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Enhanced liver fibrosis (ELF) score: Reference ranges, biological variation in healthy subjects, and analytical considerations

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ABSTRACT

Background: The Enhanced Liver Fibrosis score has been recognized as a non-invasive test for liver fibrosis. However, reference intervals, biological variation and analytical performance have not been studied in detail so far. The aim was to acquire data that are essential for correct interpretation.

Methods: A total of 40 apparently healthy volunteers were evaluated for reference ranges of serum concentration of hyaluronic acid, aminoterminal propeptide of type III collagen, and tissue inhibitor of metalloproteases-1, and calculated ELF score. A subgroup of 20 subjects was evaluated repeatedly for 7 weeks. For all variables, reference intervals, within-subject and between-subject biological variations, reference change values, and the indexes of individuality were assessed. Analytical performance (intermediate precision) and interlaboratory comparison were also evaluated.

Results: The reference ranges were 5.1–62.7 μg/L for HA, 3.56–12.6 μg/L for PIIINP, 143.6–265.3 μg/L for TIMP-1, and 7.14–9.55 for the ELF score. The within-subject variations were 32.7, 10.6, 4.2, and 3.2% for HA, PIIINP, TIMP-1, and ELF score, respectively. Similarly, the between-subject variations were 59.0, 13.3, 12.8, and 5.2%. For the ELF score, RCV was 10.1% and II was 0.62. The intermediate precisions were < 5%, < 6%, and < 10% for HA, PIIINP, and TIMP-1, respectively.

Conclusion: The reference range of the ELF score overlap with the area defined as moderate fibrosis by the manufacturer. High biological variation of HA was diminished by the natural logarithm in the calculation of the ELF score. The use of the ELF score has suitable analytical and acceptable biological performance characteristics for clinical practice. However, the transfer of results evaluated in healthy persons to the populations with chronic liver diseases deserves caution.

1. Introduction

Liver fibrosis can be assessed by a number of biomarkers and scoring systems. The Enhanced Liver Fibrosis (ELF) score is a commercially available index (ADVIA Centaur, Siemens Healthcare Diagnostics, NY, USA), based on serum concentrations of hyaluronic acid (HA), aminoterminal propeptide of type III collagen (PIIINP), and tissue inhibitor of metalloproteases-1 (TIMP-1). As a non-invasive test, the ELF score has been tested in the diagnosis of liver fibrosis, e.g., in nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), chronic hepatitis B and C, liver cirrhosis, systemic diseases, and others [1–7]. Correct interpretation of every non-invasive laboratory test is based on detailed information on analytical performance, reference ranges, biological variation, and diagnostic efficiency. In spite of this necessary approach, such data on the ELF score are rather scarce. Neuman [8] stated in her excellent review that "many critical gaps exist in the reference interval database of most of the biomarkers that have been identified for evaluation of hepatic fibrosis." The aim of our study was to evaluate the analytical performance, reference ranges, and biological variation of the ELF score and its components, i.e., HA, PIIINP, and TIMP-1.

Abbreviations: ALT, alanine aminotransferase; AP, alkaline phosphatase; PIIINP, aminoterminal propeptide of type III collagen; CV_A, analytical variation; AST, aspartate aminotransferase; CV_G, between-subject biological variation; CI, confidence interval; ELF, enhanced liver fibrosis; GGT, gamma-glutamyl transferase; HA, hyaluronic acid; II, index of individuality; LoD, limits of detection; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; RCV, reference change value; TIMP-1, tissue inhibitor of metalloproteases-1; CV_I, within-subject biological variation

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2. Patients and methods

2.1. Study subjects

A total of 40 apparently healthy persons (20 men, 20 women, Caucasian race) were invited to take part in a study for the evaluation of reference limits and biological variations (within- and between-subject variations) of the ELF score and its components, HA, PIIINP, and TIMP-1.

All study subjects were 20–50-year-old healthy non-smokers with common lifestyles [10]. All study subjects met the conditions necessary for inclusion in the study, including subjectively good health, no acute or chronic illness, no medication, and no excesses in diet and lifestyle. All subjects signed an informed consent. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the IKEM Ethics Committee. Blood samples were taken in equidistant intervals; the study lasted for 6 weeks, and thus, 7 venous blood samples were available from every study subject. The first sample drawn from each subject was used for ELF reference range determination. All participants were instructed to avoid any physical exercise for at least 24 h prior to sampling and to be in a fasting state (no food intake for at least 12 h before sampling). Basic characteristics of the study subjects are given in Table 1. Complete descriptions of the health status of all subjects were available as well. Altogether, 40 subjects were tested for the derivation of reference ranges and 20 of them for biological variation. Liver function tests of the participants of the study are given in Table 1.

2.2. Sampling and sample preparation

The study subjects were in a sitting position for at least 5 min (but not > 10 min) before and during sampling. Two randomly assigned phlebotomists were responsible for the sampling. Venous blood was taken between 08:00 and 10:00 a.m. The Vacuette system (VACUETTE® TUBE 8 mL Z Serum Separator, Clot Activator cat. No. 455071, Greiner Bio-One GmbH, Germany) was used together with 21-gauge needles (Greiner Bio-One GmbH, Germany). Separation of blood corpuscles was done within 60 min after sampling at 3000 *g* for 10 min (centrifuge Beckman Allegra, Beckman, USA). Five serum aliquots of 500 μL were prepared within 60 min after centrifugation. CryoKing tubes were used (Biologix Group Limited, cat. No. 89–3101, China). The serum aliquots were stored at −80 °C before analysis. The samples were inspected for hemolysis, icterity and chylosity prior to storage.

2.3. Analytical methods

Serum concentrations of HA, PIIINP, and TIMP-1 were measured by The ADVIA Centaur® Hyaluronic Acid (HA) assay, lot 25,215,019, The ADVIA Centaur® Amino-Terminal Propeptide of Type III Procollagen (PIIINP) assay, lot 26,290,023, and The ADVIA Centaur® Tissue Inhibitor of Matrix Metalloproteinase 1 (TIMP-1) assay, lot 28,900,016, respectively (Siemens Healthcare Diagnostics, NY, USA).

The calibrator used for HA, PIIINP, and TIMP-1 was ADVIA Centaur ELF calibrator, lot 69948A12.

Control materials used were ADVIA Centaur ELF quality control materials: 1/low - 2,427,371; 2/medium - 2,427,372; and 3/high - 2,427,373, respectively. Repeatabilities (within-run CV) as declared by the manufacturer were < 5.6, < 4.2, and < 3.3%, for HA, PIIINP, and TIMP-1, respectively. Intermediate precisions (between-run CV) were < 3.2, < 5.1, and < 5.5% for HA, PIIINP, and TIMP-1, respectively.

The measurement ranges for HA, PIIINP, and TIMP-1 were 1.6–1000, 0.5–150, and 3.5–1300 μg/L, respectively. Traceability was not provided by the manufacturer. Limits of detection (LoD) of HA, PIIINP, and TIMP-1 were 1.6, 0.5, and 3.5, respectively.

All measurements were performed in one run during one day by the same laboratory technician. We used the Centaur CP immunochemistry analyzer (Siemens Healthcare Diagnostics, NY, USA). ELF was calculated according to the Centaur CP formula: 0.846*ln(HA) + 0.735*ln (PIIINP) + 0.391*ln(TIMP-1) + 2.494.

Because of the high number of samples (seven samples drawn from each of the 20 study subjects) we decided to perform a single measurement of analyte concentration with repeated measurements of control samples (low, medium, high) before the analytical run and after every 35th serum sample from study subjects. Therefore, it was possible to assess repeatability (Table 3), which was used for the calculation of biological variability. All of the 140 study subjects´ samples were measured in a random order generated by an Excel function.

2.4. Statistical evaluation

Reference ranges (95%) were calculated with a robust technique according to the CLSI C28-A3 protocol, and the 90% confidence interval (CI) of the lower and upper reference limits was calculated as well. The distribution of HA and PIIINP was not Gaussian; therefore, the Box-Cox transformation was used for these two analytes.

Components of variance were transformed to the CV using overall means. The Iglewicz-Hoaglin method (NIST/SEMATECH) for outlier detection was used for HA (as the Cochran test would eliminate an unrealistic number of subjects). Next, an algorithm recommended by Braga was used. The only difference was the use of the Fligner-Kileen test instead of the Cochran test due to the heterogeneity of variances, as recommended by Roraas [10–12]. Components of biological variation (CV_I, within-subject variation; CV_G, between-subject variation) were calculated with a mixed linear regression model with the aid of R software version 3.4.0. The reference change value (RCV) and index of individuality (II, based on CV_I and CV_G, without CV_A taken into account) were calculated as well. We added the calculation of lognormal RCV for decrease and lognormal RCV for increase according to Fokkema [13].

Table 1
Basic characteristics of the 40 apparently healthy subjects with common lifestyles, Caucasian race [10]. The Mann-Whitney test was used for testing the difference between men and women.

	Men (N = 20)		Women (N = 20)			All (*N* = 40)	
	Median	IQR	Median	IQR	p	Median	IQR
Bilirubin (μmol/L)	14.3	11.4–20.6	11.2	8.9–19.7	< 0.0001	13.7	9.5–20.3
ALT (U/L)	32.1	27.6–40.2	27.9	24.6–32.7	0.036	29.4	25.8–36.3
AST (U/L)	28.5	24.6–36.9	24.0	22.2–30.0	N.S. (0.0547)	26.4	22.5–32.4
AP (U/L)	72.9	60.6–81.3	66.0	54.3–80.1	N.S. (0.4171)	69.6	58.8–81.0
GGT (U/L)	21.3	19.5–25.8	18.6	13.8–23.4	N.S. (0.1719)	20.4	16.5–24.9
Albumin (g/L)	53.2	51.1–54.8	49.3	47.9–50.8	0.0007	51.0	49.2–53.8

ALT = alanine aminotransferase (GPT), AST = aspartate aminotransferase (GOT), AP = alkaline phosphatase, GGT = gamma-glutamyl transferase.