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The Enhanced Liver Fibrosis (ELF) score: Normal values, influence factors and proposed cut-off values

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Background & Aims: Progressive fibrosis is a major cause of morbidity and mortality in chronic liver disease. To replace liver biopsy for disease staging, multiple serum markers are under evaluation with multiparametric panels yielding the most promising results. The Enhanced Liver Fibrosis (ELF) score is an ECM marker set consisting of tissue inhibitor of metalloproteinases 1 (TIMP-1), amino-terminal propeptide of type III procollagen (PIIINP) and hyaluronic acid (HA) showing good correlations with fibrosis stages in chronic liver disease.

Methods: The ELF score was measured in 400 healthy controls and 79 chronic hepatitis C patients using an ADVIA Centaur automated system. The ELF score was calculated using the published algorithm combining TIMP-1, PIIINP and HA values. Patients' fibrosis stage was defined histologically. ROC analyses were performed to study marker validity. Reference values and influence factors for the ELF score were validated.

Results: ELF score reference values ranged from 6.7 to 9.8 and were significantly higher for men *vs.* women (7.0–9.9 *vs.* 6.6–9.3, respectively). Afternoon values were slightly higher than morning values (6.7–9.9 *vs.* 6.6–9.5, respectively). Age was a notable influence factor. We identified three cut-off values: 7.7 for a high sensitivity exclusion of fibrosis, 9.8 for a high specificity identification of fibrosis (sensitivity 69%, specificity 98% for moderate fibrosis), and 11.3 to discriminate cirrhosis (sensitivity 83%, specificity 97%). ELF score validity was superior to the results of the single tests.

Conclusions: The ELF score can predict moderate fibrosis and cirrhosis. However, influence factors such as gender and age need to be taken into account.

Introduction

The stage of fibrosis is the most important single predictor of significant morbidity and mortality in chronic liver disease [1]. The mechanisms leading to fibrosis and eventually cirrhosis are thought to be similar, irrespective of the underlying etiology. At cellular level, hepatic stellate cells (HSC) undergo a phenotypic switch usually addressed as transactivation. Activated HSC are regarded as the main source of extracellular matrix (ECM) in the fibrotic liver [2,3]. Additional cell types namely fibroblasts and myofibroblasts may also contribute to ECM deposition [4]. Despite the similarities in pathophysiology at cellular level, morphogenesis and histologic appearance of the fibrotic liver may differ according to the etiology.

Liver biopsy remains the gold standard to evaluate liver fibrosis. Not least, one has to keep in mind that liver biopsy provides additional information like histological grading and etiology that may be overlooked when surrogate markers are used [5–9].

Despite detailed insight into cellular mechanisms that lead to liver fibrosis and cirrhosis, current availability of established non-invasive tests to monitor fibrosis is limited so far. Ideally, those tests should answer two questions. Firstly, what is the stage of fibrotic organ damage (i.e., the amount of deposited ECM and the disturbed balance of hepatic microarchitecture)? Secondly, what is the net balance between ECM deposition and degradation (i.e., the dynamics of ECM turnover)? The former serves to evaluate the prognosis and indicate therapy, while the latter might be used to control the efficacy of treatment with regard to disease progression.

Many different parameters including standard clinical chemistry and parameters of matrix metabolism have been evaluated [9,10]. In the last decade, markers were assembled to multiparametric scores. Here, we can distinguish scores assembled of standard clinical chemistry markers (e.g., aspartate aminotransferase-to-platelet ratio index, FibroTest, Forns' index) [11–14]

Keywords: ELF score; TIMP; Hyaluronic acid; Hyaluronate; PIIINP; Liver fibrosis; Cirrhosis.

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Abbreviations: ELF, enhanced liver fibrosis; ECM, extracellular matrix; TIMP-1, tissue inhibitor of metalloproteinases 1; PIIINP, amino-terminal propeptide of type III procollagen; HA, hyaluronic acid; HSC, hepatic stellate cells; CHC, chronic hepatitis C; HCV, hepatitis C virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AP, alkaline phosphatase; γGT, gamma-glutamyltransferase; CI, confidence interval; Kcal, kilocalories; ROC, receiver operating characteristics; AUC, area under the curve.

from scores using circulating markers of hepatic matrix metabolism like hyaluronic acid (HA), tissue inhibitor of metalloproteinases-1 (TIMP-1), matrix metalloproteinase-2, propeptide of type III procollagen (PIIINP) [15].

The ELF score consisting of single markers of hepatic matrix metabolism is one of the first commercially available serum multimarker fibrosis tests. However, there is a need for independent validation of the ELF score with regard to validated reference values, influencing factors and suitable cut-off values.

Materials and methods

Study subjects

Four hundred healthy volunteers (m/w 0.94:1; 13–72 years, mean 33 ± 13 years) were recruited as controls from blood donors at Hannover Medical School. The volunteers were examined thoroughly to exclude acute or chronic liver disease and other relevant health problems like morbid obesity, substance abuse or alcoholic consumption. All had normal liver function tests, a normal differential blood count, unimpaired renal function and normal concentrations of C-reactive protein. Additionally HBV, HCV, and HIV were excluded serologically.

Seventy-nine patients suffering from chronic hepatitis C virus (HCV) infection or histologically proven HCV-induced end stage liver cirrhosis in Child-Pugh stage C were studied (CHC). Histological activity of inflammation and fibrosis were assessed in liver biopsies of CHC patients taken at the same time as the blood samples. The hepatic activity index proposed by Ishak *et al.* [5] was used for quantification of inflammation and fibrosis. All patients were informed about the rationale and possible risks of the study and gave their written informed consent before inclusion. The study protocol has been approved by the ethics committee of Hannover Medical School.

Sample collection

Blood samples were collected in evacuated tubes (Monovette 02.1063, Sarstedt, Germany), allowed to clot for 30 min at room temperature and centrifuged at 1600*g* for 15 min at 4 °C. Sera were frozen at −80 °C within 2 h after collection.

Biochemical measurands

The ELF score provides a single value using an algorithm combining quantitative serum measurements of tissue inhibitor of metallo-proteinases-1 (TIMP-1), amino-terminal propeptide of type III procollagen (PIIINP) and hyaluronic acid (HA). We used the ADVIA Centaur CP immunochemical analyzer according to manufacturer's instructions. The ELF score was calculated directly by the instrument employing the following equation:

$$\text{ELF score} = 2.494 + 0.846\ \ln(C_{HA}) + 0.735\ \ln(C_{PIIINP}) + 0.391\ \ln(C_{TIMP-1})$$

Enzyme activities (U/L) were measured on a Modular P800 automatic analyzer using standard methods (Roche Diagnostics, Mannheim, Germany): alanine aminotransferase (ALT: female <34, male <45), aspartate aminotransferase (AST: female <31, male <35), alkaline phosphatase (AP: female 35–104, male 40–129), gamma-glutamyltransferase (γGT: female <38, male <55). Serum albumin (normal range 35–52 g/L) was routinely measured immunonephelometrically using the Modular P 800 (Roche Diagnostics, Mannheim, Germany).

Statistics

Data were analyzed using IBM SPSS 18 for Windows (IBM, Ehningen, Germany). Mann-Withney's *U* test, Kruskal-Wallis test, and Spearman rank correlation coefficients were performed. Differences were considered significant at $p < 0.05$. For validation of reference intervals, we used IBM SPSS for non-parametrical statistics in groups with n >120. In smaller subgroups, we estimated age and gender related reference intervals using the Trillium Reader software package (Trillium GmbH, Grafrath, Germany) developed especially for limited data sets by the German Society of Clinical Chemistry and Laboratory Medicine (DGKL) [16]. The Trillium Reader performs automatic skewness checks and applies either parametric or non-parametric statistics to suggest the most plausible lower and upper limits of reference intervals (2.5 and 97.5 percentiles). Further validation of the reference values was performed by the robust calculation method [17]. The partition of subgroups was evaluated by the standard normal deviate test according to CLSI-standard C28-A3 [18]. Receiver operating characteristics (ROC) calculations were performed using Sigmaplot 12.0 for Windows (Systat Software, USA).

Results

The multiparametric ELF score was evaluated using sera from 400 healthy non-fasting blood donors. The total ELF score reference range was calculated non-parametrically as 6.72 (90% CI 6.58–6.84) to 9.79 (90% CI 9.45–10.01). However, different influencing factors need to be considered for predefinition of cut-off values.

Influencing factors

Different factors influencing the ELF score are shown in Fig. 1 and Table 1. We could demonstrate intraday variation of ELF score values. Blood donors were divided into a morning (n = 129, m/w: 1:1, mean age 30 ± 12 years) and afternoon group (n = 236, m/w: 0.8:1, mean age 33 ± 12 years). Significant differences were shown for HA and ELF score values, which were slightly higher in the afternoon (mean values: 19.5 *vs.* 22.1 for HA; 7.9 *vs.* 8.1 for ELF, $p < 0.05$, resp., see Fig. 1A).

To explain whether those differences were more likely to derive from circadian rhythms or from food uptake, we analyzed a time course with five blood collections over eight hours with standardized food supply from four healthy volunteers. All volunteers had a standardized continental breakfast (300 kcal) between the first (fasting) and second blood collection and a standardized high-calorie lunch (1500 kcal) between the third and fourth blood collection. Time courses of TIMP-1, PIIINP, HA, and ELF score values are shown in Fig. 1B. Simultaneously measured standard laboratory tests like enzymes, cholesterol, bilirubin, and total protein showed an intraday variability from 1.6% to 10.6%. Interestingly, the highest nutrition related coefficients of variation with values up to 70% were found for HA, while the ELF score variability (2.9–7.7%) did not exceed those of standard laboratory tests. In this context, the z-statistics results (standard normal deviate test) revealed no significance, which indicates not to partition reference intervals by those subclasses.

In sera from healthy men (n = 187, mean age: 34 ± 13 years), ELF score values were slightly but significantly higher than in sera from healthy women (n = 200, mean age: 33 ± 12 years; $p < 0.001$) with mean ELF scores of 8.2 *vs.* 7.9 (see also Fig. 1A). At single assay level, PIIINP and HA values showed significant gender dependent differences (mean values men *vs.* women: 5.77 *vs.* 5.29 ($p < 0.05$) for PIIINP; 24.2 *vs.* 18.0 for HA ($p < 0.01$)). Results of standard normal deviate test recommended to partition reference intervals by gender subclasses. Non-parametrically calculated gender specific reference values (90% confidence intervals in parentheses) were: 7.04 (6.98–7.12) to 9.85 (9.45–10.17) for men and 6.58 (6.43–6.72) to 9.31 (9.11–9.45) for women.

Furthermore, age dependency was identified as the most relevant influencing factor (see Fig. 1A and Table 1). PIIINP, HA and ELF score values are significantly ($p < 0.0001$) different in a comparison of the two subgroups ⩽30 years *vs.* >30 years. In addition, the whole collective was divided into decades, which showed a significant ELF score increase from <20 up to >60 years with a

mean Δ-value of 0.3 between the decade-specific 97.5th percentiles (8.8, 9.1, 9.5, 9.5, 10.0, 10.2) (see Table 1).

ELF score values in chronic hepatitis C (CHC)

ELF scores were determined in 79 CHC sera. The relation between ELF scores and histological staging is shown in Table 2. We found increasing levels of ELF score ranges from normal controls over moderate fibrosis to end-stage cirrhosis. Using Spearman ranking, we could demonstrate a significant correlation with histological staging (r = 0.54, p <0.001). Receiver operating characteristics (ROC) analyses revealed the diagnostic validity of the ELF score in CHC (see Fig. 2 and Table 3).

The manufacturer of the ELF score stated a lower cut-off value of 7.7 for the discrimination of mild fibrosis. We found a 93% (100%) diagnostic sensitivity with a specificity of 33% for the detection of fibrotic (cirrhotic) stages in chronic hepatitis C. For the upper cut-off value of 9.8, we could demonstrate a diagnostic sensitivity of 41% (97%) for the detection of fibrosis stages F1–4 (cirrhosis stages F5/6) with a specificity of 98%. We like to suggest a new cut-off value for the discrimination between fibrotic and cirrhotic stages of 11.3 with a diagnostic sensitivity of 83% and a specificity of 97% (Table 3).

Different receiver operating curve analyses were performed to demonstrate the diagnostic validity of the multiparametric ELF score in comparison to the single serum markers TIMP-1, PIIINP, and HA. Every single marker has its individual power for the detection of different degrees of fibrosis stages and may be differentially affected by inflammatory activity. As shown in Fig. 2, any marker has a high power to differentiate patients with advanced fibrosis/cirrhosis from normal individuals. ELF score and HA demonstrate the best diagnostic efficacy to discriminate cirrhotic from pre-cirrhotic stages (area under the curves (AUC) = 0.95/0.93). However, HA is inferior for the discrimination of early fibrotic changes from non-fibrotic chronic liver disease or normal controls in comparison to TIMP-1, PIIINP, and ELF score. In summary, the ELF score combines the pros of three markers, while it avoids some diagnostic cons of single marker tests. All single markers were affected by the inflammatory activity, which makes it difficult to distinguish a mild/moderate fibrosis from a non-fibrotic stage with a higher degree of histological grading. Therefore, a ROC analysis for normal controls versus the histological staging F0 results in inappropriate high AUC of 0.7–0.8 (Fig. 2). In this context, we also found a significant correlation for ELF score values with the histological grading (r = 0.52, p <0.001) by Spearman ranking analysis.

Fig. 3 summarizes the importance of age and gender dependency for the percentage of ruling in or out potential cases of fibrosis. As an example, the proposed ELF score for ruling out fibrosis is 7.7: patients below this value are most prevalent in young women between 21 and 30 years (54%). The prevalence is lower in men (mean 23%), and the lowest

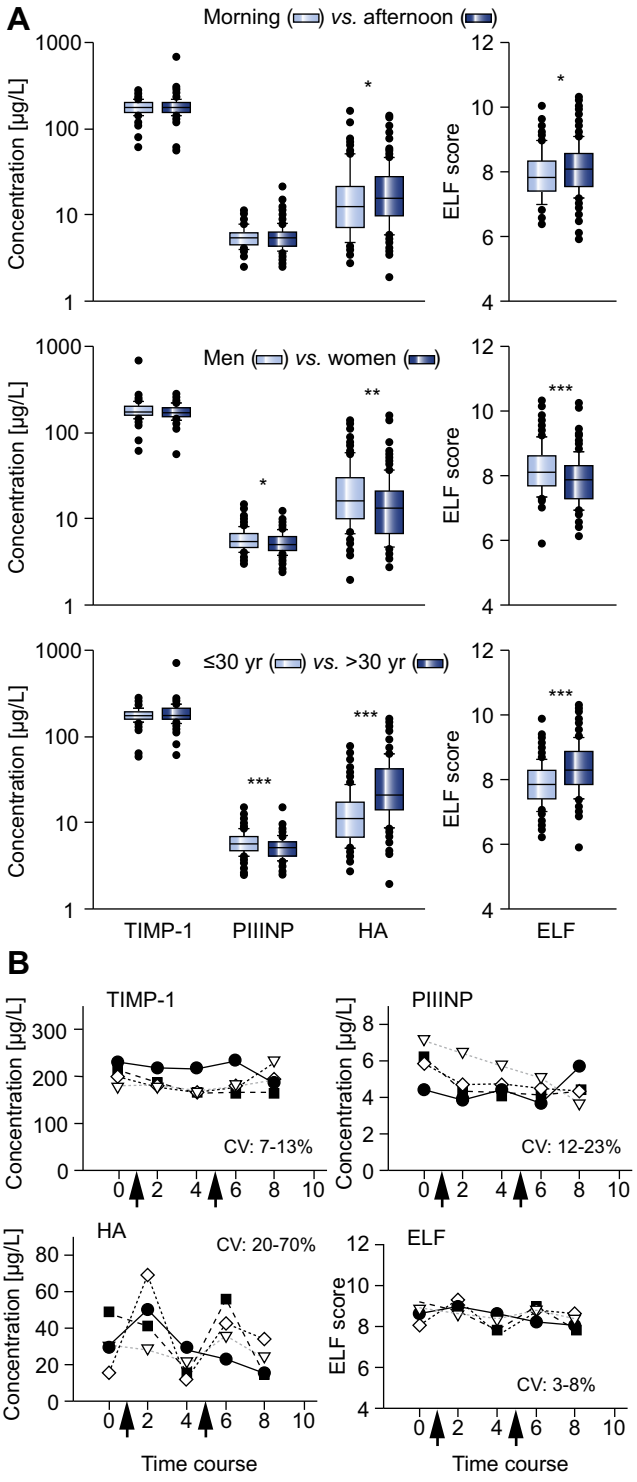

Fig. 1. Factors influencing the ELF score. (A) Box plot comparison of circulating TIMP-1, PIIINP, and HA levels and the respective ELF scores in healthy, non-fasting blood donors. Subjects were analyzed according to subgroups: blood donors coming in the morning (n = 129) *vs.* the afternoon (n = 236), men (n = 187) *vs.* women (n = 200) and age ⩽ 30 years (n = 213) *vs.* >30 years (n = 175). Mann Whitney's *U* test revealed statistical differences (men *vs.* women, morning *vs.* afternoon, ⩽30 years *vs.* >30 years) for PIIINP, HA, and the ELF score; $^{*}p$ <0.05; $^{**}p$ <0.01; $^{***}p$ <0.0001. (B) Four healthy volunteers had five serum samples taken from 8.00 to 16.00 o'clock. Individuals started fasting in the morning (time point 0). Blood samples were taken every two hours. Breakfast and lunch time points are indicated by arrows. The intraday course is shown for the ELF score, TIMP-1, PIIINP, and HA. Intraday coefficients of variation (CV) were calculated separately for each volunteer. The range of CV is indicated. For comparison, biochemical measurands (albumin, ALT, AP, AST, bilirubin, cholesterol, total protein) were determined simultaneously, ranges of CV were from 2% to 11%.

Table 1. Calculation of ELF score reference values.

Controls	N	Reference interval	Mean	Median	Min	Max	Skewness
Total	400	6.6-9.5 (6.7-9.8)	8.06	8.03	5.88	10.30	0.29
Male (M)	187	6.8-9.6 (7.0-9.8)	8.22	8.15	5.88	10.30	0.35
Female (F)	200	6.5-9.3 (6.6-9.3)	7.88	7.90	6.13	10.17	0.21
Morning	129	6.5-9.3 (6.6-9.5)	7.91	7.83	6.33	10.08	0.39
Afternoon	236	6.6-9.6 (6.7-9.9)	8.10	8.07	5.88	10.30	0.29
≤30 yr	213	6.6-9.1 (6.6-9.2)	7.81	7.80	6.13	9.83	0.16
>30 yr	175	6.9-9.8 (6.9-10.1)	8.32	8.30	5.88	10.30	0.18
≤20 yr	32	6.9-8.8	7.88	7.79	6.68	8.97	0.03
21-30 yr	181	6.5-9.1	7.80	7.80	6.13	9.83	0.19
31-40 yr	64	6.6-9.5	8.07	8.07	5.88	10.08	-0.02
41-50 yr	57	7.1-9.5	8.28	8.25	6.95	10.18	0.35
51-60 yr	41	7.2-10.0	8.55	8.48	7.50	10.30	0.53
>60 yr	13	7.7-10.2	8.95	8.96	8.05	10.16	0.53
M ≤20 yr	16	7.3-8.3	7.95	7.79	7.45	8.98	1.02
M 21-30 yr	79	6.9-9.3	8.06	8.04	7.00	9.83	0.46
M 31-40 yr	42	6.7-9.5	8.11	8.07	5.88	9.87	-0.39
M 41-50 yr	23	6.9-9.9	8.40	8.35	6.95	10.18	0.11
M 51-60 yr	16	7.2-10.3	8.76	8.82	7.59	10.30	0.25
M >60 yr	11	7.7-10.3	9.00	8.96	8.05	10.16	0.37
F ≤20 yr	16	6.7-8.9	7.80	7.82	6.86	8.64	-0.24
F 21-30 yr	102	6.4-8.8	7.60	7.56	6.13	9.29	0.14
F 31-40 yr	22	6.5-9.5	8.00	8.03	6.84	10.08	0.60
F 41-50 yr	34	7.2-9.3	8.20	8.20	7.25	9.31	0.40
F 51-60 yr	24	7.2-9.7	8.45	8.45	7.50	10.17	0.66
F >60 yr	2	-	-	-	-	-	-

Reference intervals, mean, median, minimum, and maximum values for the ELF score are calculated for both the entire group of 400 healthy controls and for defined subgroups. Calculations were performed using the *Trillium-Reader* freeware. Additional non-parametric calculations of reference intervals (2.5th–97.5th percentiles) for subgroups with n >120 are shown in parentheses.

Table 2. ELF score in chronic hepatitis C.

Staging	N	2.5^{th} -97.5^{th} percentile	Mean	Median	Min	Max	Skewness
F0	21	6.7-11.1	8.9	8.9	7.3	13.2	2.08
F1/2	19	7.4 -11.3	9.4	9.6	7.3	11.1	-0.67
F3/4	10	7.8-12.2	10.0	9.8	8.4	12.2	0.72
F1-4	29	7.5-11.7	9.6	9.7	7.3	12.2	0.04
F5/6	29	10.0-14.3	12.2	12.4	9.7	13.6	-0.66

ELF scores were calculated from 79 patients with chronic hepatitis C, which were histologically staged according to Ishak *et al.* [5] (F0 n = 21, F1–4 n = 29, F5/6 n = 29 (among these 22 with decompensated end-stage cirrhosis)). Assessed 2.5th to 97.5th percentiles, mean, median, minimum and maximum values and skewness of the subgroups were calculated. Calculations were performed using the *Trillium-Reader* freeware.

prevalence was observed in the elderly population (0% above 60 years).

Discussion

Much effort has been put in the search of non-invasive replacement of liver biopsy for the staging of chronic liver disease [10]. Currently, we are facing a number of different clinical chemical as well as modified imaging techniques, none of which has found widespread and universal acceptance yet [19–21]. Device-dependent techniques such as FibroScan® are largely limited to high-volume centers. In contrast, biochemical methods allow for a more widespread use. However, most of the currently endorsed multiparametric scoring systems lack a full clinical chemical evaluation.

The ELF score was originally assembled from an array of measurands of extracellular matrix metabolism. It was validated in a large group of patients with chronic liver disease of different etiologies [15]. Afterwards, ELF score performance was confirmed in a number of different patient groups [22–28]. Recently, ELF score measurement was made commercially available for clinical use (ADVIA Centaur CP immunochemical analyzer). However, currently there is no appropriately published evaluation, which describes the behavior of the ELF score in the assumingly healthy population. In this study, we have therefore defined the reference

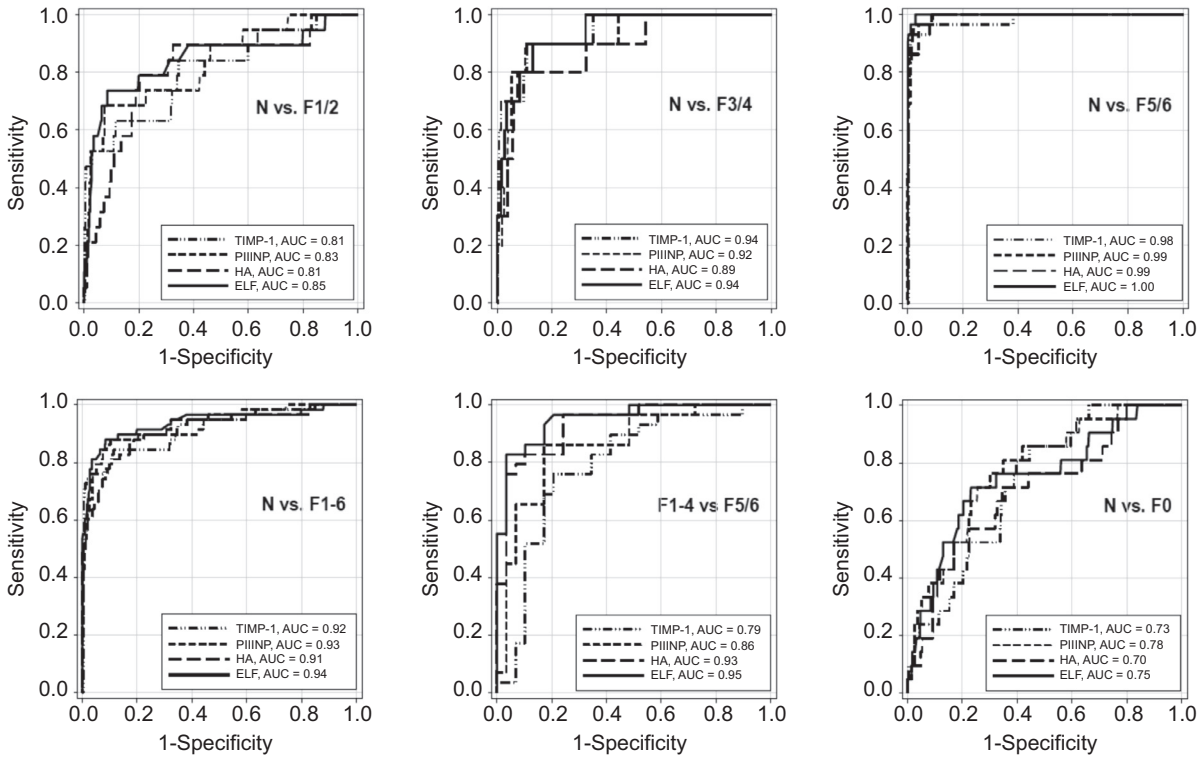

Fig. 2. ROC analysis: ELF score *vs.* single measurands. The diagnostic value of the ELF score in comparison to the single measurands TIMP-1, PIIINP, and HA is analysed by receiver operating characteristics (ROC) analysis. The ROC analyses included 79 CHC patients (for details see Fig. 4 legend) and 400 healthy controls. Areas under the curves (AUC) are indicated for all parameters.

ranges of the ELF score and reevaluated the cut-off values, which have been suggested for the detection of liver fibrosis and cirrhosis.

Analysis of ELF scores in healthy controls revealed slight intra-day variation, which appeared to depend on food uptake. Most variability was caused by differences in hyaluronic acid concentrations. However, the ELF score only partially mirrored HA fluctuations. In total, ELF score variability did not exceed that of standard laboratory measurands such as aminotransferases. Therefore, measurement of the ELF score using fasting conditions in the morning does not appear necessary.

ELF scores significantly depended on gender and age of the tested subjects. Women exhibited significantly lower ELF scores than men. Even more, ELF scores increased with age. We have therefore calculated sex and age-dependent reference intervals, which may prevent misinterpretation especially in cases close to the proposed cut-off values. Even in healthy controls, a small proportion showed ELF scores beyond the high specificity fibrosis cut-off, thus emphasizing that scores always need to be interpreted with caution.

Further, we preliminary evaluated the potential influence of factors predisposing to fatty liver on the ELF score. Therefore, we included an analysis of BMI, ALT and ELF score (n = 136, data not shown). Because of the strong age-dependency of the ELF score, correlation analyses were restricted to age decades rather than examining the entire study group. The sizes of two age–matched subgroups appeared appropriate for statistical evaluation (21–30 years, n = 56, BMI 24.8 ± 3.9; 31–40 years, n = 30, BMI 26.4 ± 5.7). While ALT correlated with BMI (r = 0.54, r = 0.26; resp.), no significant correlations between BMI and ELF were discovered (r = 0.11, r = 0.05; resp.). Similarly, Wahl *et al.* previously showed no correlation between the ELF score and the percentage of liver steatosis (r = 0.01) [29].

In a second step, we have applied the ELF score to a histologically characterized group of chronic hepatitis C patients. ROC analysis revealed that persons unaffected by hepatitis C can be distinguished, with high sensitivity and specificity, from CHC. Furthermore, early stages of fibrosis were clearly separated from late stage fibrosis or cirrhosis using ELF score cut-offs of 7.7/9.8/11.3. However, overlap of the 2.5–97.5% CI was considerable, especially in early stages of fibrosis.

This might be attributed to the fact that markers of ECM metabolism represent both the amount of deposited matrix as well as the rate of matrix deposition. Thus, the ELF score is also influenced by disease activity as shown by its correlation with markers of inflammation. Particularly in early fibrosis, matrix turnover will have a relatively higher influence on fibrosis markers than the amount deposited matrix, explaining the difficulties of separating early fibrosis stages by biomarkers if significant inflammation is present.

Which patients remain candidates for liver biopsy and how should patients be stratified according to the individual ELF scores? As summarized in Fig. 4, a cut-off value of 7.7 is recommended for high sensitivity exclusion of fibrosis. Patients below this level do not require liver biopsy and occurrence of liver fibrosis is highly unlikely. However, as shown in our study, scores below 7.7 are mostly found in young women. In patients above

Table 3. Evaluation of ELF score cut-off values.

ELF score	AUC	Cut-off	Sensitivity	Specificity
N *vs.* F1/2	0.85 ± 0.06	7.7 9.8	89.5 (66.9-98.7) 36.8 (16.3-61.6)	33.3 (28.7-38.2) 97.8 (95.8-99.0)
N *vs.* F3/4	0.94 ± 0.03	7.7 9.8	100 (69.2-100) 50.0 (18.7-81.3)	33.3 (28.7-38.2) 97.8 (95.8-98.8)
N *vs.* F5/6	1.00 ± 0.001	7.7 9.8 11.3	100 (88.1-100) 96.6 (82.2-99.9) 81.0 (62.3-93.1)	33.3 (28.7-38.2) 97.8 (95.8-98.8) 100 (99.1-100)
N *vs.* F1-4	0.88 ± 0.04	7.7 9.8 11.3	93.1 (77.2-99.2) 41.4 (23.5-61.1) 6.9 (0.8-22.8)	33.3 (28.7-38.2) 97.8 (95.8-99.0) 100 (99.1-100)
N *vs.* F1-6 (F5/6: 50%)	0.94 ± 0.02	7.7 9.8 11.3	96.6 (88.1-99.6) 69.0 (55.5-80.5) 43.1 (30.2-56.8)	33.3 (28.7-38.2) 97.8 (95.8-99.0) 100 (99.1-100)
N *vs.* F0-6 (F0: 27%, F5/6: 37%)	0.89 ± 0.02	7.7 9.8 11.3	94.9 (87.5-98.6) 54.4 (42.8-65.7) 32.9 (22.8-44.4)	33.3 (28.7-38.2) 97.8 (95.8-99.0) 100 (99.1-100)
F1-4 *vs.* F5/6	0.95 ± 0.03	7.7 9.8 11.3	100 (88.1-100) 96.6 (82.2-99.9) 82.8 (64.2-94.1)	5.2 (0.8-20.3) 58.6 (38.9-76.5) 96.6 (82.2-99.9)
F0-4 *vs.* F5/6	0.95 ± 0.02	7.7 9.8 11.3	100 (88.1-100) 96.6 (82.2-99.9) 82.8 (64.2-94.1)	10.0 (3.3-21.8) 70.0 (55.4-82.1) 96.0 (86.3-99.5)
F0-2 *vs.* F3-6	0.90 ± 0.04	7.7 9.8 11.3	100 (91.0-100) 84.6 (69.5-94.1) 64.1 (47.2-78.8)	12.5 (4.2-26.8) 75.0 (58.8-87.3) 97.5 (86.8-99.9)

Area under the curves (AUC), diagnostic sensitivities and specificities were calculated for different ELF score cut-off values using receiver operating characteristics (ROC) analyses for the discrimination of cirrhotic, fibrotic, non-fibrotic chronic liver disease and normal individuals. For the description of study subjects see also Table 2 legend.

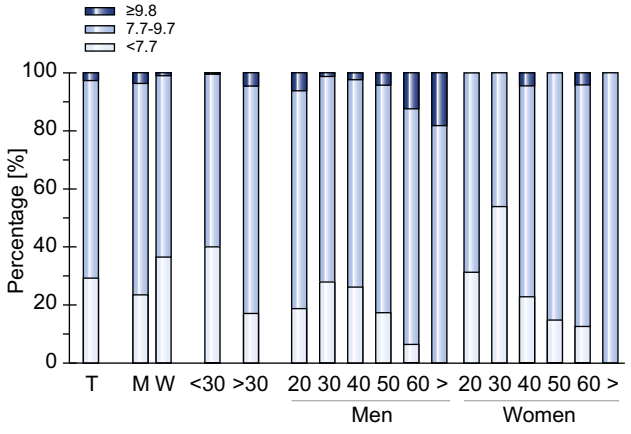

Fig. 3. ELF score cut-offs: value distribution in healthy controls. The proportional distribution of ELF score values in healthy controls is shown for the total group (T) and for men (M), women (W), <30 years, >30 years, and separately, according to age decades (11–20, 21–30, 31–40, 41–50, 51–60, 61–70) and sex. Stacked vertical bar charts with light blue bars representing an ELF score <7.7, blue bars representing 7.7–9.7, and dark blue bars representing values ⩾9.8 are shown.

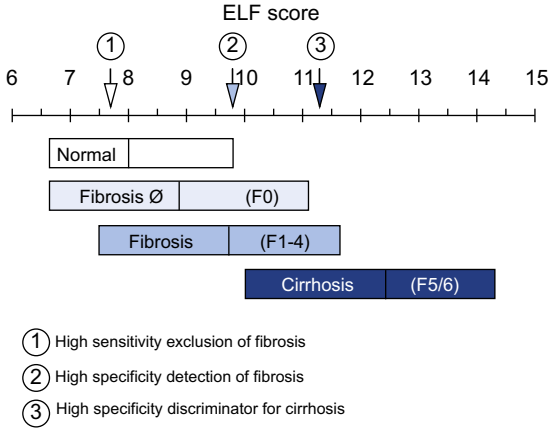

Fig. 4. ELF score discrimination between different fibrosis stages in chronic hepatitis C. ELF scores were calculated in 400 healthy controls (white) and 79 CHC patients according to fibrosis stage (F0: n = 21, light blue; F1–4: n = 29, blue; F5/6: n = 29, dark blue, 22 of the latter with decompensated cirrhosis). Boxes represent the 2.5th to 97.5th percentile range with horizontal lines for the median values (Table 1). Arrows indicate the cut-off values proposed for the ELF score: white arrow (①) for a high sensitivity exclusion of fibrosis (ELF score 7.7), blue arrow (②) for the high specificity detection of fibrosis (ELF score 9.8), and dark blue arrow (③) for the discrimination of cirrhosis (ELF score 11.3). Applying the lower cut-off ①, 4% of our CHC patients were below this value. Applying the upper cut-off ③, 29% of our CHC patients were above this value.

60 years of age, ELF scores below 7.7 are rather rare events and an adopted cut-off may be preferred.

In contrast to the actual manufacturer's manual, ELF score values between 9.8 and 11.3 cannot be clearly attributed to a certain stage of fibrosis. Cirrhosis is still unlikely, as is freedom of fibrosis. However, higher degrees of inflammatory activity may blur the picture. Therefore, liver biopsy may be required in these patients if reliable fibrosis staging is needed.

An ELF score value above 11.3 is a high specificity discriminator for cirrhosis. For fibrosis staging, no liver biopsy will be needed in these patients.

In conclusion, the ELF score appears to be a valuable tool for fibrosis staging in chronic liver disease. However, influence fac-

tors such as gender and age need to be taken into account. This is of particular relevance in the evaluation of low level fibrosis.

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Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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