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FULL PAPERS

Diagnostic accuracy of APRI, FIB-4 and Forns for the detection of liver cirrhosis in HIV/HCV-coinfected patients

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SUMMARY

We determined the diagnostic accuracy and optimal cut off of three indirect fibrosis biomarkers (APRI, FIB-4, Forns) compared with liver stiffness (LS) for the detection of liver cirrhosis in HIV/HCV-coinfected patients. An observational retrospective study on HIV/HCV-coinfected patients with concomitant LS measurement and APRI, FIB-4 and Forns was performed. The presence of liver cirrhosis was defined as a LS ≥13 KPa. The diagnostic accuracy and optimal cut-off values compared with LS categorization (<13 vs ≥13 KPa), were determined by receiver operating characteristics (ROC) curves.

The study sample included 646 patients. The area-under-the ROC curve (95% confidence interval) for the detection of liver cirrhosis was 0.84 (0.81-0.88), 0.87 (0.84-0.91) and 0.87 (0.84-0.90) for APRI, FIB-4 and Forns, respectively. According to the optimal cut off values for liver cirrhosis (≥0.97 for APRI, ≥2.02 for FIB-4 and ≥7.8 for Forns), 80%, 80% and 82% of subjects were correctly classified by the three indirect fibrosis biomarkers, respectively. Misclassifications were mostly due to false positive cases.

The study suggests that indirect fibrosis biomarkers can help clinicians exclude liver cirrhosis in the management of HIV/HCV co-infected patients, reducing the frequency of more expensive or invasive assessments.

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INTRODUCTION

Non-invasive assessment of liver fibrosis represents an appealing method to monitor liver disease in HCV-infected patients. Currently, transient elastography (TE) is the most accurate non-invasive tool to measure liver stiffness (LS), with the diagnostic accuracy increasing together with the stage of fibrosis (Friedrich Rust *et al.,* 2008; Degos *et al.,* 2010). Stiffness measurement is widely used in the assessment of fibrosis in patients with chronic hepatitis C given its good reproducibility (Fraquelli *et al.,* 2007) and its association with the risk of liver-related complications and death in HIV/HCV-coinfected patients (Fernandez-Montero *et al.,* 2013) and also in patients with compensated HCV-related liver cirrhosis (with or without concomitant HIV-coinfection) (Pérez-Latorre *et al.,* 2014).

In the last decade, direct and indirect biomarkers for predicting liver fibrosis have also been developed. Direct fibrosis biomarkers (e.g. FibroTest, FibroMeter) are calculated using serum molecules produced in the presence of liver fibrosis and released in the circulatory system while indirect biomarkers (e.g. APRI, FIB-4, Forns) result from the combination of routine blood tests. Even though indirect biomarkers had a lower diagnostic performance than direct biomarkers and especially TE (Sánchez-Conde *et al.,* 2010; Degos *et al.,* 2010; Castéra *et al.,* 2014), the absence of additional costs and the ready availability make indirect biomarkers a quick and easy non-invasive method to periodically assess liver fibrosis.

Since the early detection of liver cirrhosis has a significant impact on both clinical management and treatment decision regarding chronic hepatitis C, we evaluated the threshold and the diagnostic accuracy of APRI, FIB-4 and Forns for the diagnosis of liver cirrhosis in HIV/HCV-coinfected patients.

MATERIALS AND METHODS

This is an observational, retrospective study on HIV/HCV co-infected subjects who concomitantly performed at our clinic TE for the evaluation of LS and blood test (less than two months apart from TE) between September 2007 and May 2013. The presence of HCV co-infection was defined by a positive HCV antibody. None of the patients had transaminases flares [alanine amino transferase (ALT) and/or aspartate amino transferase (AST) >5x previous determination or >10x upper limit of normality] or was on anti-HCV treatment at the time of LS measurement.

LS was measured using FibroScan (EchoSens®, Paris, France) and expressed in kilopascal (KPa). All measures were performed after a 12 hour fast. A median value of ten successful measurements was considered to be the representative measurement of LS, given a success rate of at

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least 60% and an interquartile range (IQR) of <30%. Liver cirrhosis was defined as a LS value ≥13 kPa (Friedrich Rust *et al.,* 2008). TE was used as the reference standard.
Three indirect fibrosis biomarkers were evaluated: APRI (Wai *et al.,* 2003), Forns (Forns *et al.,* 2002) and FIB-4 (Sterling *et al.,* 2006). The occurrence of major liver events (ascites, porto-systemic encephalopathy, oesophageal varices bleeding, hepatocellular carcinoma) before TE examination was also assessed.
Continuous variables were described by median and interquartile range (IQR), while categorical variables by absolute counts and percentages (%).
The ability of the three indirect fibrosis biomarkers to detect advanced liver fibrosis (as resulted from TE) was determined by the area-under-the-curve (AUC) of receiver operating characteristics (ROC) curves. For each indirect fibrosis biomarker, the optimal cut-off value, predicting the presence or absence of advanced liver fibrosis, was calculated. To evaluate the diagnostic accuracy of each indirect fibrosis biomarker, sensitivity, specificity, negative predictive value and positive predictive value with the corresponding 95% confidence intervals (95% CI) were estimated for each cut-off value; the accuracy characteristics were determined for dichotomous categorization of each indirect fibrosis biomarker (according to the optimal cut-off value estimated by ROC curve) compared with dichotomous LS categorization (<13 vs ≥13 KPa). Total accuracy was also assessed by the percentage of patients that were correctly classified by each indirect fibrosis biomarker according to the corresponding optimal cut-off value.
The statistical analyses were carried out using SAS System, version 9.2 (SAS Institute, Cary, NC).

RESULTS

We included in the analysis 646 patients. Main demographic, clinical and biochemical characteristics are described in *Table 1*.
Overall, the median LS value was 7.5 (5.6-13.8) KPa and 172 (27%) patients showed LS ≥13 KPa. Patients with LS ≥13 KPa as compared to those with LS <13 KPa were older, had a longer duration of HIV infection, a lower nadir CD4+ cell count, higher AST and ALT values, lower albumin, platelet count and pseudocholinesterases.
At the time of TE evaluation, 27 (4%) patients had previously developed at least one major liver event (ascites n=12, porto-systemic encephalopathy n=2, hepatocellular carcinoma n=3, oesophageal varices n=10) for an overall incidence rate of 2.92 per 1000-person-years of follow-up (PYFU) since the beginning of antiretroviral treatment (95% CI: 1.82-4.03). Twenty-three patients with an LS value ≥13 KPa had ≥1 major liver event [over 2270 PYFU, the incidence rate of liver events was 10.13 per 1000-PYFU (95% CI: 5.99-14.27)] and 4 patients with an LS value <13

Table 1 - *Main characteristics of the 646 HIV/HCV co-infected subjects according to liver stiffness.*

	All *n = 646*	*LS <13 kPa* *n = 474*	*LS ≥13 kPa* *n = 172*	*p-value*
Gender, n male	 463 (72%)	 329 (69%)	 134 (78%)	0.038
Age, years	48 (45-51)	48 (44-51)	49 (46-52)	0.001
Duration of HIV infection, years	22 (15-26)	21 (14-26)	26 (23-31)	0.001
On antiretroviral therapy, n NRTI-based NNRTI-based PI-based INI- or EI-based	616 (95%) 23 (4%) 88 (14%) 422 (68%) 84 (14%)	448 (94%) 17 (4%) 72 (16%) 299 (67%) 60 (13%)	168 (98%) 6 (4%) 16 (9%) 123 (73%) 24 (14%)	0.010 0.214
Time on antiretroviral therapy, years	14 (9-18)	14 (8-18)	14 (11-17)	0.345
Nadir CD4 cells count, cells/µl	172 (85-280)	187 (100-297)	137 (58-235)	<0.0001
CD4 cells count cells/µl %	 525 (373-733) 27 (21-33)	 552 (407-739) 27 (22-33)	 448 (302-720) 27 (19-33)	 0.001 0.395
Plasma HIV-RNA, n <50 copies/µl	 500 (83%)	 368 (82%)	 132 (85%)	0.458
Alanine aminotransferase, IU/L	63 (41-103)	57 (38-91)	82 (55-135)	<0.0001
Aspartate aminotransferase, IU/L	45 (31-75)	28 (28-58)	83 (51-122)	<0.0001
Albumin, g/L	42 (39-44)	43 (41-44)	39 (36-42)	<0.0001
Platelet count, 103/µl	175 (131-213)	190 (154-223)	113 (81-151)	<0.0001
Pseudocholinesterases, KU/L	7.5 (5.7-9.2)	8.1 (6.6-9.5)	5.1 (3.2-7.3)	<0.0001
International normalized ratio (INR)	0.99 (0.94-1.06)	0.97 (0.93-1.02)	1.08 (1.00-1.18)	<0.0001
Plasma HCV-RNA, Log IU/mL	5.88 (5.04-6.40)	5.91 (5.11-6.44)	5.79 (4.87-6.32)	0.100

Abbreviations: LS = liver stiffness; NRTI = nucleos(t)idic reverse transcriptase inhibitors; NNRTI = non-nucleosidic reverse transcriptase inhibitors; PI = protease inhibitors; INI-EI = integrase inhibitors - entry inhibitors.

Table 2 - *Diagnostic accuracy and optimal cut off values of fibrosis biomarkers as compared with TE for the detection of liver cirrhosis among 646 HIV/HCV co-infected patients.*

	APRI	*FIB-4*	*Forns*
AUC (95% CI)	0.84 (0.81-0.88)	0.87 (0.84-0.91)	0.87 (0.84-0.90)
Optimal cut-off	0.97	2.02	7.08
Total accuracy %	80.3	80.0	82.4
Sensitivity (95% CI) %	76.1 (69.2-81.9)	81.4 (74.9-86.5)	71.5 (64.3-77.8)
Specificity (95% CI) %	81.9 (78.1-85.1)	79.5 (75.7-82.9)	86.3 (82.9-89.1)
PPV (95% CI) %	60.4 (53.7-66.6)	59.1 (52.7-65.1)	65.4 (58.4-71.9)
NPV (95% CI) %	90.4 (87.3-92.9)	92.2 (89.1-94.4)	89.3 (86.1-91.8)
Correctly classified patients n (%)	519 (80)	517 (80)	532 (82)
False positive cases n (%)	87 (14)	97 (15)	65 (10)
False negative cases n (%)	40 (6)	32 (5)	49 (8)

Abbreviations: TE, transient elastography; AUC = area under the curve; PPV = positive predictive value; NPV = negative predictive value; 95% CI = 95% confidence interval.

KPa had ≥1 major liver event [over 6965 PYFU, the incidence rate of liver events was 0.57 per 1000-PYFU (95% CI: 0.15-1.27)].

The median values of indirect fibrosis biomarkers were: 0.63 (0.37-1.30) for APRI, 1.62 (1.07-2.70) for FIB-4 and 6.06 (4.91-7.46) for Forns. Higher values of APRI, FIB-4 and Forns were observed in patients with LS ≥13 KPa compared to those with LS <13 KPa [APRI: 1.71 (1.01-2.99) *vs* 0.50 (0.35-0.80), p<0.0001; FIB-4: 3.77 (2.29-5.91) *vs* 1.33 (0.98-1.83), p<0.0001; Forns: 7.97 (6.91-9.34) *vs* 5.52 (4.60-6.51), p<0.0001].

The ability, optimal cut-off value and diagnostic accuracy of the three indirect fibrosis biomarkers in detecting liver cirrhosis are reported in *Table 2*. The values for the area under the ROC curve of APRI, FIB-4 and Forns for the prediction of LS ≥13 KPa were different (overall comparison: p=0.006; FIB-4 *vs* APRI: p=0.001; FIB-4 *vs* Forns: p=0.924; APRI *vs* Forns: p=0.078).

According to the estimated optimal cut-off of APRI, a value of 0.97 correctly classified 519 patients (80%), with 76.1% of sensitivity and 81.9% of specificity; the number of subjects correctly classified as having liver cirrhosis was 517 (80%) when considering the cut-off of FIB-4 (equal to 2.02; sensitivity =81.4%, specificity =79.5%) and 532 (82%) when considering the cut-off of Forns (equal to 7.08; sensitivity =71.5%, specificity =86.3%). The majority of misclassified subjects were false positive cases [87 (14%), 97 (15%) and 65 (10%) for APRI, FIB-4 and Forns, respectively] rather than false negative ones [40 (6%), 32 (5%) and 49 (8%) for APRI, FIB-4 and Forns, respectively].

DISCUSSION

In our study, APRI, FIB-4 and Forns indexes were significantly higher in subjects with liver cirrhosis, as resulted by TE; these indexes had different percentages of total accuracy (number of cases correctly classified) ranging from 80% (FIB-4) to 82% (Forns). To our knowledge, this is the first study evaluating the ability of the three indirect fibrosis biomarkers in comparison to LS on a large number of HIV/HCV co-infected subjects.

On the basis of our results, the three indirect fibrosis biomarkers appeared to be better in excluding rather than detecting liver cirrhosis, since specificity and negative predictive values of the three biomarkers were generally high, while sensitivity and positive predictive values were rather low.

A value of APRI, FIB-4 or Forns below the threshold for defining advanced liver fibrosis will reasonably predict its absence (negative predictive value 89-92%), though confirmation with a more accurate method is advisable given the possibility to misclassify some patients. On the contrary, a value above the cut-off for advanced liver fibrosis warrants further investigations to confirm the diagnosis given the frequent occurrence of false positive cases (35-41%).

The estimated cut-offs appear to be lower than those previously published reported (Forns *et al.,* 2002; Wai *et al.,* 2003; Vallet-Pichard *et al.,* 2007; Tural *et al.,* 2009). The use of LS instead of liver histology as a reference standard might have affected these results: in some circumstances (high necroinflammatory activity, steatosis, high IQR/LS median ratio) TE may overestimate liver fibrosis (Lucidarme *et al.,* 2009; Fraquelli *et al.,* 2011b), even though overestimation was more likely in patients experiencing transaminases flares (Arena *et al.,* 2008), who were indeed excluded in our study. On the other hand, liver biopsy may underestimate fibrosis, either by inadequate sampling (eg fragmented biopsy) (Everhart *et al.,* 2010) or by sampling error (Manning *et al.,* 2008; Regev *et al.,* 2002; Maharaj *et al.,* 1986), which is lower with TE because of the larger liver volume explored (Ziol *et al.,* 2005).

Although APRI, Forns and FIB-4 showed similar performances in the ability to detect advanced liver fibrosis, APRI performed (AUC) worse than the others in terms of accuracy, maybe as a consequence of the small number of variables considered (only AST and platelet), which may be frequently affected by inflammation and confounding factors (e.g. thrombocytopenia in advanced HIV infection) (Rohrbach *et al.,* 2014).

These indirect scores would not actually give us the possibility to avoid more expensive and accurate evaluations

like TE, ultrasound and biopsy in those with indirect fibrosis biomarkers above the estimated cut offs, since the occurrence of false positive is still too high (10-15%). Nonetheless, given their good performance in excluding liver cirrhosis, their non-invasiveness and wide availability, these indexes could be used for regular assessment of fibrosis, as also recently suggested by others (Holmberg *et al.,* 2013).

Our study has some limitations. Firstly, the cross-sectional design does not offer us the possibility to assess the evolution of LS and indirect biomarkers and correlate it with clinical progression. Moreover, as already observed in the majority of other studies, men represent the majority of the study population, so these results may be generalized to the male population.

In conclusion, APRI, FIB-4 and Forns may be regularly used in HIV/HCV co-infected patients to exclude liver cirrhosis. Even though the assessment with more accurate techniques is recommended to better define the stage of liver fibrosis, their ready availability could improve liver fibrosis staging in primary referral centres and in resource-limited settings.

The authors have nothing to disclose.

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