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ORIGINAL PAPER

Suboptimal performance of APRI and FIB-4 in ruling out significant fibrosis and confirming cirrhosis in HIV/HCV co-infected and HCV mono-infected patients

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Abstract

Purpose We aimed to assess the diagnostic reliability of two indirect biomarkers, APRI and FIB-4, for the staging of liver fibrosis using transient elastography (TE) as reference standard, among HIV/HCV co-infected and HCV mono-infected patients.

Methods This is an observational, retrospective study on subjects who had access to the RESIST HCV from October 2013 to December 2016, a regional network encompassing 22 hospitals and academic centers throughout Sicily. Sensitivity, specificity and diagnostic accuracy of indirect biomarkers for liver stiffness measurement (LSM) < 9.5 kPa (significant fibrosis) and LSM ≥ 12.5 kPa (cirrhosis) were determined by receiver operator characteristics (ROC) curves.

Results 238 HIV/HCV co-infected and 1937 HCV mono-infected patients were included. Performances of FIB-4 and APRI for the detection of significant fibrosis and cirrhosis proved to be unsatisfactory, with very high false negative and false positive rates among both cohorts. No significant differences were found after stratification of HIV/HCV co-infected patients for BMI < or ≥ 25, ALT < or ≥ 40 IU/L, ALT < or ≥ 80 IU/L, and presence/absence of a bright liver echo pattern on ultrasonography.

Conclusions Differently from other studies, we detected the unreliability of APRI and FIB-4 for the assessment of liver fibrosis in both HCV mono-infected and HIV/HCV co-infected patients.

Keywords APRI · FIB-4 · HIV · HCV · Noninvasive biomarkers · Transient elastography

Introduction

The evaluation of liver fibrosis by noninvasive methods is broadly performed and has almost replaced liver biopsy in the management of patients with viral hepatitis. Similarly, existing noninvasive methods are widely used in clinical practice in patients with HIV/HCV co-infection [1].

Treatment guidelines emphasize the great clinical importance of estimating the degree of liver fibrosis, as this impacts treatment strategies and prognosis in patients with liver disease [2]. Both European Association for the Study of the Liver (EASL) and Latin American Association for the study of the Liver (ALEH) assert that fibrosis stage can be assessed by noninvasive methods initially, and that liver biopsy should be reserved for cases where there is diagnostic uncertainty or potential additional aetiologies [3, 4]. In this context, a well-established panel of biomarkers of fibrosis or

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liver stiffness measurement (LSM) by transient elastography (TE) can be applied, as both perform well in the identification of cirrhosis or absence of fibrosis, even if they perform less well in identifying the intermediate stages of fibrosis.

Two of the most validated indirect biomarkers of liver fibrosis are aspartate aminotransferase-to-platelet ratio index (APRI) and FIB-4. APRI—[AST/ULN (upper limit of normal of AST) × 100]/platelets (10^9/L)—was developed in HCV patients for the diagnosis of Metavir F2 (cutoffs of 0.5 and 1.5) or F4 (cutoffs of 1.0 and 2.0) [5]. FIB-4—(age × AST)/(platelets (10^9/L) × $ALT^{1/2}$)—was specifically developed in HIV/HCV co-infected patients for the diagnosis of Metavir F3 and subsequently validated for HCV mono-infection. The cutoffs of interest are 1.45 for ruling out and 3.25 for diagnosing at least F3. Using these cutoffs, 70% of liver biopsies could be avoided, whereas 87% of patients were correctly classified [6]. Nonetheless, data from literature on the performance of these tests in the population of co-infected HIV/HCV patients were widely variable according to the different cohorts and settings that were taken into account [7, 8].

On these premises, the aim of this study was to evaluate the accuracy of APRI and FIB-4 for ruling out significant fibrosis and confirming cirrhosis, using transient elastography (TE) as reference standard, in two different cohorts composed by HIV/HCV co-infected and HCV mono-infected patients.

Materials and methods

Patients and instrumental assessment

This is an observational, retrospective study on subjects who had access between October 2013 and December 2016 to the RESIST HCV (Rete Sicilia Selezione Terapia HCV), a regional web-based network acknowledged by the Regional Health Authority encompassing 22 public hospitals and academic centers throughout Sicily which registers all patients with chronic HCV infection (both mono- and HIV/HCV co-infected) that are evaluated for direct-acting antiviral treatments [9]. A diagnosis of cirrhosis was established if at least one of the following features was present: LSM ≥ 12 kPa, previous liver biopsy with stage 4 fibrosis by METAVIR score, esophageal and/or gastric varices at esophagogastroduodenoscopy, and platelet count less than 100×10^9/L [10].

Among the entire population, we first chose patients who presented complete blood tests and who underwent TE within one month from the inclusion. Second, we selected patients according to their age, ≥ 40 and ≤ 60 years old. None of the patients were on anti-HCV treatment at the time of evaluation. LSM was obtained using Fibroscan (FibroScan®, Echosens, Paris, France) and expressed in kiloPascal (kPa): a median value of ten successful measurements was considered to be the representative LSM, given a success rate of at least 60% and an interquartile range of < 30%. TE was considered as the reference standard in relation to the two indirect biomarkers of fibrosis that we calculated, APRI and FIB-4. Another data that we extracted was the detection of a bright liver echo pattern—the ultrasonographic sign of hepatic steatosis, i.e., fine, packed and high amplitude echoes, with consequent brightness of liver, increase in liver–kidney contrast and possible evidence of vascular blurring and deep attenuation signs [11].

Statistical analysis

Data were collected with a predefined pro-forma. Clinical, biochemical and ultrasonographic results were entered into an electronic database together with data on LSM and endoscopic features for each patient.

In the descriptive analysis, false positive and false negative rates of the two indirect biomarkers were determined for both stages. The concordance level of APRI and FIB-4 to rule out significant fibrosis (LSM < 9.5 kPa) and confirm cirrhosis (LSM ≥ 12.5 kPa) was determined by Cohen's kappa. A vibration controlled transient elastography cutoff of 9.5 kPa is considered as the cutoff value to rule out advanced liver fibrosis and 12.5 kPa the cutoff value to confirm cirrhosis by the American gastroenterology Association Guidelines [12]. Furthermore, we have determined the Area Under ROC curves (AUROCs) among the two cohorts of mono- and co-infected patients, for each biomarker and for each cutoff of LSM. We compared AUROCs among the two cohorts by DeLong's test. In the cohort of HIV/HCV co-infected patients, the performance of the tests was also assessed after stratifying for BMI < or ≥ 25, ALT < or ≥ 40 IU/L, ALT < or ≥ 80 IU/L, and the presence or absence of a bright liver echo pattern on ultrasonography.

All statistical analyses were carried out using RStudio (version 0.98.945, RStudio Inc., Boston, MA, USA) for the software R (3.1.—2014-04-10, R Foundation for Statistical Computing, Vienna, Austria) with package "pROC".

Results

The study population consisted of 238 HIV/HCV co-infected and 1937 HCV mono-infected patients, selected from a total of 369 HIV/HCV co-infected and 8591 HCV mono-infected patients who had access to the RESIST HCV at the time of the study. A total of 6785 patients could not be included in analyses for the following reasons: no TE performed ($n = 2312$), missing recent blood tests—less than one month apart from TE—($n = 410$), age ≥ 60 years old or

≤40 years old ($n=4063$). According to RESIST-HCV criteria, the diagnosis of cirrhosis was made in 125 (52.5%) HIV/HCV co-infected and in 756 (39.0%) HCV mono-infected patients. Main demographic and clinical characteristics, and the overall estimates of fibrosis are summarized in Table 1.

In the cohort of mono-infected patients, AUROCs of APRI for the detection of LSM < 9.5 kPa and LSM ≥ 12.5 kPa were 0.782 and 0.806, respectively, while AUROCs of FIB-4 for the detection of LSM < 9.5 kPa and ≥ 12.5 kPa were 0.760 and 0.799, respectively. In the cohort of co-infected patients, AUROCs of APRI for the detection of LSM < 9.5 kPa and ≥ 12.5 kPa were both 0.771, while AUROCs of FIB-4 for the detection of LSM < 9.5 kPa and ≥ 12.5 kPa were 0.776 and 0.771, respectively. The selected cutoff values for FIB-4 inferior to 1.21 to rule out significant fibrosis and superior to 5.88 to confirm cirrhosis were extracted from the study by Li et al. [13]. APRI cutoff inferior to 1.0 is indicated in the study by Lin et al. where it is equivalent to LSM < 9.5 in ruling out fibrosis F3 [14]; APRI cutoff superior to 2.0 in detecting cirrhosis (LSM ≥ 12.5 kPa) was validated by Castera et al. [15]. Sensitivity, specificity, false negative and false positive rates of indirect biomarkers for ruling out significant fibrosis and detecting cirrhosis among the two cohorts of patients are shown in Tables 2 and 3.

Comparison of AUROCs of the two tests among the two different cohorts, for both stages of liver fibrosis, showed no significant difference in their performance, as depicted in Figs. 1 and 2. Similarly, no significant difference was found after stratification of HIV/HCV co-infected cirrhotic patients (LSM ≥ 12.5 kPa) for BMI < or ≥ 25, ALT < or ≥ 40 IU/L, ALT < or ≥ 80 IU/L, and presence or absence of a bright liver echo pattern, as summarized in Tables 4 and 5.

Table 1 Main demographic and clinical characteristics, and the overall estimates of fibrosis of patients included in the study

Variable	HCV mono-infected ($n=1937$)	HIV/HCV co-infected ($n=238$)
Males	1352 (69.8%)	183 (76.9%)
Females	585 (30.2%)	55 (23.1%)
Mean age (years)	51	52
Main HCV genotypes	1b (52.6%)	1b (21.3%) 3 (31.5%)
Mean LSM value (kPa)	16.5	16.2
LSM ≥ 12.5 kPa	741 (38.2%)	118 (49.6%)
Mean PLT value (/mmc)	181.000	155.700
Mean Fib4 value	3.08	3.70
Mean APRI value	1.43	1.50
Histological/clinical diagnosis of cirrhosis	756 (39.0%)	125 (52.5%)

Discussion

Although transient elastography is a well-validated noninvasive test of fibrosis, it is not broadly available.Therefore, we need to simplify the management of HCV mono-infected and HIV/HCV co-infected patients who emerged. In keeping with this, the aim of our real-life study was to assess and compare the performance of two noninvasive tests—APRI and FIB-4—in ruling out significant fibrosis and confirming cirrhosis using TE as reference standard, in

Table 2 Performances of FIB-4 and APRI for the detection of TE < 9.5 kPa (rule out significant fibrosis) and ≥ 12.5 kPa (confirm cirrhosis) in HCV mono-infected patients

FIB-4	LSM < 9.5 kPa	LSM ≥ 12.5 kPa
AUROC	0.760	0.799
Cutoff value	1.21	5.88
Sensitivity	85%	25%
Specificity	42%	98%
False negative rate	58.4%	74.7%
False positive rate	15.1%	1.7%
Cohen's kappa	0.24	0.22
APRI	LSM < 9.5 kPa	LSM ≥ 12.5 kPa
AUROC	0.782	0.806
Cutoff value	1.0	2.0
Sensitivity	54%	41%
Specificity	87%	95%
False negative rate	12.8%	44.2%
False positive rate	45.9%	5.3%
Cohen's kappa	0.29	0.33

Table 3 Performances of FIB-4 and APRI for the detection of TE < 9.5 kPa (rule out significant fibrosis) and ≥ 12.5 kPa (confirm cirrhosis) in HIV/HCV co-infected patients

FIB-4	LSM < 9.5 kPa	LSM ≥ 12.5 kPa
AUROC	0.776	0.771
Cutoff value	1.21	5.88
Sensitivity	91%	26%
Specificity	32%	97%
False negative rate	67.9%	73.7%
False positive rate	9.3%	3.3%
Cohen's kappa	0.13	0.14
APRI	LSM < 9.5 kPa	LSM ≥ 12.5 kPa
AUROC	0.771	0.771
Best cutoff value	1.0	2.0
Sensitivity	60%	40%
Specificity	82%	91%
False negative rate	17.9%	60.2%
False positive rate	40.6%	9.2%
Cohen's kappa	0.25	0.20

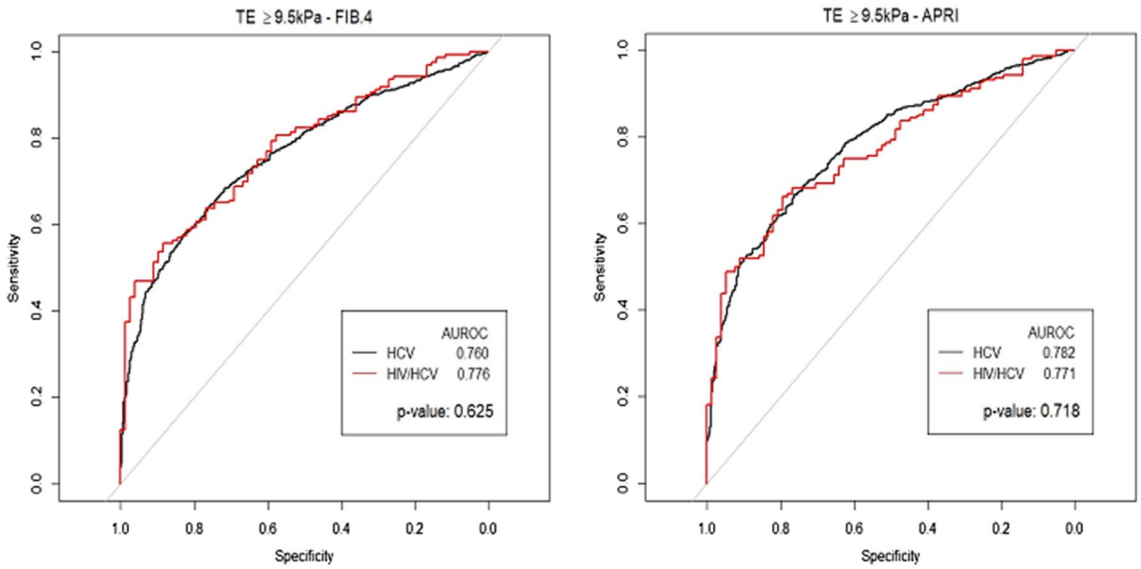

Fig. 1 Comparison of the performance of each biomarker (FIB-4 and APRI) among the two cohorts of mono- and co-infected patients for LSM ≥ 9.5 kPa

a large population of mono-infected HCV patients and co-infected HIV/HCV patients (total sample: 2175 patients). Overall, the diagnostic performances of APRI and FIB4 were disappointing not only in ruling out significant fibrosis (LSM < 9.5 kPa)—an issue which was already reported by data from literature [4]—but also in the assessment of cirrhosis, a setting in which the use of such biomarkers is recommended also by EASL guidelines [3]. Interestingly, these poor performances were reported not only among co-infected HIV/HCV patients, but also among the mono-infected HCV population.

In this line, literature on performance of these tests in the population of co-infected HIV/HCV patients is not particularly extensive, and sample sizes and results among the different studies were widely variable [7, 8]. Schmid et al. [16] compared the diagnostic performance of noninvasive tests, including TE, APRI, and FIB-4 vs liver biopsy to assess fibrosis progression over 3 years in 105 co-infected patients.

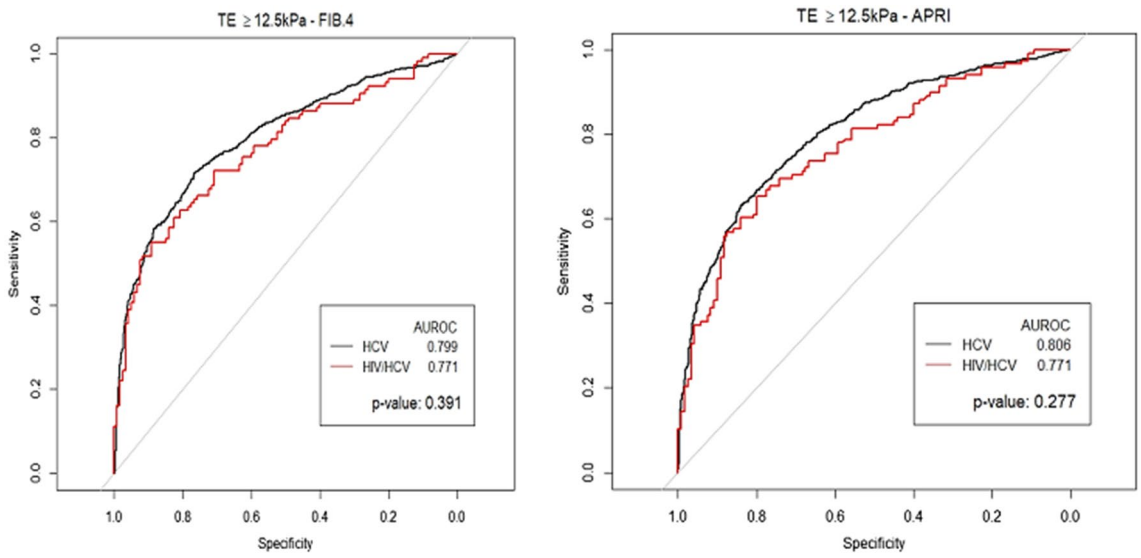

Fig. 2 Comparison of the performance of each biomarker (FIB-4 and APRI) among the two cohorts of mono- and co-infected patients for LSM ≥ 12.5 kPa

Table 4 Stratification of HIV/HCV co-infected patients in subgroups and *p* value of FIB-4 for LSM ≥ 12.5 kPa

FIB-4			
Subgroups	Patients (%)	LSM ≥ 12.5 kPa	
		AUROCs	*p* value
BMI < 25 vs BMI ≥ 25	73.1 vs 26.9	0.72 vs 0.75	0.688
ALT < 40 IU/L vs ALT ≥ 40 IU/L	27.4 vs 72.6	0.66 vs 0.75	0.312
ALT < 80 IU/L vs ALT ≥ 80 IU/L	67.8 vs 32.2	0.72 vs 0.71	0.875
Bright liver echo pattern, yes vs no	32.2 vs 67.8	0.64 vs 0.76	0.159

Table 5 Stratification of HIV/HCV co-infected patients in subgroups and *p* value of APRI for LSM ≥ 12.5 kPa

APRI			
Subgroups	Patients (%)	LSM ≥ 12.5 kPa	
		AUROCs	*p* value
BMI < 25 vs BMI ≥ 25	73.1 vs 26.9	0.71 vs 0.72	0.958
ALT < 40 IU/L vs ALT ≥ 40 IU/L	27.4 vs 72.6	0.67 vs 0.74	0.375
ALT < 80 IU/L vs ALT ≥ 80 IU/L	67.8 vs 32.2	0.71 vs 0.68	0.693
Bright liver echo pattern, yes vs no	32.2 vs 67.8	0.69 vs 0.72	0.703

TE showed the higher diagnostic accuracy in detecting both Metavir ≥ F2 and Metavir F4, while APRI and FIB-4 performed as good as or even better than the more sophisticated and expensive composite markers (Fibrotest and ELF). Merli et al. [17] compared the diagnostic accuracy of APRI, FIB-4, and Forns Index with TE for the detection of liver cirrhosis in 646 co-infected patients, concluding that indirect biomarkers could be routinely used in this setting of patients to exclude liver cirrhosis and that, even though the assessment with more accurate technique is recommended, their ready availability could improve liver fibrosis staging in primary referral centres and in resource-limited settings. In our real-life study, we were not able to replicate these good performances of APRI and FIB-4. Furthermore, the most unexpected findings were the extremely high false negative rates of both biomarkers in both cohorts. Among the current evidences, the study of Merli et al. [17] is the one with the highest rate of misclassification (in co-infected patients for TE ≥ 13 kPa), however, much lower than ours: for APRI, the false negative rate was 6%, versus 60.2% in our study; similarly, for FIB-4, the false negative rate was 5%, versus 73.7% in our study.

To avoid the comparison between groups with different median age, patients have been selected according to age ranges of ≥ 40 and ≤ 60 years old. Thus, we divided the cohort of co-infected patients into several subgroups, according to features that represent notorious risk factors among this population (elevated AST/ALT, body mass index and presence of steatosis, evaluated by ultrasonography), to assess their possible influence on the diagnostic performance of indirect biomarkers compared to the cohort of mono-infected patients. Anyway, the analysis did

not show evidence of any significant difference, and the overall performance of the two tests was unsatisfactory.

The characteristic of being a real-life study, without any patients' initial selection, and the very large number of included subjects allow us to consider these data as reliable. Nevertheless, our study has some limitations: the analysis of the data was retrospective, and we did not evaluate post-treatment data, that could present evidence of different behaviour of biomarkers compared to TE.

It is now recognized that HIV/HCV co-infected patients are a special population who need special attention in monitoring and follow-up of liver disease: while approximately 20% of HCV untreated patients have a natural progression of liver disease in about 20–30 years, HIV/HCV co-infected patients have a seven times faster progression of the disease [18, 19]. Furthermore, steatosis seems more frequent and severe among co-infected patients compared with HCV mono-infected patients, and it is associated with significant liver fibrosis, which may contribute to a more rapid progression of liver disease [20, 21]. Consequently, the survival rate in this population is considerably reduced because of complications, such as gastrointestinal bleeding, ascites, hepatic encephalopathy and hepatocellular carcinoma [22, 23]. All these data pointed out the need for a reliable tool to assess liver damage and predict liver fibrosis progression. Among the Sicilian population of co-infected HIV/HCV and mono-infected HCV patients selected from the RESIST HCV, we demonstrated the inapplicability of APRI and FIB-4 for the detection of cirrhosis and the possibility to exclude advanced fibrosis. Indeed, the high false negative rate would result in a dangerous under-diagnosis of cirrhosis and therefore patients may not undergo adequate follow-up. On the other side, APRI and FIB-4 performed suboptimally in ruling out significant fibrosis and as such it would not be possible to determine the most appropriate follow-up in patients with mild disease. Consequently, TE remains essential for an adequate risk assessment of disease progression, hepatic decompensation and death, and currently it cannot be replaced, according to our results, by serological markers. All these issues will be of relevance also in this post-DAAs era, as the importance of the assessment of fibrosis still resides in the need to subject patients to periodic follow-up and screening of complications, especially in advanced fibrosis. This is even more important in co-infected patients, where liver fibrosis progression is accelerated and it also depends on other factors, such as concurrent potentially hepatotoxic therapies, aging, comorbidities, HIV-related factors (duration of HIV viremia and low CD4 count), immune activation, and inflammation.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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