Validation of APRI and FIB-4 score in an Antwerp cohort of chronic hepatitis C patients

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Abstract

Background and aims : **Evaluation of liver fibrosis in chronic hepatitis C patients guides clinical decision-making. The aim of this study is to validate APRI and FIB-4, two easily calculated non-invasive tests to predict fibrosis, in chronic HCV patients using biopsy as a gold standard and to compare accuracy between HCV monoinfected and HIV/HCV coinfected patients.**

Patients and methods : **We retrospectively studied HCV patients of two centres who underwent liver biopsy. Liver fibrosis was staged according to METAVIR.**

Results : **136 patients were included. The AUROC of FIB-4 (0.896) to discriminate F0-F2 vs. F3-F4 was significantly higher (p = 0.0186) than the AUROC of APRI (0.842). The difference in AUROC between HIV-negative and positive patients was not significant for APRI (p = 0.471), nor for FIB-4 (p = 0.495). Performance status was lower in HIV-positive patients with 46.7% and 69.0% of patients correctly classified using APRI and FIB-4, compared to 56.6% and 73.6% in HIV-negative patients, respectively. Conversion of transaminase values from one hospital to the other did not significantly change the AUROC of FIB-4 (p = 0.928).**

Conclusions : **APRI and FIB-4 have a better performance status in HCV monoinfected patients compared to HIV/HCV coinfected patients. FIB-4 has a better AUROC compared to APRI and is the preferred noninvasive fibrosis score to discriminate between F0-F2 and F3-F4. Different hospitals should use their local absolute serum transaminase values without conversion.** (Acta gastroenterol. belg., **2015**, 78, **373-380**).

Key words : noninvasive, liver fibrosis, FIB-4, APRI, hepatitis C, coinfection.

Abbreviations : A, activity grade (METAVIR) ; ALT, alanine aminotransferase ; AP, alkaline phosphatase **;** AST, aspartate aminotransferase ; AUROC, area under receiver operating characteristic ; BMI, body mass index ; CI, confidence interval ; F, fibrosis stage (METAVIR) ; GT, genotype ; HCV, hepatitis C virus ; HIV, human immunodeficiency virus ; INR, international normalized ratio ; IQR, interquartile range ; LDH, lactate dehydrogenase ; NPV, negative predictive value ; PPV, positive predictive value ; PT, prothrombin time ; Q1, first quartile ; Q3, third quartile ; ROC, receiver operating characteristic ; SD, standard deviation ; SENS, sensitivity ; SPEC, specificity.

Introduction

Evaluation of liver fibrosis in chronic hepatitis C patients (HCV) guides further treatment decisions and is prognostically relevant. The gold standard for assessing hepatic fibrosis is liver histology. However, liver biopsy is hampered by complications (1,2), availability and costs, intra- and interobserver variability (3,4) and sampling errors (5,6).

Due to these limitations noninvasive tests to assess hepatic fibrosis have been developed. The Belgian reimbursement criteria (from January 1, 2015) for the new antivirals for the treatment of chronic hepatitis C allow noninvasive fibrosis staging of METAVIR F3 or F4. Both ultrasound-based elastography (7-12) and biochemical tests are approved in this context. In this study, we will focus on the latter.

Several scores have been developed and use serum markers, either directly involved in fibrosis remodelling or altered by its consequences. Fibrotest combines α2-macroglobulin, haptoglobin, gamma-glutamyl transferase (γGT), bilirubin and apolipoprotein A1 (13). MP3 combines PIIINP (Procollagen III N-Terminal Propeptide), a marker of fibrogenesis, and the matrix metalloproteinase MMP-1 that is involved in fibrinolysis (14). Fibrometer combines hyaluronic acid, prothrombin time, platelets, aspartate aminotransferase (AST), α2-macroglobulin, urea and age (15). Hepascore combines bilirubin, γGT, hyaluronic acid, α2-macroglobulin, age and gender (16). Forns' score combines age, γGT, cholesterol and platelet count (17). These scores however, use serum markers that are not routinely used in standard follow-up blood tests of HCV patients and/or have complex mathematical formulas. APRI and FIB-4 on the other hand are two validated, easily calculated scores based on routine biochemical tests. APRI (Aspartate aminotransferase to Platelet Ratio Index) combines AST and platelets and uses cut-off values to predict absence (< 0.5) or presence (> 1.5) of significant (≥ F2) or advanced (≥ F3) fibrosis, and cut-off values to predict absence (< 1) or presence (> 2) of cirrhosis (18). FIB-4 combines AST, alanine aminotransferase (ALT), platelets and age and uses cut-off values to exclude (< 1.45) or predict (> 3.25) advanced fibrosis (19). HIV/HCV coinfected patients are often considered as a distinct

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population and excluded from studies developing or validating noninvasive scores. In the original article of APRI, HIV status is not mentioned. FIB-4, on the other hand, was initially developed for HIV/HCV coinfected patients and later validated for HCV monoinfected patients.

The aim of the current study was to validate APRI and FIB-4 for the diagnosis of advanced fibrosis in an Antwerp cohort of chronic hepatitis C patients and to directly compare between the HCV mono- and HIV/HCV coinfected in the same setting, using biopsy as the gold standard.

Patients and methods

Patients

We performed a retrospective analysis of histology and clinical parameters in HCV-infected patients from two different centres (one academic centre, one non-academic hospital) in Antwerp, Belgium. Data were collected from September 2010 to September 2014. Eligibility criteria included chronic hepatitis C infection, age above 18 years, a liver biopsy consistent with chronic HCV and availability of serum tests within two months of the biopsy. All patients were positive for antibodies to hepatitis C and persistent HCV RNA for more than six months. Patients were excluded if they had a concurrent liver disease other than HCV, liver failure, systemic inflammatory disease, malignancy or excessive alcohol consumption (≥ 3 daily units or ≥ 21 weekly units for men, ≥ 2 daily units or ≥ 14 weekly units for women).

Histology

Liver biopsy was performed either via the transjugular or percutaneous intercostal route. One hospital used 14G tru-cut needles for percutaneous biopsies. The other hospital used 16G Menghini needles for transjugular and transcutaneous biopsies. Biopsy specimens were fixed in formalin, embedded in paraffin and stained by Haematoxylin-eosin, Fouchet, Periodic Acid-Schiff and Perl's iron. The histological lesions were described using the METAVIR scoring system by the local pathologist (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 ; no obvious cirrhosis (bridging fibrosis) ; and F4, definite cirrhosis. Significant fibrosis was defined as a METAVIR fibrosis score of 2 or higher. Advanced fibrosis was defined as a METAVIR fibrosis score of 3 or 4.

Serum fibrosis markers

Normal values of AST and ALT were respectively < 35 U/L and < 45 U/L for men, and < 31 U/L and < 34 U/L for women in hospital 1 (Dimension Vista, Siemens). Normal values of AST and ALT were respectively < 60 U/L and < 50 U/L for men, and < 37 U/L and < 42 U/L for women in hospital 2 (Vitros, OCD). If multiple laboratory values were available, the results closest to the time of biopsy were used. APRI score was calculated as follows : (AST (ULN) × 100/Platelet count (10^9/L)). FIB-4 was calculated as follows : (age (yrs) × AST (U/L))/(Platelet count (10^9/L) × (ALT (U/L))$^{1/2}$). For the interpretation of the FIB-4 score, the cut-off values were chosen according to the original publication (19). For the interpretion of the APRI score, the cut-off values 1 and 2 were similar to the original article (18). The cut-off values 0.5 and 1.5, on the other hand, were used to discriminate between F0-F2 and F3-F4, instead of between F0-F1 and F2-F4 in the original article.

Statistical analysis

The data analysis was performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, United States). Distribution of normality was evaluated using the Shapiro-Wilk test. Values with a normal distribution were expressed as mean with standard deviation. Values with normal distribution were compared between fibrosis stages using Student's t-test. Values with a non-normal distribution were expressed as median with quartiles Q1 and Q3. Values with a non-normal distribution were compared between fibrosis stages using Mann-Whitney U test. The effect of biopsy length on diagnostic performance of serum markers was assessed using a Kruskal-Wallis test. The association between categorical values was calculated by Pearson Chi-square. Spearman's rho two-tailed test was used to assess correlations. Predictivity of non-invasive scores for fibrosis was determined by the receiver operating characteristic (ROC) curve analysis. The Hanley and McNeil method was used to compare area under the ROC (AUROC) curves of different populations (VassarStats, Vassar College, Poughkeepsie, NY, United States) (21). AUROC curves of different tests within the same population were compared according to Delong (MedCalc version 14.12.0, MedCalc Software, Ostend, Belgium) (22). A p value of < 0.05 was considered statistically significant.

Results

Patient characteristics

One hundred seventy-four files were reviewed. Thirty-eight patients were excluded, because of autoimmune hepatitis (2), primary biliary cirrhosis (1), hepatitis B coinfection (7), non-alcoholic fatty liver disease (2), excessive alcohol use or signs of alcoholic steatohepatitis (17), malignancy (3), systemic inflammatory disease (3), liver failure (1) or uninterpretable biopsy (2). One hundred thirty-six patients were included in the analysis. The characteristics of these patients are summarized in Table 1. Overall median biopsy length was 18 mm (Q1 : 15, Q3 : 21 ; mean 21.2 ± 10.5 mm). Median biopsy length of the hospital using 16G needles was 30 mm (Q1 : 20, Q3 : 36.3 ; mean 29.9 ± 13.0 mm) and of the hospital using 14G needles 17 mm (Q1 : 14, Q3 : 19 ; mean

Table 1. — **Main characteristics of patients**

Characteristics	N = 136
Sex, men (n, %)	106 (77.9%)
Age (years) SD	42.8 ± 10.9
BMI (kg/m²) IQR	25.5 (22.9-28.4)
HIV-negative (n, %)	106 (77.9%)
Genotype (n, %)	
GT 1	76 (56.3%)
GT 2	7 (5.2%)
GT 3	33 (24.4%)
GT 4	19 (14.1%)
AST (U/L) IQR	47.0 (34.3-84.8)
ALT (U/L) IQR	75.0 (52.0-120.0)
Platelets (10^9/L) SD	216.4 ± 69.3
METAVIR F stage (n, %)	
F0	22 (16.2%)
F1	55 (40.4%)
F2	32 (23.5%)
F3	11 (8.1%)
F4	16 (11.8%)
METAVIR A grade (n, %)	
A0	11 (8.1%)
A1	91 (66.9%)
A2	29 (21.3%)
A3	4 (2.9%)

Main characteristics of patients. SD values represent mean with standard deviation, IQR values represent median with quartiles Q1 and Q3.

Abbreviations : A, activity grade ; ALT, alanine aminotransferase ; AST, aspartate aminotransferase ; BMI, body mass index ; F, fibrosis stage ; GT, genotype ; HIV, human immunodeficiency virus.

16.3 ± 3.6 mm). Of all biopsies, 21 (15.4%) were smaller than 15 mm, almost all (18) from the 14G needle group. There was no significant difference in biopsy length between correctly classified, misclassified or unclassified patients (p = 0.804). Our population was quite evenly distributed over the different stages of fibrosis, with 23.5% F2 and 19.9% F3 or F4. Advanced fibrosis was more prevalent in the HIV-negative population (20.7%) compared to the HIV-positive population (16.7%), but the difference was not statistically significant (p = 0.620). Characteristics of patients with F0-F2 (no advanced fibrosis) compared to patients with F3-F4 (advanced fibrosis) are summarised in Table 2. Patients with advanced fibrosis had significantly higher age, AST, ALT, bilirubin, INR, APRI and FIB-4, and significantly lower platelets, leucocytes, INR and albumin. They did not differ significantly in gender, HIV status, BMI, LDH, alkaline phosphatase, hemoglobin level or cholesterol.

Correlation between serum markers and fibrosis stage

Highly significant correlations were found between fibrosis stage and APRI ($p < 0.001$, $r_s = 0.522$) and FIB-4 ($p < 0.001$, $r_s = 0.623$). Figure 1 shows the box-plots of fibrosis scores according to METAVIR fibrosis stage.

Overall diagnostic performance

The AUROCs with 95% confidence interval for discrimination between fibrosis stages are listed in Table 3. The AUROC of FIB-4 to discriminate F0-F2 vs. F3-F4 was significantly higher than the one of APRI in all patients (p = 0.0186) (Fig. 2) and in HIV-negative patients (p = 0.0119), but did not reach statistical significance in HIV-positive patients (p = 0.4002).

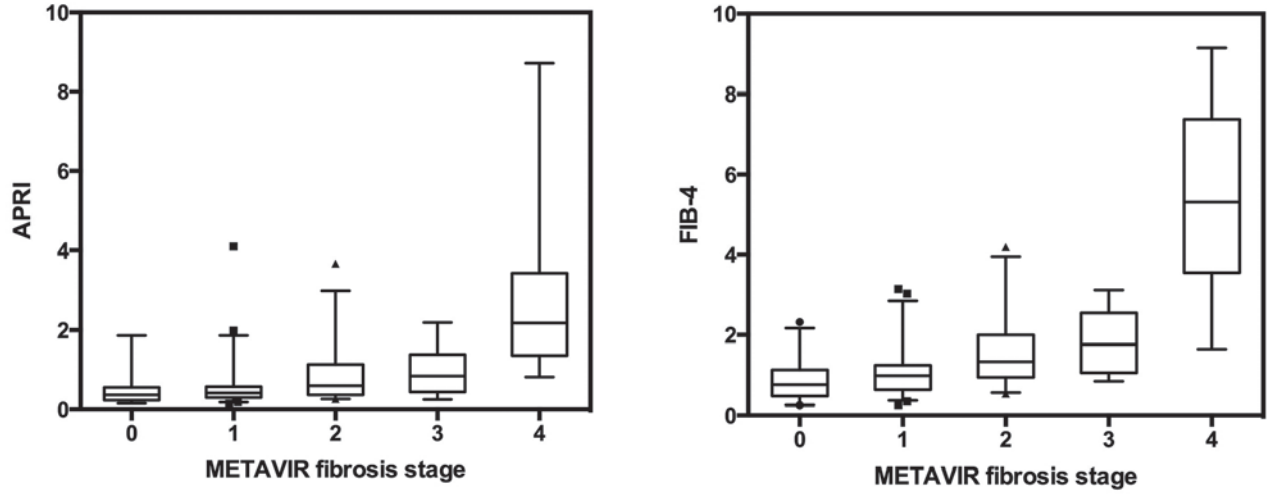
The difference between AUROCs of APRI to discriminate F0-2 vs. F3-4 in men (0.857, 95% CI 0.759-0.955) and women (0.790, 95% CI 0.636-0.945) was not statistically significant (p = 0.693), nor was the difference between AUROCs of FIB-4 to discriminate F0-2 vs. F3-4 in men (0.898, 95% CI 0.818-0.978) and women (0.827, 95% CI 0.675-0.980) (p = 0.653).

Table 2. — **Comparison of patients by fibrosis stages**

Characteristic	No advanced fibrosis (F0-F2) n = 109	Advanced fibrosis (F3-F4) n = 27	P-value
Sex, male (n, %)	82 (75.4%)	24 (88.9%)	0.125
Age (years) SD	40.9 ± 10.5	50.7 ± 8.3	< 0.001
BMI (kg/m²) IQR	25.5 (22.9-28.7)	25.5 (23.0-28.4)	0.736
HIV-negative (n, %)	84 (77.1%)	22 (81.5%)	0.797
AST (U/L) IQR	42 (33-67)	96 (52-124)	< 0.001
ALT (U/L) IQR	70 (50.3-106.8)	112 (58.0-152.0)	0.023
LDH (U/L) IQR	267 (182-496)	308 (212-528)	0.408
AP (U/L) IQR	79 (60-99)	93 (66-124)	0.082
Bilirubin (mg/dL) IQR	0.5 (0.4-0.7)	0.89 (0.6-1.1)	< 0.001
Platelets (10^9/L) SD	232.7 ± 60.0	150.3 ± 66.0	< 0.001
Hemoglobin (g/dL) IQR	14.5 (13.5-15.6)	14.8 (12.6-15.4)	0.379
Leucocytes (10^9/L) IQR	6.57 (5.56-8.20)	5.77 (4.30-6.90)	0.026
PT (INR) IQR	1.02 (1.00-1.06)	1.09 (1.04-1.18)	< 0.001
Albumin (g/L) IQR	4.25 (3.99-4.62)	4.02 (3.62-4.29)	0.012
Cholesterol (mg/dL) SD	171.2 ± 41.4	201.3 ± 44.4	0.154
APRI (value) IQR	0.467 (0.307-0.711)	1.470 (0.839-2.442)	< 0.001
FIB-4 (value) IQR	1.02 (0.66-1.33)	3.20 (1.77-5.72)	< 0.001

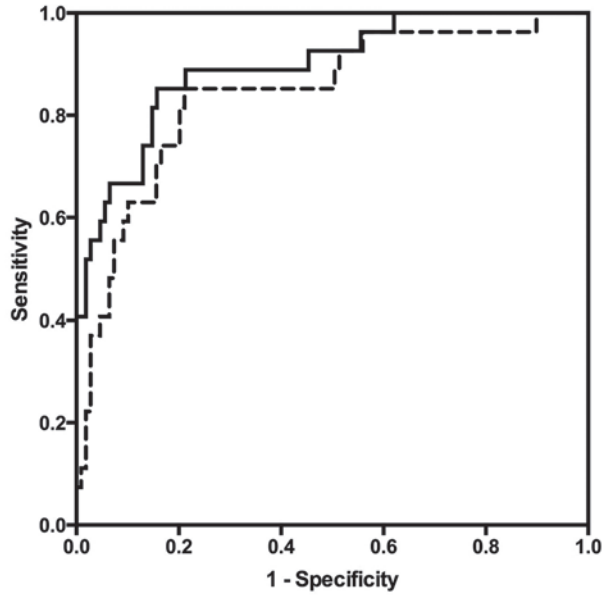
Comparison of patient characteristics between the group of no advanced fibrosis and the group of advanced fibrosis. SD values represent mean with standard deviation, IQR values represent median with quartiles Q1 and Q3.

Abbreviations : ALT, alanine aminotransferase ; AP, alkaline phosphatase ; AST, aspartate aminotransferase ; BMI, body mass index ; HIV, human immunodeficiency virus ; INR, international normalized ratio ; LDH, lactate dehydrogenase ; PT, prothrombin time.



Boxplots of fibrosis score values of APRI and FIB-4 according to METAVIR fibrosis stages. The top and bottom of each box are the 25th and the 75th centiles. The line through the box is the median and the error bars are the 5th and the 95th centiles.

Fig. 1. — Fibrosis score values of APRI and FIB-4 according to METAVIR fibrosis stages



ROC curves of APRI (dashed line) and FIB-4 (continuous line) to discriminate fibrosis stage F0-F2 versus F3-F4.

Fig. 2. — ROC curves of APRI and FIB-4 to discriminate fibrosis stage F0-F2 versus F3-F4.

Sensitivity, specificity and positive and negative predictive values

Table 4 shows the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of FIB-4 according to cut-off values and fibrosis stage, for all patients and for HIV-negative and HIV-positive patients separately. Table 5 shows the same for APRI. Note that there is a difference of interpretation between low and high cut-offs. For example, the PPV of a value below the low cut-off value 1.45 in FIB-4 is to predict the presence of no to moderate fibrosis (F0-F2), in other words to predict the absence of advanced cirrhosis (F3-F4). The PPV of a value above the high cut-off value 3.25 is to predict the presence of advanced fibrosis, in other words to predict the absence of no to moderate fibrosis.

When using the FIB-4 score in all patients, 98 patients (72.6%) were correctly classified, 32 (23.7%) unclassified and 5 (3.7%) misclassified. The three patients that were underclassified all had fibrosis F3. The two patients that were overclassified had fibrosis F2. There was no obvious difference between characteristics of correctly classified and misclassified patients. When using the APRI score to differentiate between F0-F2 vs. F3-F4 with cut-off values 0.5 and 1.5 in all patients, 74 patients (54.4%) were correctly classified, 50 (36.8%) unclassified and 12 (8.8%) misclassified. The four patients that were underclassified all had fibrosis F3. Of the 8 patients that were overclassified, one had F0, three had F1 and four had F2. The overclassified patients all had normal platelets, but very high AST.

HIV status

The differences of AUROCs between HIV-positive and negative patients have been described above. Significant correlations were also present in HIV-positive patients between fibrosis stage versus APRI ($p = 0.012$, $r_s = 0.451$) and versus FIB-4 ($p = 0.003$, $r_s = 0.528$), although lower compared to HIV-negative patients. The comparison of fibrosis score between HIV-negative and positive patients is listed in Table 6. When using the APRI score to differentiate between F0-F2 and F3-F4 with cut-off values 0.5 and 1.5, 56.6% and 46.7% were correctly classified, 34.9% and 43.3% unclassified and 8.5% and 10% misclassified, in HIV-negative and HIV-positive patients, respectively. When using the FIB-4 score to differentiate between F0-F2 and F3-F4, 73.6% and 69.0% were correctly classified, 22.6% and 27.6%

Table 3. — **Comparison of AUROCs of APRI and FIB-4 for discriminating between fibrosis stages**

Score	Prediction of fibrosis stage	All (AUC, 95% CI)	HIV- (AUC, 95% CI)	HIV+ (AUC, 95% CI)	P-value
APRI	F0-1 vs F2-4	0.777 (0.697-0.858)	0.823 (0.739-0.906)	0.597 (0.379-0.815)	0.052
	F0-2 vs F3-4	0.842 (0.754-0.930)	0.856 (0.761-0.951)	0.752 (0.505-0.999)	0.471
	F0-3 vs F4	0.936 (0.892-0.981)	0.932 (0.881-0.982)	0.964 (0.896-1.000)	0.602
FIB-4	F0-2 vs F3-4	0.896 (0.827-0.965)	0.912 (0.839-0.985)	0.825 (0.644-1.000)	0.495

Comparison of AUROCs of APRI and FIB-4 for discriminating between fibrosis stages. AUROC (AUC) with 95% confidence interval (95% CI) of APRI and FIB-4 for all patients, HIV-negative (HIV–) and HIV-positive (HIV+) patients for the prediction of significant fibrosis (F0-1 vs F2-4), advanced fibrosis (F0-2 vs F3-4) and cirrhosis (F0-3 vs F4). P-value is given for the comparison between AUROCs of HIV – and HIV + patients.

Table 4. — **Sensitivity, specificity, positive predictive value and negative predictive value of FIB-4**

Patients	Cut-off	%	Sens	Spec	PPV	NPV
All (n = 135)	< 1.45	65.2	78.7	64.9	96.6	51.1
	> 3.25	11.1	48.1	98.1	86.7	51.1
HIV – (n = 106)	< 1.45	65.1	79.8	90.9	97.1	54.1
	> 3.25	12.3	50.0	97.6	84.6	88.2
HIV + (n = 29)	< 1.45	65.5	75.0	80.0	94.7	66.7
	> 3.25	6.9	40.0	100	100	88.9

Sensitivity, specificity, positive and negative predictive value to predict the absence of advanced fibrosis (score below 1.45) and to predict the presence of advanced fibrosis (score above 3.25) for all patients (all), HIV-negative (HIV –) and HIV-positive (HIV +) patients.

Abbreviations : HIV, human immunodeficiency virus ; NPV, negative predictive value ; PPV, positive predictive value ; Sens sensitivity ; Spec, specificity ; %, percentage of patients within patient group based on the cut-off.

Table 5. — **Sensitivity, specificity, positive predictive value and negative predictive value of APRI**

Patients	Cut-off	%	Sens	Spec	PPV	NPV
All (n = 136)	< 0.5	47.8	56.0	85.2	93.8	32.4
	< 1	74.3	83.3	93.8	99.0	42.9
	> 1.5	15.4	48.1	92.7	61.9	87.1
	> 2	9.6	56.3	96.7	69.2	94.3
HIV – (n = 106)	< 0.5	49.1	59.5	90.9	96.2	37.0
	< 1	73.6	92.8	92.9	98.7	45.4
	> 1.5	16.0	45.5	91.7	58.8	85.5
	> 2	8.5	50.0	97.8	77.8	92.7
HIV + (n = 30)	< 0.5	43.3	44.0	60.0	84.6	82.4
	< 1	76.7	82.1	100	100	28.6
	> 1.5	13.3	60.0	96.0	75.0	92.3
	> 2	13.3	100	92.9	50.0	100

Sensitivity, specificity, positive and negative predictive value to predict the absence of advanced cirrhosis (score below 0.5), to predict the absence of cirrhosis (score below 1), to predict the presence of advanced fibrosis (score above 1.5) and to predict the presence of cirrhosis (score above 2) for all patients (all), HIV-negative (HIV –) and HIV-positive (HIV +) patients.

Abbreviations : HIV, human immunodeficiency virus ; NPV, negative predictive value ; PPV, positive predictive value ; Sens, sensitivity ; Spec, specificity ; %, percentage of patients within patient group based on the cut-off.

unclassified and 3.8% and 3.4% misclassified, in HIV-negative and HIV-positive patients, respectively.

Optimal cut-off values

As mentioned before, cut-off values were chosen based on previous studies. Based on our analysis, ideal cut-off values for our population have been calculated. The optimal cut-off for APRI was 0.8 to detect F3-F4 and 1.0 to detect F4, with a sensitivity, specificity, PPV, NPV and accuracy of 85.2%, 78.9%, 50.0%, 95.6% and 80.1% ; and of 93.8%, 83.3%, 42.9%, 99.0% and 84.6%, respectively. The optimal cut-off for FIB-4 was 1.63 to detect F3-F4 and 2.57 to detect F4, with a sensitivity, specificity, PPV, NPV and accuracy of 85.2%, 84.3%, 57.5%, 95.8% and 84.4% ; and of 93.8%, 93.3%, 65.2%, 99.1% and 93.3%.

Laboratory difference

This study has been performed in two different hospitals with different laboratories. APRI uses upper limit of normal (ULN), hence there is no influence of this difference on the final results. FIB-4 uses absolute values, which might influence the scores between hospitals. When comparing the ROC curves of FIB-4 between hospitals to differentiate between F0-F2 and F3-F4, the AUROC of hospital 1 was 0.947 (95% CI 0.882-1.000) and AUROC of hospital 2 was 0.842 (95% CI 0.720-0.965). Both hospitals had a similar distribution of fibrosis and the difference between AUROCs did not reach statistical significance (p = 0.198). When converting the AST and ALT values of hospital 2 to those of hospital 1 using a conversion calculator, 4 patients (5.7%) of hospital 2 got a different classification, compared to their

Table 6. — **Comparison of noninvasive fibrosis scores of patients with no to mild fibrosis and patients with advanced fibrosis by HIV status**

Score		HIV –	HIV +	P-value
FIB-4	F0-F2	1.01 (0.67-1.31)	1.02 (0.64-150)	0.615
	F3-F4	3.36 (1.87-6.04)	1.93 (1.26-4.59)	0.284
APRI	F0-F2	0.44 (0.31-0.65)	0.57 (0.29-0.83)	0.489
	F3-F4	1.42 (0.92-2.43)	2.19 (0.45-3.24)	0.928

Comparison of median fibrosis score of FIB-4 and APRI for the patient groups with no to mild fibrosis and advanced fibrosis between HIV-negative (HIV-) and HIV-positive (HIV+) patients. Values are expressed as median with quartiles Q1 and Q3 between brackets.

initial values (23). One patient (F3) went from unclassified to misclassified, one (F1) from correctly classified to unclassified and two (both F2) went from unclassified to correctly classified. The AUROC of hospital 2 with the converted values was 0.833 (95% CI 0.709-0.956) and was not statistically significantly different from the AUROC with the original values (p = 0.928).

Discussion

Several noninvasive tests combining biological parameters have recently been developed with the objective of replacing or reducing the need for liver biopsy. In this study, we assessed the diagnostic performance of APRI and FIB-4 for the differentiation between fibrosis stages F0-F2 versus F3-F4. Other scores have not been used in this study, because of known lower diagnostic performance (e.g. AST/ALT ratio), non-routinely used serum markers (e.g. Fibrometer) or patented calculations (e.g. Fibrotest). Our choice to differentiate between F0-F2 and F3-F4 was based on the need to prioritize patients with F3-F4 fibrosis, which is incorporated in the Belgian reimbursement criteria for the new direct acting antivirals for the treatment of hepatitis C (24,25). For this reason, we used the cut-off values 0.5 and 1.5 of APRI not to discern significant fibrosis as in the original study, but to identify advanced fibrosis as did one other previous study (26). Our population had slightly lower significant fibrosis (≥ F2) compared to the original article of APRI, and a comparable to higher significant fibrosis compared to the initial articles of FIB-4 (18,19,27).

FIB-4 was created and validated for the differentiation between F0-F2 versus F3- F4 (19). Although FIB-4 was first described in patients with HIV/HCV coinfection, it has also been validated as an accurate marker of fibrosis in the context of HCV monoinfection (27). APRI on the other hand was created by Wai *et al*. to predict the presence or absence of significant fibrosis (≥ F2) and to predict the presence or absence of cirrhosis in HCV monoinfected patients (18). Significant fibrosis and cirrhosis are often used in the development of noninvasive scores because the first is often considered as an indication to start treatment and the latter dictates closer clinical follow-up. Many studies using the APRI score usually focus on the detection of significant fibrosis or cirrhosis and cannot be compared with our study, as we focus on the diagnosis of advanced fibrosis (28-31).

In this retrospective study, FIB-4 was superior to APRI to discriminate between fibrosis stages F0-F2 and F3-F4. FIB-4 had a significantly better AUROC both in the overall cohort as in HIV-negative patients. In the HIV-positive population, the AUROC of FIB-4 was higher, though not statistically significant.

Since we used the cut-off values proposed in the literature (which might, however, not be the ideal cut-off values for the studied group based on their specific ROC curve), a comparison between tests cannot be solely based on the AUROC. Hence, we also looked at the performance accuracy of both tests, in which FIB-4 likewise was superior. Of all patients, 72.6% was correctly classified and 3.7% misclassified compared to 63.2% and 8.8% with APRI, respectively. These results are in line with other reports showing a higher AUROC of FIB-4 compared to APRI (32-37).

Other studies have proposed different cut-off values for APRI and FIB-4. Based on our analysis, the optimal single cut-off values were 0.8 and 1.0 for APRI, and 1.63 and 2.57 for FIB-4 to predict the presence of advanced fibrosis and cirrhosis, respectively. Trang *et al*. calculated two ideal cut-offs for APRI to differentiate between F0-F2 and F3-F4 (< 0.71 and > 1.85) and Holmberg *et al*. proposed a single cut-off for FIB-4 (1.81) (34,38). Larger studies are needed to validate the original cut- off values or to evaluate new cut-off values.

We did not study the diagnostic performance of combined blood tests, or the combination of noninvasive biological and radiological tests. Several studies have already shown an improvement of diagnostic accuracy when combining two or more noninvasive fibrosis tests (30,39-46). The combination of a biological and radiological test has also been implemented in the Belgian reimbursement criteria.

In this study, populations with different reference values both between hospitals and gender have been included. APRI uses upper limit of normal, hence these differences have no effect on the result of the score. FIB-4, on the other hand, uses absolute values. The majority of the population in the original article of Sterling *et al*. was male (81 %) and gender was not a significant factor in their model, hence no gender coefficient was used (19). This is in line with our results, which show no significant difference of the AUROC according to gender. Regrettably, in the original paper the applied upper limits of normal were not mentioned. The unicenter study of Val-

let-Pichard *et al*. to validate FIB-4 in the monoinfected population did describe the upper normal values in their hospital (45 IU/L for men and 40 IU/L for women, and 65 IU/L for men and 50 IU/L for women, for AST and ALT respectively) (27). Other studies, uni- or multicenter, often do not mention local upper limits of normal, nor do they mention any conversion measures in case of multiple centres/laboratories to uniform the data (26,28, 34,36,47). In our initial analysis, we first pooled all the data without conversion, since no reference values exist to calculate FIB-4. We subsequently converted the values of AST and ALT of hospital 2 to those of hospital 1. Although the classification changed in four cases (5.7%), the ROC curve did not differ significantly, nor did the overall diagnostic accuracy. Considering the conversion of values did not change the overall diagnostic performance and considering no reference values are given in the calculation of FIB-4, we conclude that until larger studies, incorporating the difference of normal values in their analyses, are conducted, each hospital should use its own values without conversion. A strength of this study is the evaluation of the effect of conversion, which to our knowledge, is the first time this is performed in a study in this setting.

To our knowledge, few studies have evaluated the diagnostic accuracy of noninvasive scores in HIV/HCV coinfected patients and studies directly comparing HCV monoinfected and HIV/HCV coinfected patients are even scarcer. Human immuodeficiency virus infection modifies the natural history of chronic hepatitis C with faster progression of fibrosis and a higher risk of cirrhosis and end-stage liver disease in HIV/HCV coinfected patients compared to HCV monoinfected patients (48-51). FIB-4 was initially developed for HIV/HCV coinfected patients and in the original publication of APRI, patients were presumably HCV monoinfected, as HIV status was not mentioned. There are several reasons why the diagnostic performance of both tests may be altered in HIV-positive cohorts, including HIV-induced alterations in bone marrow and immune function, and the potential hepatotoxic effect of HAART (highly active antiretroviral therapy).

In our study, APRI and FIB-4 both showed a significant correlation with fibrosis stage in HIV-positive patients, though less compared to HIV-negative patients. AUROCs were also lower in HIV-positive patients compared to HIV-negative patients without reaching statistical significance. It is possible that our study was not sufficiently powered to dismiss statistical significance. Performance status was lower for both tests with 46.7% and 69% of patients classified correctly for APRI and FIB-4, respectively. Published data are inconclusive regarding this issue with different results from Nunes *et al*., who showed a trend to an improved perfomance in the HIV/HCV coinfected population (29). Still, the majority of studies show lower diagnostic performance of these tests in HIV/HCV coinfected patients compared to HCV monoinfected patients (52-54).

Our study has several limitations. It is a retrospective analysis with small sample size and results are only compared to liver biopsy, which was evaluated by different pathologists. Although still the gold standard for fibrosis evaluation, biopsy can also over- or underestimate the degree of fibrosis (1,3-6). Bedossa *et al*. suggest that a specimen length of at least 25 mm is necessary to evaluate fibrosis accurately, where in our study the median length was only 18 mm, with a mean of 21.2 mm (6). Although, when considering the needle diameter, the median length was 30 mm for the 16G biopsies and 17 mm for the 14G biopsies.

Conclusion

FIB-4 has a better diagnostic performance compared to APRI to differentiate between fibrosis stages F0-F2 versus F3-F4 in patients with chronic hepatitis C. It is an easily calculated score using routine serum parameters. Different hospitals should use their local absolute serum transaminase values without conversion. FIB-4 can also be used for HIV/HCV coinfected patients, though with a lower diagnostic accuracy compared to monoinfected patients.

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