DOI: 10.1111/j.1468-1293.2009.00745.x
HIV Medicine (2010), 11, 64–73

ORIGINAL RESEARCH

Diagnosis of advanced fibrosis in HIV and hepatitis C virus-coinfected patients via a new noninvasive index: the HGM-3 index

S Resino,[1] D Micheloud,[1] P Miralles,[2] JM Bellón,[3] A Vargas,[1] P Catalán,[4] E Álvarez,[5] J Cosín,[2] R Lorente,[6] JC López,[2] MA Muñoz-Fernández[6] and J Berenguer[2]

[1]*Laboratory of Molecular Epidemiology of Infectious Diseases, National Centre of Microbiology, Instituto de Salud Carlos III, Majadahonda, Madrid,* [2]*Infectious Diseases-HIV Unit,* [3]*Biomedical Research Foundation,* [4]*Microbiology Department,* [5]*Pathology Department and* [6]*Molecular Immunobiology Laboratory, Hospital General Universitario 'Gregorio Marañón', Madrid, Spain*

Background

Noninvasive tests are increasingly being used for the assessment of liver fibrosis. We aimed to develop a serum index for the identification of advanced fibrosis ($F \geq 3$) in HIV/hepatitis C virus (HCV)-coinfected patients.

Methods

We carried out a cross-sectional study on a group of 195 patients comprised of an estimation group (EG; $n = 127$) and a validation group (VG; $n = 68$) who all underwent liver biopsy and had not received previous interferon therapy. Liver fibrosis was estimated using the METAVIR score. We developed a new serum index (HGM-3) dependent on levels of platelets, alkaline phosphatase, hepatic growth factor, tissue inhibitor of metalloproteinase-1 and hyaluronic acid.

Results

In the EG, the area under the receiver operating characteristic curve (AUC-ROC) of HGM-3 for identification of $F \geq 3$ was 0.939 [95% confidence interval (CI) 0.899, 0.979] which was significantly higher than the AUC-ROC of the HGM-2, FIB-4, aspartate aminotransferase to platelet ratio (APRI) and Forns' indexes. With HGM-3 <0.135 for $F<3$, 57 patients were correctly identified and two patients were misclassified. We found the presence of $F<3$ with 96.6% certainty. The negative likelihood ratio (LR) was <0.1 and the diagnostic odds ratio (DOR) was >40. With HGM-3 >0.570 in the EG for $F \geq 3$, 31 patients were correctly identified, and five patients were misclassified. We found the presence of $F \geq 3$ with 86.1% certainty. The positive LR was >12 and the DOR was >40. For the VG, the diagnostic accuracy values were similar to the values for the EG.

Conclusions

HGM-3 appears to be an accurate noninvasive method for the diagnosis of bridging fibrosis and cirrhosis in HIV/HCV-coinfected patients.

Keywords: liver cirrhosis, serum predictive markers, liver fibrosis, diagnostic accuracy, chronic hepatitis C

Accepted 14 May 2009

Introduction

HIV infection adversely impacts the natural pathology of hepatitis C virus (HCV) infection, causing a more rapid progression to fibrosis and the development of cirrhosis, hepatic decompensation, hepatocellular carcinoma and death [1–5]. For this reason, all HIV-infected individuals should be screened for HCV infection, and all individuals

Correspondence: Dr Salvador Resino, Laboratorio de Epidemiología Molecular de enfermedades Infecciosas, Centro Nacional de Microbiologia (Instituto de Salud Carlos III), Carretera Majadahonda-Pozuelo, Km 2.2, 28220 Majadahonda (Madrid), Spain. Tel: + 34 918 223 266; fax: + 34 915 097 946; e-mail: sresino@isciii.es

with positive results for HCV RNA should be candidates for anti-HCV treatment, provided that HIV infection is well controlled and there are no contraindications to therapy with interferon or ribavirin.

Grading and staging of liver inflammation and fibrosis are considered essential components of the management of patients with chronic hepatitis C. Patients with bridging fibrosis are at a high risk of developing cirrhosis in the ensuing decade [6], so there is little doubt that these patients as well as patients with established liver cirrhosis have a real need to initiate HCV antiviral therapy. The latter group of patients also need more careful monitoring and additional diagnostic tests including periodic oesophagogastroduodenoscopy to detect oesophageal varices as well as imaging and other techniques to screen for hepatocellular carcinoma. The survival rate of HIV/HCV-coinfected patients with cirrhosis after the first episode of hepatic decompensation is extremely poor [7,8].

Liver biopsy is still considered the 'reference standard' for the assessment of liver fibrosis [9]. However, this procedure has several limitations, including its invasive nature, which can lead to complications, inadequate biopsy size, intra- and inter-observer variability, tissue fragmentation, cost, and low acceptance by most patients [10–12]. In recent years, these limitations have led to the development of alternative noninvasive procedures to measure the degree of liver fibrosis. These methods are currently divided into two main categories: imaging methods, such as transient elastography [13], and assays based on serum biomarkers [14]. The potential advantages of these methods are that they are noninvasive, are easier to perform for patients and clinicians, and can be repeated periodically. Indirect markers associated with fibrosis such as routine biochemistry and platelet analyses have been incorporated into several fibrosis indexes such as the aspartate aminotransferase to platelet ratio (APRI), FIB-4, and Forns' indexes [15–17].

In this study, we aimed to develop a noninvasive index with markers derived from peripheral blood to estimate the diagnostic accuracy of advanced stages of fibrosis in HIV/HCV-coinfected patients.

Patients and methods

Patients

The patients for this cross-sectional study came from the HIV out-patient clinic of the Hospital Gregorio Marañón in Madrid, Spain. Patients with documented HIV/HCV coinfection who underwent liver biopsies between May 2000 and May 2007 were included in the study. Liver biopsies were performed on patients who were potential candidates for HCV therapy and had not received previous interferon therapy. The Inclusion criteria were: availability of a frozen serum sample collected on the day of liver biopsy, no clinical evidence of hepatic decompensation, detectable HCV RNA by polymerase chain reaction (PCR), negative hepatitis B surface antigen, CD4 lymphocyte count higher than 200 cells/μL, stable antiretroviral therapy or no need for antiretroviral therapy, and the absence of diabetes, active opportunistic infections, and active drug or alcohol addiction. In our cohort of patients, 297 HIV/HCV-coinfected patients had liver biopsy data by May 2007, but only 195 of these 297 patients could be included because they also had had a serum sample collected and frozen. All work was conducted in accordance with the Declaration of Helsinki. All patients gave their written consent for the liver biopsy and the Institutional Ethics Committee approved the study.

Clinical and laboratory data

On the day of the biopsy, the following information was obtained from the medical records: age, gender, risk category, weight, height, Centers for Disease Control and Prevention (CDC) clinical category, nadir CD4 T-cell count, prior antiretroviral therapy, antiretroviral treatment at the time of liver biopsy and total time on highly active antiretroviral therapy (HAART). The duration of HCV infection for patients with a history of injecting drug use was estimated to begin in the first year needles were shared. Patients were questioned in relation to alcohol consumption. We considered the consumption of >50 g of alcohol per day for ≥ 12 months as a high intake. After an overnight fast and immediately before the liver biopsy was performed, a blood sample was taken from the patient for analysis of complete blood counts, liver panel, basic metabolic panel, coagulation tests, plasma HIV RNA levels and CD4 T-cell counts. Also, a fasting serum sample was immediately stored and frozen (-70 °C) for further assays. All patients gave written consent for the samples to be collected.

HIV and HCV infections were documented in all patients by enzyme-linked immunosorbent assay (ELISA) and PCR. The HCV viral load was measured by PCR (Cobas Amplicor HCV Monitor Test; Branchburg, NJ, USA) and the results are reported in IU/mL. HCV genotype was determined by hybridization of biotin-labelled PCR products to oligonucleotide probes bound to nitrocellulose membrane strips (INNO-LiPA HCV II; Innogenetics, Ghent, Belgium).

Serum markers analysed

In our study, serum markers were measured from a blood sample taken before liver biopsy. A multiplex suspension

bead array immunoassay was performed using the Luminex 100™ analyser (Luminex Corporation, Austin, TX, USA) to identify protein expression in frozen serum samples according to the manufacturers' specifications. A multiplex kit (LINCOplex™; LINCO Research, St Charles, MO, USA) was used to specifically evaluate the following markers: insulin, leptin, hepatocyte growth factor (HGF), nerve growth factor (NGF), soluble Fas-associated death domain protein ligand (sFasL), soluble Fas-associated death domain protein (sFas), macrophage migration inhibitory factor (MIF), soluble intercellular adhesion molecule (sICAM), and soluble vascular cell adhesion molecule (sVCAM). A minimum of 100 events (beads) were collected for each protein sample, and median fluorescence intensities (MFIs) were obtained. Analyte protein concentrations were automatically calculated based on standard curve data using MasterPlex™ QT Analysis version 2 (MiraiBio Inc., Alameda, CA, USA). A five-parameter regression formula was used to calculate the sample concentrations from the standard curves.

Using commercially available reagents, we also tested via ELISA: hyaluronic acid (HA; HA-ELISA; Echelon Biosciences Inc., Salt Lake City, UT, USA), angiopoietin-II (Ang-2; R&D Systems, Minneapolis, MN, USA), tissue inhibitor of metalloproteinase-1 (TIMP-1), matrix metalloproteinase-1 (MMP-1) and matrix metalloproteinase-2 (MMP-2) (GE Healthcare UK Limited, Buckinghamshire, UK), and YKL-40 (Quidel Corporation, San Diego, CA, USA).

In each patient, the degree of insulin resistance (IR) was estimated by the homeostatic model assessment method (HOMA) described by Matthews *et al.* [18]. In particular, an IR score (HOMA-IR) was obtained from samples acquired from fasting patients using the formula: [plasma glucose (mmol/L) $\times$ serum insulin (mU/L)]/22.5.

Liver biopsy and histology

Liver biopsies were performed on an outpatient basis following the recommendations of the Patient Care Committee of the American Gastroenterological Association [19]. All liver biopsies were performed by the same physicians (J.B. and P.M.) with a suction needle (HISTOCUT 16G; Sterylab Srl., Milan, Italy). Ultrasound was routinely used to determine the percutaneous biopsy site. We did not record systematically the size of liver biopsy specimens; however, during the study period, five out of 297 biopsies yielded insufficient liver tissue for pathological diagnosis.

The liver tissue sections were fixed in formalin, embedded in paraffin and stained with haematoxylin-eosin, Mason's trichrome, and Perls' iron. The samples were evaluated by a pathologist (E.A.) who was unaware of the patients' clinical or laboratory data. Liver fibrosis was estimated following the criteria established by the METAVIR Cooperative Study Group [20]. Fibrosis was scored as follows: F0, no fibrosis; F1, portal fibrosis; F2, periportal fibrosis or rare portal-portal septa; F3, fibrous septa with architectural distortion but with no obvious cirrhosis (bridging fibrosis); and F4, definite cirrhosis. The researchers in charge of evaluating the biopsies, interpreting the clinical data, or calculating and analysing the reference standard all performed each function without knowledge of the results of the other evaluations.

Statistics

Overall, results are presented as medians (percentile 25, percentile 75) for continuous variables and as frequencies and percentages for categorical data. Analysis of normality was performed with the Kolmogorov–Smirnov test. Categorical data and proportions were analysed using the χ^2 test or Fisher's exact test as required. The Student *t*-test was used to compare the means of the two groups with normal distributions and the Mann–Whitney test to compare variables with nonnormal distributions. An analysis of variance (ANOVA) adjusted with the Bonferroni test was used to compare the means of three or more groups with normal distributions. Multiple association tests were performed using univariate logistic regression and forward stepwise logistic regression analyses to identify the independent variables associated with the primary endpoint (advanced fibrosis; $F \geq 3$). In the last analysis we included all variables that were statistically significant ($P < 0.05$) in the univariate analysis. A forward stepwise logistic regression analysis was conducted with *P*-values for entry and exit of 0.05 and 0.10, respectively. We developed a new index for advanced fibrosis ($F \geq 3$) diagnosis using a logistic probability function that we have called HGM-3.

We evaluated the diagnostic values of HGM-3 by calculating the areas under the receiver operating characteristic curves (AUC-ROCs) for the estimation and validation groups. For purposes of comparison, we also evaluated four simple reported models consisting of routine parameters to predict liver fibrosis: (a) HGM-1 and HGM-2 [21], (b) FIB-4 [17], (c) APRI [16] and (d) Forns' indexes [15]. We evaluated the diagnostic value of these indexes by comparing the calculated AUC-ROCs [22,23] for all patients included in this study.

Moreover, we evaluated new cut-offs for the HGM-3 index according to a sensitivity (Se) of 95% for the low cut-off used to predict liver biopsies without advanced fibrosis ($F < 3$); and a specificity (Sp) of 95% for the high cut-off used to predict liver biopsies with advanced fibrosis ($F \geq 3$). We calculated the Se, Sp, positive predictive value and

negative predictive value for each cut-off point to evaluate the diagnostic accuracy. We also calculated the diagnostic odds ratio (DOR) which expresses the strength of the association between the test result and disease: it is the ratio of the odds of a positive result in a person with the target condition compared to a person without the condition [24]. A DOR of 1 suggests the test provides no diagnostic evidence. Moreover, we also calculated the likelihood ratios (LRs), which describe how many times a person with the target condition is more likely to have a particular test result than a person without that condition. LRs affect the probability that a target condition is present after the test has been performed. Binary tests have two LRs, positive and negative (LR+ and LR−). An LR of 1 indicates no diagnostic value.

All tests were two-tailed, with *P*-values <0.05 considered to be significant. Statistical analysis was performed using SPSS 14.0 software (SPSS Inc., Chicago, IL, USA) and STATA 9.1 (StataCorp LP, College Station, TX, USA).

Results

Patients

We randomly divided the 195 patients who underwent liver biopsy into two groups: an estimation group ($n = 127$; 65%) and a validation group ($n = 68$; 35%). The two groups had similar baseline characteristics except for a lower frequency of high alcohol intake and a higher serum concentration of YKL-40 in the estimation group compared with the validation group (Table 1).

Predictive markers of advanced fibrosis ($F \geq 3$)

In the estimation group, we identified clinical and laboratory variables associated with advanced fibrosis by univariate logistic regression analysis (Table 2). Univariate analysis revealed that a high number of variables were associated with advanced fibrosis ($F \geq 3$). Eventually, six variables [platelet count, alkaline phosphatase (ALP), HGF, TIMP-1, HA and time on HAART (months)] were identified as independent predictors of advanced fibrosis by forward stepwise logistic regression analysis (Table 3). However, we only included the markers obtained from peripheral blood (platelet count, ALP, HGF, TIMP-1 and HA) to develop a new index for advanced fibrosis ($F \geq 3$) which we have called HGM-3:

$$\Pr(F \geq 3) = \frac{1}{1 + e^{-x}}$$

$$\begin{aligned} x = &- 5.0596 - (1.210 \times 10^{-2} \times \text{Platelet}) \\ &+ (1.203 \times 10^{-2} \times \text{ALP}) \\ &+ (1.220 \times 10^{-3} \times \text{HA}) + (4.526 \times 10^{-4} \times \text{HGF}) \\ &+ (6.312 \times 10^{-3} \times \text{TIMP} - 1) \end{aligned}$$

Performance of HGM-3

Figure 1(a) and (b) show that the HGM-3 index increased significantly with stage of hepatic fibrosis in both the estimation and validation groups. We found statistical differences when comparing F3–F4 with F0–F1 and F2; and when comparing F4 with F0–F1, F2 and F3 ($P<0.05$). We found similar values of AUC-ROCs for the validation and estimation groups (Fig. 1C). Moreover, the AUC-ROC values for significant fibrosis ($F \geq 2$) of the HGM-3 were similar to those of the HGM-1, FIB-4, APRI and Forns' indexes ($P<0.05$) (Table 4). However, the AUC-ROC values for advanced fibrosis ($F \geq 3$) of the HGM-3 were significantly higher than those of the HGM-2, FIB-4, APRI and Forns' indexes ($P<0.05$) (Table 4). Moreover, the AUC value of HGM-3 for the diagnosis of cirrhosis (F4) was also higher than those for the FIB-4, APRI and Forns' indexes (Table 4) but we did not find statistically significant differences between HGM-3 and HGM-2.

Diagnosis of advanced fibrosis ($F \geq 3$)

With the low HGM-3 cut-off point (<0.135) in the estimation group, 57 patients were correctly identified (true negatives without advanced fibrosis), and only two patients were misclassified (false negatives with advanced fibrosis) (Table 5). We found the presence of $F<3$ with 96.6% certainty. The LR− was very low and the DOR was >40. The percentage of patients correctly identified was $<80\%$. For the validation group, the diagnostic accuracy values were similar to the values for the estimation group (Table 5).

When we applied the high HGM-3 cut-off (>0.570) to the estimation group, 31 patients were correctly identified (true positive with advanced fibrosis), and only five patients were misclassified (false positive without advanced fibrosis) (Table 5). We found the presence of $F \geq 3$ with 86.1% certainty. The LR+ was very high and the DOR was >40. The percentage of patients correctly identified was $>80\%$. However, the diagnostic accuracy values for the validation group were slightly worse than those for the estimation group, but the difference was not statistically significant (Table 5). We found the presence of $F \geq 3$ with 76.9% certainty. The sensitivity value was lower, and the LR+ and DOR were also lower than for the estimation group.

Table 1 Characteristics of the 195 HIV/hepatitis C virus (HCV)-coinfected patients who underwent a liver biopsy

	Estimation group	Validation group	All patients
No. HIV-1-infected patients*	127	68	195
Male*	96 (75.6)	51 (75)	147 (75.4)
Age (years)†	39.5 (36.8, 43)	38.7 (36.8, 43.6)	39.5 (36.8, 43.3)
HIV acquired by IDU*	111 (87.4)	63 (92.6)	174 (89.2)
Prior AIDS diagnosis*	38 (29.9)	22 (32.4)	60 (30.8)
Years since HCV infection†	21.6 (18.1, 24.7)	20.7 (17.4, 23.6)	21.3 (17.7, 24.5)
High alcohol intake*	13 (10.3)	14 (20.6)*	27 (13.9)
Antiretroviral therapy			
No treatment*	7 (5.5)	3 (4.4)	10 (5.1)
PI-based*	27 (21.3)	20 (29.4)	47 (24.1)
NNRTI-based*	66 (52)	35 (51.5)	101 (51.8)
Triple NRTI-based*	18 (14.2)	6 (8.8)	24 (12.3)
Other*	9 (7.1)	4 (5.9)	13 (6.7)
Months on HAART ($n = 178$)†	50.7 (33.4, 63.5)	52.2 (34.9, 87.2)	51 (34.3, 65.8)
Stage of liver fibrosis*			
F0	7 (5.5)	8 (11.8)	15 (7.7)
F1	38 (29.9)	29 (42.6)	67 (34.4)
F2	39 (30.7)	13 (19.1)	52 (26.7)
F3	28 (22)	10 (14.7)	38 (19.5)
F4	15 (11.8)	8 (11.8)	23 (11.8)
HIV markers			
Nadir CD4 count (cells/μL)†	210 (100, 331)	215 (115, 325)	210 (103, 325)
Baseline CD4 count (cells/μL)†	490 (360, 660)	527 (397, 744)	493 (373, 667)
HIV RNA < 50 copies/mL*	99 (78)	51 (75)	150 (76.9)
Log_{10} VL (copies/mL) ($n = 45$)†	2.99 (2.69, 3.8)	3.66 (2.91, 4.09)	3.23 (2.71, 3.98)
HCV markers*			
HCV genotype			
1–4	102 (81.6)	48 (71.6)	150 (78.1)
3	23 (18.4)	19 (28.4)	42 (21.9)
HCV RNA > 850 000 copies/mL	77 (75.5)	43 (74.1)	120 (75)
Haematological parameters†			
Platelet count ($\times 10^9$ cells/L)	177 (142, 221)	189 (139, 231)	178 (141, 223)
Fibrinogen (mg/dL)	260 (228, 310)	256 (229, 286)	259 (228, 305)
INR	1 (1, 1.05)	1 (0.97, 1)	1 (1, 1.02)
Biochemical parameters†			
Glucose (mg/dL)	88 (79, 97)	85.5 (77, 93)	87 (78, 96)
ALP (IU/dL)	124 (85, 196)	123 (76, 194)	124 (81, 196)
AST (IU/dL)	61 (39, 92)	52 (37.5, 75.5)	57 (38, 85)
GGT (IU/dL)	107 (59, 244)	122 (53, 196)	113 (59, 211)
ALT (IU/dL)	87 (49, 137)	65.5 (49, 94.5)	77 (49, 118)
AST/ALT	0.74 (0.59, 0.96)	0.77 (0.62, 1.03)	0.76 (0.62, 0.97)
Cholesterol (mg/dL)	170 (148, 192)	173 (144, 208)	170 (148, 196)
Fibrosis markers†			
Insulin (pg/mL)	480 (336, 748)	481 (405, 780)	481 (360, 768)
HOMA	3 (2, 4)	2 (2, 5)	2 (2, 4)
Leptin (pg/mL)	3836 (1706, 9636)	3779 (1850, 9064)	3836 (1778, 9387)
HGF (pg/mL)	1535 (917, 2759)	1790 (1134, 3150)	1636 (987, 2927)
NGF (pg/mL)	9 (4, 15)	10 (6, 16)	9 (5, 15)
sFasL (pg/mL)	103 (67, 155)	89 (50, 139)	99 (55, 155)
sFas (pg/mL)	11419 (6381, 18865)	9482 (4261, 17763)	10887 (5523, 18789)
MIF (pg/mL)	1796 (895, 3167)	1516 (1137, 2812)	1653 (961, 2904)
sICAM (pg/mL)	519166 (397948, 954064)	512922 (427156, 865666)	518318 (404938, 954064)
sVCAM (pg/mL)	894728 (501227, 1720604)	925408 (392543, 1590870)	912158 (470112, 1661370)
HA (pg/mL)	1203 (624, 1742)	1125 (713, 1620)	1185 (656, 1654)
Ang-II (pg/mL)	4420 (2973, 7686)	5003 (2651, 8392)	4758 (2711, 7745)
TIMP-1 (ng/mL)	277 (177, 401)	240 (89, 351)	265 (156, 394)
YKL-40 (ng/mL)	2170 (945, 5771)	1583 (735, 3390)*	1954 (845, 4624)
MMP-1 (ng/mL)	163 (140, 280)	184 (148, 320)	165 (142, 292)
MMP-2 (ng/mL)	116 (83, 500)	105 (80, 473)	111 (82, 493)

*Absolute number (percentage).
†Median (percentile 25, percentile 75).
ALP, alkaline phosphatase; ALT, alanine aminotransferase; Ang-II, angiopoietin II; AST, aspartate aminotransferase; GGT, gamma glutamyl transpeptidase; HAART, highly active antiretroviral therapy; HCV, hepatitis C virus; HGF, hepatocyte growth factor; HIV-1, human immunodeficiency virus type 1; HOMA, insulin resistance score; IDU, injecting drug use; INR, international normalized ratio; MIF, macrophage migration inhibitory factor; MMP-1, matrix metalloproteinase-1; MMP-2, matrix metalloproteinase-2; NGF, nerve growth factor; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; HIV RNA, HIV plasma viral load; HCV RNA, HCV plasma viral load; sFasL, serum-soluble Fas-associated death domain protein ligand; sFas, serum-soluble Fas-associated death domain protein; sICAM, soluble intercellular adhesion molecule; sVCAM, soluble vascular cell adhesion molecule; HA, hyaluronic acid; TIMP-1, tissue inhibitor of metalloproteinase-1; YKL-40, also known as human cartilage 39 (HC gp-39).

Table 2 Variables assessed for association with advanced fibrosis ($F \geq 3$) in all HIV/hepatitis C virus (HCV)-coinfected patients

Clinical	Sex, age at biopsy, HIV transmission category, CDC clinical category, duration of HCV infection, and antiretroviral therapy (use of HAART, type of HAART, and years on HAART*)
Routine laboratory tests	Platelets*, INR*, ALP*, AST*, ALT*, GGT, cholesterol, CD4 cell count (nadir and at the time of biopsy), HIV RNA viral load, HCV-1 or HCV-4 genotype, and HCV RNA viral load*
Nonroutine laboratory tests	Insulin, HOMA-IR (≥ 3.8)*, leptin, HGF*, NGF, sFasL, sFas*, MIF, sICAM*, sVCAM*, HA*, Ang-2*, TIMP-1*, YKL-40*, MMP-1 and MMP-2
Pathology	Distribution of liver fibrosis: stage 0 (no fibrosis), stage 1 (portal fibrosis), stage 2 (periportal fibrosis), stage 3 (bridging fibrosis) and stage 4 (cirrhosis)

*Univariate analysis revealed that years on HAART, platelet count, INR, ALP, AST, ALT, HCV RNA viral load, HOMA-IR (≥ 3.8), HGF, sFas, sICAM, sVCAM, HA, Ang-2, TIMP-1 and YKL-40 were all associated with advanced fibrosis ($F \geq 3$).
ALP, alkaline phosphatase; ALT, alanine aminotransferase; Ang-2, angiopoietin-II; AST, aspartate aminotransferase; CDC, Centers for Disease Control and Prevention; GGT, gamma glutamyl transpeptidase, HA, hyaluronic acid; HAART, highly active antiretroviral therapy; HGF, hepatocyte growth factor; HOMA-IR, insulin resistance score; INR, international normalized ratio; NGF, nerve growth factor; sFasL, soluble Fas-associated death domain protein ligand; sFas, soluble Fas-associated death domain protein; MIF, macrophage migration inhibitory factor; MMP-1, matrix metalloproteinase-1; MMP-2, matrix metalloproteinase-2; sICAM, soluble intercellular adhesion molecule; sVCAM, soluble vascular cell adhesion molecule; TIMP-1, tissue inhibitor of metalloproteinase-1; YKL-40, also known as human cartilage 39 (HC gp-39).

Table 3 Summary of results for forward stepwise multivariate logistic regression analysis for the estimation group

Advanced fibrosis ($F \geq 3$)	OR	95% CI		*P*-value
Time on HAART (months)	0.957	0.927	0.988	0.007
Platelet count ($\times 10^9$ cells/L)	0.982	0.967	0.997	0.022
ALP (IU/dL)	1.012	1.003	1.020	0.007
HGF (pg/mL)	1.001	1.000	1.002	0.001
HA (pg/mL)	1.002	1.001	1.003	0.003
TIMP-1 (ng/mL)	1.009	1.004	1.014	0.001

ALP, alkaline phosphatase; CI, confidence interval; HA, hyaluronic acid; HGF, hepatocyte growth factor; OR, odds ratio; TIMP-1, tissue inhibitor of metalloproteinase-1.

Discussion

In this study, we aimed to develop a noninvasive index in order to identify advanced liver fibrosis in a series of 195 HIV/HCV-coinfected patients naïve to anti-HCV treatment. Patients were randomly divided into an estimation group and a validation group. We assessed routine laboratory data as well as markers of extracellular matrix (ECM) metabolism, inflammation, growth factors and IR. In the estimation group, univariate analyses revealed that platelet count, ALP, HGF, TIMP-1 and HA were all associated with advanced liver fibrosis. With these markers, we developed a new index using a logistic probability function which we have designated HGM-3. We did not included 'time on HAART' in the final model because the models with and without 'time on HAART' were not significantly different. Moreover, it is often difficult to calculate the time on HAART for patients who change their centre of reference several times or for whom the clinical history is incomplete. HGM-3 had an AUC-ROC for the identification of advanced liver fibrosis higher than 0.90, which was significantly higher than the AUC-ROC obtained with the HGM-2, FIB-4, APRI or Forns' index. These results confirm that HGM-3 is an accurate noninvasive method for the detection of bridging fibrosis and cirrhosis in HIV/HCV-coinfected patients.

Liver fibrosis is considered a dynamic process characterized by matrix remodelling and excessive deposition of ECM proteins including collagen [25,26]. Currently, two types of serum markers of liver fibrosis have been used: indirect markers that reflect alterations in hepatic function but do not directly reflect ECM metabolism (i.e. platelet count, coagulation studies, etc.), and direct markers that reflect qualitative and quantitative changes in ECM macromolecules [9].

We evaluated a variety of standard indirect markers of liver fibrosis. By multivariate analysis, we found platelet count and ALP to be independent predictive markers of advanced fibrosis. Our findings echo the results of many previous studies which showed that platelet count and ALP levels were important predictors of either significant fibrosis or cirrhosis [27]. Among the direct markers of liver fibrosis we found a good correlation between TIMP-1 and HA serum levels and the stage of fibrosis. Both markers showed an excellent predictive value for advanced fibrosis, confirming the results of other studies [28–30]. In our study, several other markers failed to show any predictive value for advanced fibrosis. These markers consisted of matrix remodelling indicators such as MMP-1, MMP-2 and YKL-40, as well as several molecules related to regulation of metabolism (leptin, insulin, and NGF) and inflammation (sICAM, sVCAM, sFas, sFasL and MIF).

Notably, we found that HGF is a good predictive marker of advanced liver fibrosis. To the best of our knowledge, this is the first study that shows that serum HGF is a good predictive marker of advanced liver fibrosis in patients with chronic hepatitis C. HGF is a factor for paracrine cellular growth, motility and morphogenesis. It is secreted by mesenchymal cells and targets and acts primarily upon epithelial and endothelial cells, but also acts on haemopoietic progenitor cells. It has been shown to have a major role in embryonic organ development, in adult organ regeneration and in wound healing. Serum HGF levels are strongly associated with liver diseases, obesity, IR, and metabolic syndrome [31]. It is possible that elevated HGF

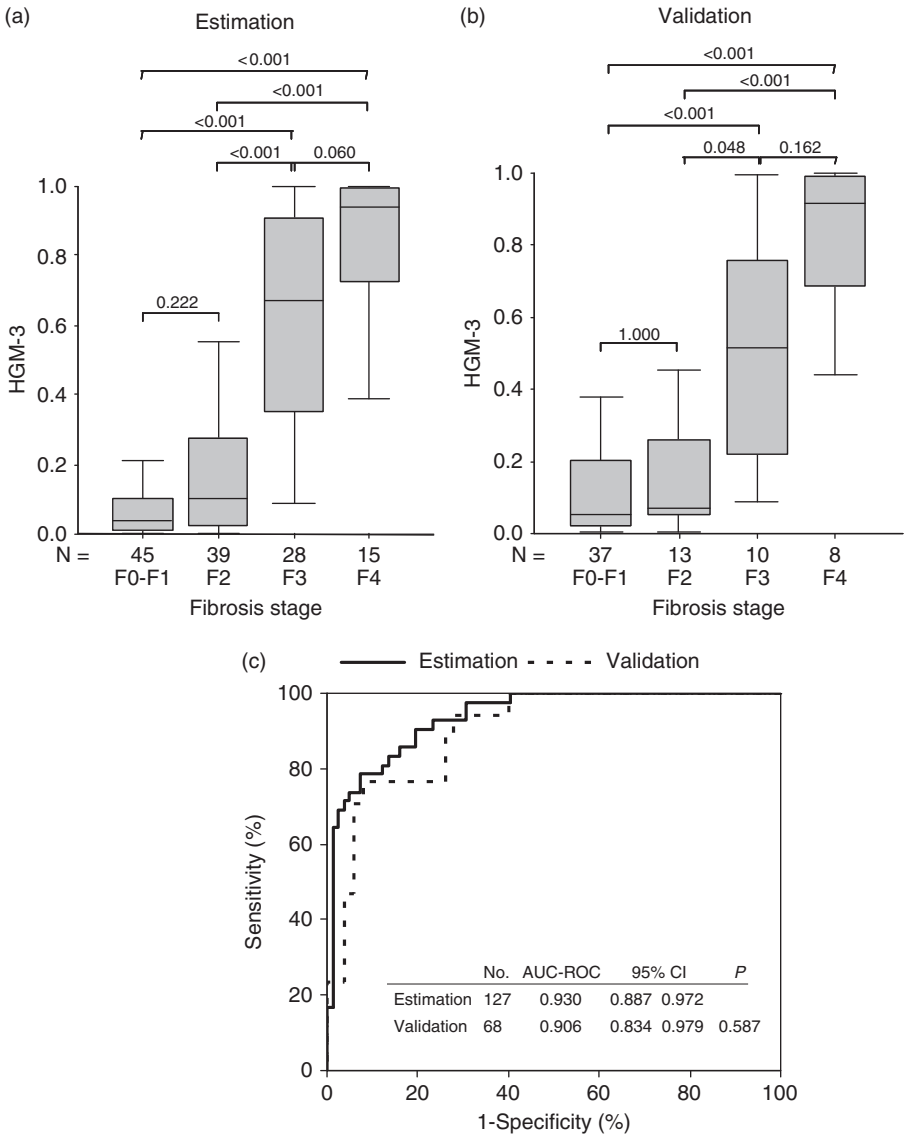

Fig. 1 Box plots illustrating the distribution of HGM-3 values against fibrosis score for the estimation (a) and validation (b) groups. Horizontal lines inside each box represent the median, and the lower and upper borders of the box encompass the interquartile range. The vertical lines from the ends of each box encompass the extreme data points. (c) Diagnostic values of the new index (HGM-3) for prediction of advanced fibrosis (F3, F4) in the estimation and validation groups. CI, confidence interval; AUC-ROC, area under the receiver operating characteristic curve.

levels reflect significant liver damage or, alternatively, an imbalance between HGF clearance and production which could be an indicator of liver dysfunction because the liver is the major organ through which HGF is eliminated from systemic circulation.

Many experts believe that current noninvasive tests of hepatic fibrosis cannot yet replace liver biopsies [27,32–34]. However, in one prospective study, comparing liver biopsies with a noninvasive index, it was found that the size of the liver biopsy was inadequate in a significant proportion of patients with chronic hepatitis C. Moreover, when biopsy and marker results were discordant, an explanation could be identified in more than two-thirds of the cases and, in those cases, biopsy failure was more than seven times more common than diagnostic failure of serum markers [35]. Some experts would consider non-invasive serum tests of fibrosis with AUC-ROC areas of 0.85–0.90 to be as good as a liver biopsy for staging fibrosis [36]. The AUC-ROC of HGM-3 for the detection of advanced fibrosis was higher than 90%; a value of

accuracy that has not been previously achieved with other markers for HIV/HCV-coinfected patients [30,37,38]. Furthermore, we found that HGM-3 had higher diagnostic accuracy than the HGM-2, APRI, FIB-4 or Forns' index [15–17,21]. It is important to note that this sample cohort is a subgroup of patients included in a previous report in which we estimated the HGM-2 index [21], and we found that the HGM-3 was more accurate than the HGM-2 index. Noninvasive serum panels such as APRI, FIB-4 and Forns' indexes are very cheap and widely available but are relatively inaccurate at diagnosing HIV/HCV coinfection [39–41]. The HGM-3 model contains some nonroutine tests that are not widely available and may be expensive if they were to perform the ELISA classic, which makes the model less attractive as it may not be possible for most clinicians to use it. However, at present, these molecules can be measured using a new Luminex-based assay in a quick and inexpensive way.

HGM-3 also gave good results for cirrhosis diagnosis, but we found similar AUC-ROC values for HGM-3 and HGM-2. In our opinion, HGM-3 is less useful for diagnosis of cirrhosis or advanced fibrosis because HGM-2 is calculated from indirect markers associated with fibrosis such as routine biochemistry and platelet data which are widely available and very inexpensive [21]. Moreover, we were unable to assess the diagnostic accuracy in the estimation and validation groups because of the low number of patients with cirrhosis included in this study.

The identification of this index in HIV-positive individuals is also of importance as HIV infection may alter the expression of many of the immune, apoptotic and ECM markers. However, this study had several limitations. (1) The low number of patients. (2) The study was limited to patients with well-preserved immune function and extrapolation to individuals with more marked immune suppression will require further study. (3) We did not compare HGM-3 with SHASTA, Fibrotest, Hepascore and Fibrometer because we did not have all the variables needed to calculate these indexes. (4) HGM-3 was derived from the majority of this combined cohort and so would be expected to perform optimally in this cohort; whereas the other indexes tested (APRI, FIB-4 and Forns' indexes) were not optimized in this cohort and would be expected to perform less well. (5) We cannot give exact information regarding biopsy length or portal tracts; however, we found that only 1.68% of biopsies were defective for pathological diagnosis and these cases were excluded from the study. In any case, the pathologist had samples of acceptable quality

Table 4 Summary of area under the receiver operating characteristic curves (AUC-ROCs) of the HGM-3, HGM-2, HGM-1, FIB-4, aspartate aminotransferase to platelet ratio (APRI) and Forns' indexes

Index	AUC-ROC	95% CI		*P*-value
Significant fibrosis ($F \geq 2$)				
HGM-3	0.779	0.714	0.844	–
HGM-1	0.788	0.723	0.854	0.999
FIB-4	0.730	0.659	0.801	0.567
APRI	0.771	0.704	0.839	0.999
Forns'	0.732	0.661	0.803	0.647
Advanced fibrosis ($F \geq 3$)				
HGM-3	0.929	0.894	0.965	–
HGM-2	0.850	0.790	0.909	0.016
FIB-4	0.757	0.679	0.835	<0.001
APRI	0.772	0.698	0.846	<0.001
Forns'	0.759	0.683	0.834	<0.001
Cirrhosis (F4)				
HGM-3	0.931	0.891	0.970	–
HGM-2	0.917	0.875	0.958	0.495
FIB-4	0.815	0.726	0.904	0.007
APRI	0.798	0.721	0.875	<0.001
Forns'	0.815	0.734	0.895	0.008

AUC-ROC, area under the receiver operating characteristic curve; CI, confidence interval.

Table 5 Diagnostic accuracy and predictive values of the new index (HGM-3) for advanced fibrosis ($F \geq 3$) and cirrhosis (F4)

Cut-off	TP	FP	TN	FN	*Se (C195)	*Sp (C195)	*PPV (C195)	*NPV (C195)	LR + (C195)	LR − (C195)	DOR (C195)	*PCI (C195)
Estimation group ($n = 127$)												
0.135	41	27	57	2	95.3 (84.5, 98.7)	67.9 (57.3, 76.9)	60.3 (48.4, 71.1)	96.6 (88.5, 99.1)	2.97 (2.16, 4.08)	0.07 (0.02, 0.27)	43.28 (9.74, 192.29)	77.2 (69.1, 83.6)
0.570	31	5	79	12	72.1 (57.3, 83.3)	94 (86.8, 97.4)	86.1 (71.3, 93.9)	86.8 (78.4, 92.3)	12.11 (5.07, 28.91)	0.30 (0.18, 0.48)	40.82 (13.28, 125.47)	86.6 (79.6, 91.5)
Validation group ($n = 68$)												
0.135	17	16	34	1	94.4 (74.2, 99.0)	68.0 (54.2, 79.2)	51.5 (35.2, 67.5)	97.1 (85.5, 99.5)	2.95 (1.94, 4.49)	0.08 (0.01, 0.56)	36.13 (4.41, 295.75)	75.0 (63.6, 83.8)
0.570	10	3	47	8	55.6 (33.7, 75.4)	94.0 (83.8, 97.9)	76.9 (49.7, 91.8)	85.5 (73.8, 92.4)	9.26 (2.87, 29.9)	0.47 (0.28, 0.81)	19.58 (4.40, 87.08)	83.8 (73.3, 90.7)

CI, confidence interval; DOR, diagnostic odds ratio; FN, false negative cases (missed cases); FP, false positive cases (over-diagnosis); LR, likelihood ratio; NPV, negative predictive value; PCI, patients correctly identified; PPV, positive predictive value; Se, sensitivity; Sp, specificity; TN, true negative cases (correct diagnosis); TP, true positive cases (correct diagnosis).
*Values are given as percentage, with 95% confidence interval in parentheses.

to make a diagnosis of fibrosis for 98.32% of obtained biopsies.

In summary, we found that platelet count, ALP, HGF, TIMP-1 and HA were independent predictive markers of advanced fibrosis in HIV/HCV-coinfected patients. The combination of these indirect markers with direct markers of fibrosis in a logistic probability function yielded a new serum index that accurately predicted bridging fibrosis and cirrhosis. However, as with most models, HGM-3 better predicts the absence of fibrosis (97% certainty for $F<3$ fibrosis) than the presence of significant fibrosis (77% certainty). HGM-3 improves upon the accuracy of other previously published indexes but still has limitations in accurately identifying patients with $F \geq 3$. This indicates that further research should be carried out to improve the ability to diagnose advanced fibrosis ($F \geq 3$) in HIV/HCV-coinfected patients.

Acknowledgements

This work was supported by grants from Fundación para la Investigación y la Prevención del SIDA en España (FIPSE) (Ref. 36650/07) and Instituto de Salud Carlos III (Ref. PI07/90201; Ref. UIPY 1467/07; PI08/0738) to SR and from FIS (Ref. ISCIII-RETIC RD06/006, PI08/0928) and FIPSE (Ref. 36443/03) to JB. DM is supported by a grant from Fundación Lair (grant 020907).

Financial disclosure

The authors do not have commercial or other associations that might pose a conflict of interest.

References

1 Soto B, Sanchez-Quijano A, Rodrigo L *et al.* Human immunodeficiency virus infection modifies the natural history of chronic parenterally-acquired hepatitis C with an unusually rapid progression to cirrhosis. *J Hepatol* 1997; **26**: 1–5.

2 Benhamou Y, Bochet M, Di Martino V *et al.* Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. The Multivirc Group. *Hepatology* 1999; **30**: 1054–1058.

3 Bica I, McGovern B, Dhar R *et al.* Increasing mortality due to end-stage liver disease in patients with human immunodeficiency virus infection. *Clin Infect Dis* 2001; **32**: 492–497.

4 Graham CS, Baden LR, Yu E *et al.* Influence of human immunodeficiency virus infection on the course of hepatitis C virus infection: a meta-analysis. *Clin Infect Dis* 2001; **33**: 562–569.

5 Garcia-Samaniego J, Rodriguez M, Berenguer J *et al.* Hepatocellular carcinoma in HIV-infected patients with chronic hepatitis C. *Am J Gastroenterol* 2001; **96**: 179–183.

6 Yano M, Kumada H, Kage M *et al.* The long-term pathological evolution of chronic hepatitis C. *Hepatology* 1996; **23**: 1334–1340.

7 Merchante N, Giron-Gonzalez JA, Gonzalez-Serrano M *et al.* Survival and prognostic factors of HIV-infected patients with HCV-related end-stage liver disease. *AIDS* 2006; **20**: 49–57.

8 Giron-Gonzalez JA, Brun F, Terron A, Vergara A, Arizcorreta A. Natural history of compensated and decompensated HCV-related cirrhosis in HIV-infected patients: a prospective multicentre study. *Antivir Ther* 2007; **12**: 899–907.

9 Manning DS, Afdhal NH. Diagnosis and quantitation of fibrosis. *Gastroenterology* 2008; **134**: 1670–1681.

10 Cadranel JF, Rufat P, Degos F. Practices of liver biopsy in France: results of a prospective nationwide survey. Group of Epidemiology of the French Association for the Study of the Liver (AFEF). *Hepatology* 2000; **32**: 477–481.

11 Regev A, Berho M, Jeffers LJ *et al.* Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol* 2002; **97**: 2614–2618.

12 Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003; **38**: 1449–1457.

13 Saito H, Tada S, Nakamoto N *et al.* Efficacy of non-invasive elastometry on staging of hepatic fibrosis. *Hepatol Res* 2004; **29**: 97–103.

14 Castera L, Vergniol J, Foucher J *et al.* Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128**: 343–350.

15 Forns X, Ampurdanes S, Sanchez-Tapias JM *et al.* Long-term follow-up of chronic hepatitis C in patients diagnosed at a tertiary-care center. *J Hepatol* 2001; **35**: 265–271.

16 Wai CT, Greenson JK, Fontana RJ *et al.* A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518–526.

17 Sterling RK, Lissen E, Clumeck N *et al.* Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43**: 1317–1325.

18 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412–419.

19 Jacobs WH, Goldberg SB. Statement on outpatient percutaneous liver biopsy. *Dig Dis Sci* 1989; **34**: 322–323.

20 Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; **24**: 289–293.

21 Berenguer J, Bellon JM, Miralles P *et al.* Identification of liver fibrosis in HIV/HCV-coinfected patients using a simple predictive model based on routine laboratory data. *J Viral Hepat* 2007; **14**: 859–869.

22 DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988; **44**: 837–845.
23 Song HH. Analysis of correlated ROC areas in diagnostic testing. *Biometrics* 1997; **53**: 370–382.
24 Glas AS, Lijmer JG, Prins MH, Bonsel GJ, Bossuyt PM. The diagnostic odds ratio: a single indicator of test performance. *J Clin Epidemiol* 2003; **56**: 1129–1135.
25 Bataller R, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209–218.
26 Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *J Clin Invest* 2007; **117**: 524–529.
27 Parkes J, Guha IN, Roderick P, Rosenberg W. Performance of serum marker panels for liver fibrosis in chronic hepatitis C. *J Hepatol* 2006; **44**: 462–474.
28 Halfon P, Bacq Y, De Muret A *et al*. Comparison of test performance profile for blood tests of liver fibrosis in chronic hepatitis C. *J Hepatol* 2007; **46**: 395–402.
29 Larrousse M, Laguno M, Segarra M *et al*. Noninvasive diagnosis of hepatic fibrosis in HIV/HCV-coinfected patients. *J Acquir Immune Defic Syndr* 2007; **46**: 304–311.
30 Kelleher TB, Mehta SH, Bhaskar R *et al*. Prediction of hepatic fibrosis in HIV/HCV co-infected patients using serum fibrosis markers: the SHASTA Index. *J Hepatol* 2005; **43**: 78–84.
31 Asano Y, Iimuro Y, Son G, Hirano T, Fujimoto J. Hepatocyte growth factor promotes remodeling of murine liver fibrosis, accelerating recruitment of bone marrow-derived cells into the liver. *Hepatol Res* 2007; **37**: 1080–1094.
32 Lichtinghagen R, Bahr MJ. Noninvasive diagnosis of fibrosis in chronic liver disease. *Expert Rev Mol Diagn* 2004; **4**: 715–726.
33 Thuluvath PJ, Krok KL. Noninvasive markers of fibrosis for longitudinal assessment of fibrosis in chronic liver disease: are they ready for prime time? *Am J Gastroenterol* 2005; **100**: 1981–1983.
34 Zeremski M, Talal AH. Noninvasive markers of hepatic fibrosis: are they ready for prime time in the management of HIV/HCV co-infected patients? *J Hepatol* 2005; **43**: 2–5.
35 Poynard T, Munteanu M, Imbert-Bismut F *et al*. Prospective analysis of discordant results between biochemical markers and biopsy in patients with chronic hepatitis C. *Clin Chem* 2004; **50**: 1344–1355.
36 Afdhal NH, Nunes D. Evaluation of liver fibrosis: a concise review. *Am J Gastroenterol* 2004; **99**: 1160–1174.
37 de Ledinghen V, Douvin C, Kettaneh A *et al*. Diagnosis of hepatic fibrosis and cirrhosis by transient elastography in HIV/hepatitis C virus-coinfected patients. *J Acquir Immune Defic Syndr* 2006; **41**: 175–179.
38 Nunes D, Fleming C, Offner G *et al*. HIV infection does not affect the performance of noninvasive markers of fibrosis for the diagnosis of hepatitis C virus-related liver disease. *J Acquir Immune Defic Syndr* 2005; **40**: 538–544.
39 Cacoub P, Carrat F, Bedossa P *et al*. Comparison of non-invasive liver fibrosis biomarkers in HIV/HCV co-infected patients: the fibrovic study–ANRS HC02. *J Hepatol* 2008; **48**: 765–773.
40 Loko MA, Castera L, Dabis F *et al*. Validation and comparison of simple noninvasive indexes for predicting liver fibrosis in HIV-HCV-coinfected patients: ANRS CO3 Aquitaine Cohort. *Am J Gastroenterol* 2008; **103**: 1973–1980.
41 Macias J, Giron-Gonzalez JA, Gonzalez-Serrano M *et al*. Prediction of liver fibrosis in human immunodeficiency virus/hepatitis C virus coinfected patients by simple non-invasive indexes. *Gut* 2006; **55**: 409–414.