Clinica Chimica Acta 461 (2016) 151–155

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Enhanced liver fibrosis (ELF) score: Analytical performance and distribution range in a large cohort of blood donors

Alessandra Dellavance [a], Flávia Fernandes [b,c,*], Natália Shimabokuro [d], Flavia Latini [e], Danielle Baldo [a], José Augusto Barreto [e], Valdecir Marvulle [f], Luis Eduardo Andrade [g], Maria Lucia Ferraz [h,i]

[a] Research and Development Division, Fleury Group, São Paulo, Brazil
[b] University of the State of Rio de Janeiro, Gastroenterology and Hepatology Department, Rio de Janeiro, Brazil
[c] Bonsucesso Federal Hospital, Gastroenterology and Hepatology Department, Rio de Janeiro, Brazil
[d] Federal University of São Paulo, Rheumatology Division, UNIFESP, São Paulo, Brazil
[e] Benevolent Association of Blood Collection, Colsan, São Paulo, Brazil
[f] Federal University of São Paulo, Statistics Department, UNIFESP, São Paulo, Brazil
[g] Federal University of São Paulo, Rheumatology Department, São Paulo, Brazil
[h] Federal University of São Paulo, Gastroenterology Department, São Paulo, Brazil
[i] D'Or Institute for Research and Education, Rio de Janeiro, Brazil

ARTICLE INFO

Article history:
Received 28 July 2015
Received in revised form 21 July 2016
Accepted 8 August 2016
Available online 9 August 2016

Keywords:
Elf
Coefficient of variation
Liver fibrosis
Hyaluronic acid
Metalloproteinases

ABSTRACT

Background: The Enhanced Liver Fibrosis (ELF) is a serological score that includes hyaluronic acid (HA), tissue inhibitor of matrix metalloproteinases-1(TIMP-1), and aminoterminal propeptide of type III procollagen (PIIINP) and shows good performance for detecting liver fibrosis. There are few studies evaluating ELF's intra and inter-assay variation and stability of the samples. The influence of host variables, such as age, gender and body mass index (BMI) is also not well known. We determined ELF's analytical performance and possible influences of gender, age and BMI.
Methods: The study included 958 healthy blood donors evaluated for age, gender, and BMI.
Results: Mean ELF scores were significantly different between female (8.53 ± 0.75) and male groups (8.76 ± 0.76) and also according to age strata ($p < 0.001$). For both genders, ELF significantly varied in individuals with BMI under 25 ($p < 0.001$). Analytes remained stable after freezing/thawing cycles and intra- and inter-assay coefficients of variation were low.
Conclusions: ELF has appropriate precision and is quite robust, due to the high stability of the analytes in fresh and frozen samples. ELF's results are influenced by gender, age and BMI which should be taken into account when analyzing its results.

1. Background

The Enhanced Liver Fibrosis (ELF) score is a mathematical index derived from the serum concentration of hyaluronic acid (HA), tissue inhibitor of matrix metalloproteinases-1(TIMP-1), and aminoterminal propeptide of type III procollagen (PIIINP) [1]. ELF score has demonstrated good performance in detecting fibrosis as well as in providing valuable prognostic information in many distinct clinical scenarios [2–11]. Its accuracy in estimating the fibrosis stage is well established in chronic liver diseases, especially in chronic hepatitis C [12–17]. Recently, it has also demonstrated to be a useful marker of fibrotic involvement in systemic sclerosis [18]. It also has shown to be of prognostic value in primary sclerosing cholangitis [19] and in chronic hepatitis B [20].

ELF is commercially available for clinical use since 2010 (ADVIA Centaur CP immunochemical analyzer, Siemens Healthcare Diagnostics). Since then, many studies have been performed but they usually do not evaluate the performance of the ELF score regarding intra and inter-assay precision and stability of the samples. Both in patients with or without fibrosis, few studies have evaluated the influence of host variables, such as age, gender and body mass index (BMI) [4,21–23].

To the best of our knowledge only three groups have evaluated the range of ELF panel in healthy individuals [21–23]. Lichtinghagen et al. [21] studied 400 healthy individuals and observed that gender and age influenced ELF score. In contrast, an Asian study by Yoo et al. [22]

Abbreviations: ELF, enhanced liver fibrosis; HA, hyaluronic acid; TIMP-1, tissue inhibitor of matrix metalloproteinases-1; PIIINP, propeptide of type III procollagen; F/T, freezing/thawing..

* Corresponding author at: Bonsucesso Federal Hospital, Gastroenterology and Hepatology Dept., Av. Londres, 616 - Bonsucesso, Rio de Janeiro 21041-030, Brazil.
E-mail address: ffernandes.flavia@gmail.com (F. Fernandes).

http://dx.doi.org/10.1016/j.cca.2016.08.006
0009-8981/

did not find age as an interfering factor, but confirmed gender and BMI as modulating factors of ELF score.

2. Material and methods

Blood donors were sequentially retrieved in the period of 06/01/2013 to 07/01/2013 to build a large collection with comparable representation of gender and age decades from 16 to 60 y. Healthy blood donors from Colsan - Benevolent Association of Blood Collection in São Paulo, Brazil, were invited for the study. Initially 2000 blood donors were recruited in the blood bank in order to compose a group of up to 1000 serum samples distributed according to gender and age. The 2000 serum samples were grouped according to gender and age (≤20; 21–30; 31–40; 41–50; 51–60 and finally ≥61 y). From this total we randomly selected 958 samples with a balanced distribution among different ages and genders strata, respecting the recommendations of Clinical and Laboratory Standards Institute's (CLSI) for determination of reference values. [24].

The exclusion criteria were age <16 y and >69 y, weight lower than 50 kg and individuals infected with HIV and hepatitis B or C. Besides age and gender, volunteers were also evaluated concerning the body mass index. Blood samples were collected in the morning after a light meal and allowed to clot for 30 min at room temperature. Afterwards they were centrifuged at 1600*g* for 15 min at 4 °C. Sera were coded and frozen at −80 °C within 2 h after collection until the time of the use.

Analyte stability was verified in three samples that were divided into several aliquots that were subjected to progressively increasing number of freezing/thawing cycles at 24-h intervals. Each aliquot received a label specifying the number of freezing/thawing cycles. Aliquot F/T#1 was subjected to the process only once; aliquot F/T#2 was subjected twice to the process and so on up to aliquot F/T#9. All aliquots were kept at -80 °C until processing. Two additional aliquots from each sample were kept at room temperature for 24 h. PIIINP, HA, and TIMP-1 were determined in the three fresh samples before distribution into aliquots and in all aliquots of these three samples. Rules recommended by the Clinical and Laboratory Standards Institute (CLSI) according EP5-A2 were followed to evaluate the inter and intra-assay precision. [25]. Aliquots of sera pools with values strictly pre-defined by the manufacturer as high and intermediate were evaluated in quadruplicate in a same batch routine testing and over five consecutive days.

PIIINP, HA, and TIMP-1 were measured in all patients in a random-access automated clinical immunochemistry analyzer that performs magnetic separation enzyme immunoassay tests (ADVIA Centaur™, Siemens Healthcare Diagnostics). The ELF score was calculated using the algorithm: $ELF = 2.278 + 0.851 \ln(HA) + 0.751 \ln(PIIINP) + 0.394 \ln(TIMP\text{-}1)$ as previously established [1].

Data were analyzed using IBM SPSS 18 for Windows (IBM, Ehningen, Germany). Continuous variables were reported as mean ± standard deviation (SD). Discrete variables were reported as absolute and relative frequency. Significance level was determined when $p \le 0.05$ assuming two-tailed tests. Distribution analysis were performed using Kolmogorov-Smirnov Test to check whether the variables exhibited a normal distribution pattern. For means comparisons test and ANOVA (Bonferroni) were applied. To evaluate possible interactions between age and BMI Pearson Correlation coefficient and linear regression were applied. The study protocol was conducted in accordance with Helsinki Declaration, and was approved by the local Ethics Committee. All blood donors signed an informed consent upon enrollment in the study.

3. Results

Results of ELF score are shown in Table 1 and Fig. 1. Mean ELF value was 8.68 ± 0.80, with the following percentile distribution: 8.11 for percentile 25, 8.67 for percentile 50, and 9.23 for percentile 75. The range was from 6.25 to 11.98. After the initial distribution nine donors behaved as outliers and were excluded from the subsequent analysis. For the remaining 949 healthy blood donors ELF value was 8.65 ± 0.76, with the following percentile distribution: 8.10 for percentile 25, 8.66 for percentile 50, and 9.21 for percentile 75. The range was from 6.25 to 10.85.

Table 1
ELF Results according to gender, age and body mass index.

Blood donors	N	ELF min.	ELF max.	Mean	p value
Total	949	6.52	10.85	8.65 ± 0.76	
Men	501 (53%)	6.54	10.85	8.76 ± 0. 76	<0.001
Women	448 (47%)	6.52	10.6	8.53 ± 0. 75	
Comparison among age strata (y)					
≤30 (a)	222 (23.3%)	6.79	10.45	8.20 ± 0.05	<0.001
31–50 (b)	403 (42.5%)	6.52	10.37	8.53 ± 0.03	
≥51 (c)	324 (34.2%)	7.58	10.85	9.12 ± 0.03	
Comparison among age strata for men (y)					
≤30 (a)	111 (22.1%)	7.09	10.45	8.41 ± 0.69	<0.001*
31–50 (b)	206 (41.2%)	6.54	10.32	8.57 ± 0.73	
≥51 (c)	184 (36.7%)	7.58	10.85	9.21 ± 0.62	
Comparison among age strata for women (y)					
≤30 (a)	111 (24.8%)	6.79	9.46	8.00 ± 0.69	<0.001
31–50 (b)	197 (44.0%)	6.52	10.37	8.49 ± 0.69	
≥51 (c)	140 (31.2%)	7.94	10.60	9.01 ± 0.55	
Comparison among three BMI strata					
≤25 (d)	340 (35.8%)	6.79	10.70	8.42 ± 0.04	<0.001
25–30 (e)	407 (42.9%)	6.54	10.85	8.75 ± 0.03	
≥30 (f)	202 (21.3%)	6.52	10.53	8.86 ± 0.05	
Comparison among BMI strata (only men)					
≤25 (d)	151 (30.1%)	6.98	10.70	8.62 ± 0.73	<0.001**
25–30 (e)	241 (48.1%)	6.54	10.85	8.79 ± 0.75	
≥30 (f)	109 (21.8%)	7.00	10.53	8.90 ± 0.79	
Comparison among BMI strata (only women)					
≤25 (d)	189 (42.2%)	6.79	10.37	8.26 ± 0.75	<0.001**
25–30 (e)	166 (37.0%)	6.93	10.6	8.68 ± 0.66	
≥30 (f)	93 (20.8%)	6.52	10.37	8.81 ± 0.72	

Data expressed as mean, ± standard deviation or absolute (%). BMI, body mass index; ELF, enhanced liver fibrosis; min, minimum; max, maximum. Significance level 0.05.

* Significantly difference observed only among groups (c) and (b) and groups (c) and (a), but not for groups (a) and (b).

** Significantly difference observed only for groups (d) and (e) and groups (d) and (f), but not for groups (e) and (f).

The studied population had 53% males and a mean age of 42.51 ± 13.71 y (range 16–68). The mean BMI was 27.06 ± 4.32 (range 18.65–47.18), with 35.8% below 25, 42.9% between 25 and 30, and 21.3% over 30. For analysis purposes, the studied individuals were divided into 3 groups: (a) <30 y (23.3%), (b) between 30 and 50 y (42.5%) and (c) >50 y (34.2%).

Mean ELF scores were significantly different between female (8.53 ± 0.75) and male groups (8.76 ± 0.76), ($p < 0.001$) (Fig. 1). In men and women, the mean ELF score differed significantly according to age strata (Fig. 1). Mean ELF score in individuals <30 y was 8.00 (female) and 8.41 (male); between 31 and 50 y, the mean ELF score was 8.49 (female) and 8.57 (male); and for those >50 y, the mean score was 9.01 (female) and 9.21 (male). Among women there were significant differences in ELF panel throughout all ages. For men the significance was observed only for individuals >51 y. There was no significant difference between groups (a) and (b) (Table 1).

For analysis purposes, the studied individuals were divided into three groups: (d) BMI under 25, (e) BMI between 25 and 30 and (f) BMI >30. The mean ELF score in individuals with BMI under 25 was 8.26 (female) and 8.62 (male); for those with BMI between 25 and 30, the mean ELF score was 8.68 (female) and 8.79 (male); and for those with BMI above 30, the mean ELF score was 8.81 (female) and 8.90 (male). For both genders, individuals with BMI <25 differed significantly from the other two BMI subgroups ($p < 0.001$), but no significant difference was observed among groups (e) and (f) (Table 1).