Evaluation of Liver Fibrosis: Concordance Analysis between Noninvasive Scores (APRI and FIB-4) Evolution and Predictors in a Cohort of HIV-Infected Patients without Hepatitis C and B Infection

Monia Mendeni,[1,a] Emanuele Focà,[1,a] Daria Gotti,[1] Nicoletta Ladisa,[3] Gioacchino Angarano,[3] Laura Albini,[1] Filippo Castelnuovo,[2] Giampiero Carosi,[1] Eugenia Quiros-Roldan,[1] and Carlo Torti[1]

[1]Institute of Infectious and Tropical Diseases, University of Brescia, and [2]Spedali Civili di Brescia, Brescia, and [3]Institute of Infectious Diseases, Policlinico di Bari, Bari, Italy

Background. There is lack of data on the incidence of liver fibrosis (LF) progression in patients with human immunodeficiency virus (HIV) monoinfection and risk factors for LF.

Methods. We performed an observational prospective study in a cohort of HIV-infected patients who had initiated highly active antiretroviral therapy (HAART). FIB-4 and aspartate aminotransferase (AST)-to-platelet ratio index (APRI) were assessed. The concordance between the 2 scores was assessed by weighted kappa coefficient. Kaplan-Meier analysis was used to estimate the incidence of LF. Cox regression analysis was used to assess the predictors of transition.

Results. A total of 1112 patients were observed for a mean of 2249 days of follow-up. The concordance between FIB-4 and APRI was moderate (kappa = .573). The incidence of transition to higher FIB-4 classes was 0.064 (95% confidence interval [CI], 0.056–0.072) per person-year of follow-up (PYFU), whereas the incidence of transition to higher APRI classes was 0.099 (95% CI, 0.089–0.110) per PYFU. The incidence of transition to FIB-4 >3.25 was 0.013 per PYFU (95% CI, 0.010–0.017) and 0.018 per PYFU (95% CI, 0.014–0.022) for APRI >1.5. In multivariate analyses, for transition to higher classes, HIV RNA level <500 copies/mL was found to be protective for both scores, and higher CD4+ T cell count was found to be protective for FIB-4. Additional risk factors were age ⩾40 years, male sex, intravenous drug use as an HIV infection risk factor, higher degree of LF, higher gamma-glutamyl transpeptidase (γGT) at baseline, and use of dideoxynucleoside-analogue drugs (DDX). Consistent results for the main study outcomes were obtained for confirmed LF transition and transition to FIB-4 >3.25 and APRI>1.5 as study outcomes.

Conclusions. Overall, our results suggest a possible benefit associated with earlier HAART initiation, provided that the effectiveness of HAART is sustained and treatment with DDX is avoided.

Because the introduction of highly active antiretroviral therapy (HAART) has increased the life expectancy of human immunodeficiency virus (HIV)–infected individuals, progression to end-stage liver disease has become an important cause of mortality and morbidity in this population, most often in those individuals with hepatotropic co-infections (infections due to hepatitis B virus (HBV) or hepatitis C virus (HCV) [1, 2]. In addition, HIV-monoinfected patients have also displayed liver diseases [3–5].

Today, different noninvasive markers of liver fibrosis (LF) are available, such as FIB-4 score and the aspartate aminotransferase (AST)-to-platelet ratio index (APRI)

Received 6 August 2010; accepted 13 January 2011.

[a]M.M. and E.F. contributed equally to the work.

Presented in part: 2nd Italian Conference on AIDS and Retroviruses, Brescia, Italy, 20–22 June 2010 [Abstract CO30], and the 10th International Congress on Drug Therapy in HIV Infection, Glasgow, UK, 7–11 November 2010 (Abstract 92).

Correspondence: Carlo Torti, MD, Institute of Infectious and Tropical Diseases, University of Brescia, School of Medicine, P.le Spedali Civili 1, 25123 Brescia, Italy (torti@med.unibs.it).

Clinical Infectious Diseases 2011;52(9):1164–1173

1058-4838/2011/529-0001$37.00
DOI: 10.1093/cid/cir071

[6, 7], when liver biopsy is not indicated or is not accepted by patients [8]. FIB-4, which consists of alanine aminotransferase (ALT) level, AST level, platelet count and age [6], appears to be a strong predictor of de-compensated cirrhosis or death [9]. Although no formal validations are available for APRI in HIV-monoinfected patients, it has been previously validated in individuals with HIV and HCV co-infection [10, 11].

Three cross-sectional studies have found a significant correlation between lower or undetectable HIV RNA level and lower stages of LF, with 2 of these studies finding a correlation at multivariable analysis in the overall population [4, 12] and 1 study finding a correlation only in a subset of 72 mono-infected women [13]. Because LF is the result of a chronic process influenced by factors that are not always captured at a single time point, longitudinal studies may be more powerful than these cross-sectional studies.

Therefore, the main aim of our study was to estimate, in a cohort of HIV-monoinfected patients, the incidence and predictors of LF progression as evaluated by both FIB-4 and APRI scores. Moreover, the concordance between these 2 methods was assessed.

METHODS

Patient Cohort

The prospective Hepatotoxicity of Different Kinds of Antiretrovirals study within the collaborative network of the Italian Standardized Management of Retroviral HIV Infection cohort aimed at studying liver complications after combination antiretroviral therapy (cART). Briefly, HIV-positive patients who started cART in 2 large Italian referral centers from 1996 through 2006 were enrolled, and data were recorded through a common electronic chart (NetCare; Healthware). Patients underwent blood testing (including liver function tests, kidney function tests, and determination of HIV RNA levels and CD4+ T lymphocyte counts) every 3 months. Data were periodically verified for consistency and accuracy every 6 months. The results of the hepatic toxicity in patients with HBV-HCV co-infection were previously published [14].

For the present study, we excluded patients with positive HBV surface antigen (HBsAg), HBV surface antibody (HBsAB), HBV core antibody (HBcAb), and HCV antibody (HCVAb) test results, liver cirrhosis (diagnosed by clinical work-up, including histological testing), and hepatocarcinoma (as determined by findings of conventional imaging). The start date of follow-up was the date of cART initiation. Baseline data were obtained within 6 months prior to cART initiation. The type of cART prescribed was considered as one of the baseline factors either for cohort description or for analyses of baseline predictors following an intention-to-treat approach. The database was frozen as of December 2009.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration. Informed consent was obtained from all patients.

Study Outcomes

The FIB-4 score was calculated using Sterling's formula [6], as follows: age [years] $\times$ AST [IU/L]/platelet count [expressed as platelets $\times$ 10^9/L] $\times$ (ALT$^{1/2}$[IU/L]). The APRI score was calculated using Wai's formula [7]: (AST/upper limit of normal considered as 40 IU/L)/platelet count (expressed as platelets $\times$ 10^9/L) $\times$ 100. LF was ranked using standard cut-off values [15–17] into 3 classes: FIB-4 class 1, $\leq$ 1.45; FIB-4 class 2, from 1.46 through 3.25; FIB-4 class 3, $>$ 3.25; APRI class 1, $\leq$ 0.5; APRI class 2, from 0.51 to 1.5; and APRI class 3, $>$ 1.5.

Any transition from FIB-4/APRI classes at baseline to any higher classes during the follow-up period (ie, from class 1 to class 2 or 3; or from class 2 to class 3) was chosen as the primary study outcome to increase the sensitivity for possible progression of LF. With this outcome, the precisions of the estimate of the confidence interval 95% (95% CI) for incidence of fibrosis progression were $\pm$2.6% (for FIB-4) and $\pm$2.8% (for APRI). However, we recognized that both FIB-4 and APRI showed the highest positive predictive value for the most-severe stages of hepatic fibrosis at liver biopsy [18]. Therefore, to enhance the specificity of our results, we also used transition to FIB-4 $>$3.25 and to APRI $>$1.5 as study outcomes. Lastly, owing to the fact that these scores may fluctuate or revert back to lower classes after initial worsening, confirmed transition to higher FIB-4 or APRI classes in at least 2 subsequent measurements (unless follow-up was truncated) was also analyzed.

Data Collection

Baseline data included date of birth, sex, ethnicity, date of HIV infection diagnosis, risk factors for HIV acquisition, and cART prescribed as first-line regimens. The following parameters were collected at baseline and scheduled every 3 months: CD4+ T cell count, HIV RNA level, AST level, ALT level, total bilirubin, γGT level as a marker of alcohol intake [19, 20], and platelet count. Both FIB-4 and APRI scores were calculated using all data at the same time points. Specifically, a mean ($\pm$ standard deviation [SD]) of 4.15 $\pm$ 2.06 measurements per person-year of follow-up (PYFU) were available for FIB-4 and 4.16 $\pm$ 2.08 measurements per PYFU were available APRI. In 36 instances, an APRI score was obtained without a FIB-4 score. Lastly, the actual dates of drug switch or cessation of cART were recorded at each visit.

Statistical Analysis

Descriptive analysis was conducted as appropriate. Quantitative variables have been summarized with a simple size (n), mean, median, SD, minimum, and maximum values, and qualitative

Table 1. Baseline Characteristics of Patients Divided by Stability or Improvement in the FIB-4 and APRI Scores (Group 1) or Progression to Higher Classes of These Scores (Group 2)

Variable	Total (*n*=1112)	FIB-4 score (*n*=1074)			APRI score (*n*=1079)		
		Group 1 (*n*=815)	Group 2 (*n*=259):	*P*	Group 1 (*n*=743)	Group 2 (*n*=336)	*P*
Qualitative variables							
Sex							
Male	767 (69)	545 (66.9)	189 (73)	.066	498 (67)	241 (71.7)	.124
Female	345 (31)	270 (33.1)	70 (27)		245 (33)	95 (28.3)	
Risk factor for HIV infection							
Heterosexual	726 (65.3)	531 (65.2)	164 (63.3)	.104	475 (63.9)	221 (65.8)	.563
sex	275 (24.7)	212 (26)	60 (23.2)		194 (26.1)	81 (24.1)	
MSM	56 (5)	34 (4.2)	20 (7.7)		34 (4.6)	20 (6)	
IVDU	55 (4.9)	38 (4.7)	15 (5.8)		40 (5.4)	14 (4.2)	
Other/unknown							
HIV RNA level, copies/mL							
⩽100,000	574 (51.6)	436 (53.5)	123 (47.5)	.007	385 (51.8)	175 (52.1)	.059
>100,000	448 (40.3)	326 (40)	104 (40.2)		308 (41.5)	125 (37.2)	
NA	90 (8.1)	53 (6.5)	32 (12.4)		50 (6.7)	36 (10.7)	
AST or ALT level (ACTG criteria)[a]							
Grade 0	735 (66.1)	550 (67.5)	166 (64.1)	.615	485 (65.3)	245 (72.9)	.021
Grade 1	332 (29.9)	236 (29)	84 (32.4)		233 (31.4)	88 (26.2)	
Grade 2	36 (3.2)	23 (2.8)	7 (2.7)		23 (3.1)	3 (0.9)	
Grade 3	7 (0.6)	5 (0.6)	1 (0.4)		1 (0.1)	0	
Grade 4	2 (0.2)	1 (0.1)	1 (0.4)		1 (0.1)	0	
HAART							
2 NRTIs+PI ± r	623 (56)	444 (54.5)	155 (59.8)	.097	408 (54.9)	193 (57.4)	.654
2 NRTIs+NNRTI	446 (40.1)	343 (42.1)	91 (35.1)		307 (41.3)	129 (38.4)	
Other[b]	43 (3.9)	28 (3.4)	13 (5)		28 (3.8)	14 (4.2)	
DDX							
Yes	271 (24.4)	167 (20.5)	91 (35.1)	<.001	153 (20.6)	105 (31.3)	<.001
No	841 (75.6)	648 (79.5)	168 (64.9)		590 (79.4)	231 (68.8)	
Quantitative variables							
Age							
Mean years ± SD	38 ± 10.4	36.9 ± 10.1	40.1 ± 10.5	<.001	37.9 ± 10.6	38.2 ± 10.2	.710
Median years	36	35	38		36	36	
CD4+ cell count							
Mean cells/mm^3 ± SD	218.9 ± 180.3	225 ± 178.8	214.3 ± 187.4	.420	214 ± 172.4	238.1 ± 196.1	.053
Median cells/mm^3	201	213	176		200.5	230.5	
HIV RNA level							
Mean copies/mL ± SD	215,105.5 ± 516,184.8	195,442.6 ± 406,865	242,778.7 ± 701,507.3	.333	218,945.6 ± 542,240.1	177,755.2 ± 331,247	.143
Median copies/mL	79,000	78,050	79,000		82,000	73,500	
γGT level							
Mean IU/L ± SD	52.4 ± 85.9	48.4 ± 78.1	60.4 ± 102.6	.096	49.2 ± 85.4	50.5 ± 66.5	.783
Median IU/L	29	27	32.5		28	30	
APRI score							
Mean score ± SD	0.5 ± 0.5	0.4 ± 0.3	0.4 ± 0.2	.023	0.4 ± 0.3	0.3 ± 0.2	<.001
Median score	0.4	0.3	0.4		0.4	0.3	
FIB-4 score							
Mean score ± SD	1.2 ± 1.3	1.1 ± 0.6	1.1 ± 0.4	.142	1.2 ± 0.8	1 ± 0.5	<.001
Median score	1	0.9	1		1	0.9	

NOTE. Data are no. (%) of patients, unless otherwise indicated. ACTG, AIDS Clinical Trials Group; ALT, alanine aminotransferase; AST, alanine aspartate aminotransferase; DDX, dideoxynucleoside-analogue drugs; HAART, highly active antiretroviral therapy; IVDU, intravenous drug users; MSM, men who have sex with men; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PI±r, PI with or without ritonavir; SD, standard deviation; ULN, upper limit of normal; γGT, gamma-gutamyl transpeptidase; APRI, AST-to-platelet ratio index; HIV, human immunodeficiency virus.

[a] ALT and/or AST levels were defined in accordance with ACTG criteria: grade 0, values at the ULN (40 IU/L); grade 1, 1.25–2.5 × the ULN; grade 2, 2.5–5.0 × ULN; grade 3, 5–10 × ULN; grade 4, >10 × ULN [27]

[b] Other included NRTI only (*n* = 27), NRTI+PI+NNRTI (*n* = 2), regimens including 2 full-dose PIs (*n* = 3), enfuvirtide-containing regimens (*n* = 3), and 3 NRTIs+PI±r or NNRTI (*n* = 8).

Table 2. Percentage Agreement between FIB-4 and APRI Scores at Baseline

	FIB-4, no. (%) of patients		
APRI	Class 1	Class 2	Class 3
Class 1	**718 (64.6)**	66 (5.9)	1 (0.1)
Class 2	110 (9.9)	**164 (14.7)**	20 (1.8)
Class 3	4 (0.4)	12 (1.1)	**17 (1.5)**

NOTE. For FIB-4, the 3 classes were as follows: class 1, ⩽ 1.45; class 2, 1.46–3.25; class 3, > 3.25. For APRI, the classes were as follows: class 1, ⩽0 .5; class 2, 0.51–1.5; class 3, > 1.5. The boldface type indicates percentages of concordant cases.

variables have been summarized with frequency distributions and percentages.

Concordance between FIB-4 and APRI scores was assessed using the kappa score, which is interpreted as follows: 0.8–1.0, almost perfect agreement; 0.6–0.79, substantial agreement; 0.4–0.59, moderate agreement; and <0.4, fair to poor agreement [21–23].

The incidence of FIB-4 and APRI transitions was assessed by Kaplan-Meier analysis. The at-risk population consisted of patients in class 1 or 2 with either FIB-4 or APRI scores at baseline. Patients were followed up until death, loss to follow-up (defined as lack of controls for ⩾1 year), improvement of LF, or achievement of the study outcome, whichever came first.

Cox regression analysis was used to explore possible predictors of LF progression, with a focus on modifiable factors. In a first set of analyses, only baseline fixed (non–time-dependent) covariates were used, as follows: sex, age, risk factor for HIV transmission, HIV RNA level, γGT level, CD4+ T cell count, and type of cART. In a second set of analyses, time-dependent covariates were also input into the models (HIV RNA level, γGT level, CD4+ T cell count, receiving vs not receiving therapy, and type of cART).

Although we recognized that it was difficult to disregard the impact of single drugs in the context of a combination therapy, a sensitivity analysis was conducted to explore the impact of dideoxynucleoside reverse-transcriptase inhibitors (DDX; ie, didanosine, stavudine and zalcitabine) on the risk of LF. These analyses were performed because several studies have indicated that these drugs may influence liver diseases [24–26].

The study was designed to generate hypotheses; thus, a formal sample size was not feasible, because the aim of the study was to explore the presence of any prognostic factors that were not known in advance. However, the likelihood ratio (LR) test has been used to test the global null hypothesis H_0:β = 0 (all regression coefficients equal to zero). Because the LR results were statistically significant, the proposed models fit the data significantly better than the null model. Data were analyzed using SAS software, version 9.2 (SAS) on a Windows XP Pro operating system. *P* values <.05 were considered statistically significant.

RESULTS

Patient Characteristics at Baseline and Concordance Study

Baseline characteristics of the 1112 patients included in the concordance study are shown in Table 1. Most patients were men and had acquired HIV infection through sexual intercourse. Half of them (49.7%) were severely immunosuppressed (CD4+ cell count, <200 cells/mm^3). Slightly more patients received protease inhibitors (PIs), either not boosted or boosted by low-dose ritonavir (PI±r), than received nonnucleoside reverse-transcriptase inhibitors (NNRTIs). The remaining 43 patients received mixed regimens based on nucleoside reverse-transcriptase inhibitors only or multiple-class regimens targeted to drug-resistant HIV strains. Two-thirds of the patients were in class 1 for either FIB-4 or APRI at baseline.

As shown in Table 2, 899 (80.8%) of 1112 patients belonged to the same classes for both FIB-4 and APRI. The majority of the discordant results belonged to class 1 or 2. The kappa-value was 0.573 (95% CI, 0.524–0.623).

Incidence of Liver Fibrosis Progression

Patients with class 3 for each score were excluded, leaving 1074 and 1079 patients in class 1 or 2 for the survival analyses of FIB-4 and APRI progression, respectively (Table 1). Patients were followed-up for a mean (±SD) of 2249 ± 1130 days.

Figure 1A represents the survival curves of transition from class 1 or 2 to higher classes (primary outcome). Regarding FIB-4, 259 (24.12%) of 1074 patients had LF progression, accounting for an incidence of 0.064 per PYFU (95% CI, 0.056–0.072). Transition was confirmed in 89 (8.29%) of 1074 patients, corresponding to an incidence of 0.018 per PYFU (95% CI, 0.014–0.022). Overall, among patients who experienced progression, 117 (45.17%) of 259 reverted back to lower FIB-4 classes without any subsequent worsening. Regarding APRI score, a total of 336 (31.14%) of 1079 patients had transition to higher classes, corresponding to an incidence of 0.099 per PYFU (95% CI, 0.089–0.110). Transition was confirmed in 126 (11.68%) of 1079 patients, corresponding to an incidence of 0.028 per PYFU (95% CI, 0.024–0.034). Overall 174 (51.79%) of 336 patients reverted back to lower APRI classes without worsening again during the follow-up.

Figure 1B represents the survival curves of transition from class 1 or 2 to class 3. With respect to FIB-4, 66 (6.15%) of 1074 patients had this transition, accounting for 0.013 per PYFU (95% CI, 0.010–0.017). With respect to APRI score, 83 (7.69%) of 1079 patients had transition to class 3, accounting for 0.018 per PYFU (95% CI, 0.014–0.022).

Risk Factors for Liver Fibrosis Progression

Baseline factors. Cox regression analyses for the transition from class 1 or 2 to higher classes (primary outcome) and for the

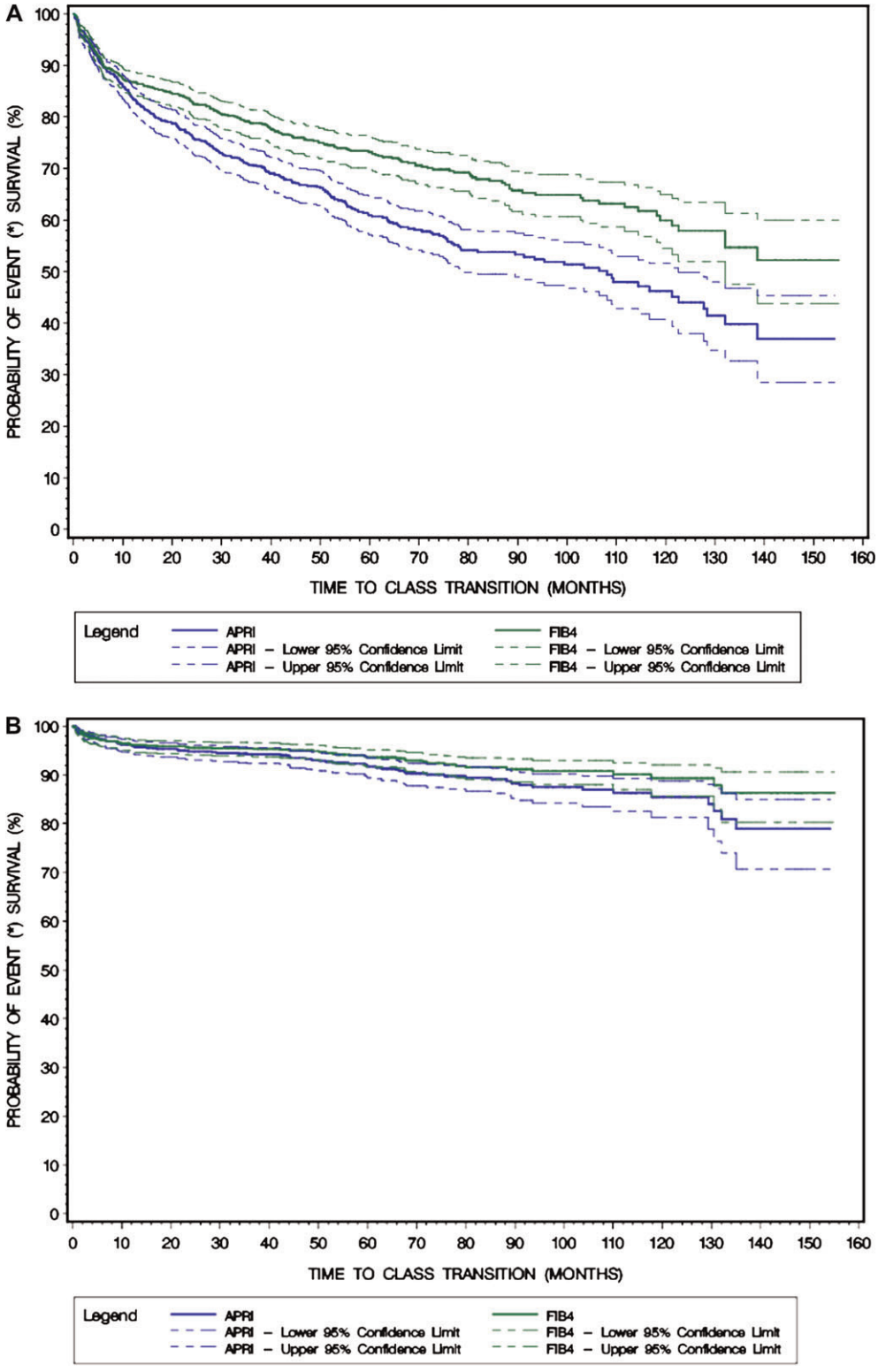


Figure 1. Survival curves of liver fibrosis progression for the primary outcome (any transition to higher classes; *A*) and for the secondary outcome (transition to class 3; *B*). An event was defined as the first class transition from class 1 or 2 to a higher class (*A*) and the first class transition from class 1 or 2 to class 3 (*B*). Patients who did not experience any class worsening were censored at the date of the last follow-up visit; patients who experienced a class improvement were censored at that date.

Table 3. Multivariate Cox Regressions Analyses of Baseline Risk Factors for Liver Fibrosis Progression (Any Transition to Higher Classes and Transition from Class 1–2 to Class 3)

	Transition to higher classes				Transition to class 3			
	FIB-4 SCORE		APRI SCORE		FIB-4 SCORE		APRI SCORE	
Risk factor	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
FIB-4 score (× 1.0) or APRI score (× 0.6)	1.526 (1.146–2.032)	.004	1.748 (1.162–2.631)	.007	2.560 (1.586–4.131)	<.001	5.023 (2.792–9.037)	<.001
Sex (M vs F)	1.134 (0.833–1.545)	.425	1.390 (1.059–1.826)	.018	1.767 (0.869–3.594)	.116	1.149 (0.686–1.926)	.598
Age (× 10 years)	1.037 (1.021–1.052)	<.001	1.000 (.987–1.012)	.938	1.033 (1.004–1.062)	.023	0.986 (.962–1.011)	.284
Risk for HIV infection (sex/others vs IVDU)	0.524 (0.290–0.944)	.031	1.051 (0.557–1.984)	.877	0.878 (0.211–3.649)	.858	0.894 (0.278–2.868)	.850
CD4+ T cell count (× 100 cells/mm^3)	0.999 (0.999–1.000)	.243	1.001 (0.999–1.001)	.798	1.000 (0.998–1.002)	.961	1.000 (0.998–1.001)	.569
HIV RNA level (× 1 $\log_{10}$ copies/mL)	1.013 (0.938–1.094)	.747	0.992 (0.926–1.063)	.822	1.021 (0.870–1.197)	.858	0.997 (0.872–1.140)	.963
Therapy with 2NRTI+NNRTI vs others	0.647 (0.362–1.157)	.142	0.725 (0.415–1.266)	.258	0.272 (0.099–0.748)	.012	0.278 (0.122–0.634)	.002
Therapy with 2NRTI+PI +/-r vs others	0.560 (0.311–1.010)	.054	0.725 (0.415–1.267)	.259	0.393 (0.148–1.042)	.061	0.312 (0.138–0.704)	.005
γGT (× 10 IU/L)	1.011 (1.000–1.023)	.059	1.015 (1.003–1.028)	.015	1.000 (.969–1.033)	.988	1.008 (.985–1.033)	.488

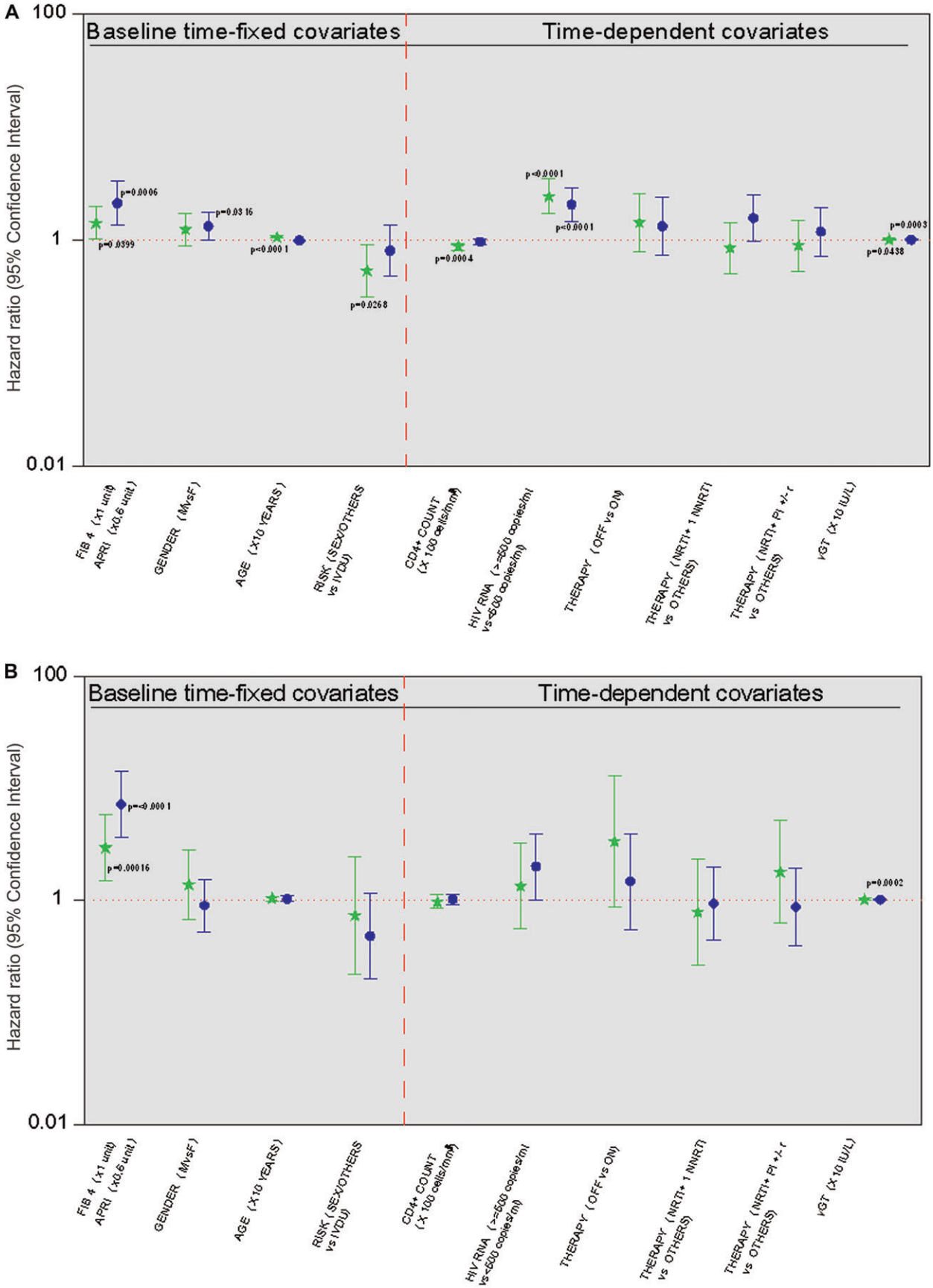
NOTE. For the primary outcome, likelihood ratio (χ^2) was 72.00 for FIB-4 transition (DF = 9; $P < .001$) and 23.13 for APRI transition (DF = 9; $P = .006$). CI, confidence interval; HR, hazard ratio; IVDU, intravenous drug users; F, female; M, male; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI+/-r, protease inhibitor with or without ritonavir; γGT, gamma-gutamyl transpeptidase.

transition to class 3 (secondary outcome) were first performed only with baseline time-fixed covariates.

At univariate analysis, the following factors were predictors of FIB-4 transition to higher classes: male sex (hazard ratio [HR], 1.453 [95% CI, 1.104–1.912]; $P = .008$), older age (per 10 years: HR, 1.050 [95% CI, 1.038–1.062]; $P < .001$), higher HIV RNA level (per $\log_{10}$ copies/mL: HR, 1.079 [95% CI, 1.002–1.162]; $P = .045$), higher γGT values (per 10 IU/L: HR, 1.014 [95% CI 1.005–1.024]; $P = .004$). By contrast, patients with higher CD4+ cell counts at baseline (per 100 cells/mm^3: HR, .999; [95% CI, 0.998–1.000]; $P = .008$) and those who acquired HIV by sexual risk or other modes of transmission versus through intravenous drug use (IVDU) appeared to be protected (HR, 0.572 [95% CI, 0.362–0.902]; $P = .016$). When the outcome was restricted to class 3 transition, older age (HR, 1.055 [95% CI, 1.032–1.078]; $P < .001$), and male sex (HR, 2.101 [95% CI, 1.145–3.854]; $P = .017$) were confirmed to be risk factors, whereas HAART therapy containing NNRTI appeared to be protective (HR, 0.341 [95% CI, 0.128–0.910]; $P = .032$), in comparison to other therapies.

Regarding APRI, male sex (HR, 1.385 [95% CI, 1.092–1.756]; $P = .007$) and higher γGT level (HR, 1.020 [95% CI, 1.010–1.030]; $P < .001$) were found to be risk factors for the primary outcome (any transition). The impact of γGT was confirmed for transition to class 3 (HR, 1.023 [95% CI, 1.006–1.040]; $P = .009$). Moreover, with this outcome, HAART (either containing PI±r or NNRTIs) was found to be protective (for NNRTI: HR, 0.290 [95% CI, 0.132–0.638]; $P = .002$; for PI: HR, 0.324 [95% CI, 0.152–0.691]; $P = .004$). Lastly, higher LF scores at baseline were associated with a greater risk of transition during the follow-up, both for FIB-4 (for each 1 unit increase for any transition: HR, 2.257 [95% CI, 1.816–2.806]; $P < .001$; for transition to class 3: HR, 3.431 [95% CI, 2.383–4.941]; $P < .001$) and for APRI (for each 0.6 unit increase for any transition: HR, 1.916 [95% CI, 1.350–2.720]; $P <= .001$; for transition to class 3: HR, 5.735 [95% CI, 3.460–9.507]; $P < .001$).

Table 3 shows the results of the multivariable analyses with both the primary (any transition) and the secondary (transition to class 3) outcome. Multivariate analysis confirmed that, in addition to higher FIB-4 and APRI scores, older age was a significant predictor of FIB-4 transition to higher classes, whereas risk factors other than IVDU and, at a borderline significance level, higher γGT level were predictors of FIB-4 transition to higher classes. Moreover, PI±r-containing regimens appeared to confer a borderline significant protection. Male sex and γGT level were confirmed as being risk factors for APRI transition to higher classes. Regarding the secondary outcome (class 3 transition), older age was confirmed as a significant predictor of FIB-4 transition, whereas HAART prescription (including either a PI±r or an NNRTI, in comparison with other regimens) appeared to confer a protection for both FIB-4 and APRI. Moreover, a sensitivity analysis including DDX showed that these drugs were associated with an independent risk of transition either to higher classes (for FIB-4: HR, 1.686 [95% CI, 1.256–2.263]; $P < .001$; for APRI: HR, 1.395 [95% CI, 1.068–1.823]; $P = .015$) or to class 3 (for APRI: HR, 1.796 [95% CI, 1.070–3.017]; $P = .027$).



Also, additional analyses exploring risk factors for confirmed transitions to higher classes indicated that receipt of a DDX prescription was significantly associated with an increased risk of progression to higher classes in the multivariate analysis for both FIB-4 (HR, 2.188 [95% CI, 1.320–3.626]; $P = .002$) and APRI (HR, 2.477 [95% CI, 1.652–3.715]; $P < .001$).

Baseline and Time-dependent Factors. Figure 2A shows the results of the multivariable Cox model with both time-fixed and time-dependent covariates for the primary outcome. Predictions of the baseline variables were confirmed, independently from the time-dependent covariates. Among the time-dependent covariates, HIV RNA>500 copies/mL and higher γGT values were independent risk factors. Moreover, for FIB-4 transition, higher CD4+ T cell count during the follow-up was protective. Sensitivity analysis including DDX as time-dependent covariate, showed that these drugs were associated with a significant risk of transition for both FIB-4 (HR, 1.662, 95% CI 1.237–2.233; $P = .0007$) and APRI (HR, 1.661, 95% CI, 1.286–2.145; $P <= .001$).

When we considered the confirmed transition to higher classes (confirmed primary outcome), higher CD4+ T cell count during the follow-up was an independent protective factor both for FIB-4 (HR, 0.882 [95% CI, 0.784–0.992]; $P = .036$) and APRI (HR, 0.908 [95% CI, 0.830–0.993]; $P = .034$). Additionally, receipt of DDX drugs was associated with a significant risk of transition for APRI.

Higher values of LF scores at baseline predicted transition to class 3 both for FIB-4 and for APRI, whereas HIV RNA level >500 copies/mL and higher γGT values were independent risk factors for APRI transition (Figure 2B). Sensitivity analysis showed that DDX use was associated with a significant risk of transition to APRI class 3 (HR, 2.612 [95% CI, 1.584–4.308]; $P <= .001$).

DISCUSSION

The concordance study showed moderate agreement between FIB-4 and APRI (kappa = 0.6). FIB-4 considers patient age and ALT level, whereas APRI does not, which produced different results with the predefined cut-off values. In particular, several patients who were in class 1 according to FIB-4 were in class 2 according to APRI. Therefore, APRI seemed to be stricter than FIB-4, but the clinical meaningfulness of this finding is questionable. In fact, both FIB-4 and APRI were shown to have the highest positive predictive value only for the most severe stages of LF [18]. For these reasons, rather than using FIB-4 and APRI as substitutes for liver histology at a single time-point for comparison of LF among different individuals, we used them to estimate the evolution of these markers and to determine associated risk factors for possible LF progression.

The cumulative incidence of LF progression ranged from 8.3% (for confirmed transition with FIB-4) to 31% (for single transition with APRI), and it was still significant for transition to grade 3 (6%–8%). Moreover, the risk was progressive over the entire follow-up period.

Predictors of LF progression were therefore assessed with attention to both baseline and time-varying factors. Interestingly, for the first time in a longitudinal study, a protective effect associated with control of HIV RNA level by HAART was demonstrated for progression both to higher classes (for FIB-4 and APRI) and to class 3 (for APRI). The effect was borderline significant for FIB-4 class 3, probably because of the lower number of patients who met this end point.

The effect of HAART as first-line treatment (including either an NNRTI or a PI±r) and the trend toward significant protection in patients who continued cART without any interruption provided consistent results. Moreover, higher CD4+ cell count over time was associated with lower risk, although the correlation was not confirmed for transition to grade 3. Overall, the positive impact of the control of HIV infection suggests that early initiation of HAART may provide an opportunity to prevent LF progression.

The strong and consistent association between higher fibrosis scores at baseline and the risk of fibrosis progression simply reflects a "regression-to-the-mean" effect, but it may be clinically relevant: LF should always be assessed, and more-proactive interventions should be performed for patients who already have a significant degree of LF.

Figure 2. Multivariable cox regression models with both time-fixed (baseline) and time-dependent (varying during the follow-up) covariates for the primary outcome (any transition to higher classes; *A*) and for the secondary outcome (transition to class 3; *B*). Green stars (FIB-4) or blue circles (APRI) represent hazard ratio; green or blue vertical lines represent 95% confidence intervals. *A*, Significant predictors for the primary outcome (transition to higher classes) were the following: HIV - RNA level >500 copies/mL for FIB-4 (HR, 2.456 [95% CI, 1.717–3.514]; $P < .001$); HIV RNA level >500 copies/mL for APRI (HR, 2.084 [95% CI, 1.495–2.907]; $P < .001$); per 10 IU/L increase of γGT for FIB-4 (HR, 1.009 [95% CI, 1.000–1.018]; $P = .0438$); per 10 IU/L increase of γGT for APRI (HR, 1.017 [95% CI, 1.008– - 1.026]; $P <= .001$); per 100 cell/mm^3 increase of CD4+ cell count for FIB-4 (HR, 0.881 [95% CI, 0.820–0.945]; $P <= .001$). Likelihood ratio (χ^2) for FIB-4=135.67; DF = 11; $P < .001$. Likelihood ratio (χ^2) for APRI = 91.90; DF = 11; $P < .001$. *B*, Significant predictors for the secondary outcome (transition to class 3) were the following: HIV RNA level >500 copies/mL for APRI (HR, 1.940 [95% CI, 0.968–3.888]; $P = .062$); per 10 IU/L increase of γGT for APRI (HR, 1.023 [95% CI, 1.011–1.036]; $P <= .001$). F, female; M, male; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI+/- r, protease inhibitor with or without ritonavir; γGT, gamma-gutamyl transpeptidase level; APRI, AST-To-platelets ratio index; HIV, human immunodeficiency virus.

Importantly, DDX use was associated with greater risk of LF progression according to both FIB-4 and APRI. In contrast, DallaPiazza et al. [4] did not find any association, but the cross-sectional design of their study may explain this apparent discrepancy. Prolonged didanosine exposure was associated with cryptogenic liver disease [25, 28]. Dideoxynucleosides were also associated with microvesicular steatosis leading to cirrhosis [29, 30]. Although DDX have been largely abandoned in developed countries, these drugs are still widely used in resource-limited settings. In view of their potential toxic effects [31, 32], including the progression of LF demonstrated herein, they must be avoided.

Increasing γGT level was associated with the risk of fibrosis progression, emphasizing the importance of reducing possible alcohol abuse. Moreover, the risk of fibrosis progression appeared greater in IVDUs, possibly reflecting liver damage attributable to the use of recreational drugs, concomitant alcohol abuse, or HCV co-infection that was not detected through HCV antibody testing [33]. Furthermore, older patients and men appeared to be more vulnerable for the risk of FIB-4 progression, which is analogous with previous results for HCV-infected patients [34]. It may be that host mechanisms against Hepatitis E virus infection were weaker in older people and that alcohol abuse was more common among men than among women.

This study has limitations that should be acknowledged. First, LF scores are not yet the gold standard, but liver biopsy is not ethically acceptable for monitoring LF, and our study would have required a much greater length of follow-up and/or a much larger cohort of patients for determination of clinical outcomes. Second, we point out that earlier HAART initiation could be beneficial, but this conclusion would have been stronger if it had been supported by analysis of fibrosis progression rates prior to and after cART or, ideally, by a randomized clinical trial. Third, we used γGT level as a surrogate marker for alcohol intake [19, 20]. Fourth, extensive work-up to find the causes of elevated FIB-4 and APRI scores (eg, HCV RNA level, liver steatosis, and Hepatitis E virus (HEV) infection) was not available for most patients. Additional studies are needed in this respect.

In conclusion, this study demonstrated that HIV-monoinfected patients display significant risk of LF progression. Importantly, control of HIV RNA levels induced by HAART may slow down this process. Although formal validations are necessary, in the meantime, FIB-4 and APRI should be used to identify those patients who are most at risk and who need further clinical work-up (such as liver biopsy) and more-proactive counseling to modify dangerous behaviors (such as alcohol abuse). Lastly, our results suggest the benefit of earlier HAART initiation for liver disease fibrosis in HIV-monoinfected patients, provided that the most dangerous drugs (ie, DDX) are avoided.

Acknowledgments

Financial support. Abbott Italia. Statistical analysis was performed by CROS NT.

Potential conflicts of interest. C.T. and G.C. have received unrestricted educational grants (as speakers or for participation to conferences) from Abbott, Gilead, Merck, GlaxoSmithKline, Bristol Myers Squibb, Schering Plough, and Roche Spa.

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