-2000 conducted

by the European Liver Fibrosis Group. The methods are discussed briefly below; however, a full description of the study methods is presented in the publications by Rosenberg et al.(29) and Parkes et al.(33). Briefly, serum samples were obtained at the time of liver biopsy from 921 patients recruited consecutively with a spectrum of liver diseases(29) at the time of their first investigation for abnormal liver function. Assays of hyaluronic acid (HA), amino-terminal peptide of procollagen III (PIIINP) and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) were performed on an Immuno-1 autoanalyser using the manufacturer's reagents and in accordance with the manufacturer's instructions (Siemens Healthineers, Tarrytown, USA). Results were entered into the ELF algorithm to calculate ELF scores. Liver biopsies were analysed by a single pathologist and staged for liver fibrosis using both the Scheuer and Ishak systems. In the present study the diagnostic performance of the ELF test was calculated and sensitivity, specificity, and areas under receiver operator characteristic curves are reported. Previously we have also reported positive and negative predictive values for the ELF test(29).

501 patients recruited to the original European Liver Fibrosis study at 7 centres in England (1998-2000) were followed up for clinical outcomes through clinical record review. The spread of aetiologies represented is displayed in Table 1. Of these, 457 were included in the data analysis as 44 patients were excluded because they had entered study with a transplanted liver.The main outcome was the performance of the Enhanced Liver Fibrosis test in predicting the incidence of the first liver-related clinical event defined as: (A) **Liver-related death** - defined as any mention of liver disease in part 1 of the death certificate (where the primary cause of death is recorded). The

performance of the Enhanced Liver Fibrosis test in predicting all-cause mortality was also assessed; or (B) Any episodes of **Decompensated Cirrhosis** after recruitment – including ascites (detected by paracentesis, ultrasound, or on clinical examination), encephalopathy (defined clinically), oesophageal variceal haemorrhage (confirmed by endoscopy), liver transplantation and hepatocellular cancer (diagnosed by one or more space-occupying lesions seen by imaging methods with typical patterns of HCC or by histology).

Participant socio-demographic and clinical characteristics were described using frequency and percentage for categorical variables, Correlation between continuous variables was assessed using Pearson's correlation. Linear regression was used to generate and fit regression curves and straight lines for ELF with both Ishak and Scheuer staging. The risks and hazard ratios for liver related events were calculated using Cox proportional hazard model after adjusting for age and sex. Kaplan Meier survival curves for survival free of liver related events including complications of portal hypertension, liver cancer, liver transplantation and death were generated using SPSS for patients with ELF scores in the ranges <7.70; 7.7 - 9.79; 9.80 - 11.29 and ≥11.30. .Data analyses were conducted using SPSS Inc version 24.0 (College Station TX: StatCorp LP; 2013).

Results

The Delphic panel expressed the view that the clinical utility of non-invasive testing (NIT) is to detect fibrosis in patients considered to be at low risk of severe liver disease; and to identify cases of advanced fibrosis and cirrhosis with accuracy amongst patients suspected of having significant fibrosis.

The clinicians' consensus was that the test sensitivity for detecting minor degrees of fibrosis should be 80-85% when seeking cases of fibrosis amongst patients suspected to be at low risk of fibrosis, acknowledging that this strategy may miss 20% of cases with minimal fibrosis whose disease may subsequently progress (false negative test). Their view was that these cases could be followed with repeated testing if this was clinically indicated. Similarly the panel opted for 80% sensitivity for the detection of cirrhosis, accepting that up to 20% of patients with either minimal fibrosis or cirrhosis might be missed They originally agreed to a minimum specificity for cirrhosis of 85% meaning that up to 15% of patients would be mis-diagnosed as having cirrhosis when they in fact had lesser degrees of fibrosis (false positive test).
Subsequently, having reviewed the test performance characteristics of ELF for the detection of cirrhosis (S6) the clinicians requested an additional threshold with greater specificity for the diagnosis of cirrhosis such that fewer than 5% of mild-advanced fibrosis (<S6) cases would be mis-classified as cirrhotic. This threshold coincides with that previously identified by Lichtinghagen et al (37). The ELF test thresholds that yielded the sensitivities and specificities for fibrosis detection identified using the Delphic process, based on the data obtained from the EUROGOLF cohort (29) and are <7.7 for none-mild fibrosis, 7.7 to <9.8 for moderate fibrosis, 9.8 to <11.3 for severe fibrosis and ≥11.3 for cirrhosis.

The ability of ELF to distinguish between different binary categorizations of stages of fibrosis is presented in Table 2. Using Ishak histological staging as the reference standard, 80% of patients with any detectable liver fibrosis

(>S0) and 85% with more than minimal fibrosis (>S1) will have an ELF score of greater than 7.70. In the mid-range between 7.71 and 9.79 the ELF test can be used to classify patients as having mild or moderate fibrosis (>S2) with 80% sensitivity. The ≥9.8 threshold has a specificity for the detection of advanced fibrosis (>S4) of 90%. In relation to cirrhosis, 76% of patients with cirrhosis (S6) on liver biopsy will have an ELF score ≥9.8, however the specificity for the detection of cirrhosis using this cut-off is only 87%. The higher threshold of ≥11.3 has 97% specificity. Thus, only 3% of patients will have a false positive diagnosis of cirrhosis.

However, the loss of sensitivity consequent on using the threshold of 11.3 results in the risk of 62% of patients with cirrhosis being mis-classified because they have an ELF score that falls below this threshold.

Examination of the relationship between ELF scores and histological stages within the categorical boundaries established by the thresholds reveals that there is a positive correlation between rising ELF score and increasing Ishak or Scheuer histology stage (Pearson's R – 0.598 $p<0.001$ for Ishak and $R^2 = 0.99527$ $p<0.001$ for Scheuer) (see Figure 1Aand B).

Fitting straight lines across these curves revealed that a 0.5 change in ELF corresponded to a change in 1 histological stage in the F1-F3 Scheuer or S2-S5 Ishak range (see Figure 1 C and D). The mean change in ELF per Ishak stage between S1 and S6 was 0.52 and the mean change in ELF per Scheuer stage between F1 and F4 was 0.53. An ELF score of less than 7.7 represents no fibrosis while a score of 8.3 correlates with moderate fibrosis, and the

difference between scores of 8.7 and 9.4 corresponds to the difference between moderate and advanced histological stages of liver fibrosis.

To evaluate the prognostic performance of the ELF test using the thresholds identified for histological staging, ELF scores were compared to the liver related events for each patient. Participants were sorted according to their liver related event status at (i) overall (ii) 5 years follow up (iii) 6 years follow up, according to one of four discrete ELF score groupings (<7.7; 7.7 to <9.8; 9.8 to <11.3; ≥11.3) (Table 3). The risk of developing a liver related event (LRE) and the hazard ratios of these outcomes adjusted for age and sex were calculated using Cox proportional hazards model and are presented in Table 4. An ELF score of 9.8-11.3 is associated with a 10 fold increase in the relative risk of a liver related event in the next 6 years compared to an ELF score of 7.7-9.8. A patient with an ELF score between 9.8 and 11.3 has a 38% chance of having a liver related event within 6 years and is 21 times more likely to experience such an event compared to a patient with little or no liver fibrosis (ELF score <7.7). At 5 years a patient with a score of >11.3 has a 57% chance of a LRE and is 70 times more likely to have a LRE than a person with score of less than 7.7.

The Kaplan-Meier Survival curves for survival without a liver related event for each of the diagnostic thresholds are presented in figure 2. The separation of the curves for 7.7-9.79, 9.8-11.3 and >11.3 each reaches statistical significance.

Discussion

The principle aim of this work is to place in the public domain the evidence that supports the use of the manufacturer's thresholds for the interpretation of ELF test results. An additional threshold has been described for more precise identification of patients with cirrhosis. Furthermore we have taken the opportunity to determine the prognostic performance of the manufacturer's published thresholds and the newly derived cirrhosis threshold in the original EUROGOLF cohort over 7 years.

The Enhanced Liver Fibrosis test has been shown to be an accurate non-invasive test for both assessing the severity of fibrosis in chronic liver disease in a wide range of aetiologies (36-39) and predicting the incidence of liver-related death and cirrhosis in patients(33). Previous studies have described similar performance to other biomarker panels and better diagnostic yield than transient elastography (36).

The ELF threshold of 9.8 identifies cirrhotic patients with approximately 80% sensitivity in line with the requirement of the expert panel. However at this threshold 13%, or approximately 1 in 8 of the patients classified as having cirrhosis would in fact have milder fibrosis. Clinicians reported that this threshold resulted in too high a proportion of their patients being classified as potentially cirrhotic and thus potentially requiring investigation and management of portal hypertension and hepatocellular cancer. A higher threshold more specific for cirrhosis was requested. Accordingly the 11.3 threshold was identified, associated with 97% specificity for cirrhosis so that

the number of false positive results is reduced considerably. Patients whose scores lie between 9.8 and 11.3 should be considered to be highly likely to have advanced fibrosis and thus warrant closer monitoring for disease progression according to clinical circumstances. We suggest that clinical judgment should be used in the management of patients with ELF scores in this range. The selection of thresholds should be dictated by circumstance so that the threshold of 9.8 should be used in case-finding strategies seeking cases of cirrhosis while the threshold of 11.3 should be used to confirm the likely presence of cirrhosis. Incidentally this threshold was identified independently by Lichtenhagen at al.(40) from analysis of a separate cohort of patients. Further studies are required to evaluate the utility of this cirrhosis specific threshold of ELF in determining the need for variceal assessment and surveillance for liver cancers.

While the manufacturer's thresholds will undoubtedly aid clinicians used to converting NIT scores to their histological equivalent we believe that this approach under-values the information contained in ELF scores as a continuous variable. Furthermore non-invasive tests have the advantage over histological staging in that they can provide further diagnostic and prognostic information even after cirrhosis is established. Liver biopsies are often reported as exhibiting histological stages of fibrosis near the boundaries of categorical variables. The near linear correlation between ELF thresholds and the corresponding Ishak stages provides the opportunity to interpret ELF scores falling between the thresholds in the differentiation between degrees of fibrosis within categorical histology stages. These finer distinctions are of particular value when attempting to monitor disease progression or regression

where NIT such as ELF may prove most informative in tracking response to treatment. Increasingly the ELF test is being used in this context(41, 42). In the mid ranges of fibrosis severity there appears to be a near linear correlation between ELF score and histological stage. The greater increase in median ELF scores associated with cirrhosis (S6) can be attributed to the fact that ELF scores continue to track increasing amounts of fibrosis within the single histological category of cirrhosis.

The biological validity of the ELF test as a surrogate measure of liver fibrosis is suggested by the correlation with metabolic changes associated with more advanced fibrosis (43) and is further supported by the good correlation between ELF scores and prognosis for liver related morbidity and mortality. The prognostic value of the ELF test has been reported in CLD caused by a range of aetiologies in the original EUROGOLF cohort(33), as well as in primary biliary cholangitis(32) and in primary sclerosing cholangitis(34). In each of these studies thresholds have been derived from original data in order to predict the risk of clinical outcomes. While these thresholds are informative, the difference between thresholds for fibrosis assessment and prognostication introduces complexity and confusion. We have used the thresholds identified for fibrosis assessment to interrogate the original EUROGOLF cohort for clinical outcomes. These three thresholds define four categories of risk for liver related outcomes that are statistically separate and carry meaningful information that can be conveyed to patients and colleagues and used to guide management decisions.

Whilst the categorical risk stratification based on Cox proportionate hazard models and Kaplan-Meier survival analysis identified statistically separate groups, as with all categorical groupings movement between categories across a single threshold does not reflect the true change in risk for the patient and so changes in ELF score should be interpreted in relation to their correlation with the biological process of fibrosis that underpins risk of morbidity and mortality. Previously we have shown that a unit change in the ELF score correlates with a doubling of the risk of a liver related outcome over 7 years(33). Thus the difference between a score of 8.4 and 9.5 represents a clinically meaningful and important difference even though both scores fall between the 7.7 and 9.8 thresholds denoting moderate fibrosis.

In the 2016 NICE guidelines the value of 10.51 has been recommended for the assessment and management of NAFLD to diagnose patients with advanced fibrosis(44). This value falls in the middle of the 9.8 – 11.3 range with an associated specificity for diagnosing advanced fibrosis >90%. Furthermore, the risk of a liver-related death in 5 years with an ELF score of 10.5 is approximately 25%, and the patient has a risk at least 20 times greater than someone with an ELF score <7.7.

It must be acknowledged that the thresholds defined in this study are based on specific cohort of patients with mixed chronic liver diseases and may not be equally applicable in all categories of chronic liver disease. Sub-group analyses failed to show any statistically significant differences in the

performance of ELF between viral, alcoholic and fatty aetiologies but larger studies in these disorders are warranted. Although this study has not investigated the performance of ELF in different aetiologies it remains a strength of the test that it can be applied to a wide range of chronic liver diseases using the same thresholds.

This study aimed to compare ELF with the reference standard tests of liver histology and clinical outcomes. Participants were not assessed with fibroelastography or with “indirect’ marker assays such as APRI and FIB4 due to variation in the availability of both AST and ALT results but such comparisons would be of interest. Furthermore it would be of interest to analyse changes of ELF over time and their relationship to clinical outcome. It is hoped that by placing these data in the public domain other investigators will be enabled to explore the utility of the ELF test in the investigation, prognostication and management of patients with chronic liver disease.

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References

1. WHO European Health for All Database. WHO. 2009.
2. D'Amico G ea. Natural history and prognostic indicators of survival in cirrhosis: A systematic review of 118 studies. Hepatology (Baltimore, Md). 2006;44(1):217–31.
3. Tsochatzis EA, Bosch J, Burroughs AK. New therapeutic paradigm for patients with cirrhosis. Hepatology (Baltimore, Md). 2012;56(5):1983-92.
4. Schuppan D, Afdhal NH. Liver cirrhosis. Lancet. 2008;371(9615):838-51.
5. Williams R, Ashton K, Aspinall R, Bellis MA, Bosanquet J, Cramp ME, et al. Implementation of the Lancet Standing Commission on Liver Disease in the UK. Lancet. 2015;386(10008):2098-111.
6. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. Journal of hepatology. 1995;22(6):696-9.
7. Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Engl J Med. 2001;344(7):495-500.
8. McGill DB, Rakela J, Zinsmeister AR, Ott BJ. A 21-year experience with major hemorrhage after percutaneous liver biopsy. Gastroenterology. 1990;99(5):1396-400.
9. Rousselet MC, Michalak S, Dupre F, Croue A, Bedossa P, Saint-Andre JP, et al. Sources of variability in histological scoring of chronic viral hepatitis. Hepatology (Baltimore, Md). 2005;41(2):257-64.
10. Colloredo G, Guido M, Sonzogni A, Leandro G. Impact of liver biopsy size on histological evaluation of chronic viral hepatitis: the smaller the sample, the milder the disease. Journal of hepatology. 2003;39(2):239-44.
11. Grant A, Neuberger J. Guidelines on the use of liver biopsy in clinical practice. British Society of Gastroenterology. Gut. 1999;45 Suppl 4:Iv1-iv11.
12. Fernandez M, Trepo E, Degre D, Gustot T, Verset L, Demetter P, et al. Transient elastography using Fibroscan is the most reliable noninvasive method for the diagnosis of advanced fibrosis and cirrhosis in alcoholic liver disease. European journal of gastroenterology & hepatology. 2015;27(9):1074-9.
13. Seo YS, Kim MY, Kim SU, Hyun BS, Jang JY, Lee JW, et al. Accuracy of transient elastography in assessing liver fibrosis in chronic viral hepatitis: A multicentre, retrospective study. Liver international : official journal of the International Association for the Study of the Liver. 2015;35(10):2246-55.
14. Tsochatzis EA, Gurusamy KS, Ntaoula S, Cholongitas E, Davidson BR, Burroughs AK. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: a meta-analysis of diagnostic accuracy. Journal of hepatology. 2011;54(4):650-9.
15. 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 (Baltimore, Md). 2003;38(2):518-26.
16. Lin CL, Liu CH, Wang CC, Liang CC, Su TH, Liu CJ, et al. Serum Biomarkers Predictive of Significant Fibrosis and Cirrhosis in Chronic Hepatitis B. Journal of clinical gastroenterology. 2015;49(8):705-13.
17. Ucar F, Sezer S, Ginis Z, Ozturk G, Albayrak A, Basar O, et al. APRI, the FIB-4 score, and Forn's index have noninvasive diagnostic value for liver fibrosis in patients with chronic hepatitis B. European journal of gastroenterology & hepatology. 2013;25(9):1076-81.
18. Teshale E, Lu M, Rupp LB, Holmberg SD, Moorman AC, Spradling P, et al. APRI and FIB-4 are good predictors of the stage of liver fibrosis in chronic hepatitis B: the Chronic Hepatitis Cohort Study (CHeCS). Journal of viral hepatitis. 2014;21(12):917-20.

19. Ma J, Jiang Y, Gong G. Evaluation of seven noninvasive models in staging liver fibrosis in patients with chronic hepatitis B virus infection. European journal of gastroenterology & hepatology. 2013;25(4):428-34.
20. 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. Journal of hepatology. 2012;57(3):541-8.
21. 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(10):1867-73.
22. 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 (Baltimore, Md). 2005;42(6):1373-81.
23. Patel K, Gordon SC, Jacobson I, Hezode C, Oh E, Smith KM, et al. Evaluation of a panel of non-invasive serum markers to differentiate mild from moderate-to-advanced liver fibrosis in chronic hepatitis C patients. Journal of hepatology. 2004;41(6):935-42.
24. Adams LA. Biomarkers of liver fibrosis. J Gastroenterol Hepatol. 2011;26(5):802-9.
25. Ngo Y, Munteanu M, Messous D, Charlotte F, Imbert-Bismut F, Thabut D, et al. A Prospective Analysis of the Prognostic Value of Biomarkers (FibroTest) in Patients with Chronic Hepatitis C. Clinical Chemistry. 2006;52(10):1887-96.
26. Liu Y, Dong CF, Yang G, Liu J, Yao S, Li HY, et al. Optimal linear combination of ARFI, transient elastography and APRI for the assessment of fibrosis in chronic hepatitis B. Liver international : official journal of the International Association for the Study of the Liver. 2015;35(3):816-25.
27. Fitzpatrick E, Dhawan A. Noninvasive biomarkers in non-alcoholic fatty liver disease: current status and a glimpse of the future. World J Gastroenterol. 2014;20(31):10851-63.
28. Boursier J, de Ledinghen V, Zarski JP, Rousselet MC, Sturm N, Foucher J, et al. A new combination of blood test and fibroscan for accurate non-invasive diagnosis of liver fibrosis stages in chronic hepatitis C. The American journal of gastroenterology. 2011;106(7):1255-63.
29. Rosenberg WM, Voelker M, Thiel R, Becka M, Burt A, Schuppan D, et al. Serum markers detect the presence of liver fibrosis: a cohort study. Gastroenterology. 2004;127(6):1704-13.
30. Abignano G, Cuomo G, Buch MH, Rosenberg WM, Valentini G, Emery P, et al. The enhanced liver fibrosis test: a clinical grade, validated serum test, biomarker of overall fibrosis in systemic sclerosis. Ann Rheum Dis. 2014;73(2):420-7.
31. Nobili V, Parkes J, Bottazzo G, Marcellini M, Cross R, Newman D, et al. Performance of ELF serum markers in predicting fibrosis stage in pediatric non-alcoholic fatty liver disease. Gastroenterology. 2009;136(1):160-7.
32. Mayo MJ, Parkes J, Adams-Huet B, Combes B, Mills AS, Markin RS, et al. Prediction of clinical outcomes in primary biliary cirrhosis by serum enhanced liver fibrosis assay. Hepatology (Baltimore, Md). 2008;48(5):1549-57.
33. Parkes J, Roderick P, Harris S, Day C, Mutimer D, Collier J, et al. Enhanced liver fibrosis test can predict clinical outcomes in patients with chronic liver disease. Gut. 2010;59(9):1245-51.
34. Vesterhus M, Hov JR, Holm A, Schrumpf E, Nygard S, Godang K, et al. Enhanced liver fibrosis score predicts transplant-free survival in primary sclerosing cholangitis. Hepatology (Baltimore, Md). 2015;62(1):188-97.
35. Dalkey N, Helmer O. An Experimental Application of the DELPHI Method to the Use of Experts. Management Science. 1963;9(3):458-67.

36. Friedrich-Rust M, Rosenberg W, Parkes J, Herrmann E, Zeuzem S, Sarrazin C. Comparison of ELF, FibroTest and FibroScan for the non-invasive assessment of liver fibrosis. BMC gastroenterology. 2010;10:103.
37. Guha IN, Parkes J, Roderick P, Chattopadhyay D, Cross R, Harris S, et al. Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: Validating the European Liver Fibrosis Panel and exploring simple markers. Hepatology (Baltimore, Md). 2008;47(2):455-60.
38. Parkes J, Guha IN, Roderick P, Harris S, Cross R, Manos MM, et al. Enhanced Liver Fibrosis (ELF) test accurately identifies liver fibrosis in patients with chronic hepatitis C. Journal of viral hepatitis. 2011;18(1):23-31.
39. Trembling PM, Lampertico P, Parkes J, Tanwar S, Vigano M, Facchetti F, et al. Performance of Enhanced Liver Fibrosis test and comparison with transient elastography in the identification of liver fibrosis in patients with chronic hepatitis B infection. Journal of viral hepatitis. 2014;21(6):430-8.
40. Lichtinghagen R, Pietsch D, Bantel H, Manns MP, Brand K, and Bahr MJ. The enhanced liver fibrosis (ELF) score: normal values, influence factors and proposed cut-off values. J Hepatol. 2013;59(2):236-242.
41. Armstrong MJ, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, et al. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. Lancet. 2016;387(10019):679-90.
42. Nevens F, Andreone P, Mazzella G, Strasser SI, Bowlus C, Invernizzi P, et al. A Placebo-Controlled Trial of Obeticholic Acid in Primary Biliary Cholangitis. N Engl J Med. 2016;375(7):631-43.
43. Sands CJ, Guha IN, Kyriakides M, Wright M, Beckonert O, Holmes E, et al. Metabolic phenotyping for enhanced mechanistic stratification of chronic hepatitis C-induced liver fibrosis. The American journal of gastroenterology. 2015;110(1):159-69.
44. Non-alcoholic fatty liver disease (NAFLD): assessment and management. NICE Guidelines. 2016.

Table 1: Demographics of the EUROGOLF cohort

Clinical Condition	Female	Male	Total
Hepatitis C Virus (HCV) Infection	54	143	197
Hepatitis B Virus (HBV) Infection	2	23	25
Non-alcoholic Fatty Liver Disease (NAFLD)	8	36	44
Alcoholic Liver Disease (ALD)	24	62	86
Autoimmune Hepatitis (AIH)	20	6	26
Hereditary Hemochromatosis (HH)	5	9	14
Primary Biliary Cholangitis (PBC)	16	2	18
Primary Sclerosing Cholangitis (PSC)	2	6	8
*Other	21	18	39
Total	152	305	457

*Includes cases of chronic liver disease of unknown aetiology and cases in which no diagnosis was made in the investigation of abnormal liver function tests.

Table 1 Legend: 457 patients recruited to EUROGOLF in the United Kingdom were followed up for clinical outcomes over 7 years following enrolment.

Table 2: The relationship between the ELF Score and liver fibrosis severity

ELF Score	Ishak Histological Staging	Fibrosis Severity	Sensitivity	Specificity
7.7	F0 v 123456	Minimal	80	42
7.7	F01 v 23456	Mild	85	38
8.0	F01 v 23456	Mild Fibrosis	80	59
8.3	F012 v 3456	Moderate Fibrosis	80	60
8.7	F0123 v 456	Moderate Fibrosis	80	72
9.0	F0123 v 456	Moderate Fibrosis	73	80
9.4	F01234 v 56	Advanced Fibrosis	73	84
9.8	F01234 v 56	Advanced Fibrosis	65	90
≥ 9.8	F012345 v 6	Cirrhosis	76	87
≥ 11.3	F012345 v 6	Cirrhosis	38	97

Table 2 Legend: ELF scores corresponding to Ishak fibrosis stage groupings as binary outcomes are presented with associated histological descriptors and the sensitivity and specificity for detection of the fibrosis stage.

Table 3: Clinical Correlation of ELF Score to Liver related outcome

(i) 5 years follow-up

ELF	**Liver related outcome at 5 years follow up number (%)**	**No liver related outcomes number (%)**	**Total number (%)**
<7.7	1 (1.1)	88 (98.9)	89 (100)
7.70 - 9.79	9 (4.1)	208 (95.9)	217 (100)
9.80 - 11.29	13 (23.6)	42 (76.4)	55 (100)
≥11.3	21 (56.8)	16 (43.2)	37 (100)
Total	44 (11.1)	354 (88.9)	398 (100)

(ii) 6 years follow-up

ELF	**Liver related outcome at 6 years follow up number (%)**	**No liver related outcomes number (%)**	**Total number (%)**
<7.7	2 (2.5)	79 (97.5)	81 (100)
7.70 - 9.79	11 (5.6)	187 (94.4)	198 (100)
9.80 - 11.29	20 (37.7)	33 (62.3)	53 (100)
≥11.3	21 (56.8)	16 (43.2)	37 (100)
Total	54 (14.6)	315 (85.4)	369 (100)

(iii) Overall (7 years follow-up)

ELF	**Liver related outcome number (%)**	**No liver related outcomes number (%)**	**Total number (%)**
<7.7	2 (1.9)	104 (98.1)	106 (100)
7.70 - 9.79	12 (4.8)	239 (95.2)	251 (100)
9.80 - 11.29	23 (37.7)	38 (62.3)	61 (100)
≥11.3	24 (61.5)	15 (38.5)	39 (100)
Total	61 (13.3)	396 (86.7)	457 (100)

Table 3 Legend: The number of liver related outcomes (including complications of portal hypertension, liver cancer, liver transplantation and death are presented (i) at 5 years; (ii) at 6 years and (iii) for the duration of follow-up to 7 years;

Table 4: The Risks and Hazard Ratios (relative to ELF <7.7) for liver related outcomes at 5 and 6 years

	Risk % (95% CI)	**Hazard Ratio *adjusted for age and sex (95% CI) Cox Proportional Hazard**
Liver related outcomes 5 years		
<7.7	1.1 (0.2, 6.1)	1.0
7.70 - 9.79	4.1 (2.2, 7.7)	3.53 (0.45, 28.12)
9.80 - 11.29	23.6 (14.4, 36.3)	21.00 (2.68,164.65)
≥11.3	56.8 (40.9, 71.3)	71.04 (9.4, 536.7)
Liver related outcomes 6 years		
<7.7	2.5 (0.7, 8.6)	1.00
7.70 - 9.79	5.6 (3.1, 9.7)	2.07 (0.44, 9.67)
9.80 - 11.29	37.7 (25.9, 51.2)	21.03 (4.60, 99.19)
≥11.3	56.8 (40.9, 71.3)	47.37 (9.75, 230.17)

Table 4 Legend: The risk and hazard ratios of a liver related event associated with ELF scores in specified ranges are presented along with the associated 95% confidence intervals. Hazard ratios are calculated using Cox proportional hazard model after adjusting for age and sex.

Figure Legends:

Figure. 1A: The relationship between ELF score and histological stage of liver fibrosis using the Ishak staging system.

Figure 1B: The relationship between ELF score and histological stage of liver fibrosis using the Scheuer staging system.

Figure 1C: Linear regression was used to fit a line to the plot of median ELF scores against Ishak histological stages. The line of best fit was used to determine the relationship between ELF and fibrosis stage. An increase of approximately 0.5 in ELF corresponds to an increase in fibrosis of 1 Ishak stage in the mid range from S1 to S5. The greater increase in median ELF scores associated with cirrhosis (S6) can be attributed to the fact that ELF scores continue to track increasing amounts of fibrosis within the single histological category of cirrhosis.

Figure 1D: Linear regression was used to fit a line to the plot of median ELF scores against Scheuer histological stages. The line of best fit was used to determine the relationship between ELF and fibrosis stage. An increase of approximately 0.5 in ELF corresponds to an increase in fibrosis of 1 Scheuer stage across the mid range from F1 to F4. The greater increase in median ELF scores associated with cirrhosis (F4) can be attributed to the fact that ELF scores continue to track increasing amounts of fibrosis within the single histological category of cirrhosis.

Figure 2: Kaplan Meier survival curves for survival free of liver related events including complications of portal hypertension, liver cancer, liver transplantation and death for patients with ELF scores in the ranges <7.70; 7.7 - 9.79; 9.80 - 11.29 and ≥11.30

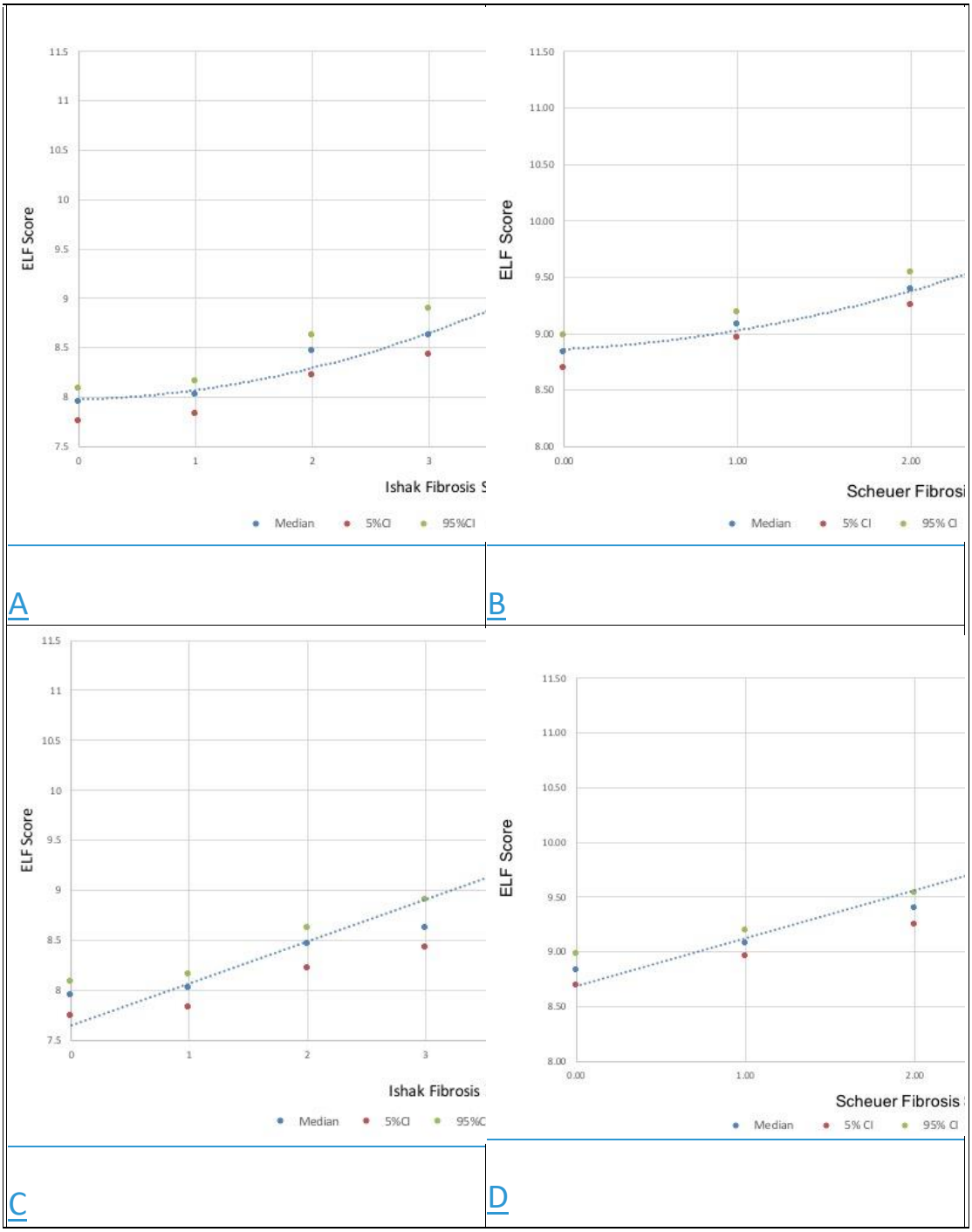
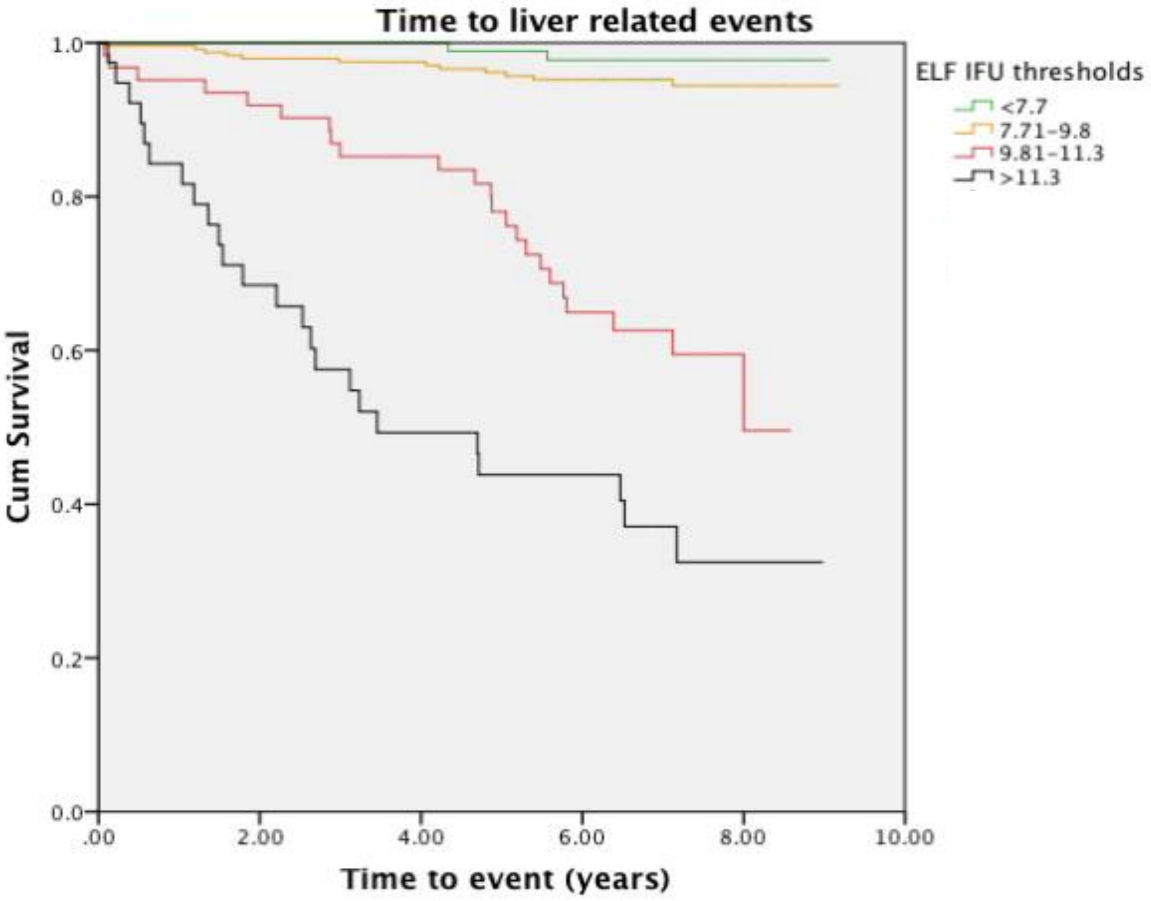

ELF Score
Ishak Fibrosis S
Median
5%CI
95%CI
A
ELF Score
Scheuer Fibrosi
Median
5% CI
95% CI
B
ELF Score
Ishak Fibrosis
Median
5%CI
95%C
C
ELF Score
Scheuer Fibrosis
Median
5% CI
95% CI
D

Figure 2


Time to liver related events
Cum Survival
1.0
0.8
0.6
0.4
0.2
0.0
.00
2.00
4.00
6.00
8.00
10.00
Time to event (years)
ELF IFU thresholds
<7.7
7.71-9.8
9.81-11.3
>11.3