

## Retrospective Study

**Fibrosis assessment using FibroMeter combined to first generation tests in hepatitis C**

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**Abstract****AIM**

To evaluate the performance of FibroMeter<sup>Virus3G</sup> combined to the first generation tests aspartate amino-transferase-to-platelet ratio index (APRI) or Forns index

to assess significant fibrosis in chronic hepatitis C (CHC).

## METHODS

First generation tests APRI or Forns were initially applied in a derivation population from Rio de Janeiro in Brazil considering cut-offs previously reported in the literature to evaluate significant fibrosis. FibroMeter<sup>Virus3G</sup> was sequentially applied to unclassified cases from APRI or Forns. Accuracy of non-invasive combination of tests, APRI plus FibroMeter<sup>Virus3G</sup> and Forns plus FibroMeter<sup>Virus3G</sup> was evaluated in the Brazilian derivation population. APRI plus FibroMeter<sup>Virus3G</sup> combination was validated in a population of CHC patients from Angers in France. All patients were submitted to liver biopsy staged according to METAVIR score by experienced hepatopathologists. Significant fibrosis was considered as METAVIR F  $\geq$  2. The fibrosis stage classification was used as the reference for accuracy evaluation of non-invasive combination of tests. Blood samples for the calculation of serum tests were collected on the same day of biopsy procedure or within a maximum 3 mo interval and stored at -70 °C.

## RESULTS

Seven hundred and sixty CHC patients were included (222 in the derivation population and 538 in the validation group). In the derivation population, the FibroMeter<sup>Virus3G</sup> AUROC was similar to APRI AUROC (0.855 *vs* 0.815,  $P = 0.06$ ) but higher than Forns AUROC (0.769,  $P < 0.001$ ). The best FibroMeter<sup>Virus3G</sup> cut-off to discriminate significant fibrosis was 0.61 (80% diagnostic accuracy; 75% in the validation population,  $P = 0.134$ ). The sequential combination of APRI or Forns with FibroMeter<sup>Virus3G</sup> in derivation population presented similar performance compared to FibroMeter<sup>Virus3G</sup> used alone (79% *vs* 78% *vs* 80%, respectively,  $P = 0.791$ ). Unclassified cases of significant fibrosis after applying APRI and Forns corresponded to 49% and 54%, respectively, of the total sample. However, the combination of APRI or Forns with FibroMeter<sup>Virus3G</sup> allowed 73% and 77%, respectively, of these unclassified cases to be correctly evaluated. Moreover, this combination resulted in a reduction of FibroMeter<sup>Virus3G</sup> requirement in approximately 50% of the entire sample. The stepwise combination of APRI and FibroMeter<sup>Virus3G</sup> applied to the validation population correctly identified 74% of patients with severe fibrosis ( $F \geq 3$ ).

## CONCLUSION

The stepwise combination of APRI or Forns with FibroMeter<sup>Virus3G</sup> may represent an accurate lower cost alternative when evaluating significant fibrosis, with no need for liver biopsy.

**Key words:** Chronic hepatitis C; Fibrosis; Liver biopsy; Non-invasive methods; FibroMeter<sup>Virus3G</sup>; Combination algorithms

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**Core tip:** Liver fibrosis assessment still poses a challenge

when prioritizing hepatitis C treatment due to logistical and financial barriers in the use of direct acting antiviral drugs. We introduced a new stepwise combination of first generation fibrosis tests - aminotransferase-to-platelet ratio index (APRI) and Forns-followed by FibroMeter<sup>Virus3G</sup> whenever results remained unclassified after first generation tests to identify significant fibrosis. This combination presented similar accuracy to FibroMeter<sup>Virus3G</sup> used as the only test, reduced APRI and Forns grey zone, and spared FibroMeter<sup>Virus3G</sup> requirement in 50% of cases. This approach represents a lower-cost alternative to assess fibrosis, with no need for liver biopsy.

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## INTRODUCTION

Fibrosis staging in chronic hepatitis C (CHC) has evolved in recent years with the introduction of blood tests for liver fibrosis as well as physical methods such as elastometry. Although liver biopsy has been classically considered the standard tool to evaluate fibrosis, it presents well-known inconveniences<sup>[1-3]</sup> and limitations<sup>[4-7]</sup> which make its use to assess fibrosis staging controversial amongst various authors<sup>[8-11]</sup>. However, even when considering the recent advances in CHC therapy, the diagnosis of significant fibrosis still represents a challenge to define which patients should have priority in treatment, mainly in resource limited countries. Thus, the development and improvement of alternative methods to identify candidates for an early treatment or intensive fibrosis monitoring is still recommended<sup>[11]</sup>. Most of the commonly used first generation non-invasive tests such as aspartate aminotransferase-to-platelet ratio index (APRI)<sup>[12]</sup>, FIB-4<sup>[13]</sup> and Forns index<sup>[14]</sup> have been constructed and evaluated as binary diagnosis tools aiming to predict or exclude significant fibrosis, advanced fibrosis or cirrhosis at specific cut-offs. Although they are all free of charge, easily accessible and well validated for CHC, these non-invasive tests are limited to classify all patients<sup>[12-14]</sup>.

The interest in detailed fibrosis class classification for non-invasive tests of fibrosis has recently grown<sup>[15-18]</sup>, representing a more comprehensive and sophisticated approach to assess liver fibrosis. In this line, FibroMeters are a group of blood tests providing classifications intended to evaluate liver fibrosis in chronic viral hepatitis, alcoholic liver disease and non-alcoholic fatty liver disease<sup>[19-21]</sup>. FibroMeter dedicated for viral aetiology has recently evolved from FibroMeter<sup>Virus2G[20]</sup> to a less costly

hyaluronic acid free test FibroMeter<sup>Virus3G[21]</sup>, which discriminates seven different fibrosis classes. FibroMeters provide scores ranging from 0 to 1 which are correlated with METAVIR staging system<sup>[22]</sup>. Although this new non-invasive test represents a better strategy to evaluate fibrosis in CHC, it may signify an economic burden hindering easy access mainly in developing countries. Thus, in order to identify patients with significant fibrosis and optimize costs, we evaluated the performance of a stepwise combination using APRI and Forns followed by FibroMeter<sup>Virus3G</sup> in cases whose results remained unclassified after use of these first generation tests, always considering liver biopsy as reference.

## MATERIALS AND METHODS

### Patients

A cross-sectional study with prospective inclusion of compensated CHC patients submitted to percutaneous liver biopsy was performed at the Federal University of Rio de Janeiro, Brazil, as part of a pre-treatment routine evaluation. This group represented the derivation population of the study. Patients with concomitant human immunodeficiency virus infection, hepatitis B virus, alcohol abuse, metabolic, autoimmune or biliary diseases, liver transplantation or those who had previously undergone antiviral treatment were excluded. The validation population was composed by an independent cohort of CHC patients from Angers in France, who fulfilled the same inclusion and exclusion criteria. All patients signed an informed consent form and the study was approved by the Ethics Committee of both Institutions.

### Liver biopsy

In the derivation population, all consecutive biopsies were guided by ultrasonography using a 14 or 16 G disposable Tru Cut needle (Surecutw, TSK Laboratory, Akasaka, Japan) obtaining a maximum length of 20 mm for each pass. In validation population, Menghini needle was used. Samples were considered inappropriate when length presented < 10 mm or contained < 6 portal tracts. Serial sections 5 µm thick were cut from each paraffin block and routinely stained with hematoxylin and eosin, periodic acid-Schiff diastase, reticulin, Masson Trichrome and Picrosirius red. Liver fibrosis was staged from F0 to F4 according to METAVIR staging system<sup>[22]</sup> by an experienced hepatopathologist in each centre, blinded for biological and clinical results. METAVIR F ≥ 2 was considered significant fibrosis. This fibrosis stage classification was used as the reference for accuracy calculation of non-invasive tests.

### Blood fibrosis tests

Serum tests of fibrosis were performed with blood samples collected from fasting patients on the same day of biopsy procedure or within a maximum 3 mo interval and stored at -70 °C. APRI and Forns were selected due to their free accessibility and their good

accuracy to discriminate significant fibrosis. The values of APRI<sup>[12]</sup>, Forns<sup>[14]</sup> and FibroMeter<sup>Virus3G[21]</sup> tests were calculated according to the original studies: (1) APRI = AST level/ULN/platelet counts (10<sup>9</sup>/L) × 100; (2) Forns index = 7.811 - 3.131 × ln(platelet count) + 0.781 × ln(GGT) + 3.467 × ln(age) - 0.014 × cholesterol; and (3) FibroMeter<sup>Virus3G</sup> = patented formula including the biologic parameters prothrombin index, AST, ALT, Urea, GGT, alpha-2-macroglobulin and platelets. The calculations were performed by Echosens (Paris, France) laboratory.

### Statistical analysis

Quantitative variables were expressed as mean ± SD values or proportions. Student's *t* test or ANOVA were used to compare continuous variables, and McNemar  $\chi^2$  test to compare paired proportions. The performance of APRI, Forns and FibroMeter<sup>Virus3G</sup> to predict significant fibrosis was expressed by the AUROC. In order to evaluate the applicability of FibroMeter<sup>Virus3G</sup>, considering an economic approach, we determined the best cut-off point of FibroMeter<sup>Virus3G</sup> to discriminate significant fibrosis using the Youden index that maximizes sensitivity and specificity. The performance of APRI and Forns were separately assessed to exclude or predict significant fibrosis respectively, at cut-offs already established in literature as follows: APRI: cut-off of 0.5 and 1.5<sup>[12]</sup>; Forns: cut-off of 4.2 and 6.9<sup>[14]</sup>. FibroMeter<sup>Virus3G</sup> was then sequentially tested in a stepwise use, considering the results allocated in unclassified APRI values (between 0.5 and 1.5) and Forns values (between 4.2 and 6.9), using histology as reference. The overall accuracy of the aforementioned approaches was calculated considering the sum of true positive and negative cases as a proportion of the total. Sensitivity, specificity, predictive positive (PPV) and negative values (NPV) of first generation tests and sequential use of APRI + FibroMeter<sup>Virus3G</sup> and Forns + FibroMeter<sup>Virus3G</sup> were evaluated.

Data were analyzed using the statistics software programs SPSS version 20 for Windows and MedCalc version 14.8.1. A *P* value < 0.05 was considered statistically significant.

## RESULTS

### Patients' characteristics

The initial series of liver specimens in Rio population consisted of 231 biopsies, of which four (1.7%) were excluded, due to evidence of other hepatic diseases associated with hepatitis C, and five (2.0%) were considered inadequate for analysis. Thus, the final Rio population included 222 biopsies of CHC patients. The validation cohort was represented by 538 French CHC patients. Excepted for gender, demographic characteristics, laboratory data and histological features of derivation and validation cohort were similar and described in Table 1.

The mean length of liver fragments was 24 ± 5 mm in derivation population vs 22 ± 10 mm in validation cohort (*P* = 0.315). The prevalence of significant fibrosis

**Table 1** Demographic, laboratory and histological features of patients with chronic hepatitis C of both populations

Variables	All (n = 760)	Rio population (n = 222)	Angers population (n = 538)
Females, n (%)	401 (54)	134 (60)	179 (35)
Age (yr, mean ± SD)	46 ± 11	51 ± 11	46 ± 11
AST, IU/L (mean ± SD)	67 ± 58	68 ± 52	66 ± 60
ALT, IU/L (mean ± SD)	101 ± 84	100 ± 67	101 ± 90
Platelet count, 10 <sup>6</sup> /mm <sup>3</sup> (mean ± SD)	208 ± 68	203 ± 63	210 ± 70
GGT, IU/L (mean ± SD)	144 ± 171	124 ± 135	110 ± 184
APRI	1.0 ± 1.2	0.9 ± 1.2	1.1 ± 1.3
Forns		6.0 ± 1.9	
FibroMeter <sup>Virus3G</sup>	0.6 ± 0.3	0.6 ± 0.3	0.6 ± 0.3
Biopsy length (mm, mean ± SD)	22 ± 9	24 ± 5	22 ± 10
METAVIR stage, n (%)			
F0	22 (3)	5 (2)	17 (3)
F1	283 (37)	91 (41)	192 (36)
F2	215 (28)	55 (25)	160 (30)
F3	145 (19)	54 (24)	91 (17)
F4	95 (13)	17 (8)	78 (14)

AST: Aspartate transaminase; ALT: Alanine aminotransferase; GGT: Glutamyl transpeptidase; APRI: Aspartate transaminase to platelet ratio index.

**Table 2** Rates of correct FibroMeter<sup>Virus3G</sup> stage classification in comparison to liver biopsy in the Rio population

FibroMeter stage	METAVIR fibrosis classification						Correct fibrosis class classification according to liver biopsy (%)
	0	1	2	3	4	n	
F0/F1	0	10	0	0	0	10	10/10 = 100
F1	0	6	1	0	0	7	6/7 = 86
F1[F1-F2]	3	21	4	2	0	30	25/30 = 83
F2[F1-F2]	1	26	10	4	1	42	36/42 = 86
F2[F1-F3]	1	15	12	10	0	38	37/38 = 97
F2/F3	0	9	17	12	3	41	29/41 = 71
F3[F2-F4]	0	4	8	22	6	40	36/40 = 90
F4[F3-F4]	0	0	3	4	7	14	11/14 = 79
Total	5	91	55	54	17	222	190/222 = 86

was 57% vs 61% ( $P = 0.399$ ) comparing derivation population to validation cohort, respectively, considering liver biopsy as reference.

### Rio (derivation) population

FibroMeter<sup>Virus3G</sup> applied as a class classification test presented an overall rate of correct diagnosis of 86% considering any of the results reported in FibroMeter<sup>Virus3G</sup> stage class in accordance with fibrosis scored by METAVIR (Table 2). The AUROCs of both tests comparing METAVIR F0-F1 vs F2-F4 were similar between FibroMeter<sup>Virus3G</sup> and APRI [0.855 (0.801-0.898) vs 0.815 (0.757-0.864)] but the difference was at the limit of significance ( $P = 0.06$ ). The FibroMeter<sup>Virus3G</sup> AUROC was higher in comparison to Forns AUROC [0.855 (0.801-0.898) vs 0.769 (0.708-0.823);  $P < 0.001$ ]. The best cut-off that predicted significant fibrosis was 0.61, demonstrating an accuracy of 80% compared to liver biopsy. The PPV, NPV and accuracy of all tests were shown in Table 3. The stepwise combination of APRI or Forns followed by FibroMeter<sup>Virus3G</sup> provided an overall accuracy of 79% (Figure 1) and 78% (Figure 2), respectively ( $P = 0.791$ ), when identifying significant fibrosis. It also enabled 49% ( $n = 109$ ) and 54% ( $n = 120$ ) of the total sample, representing the grey zone of APRI and Forns for significant fibrosis, to be correctly classified in 73% and 77% of cases, respectively.

Thus, diagnostic accuracy did not differ comparing the use of FibroMeter<sup>Virus3G</sup> test alone or combined with APRI or Forns (80% vs 79% vs 78%, respectively,  $P = 0.79$ ), but represented a lower cost alternative since this procedure led to a 51% reduction of FibroMeter<sup>Virus3G</sup> test requirement using APRI + FibroMeter<sup>Virus3G</sup> and 46% reduction using Forns + FibroMeter<sup>Virus3G</sup> ( $P = 0.25$ ). Rates of well classified patients applying the algorithm APRI + FibroMeter<sup>Virus3G</sup>, using METAVIR score as reference, were as follows: 100% for F0, 81% for F1, 67% for F2, 80% for F3 and 94% for F4.

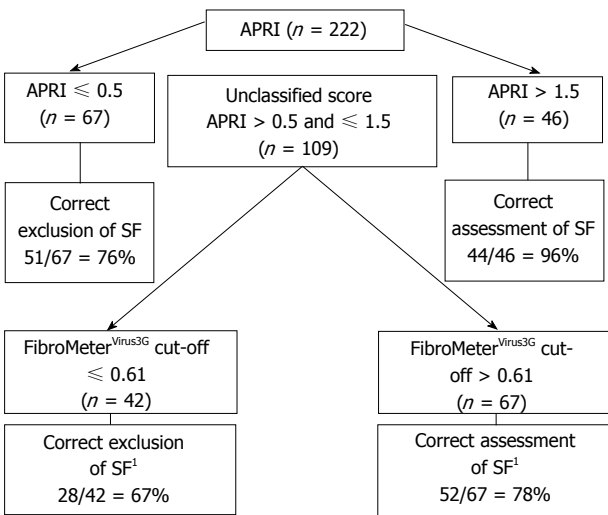
### Angers (validation) population

The cut-off of 0.61 found in derivation population presented an overall accuracy of 75% when discriminating significant fibrosis in the validation cohort in comparison to 80% in derivation population ( $P = 0.13$ ), considering histology as reference. The diagnostic accuracy of APRI + FibroMeter<sup>Virus3G</sup> combination in validation population to detect significant fibrosis and advanced fibrosis was respectively 69% and 74%. Rates of correct classification of this algorithm according to METAVIR score were as follows: 100% for F0, 88% for F1, 39% for F2, 64% for F3 and 85% for F4. When analysing the subgroup of false negative patients in this population we observed that 69% are represented by F2, 23% are F3, and only

**Table 3 Performance of aspartate transaminase to platelet ratio index, Forns, FibroMeter<sup>Virus3G</sup> and combination algorithm to discriminate significant fibrosis (FO-F1 vs F2-F4) in derivation and validation population**

Serum fibrosis tests	AUROC (95%CI)	Cut-off value	Se (%)	Sp (%)	PPV (%)	NPV (%)	OA (%)
Derivation population (n = 222)							
FM score	0.855 (0.801-0.898)	0.61	79	81	85	74	80
APRI	0.815 (0.757-0.864)	0.5 <sup>1</sup>	87	53	71	76	72
		1.5 <sup>2</sup>	35	98	96	53	62
Forns	0.769 (0.708-0.823)	4.2 <sup>3</sup>	94	31	64	79	66
		6.9 <sup>4</sup>	41	87	81	53	61
Apri + FM			76	82	85	72	79
Forns + FM			81	75	81	75	78
Validation population (n = 538)							
FM score	0.854 (0.821-0.888)	0.61	67	87	89	63	75
Apri + FM			57	88	87	57	69

<sup>1</sup>APRI ≤ 0.5 exclude significant fibrosis; <sup>2</sup>APRI > 1.5 predict significant fibrosis; <sup>3</sup>Forns ≤ 4.2 exclude significant fibrosis; <sup>4</sup>Forns > 6.9 predict significant fibrosis. FM: FibroMeterVirus3G; AUROC: Area under ROC curve; Se: Sensitivity; Sp: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; OA: Overall accuracy; APRI: Aspartate transaminase to platelet ratio index.

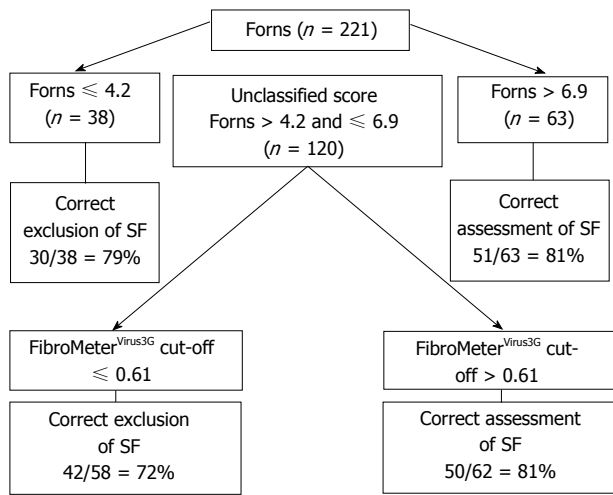


**Figure 1 Sequential algorithm of aminotransferase-to-platelet ratio index + FibroMeter<sup>Virus3G</sup> to predict significant fibrosis, in the Rio population.** Accuracy of sequential use of aminotransferase-to-platelet ratio index (APRI) + FibroMeter<sup>Virus3G</sup> was determined considering: Number of correct assessments of SF (96) + number of correct exclusions of SF (79)/total of liver biopsies (222) = 175/222 = 79%. <sup>1</sup>Considering liver biopsy as reference.

8% are cirrhotic. The PPV, NPV and accuracy of APRI + FibroMeter<sup>Virus3G</sup> combination on validation population are shown in Table 3.

**DISCUSSION**

Although new treatment regimens with very high rates of sustained virologic response are now available to treat hepatitis C patients, the logistical and financial barriers to treat all infected patients represent an important limitation, even in resource-replete countries<sup>[23]</sup>. Thus, it is necessary to determine an optimal and practical approach to prioritize these highly efficacious, but extremely costly therapies, for a selected population at risk of disease progression or for those who require immediate therapy. There is a consensus that non-invasive evaluation of liver



**Figure 2 Sequential algorithm of Forns + FibroMeter<sup>Virus3G</sup> to predict significant fibrosis, in the Rio population.** Accuracy of sequential use of Forns + FibroMeter<sup>Virus3G</sup> was determined considering: Number of correct assessments of SF [(101) + number of correct exclusions of SF (72)]/total of liver biopsies (221) = 173/221 = 78%.

fibrosis may be useful to complement or even replace liver biopsy in CHC owing to its low risk of complications and good accuracy. However, non-invasive tests also present some limitations related to cost, fibrosis discrimination and accuracy.

The present study originally evaluated the combination of a more robust patented fibrosis test, FibroMeter<sup>Virus3G</sup>, with low cost first generation tests to enhance its applicability in the clinical practice. Although APRI and Forns are well established non-invasive tests to assess fibrosis in CHC, their main limitation is that when used alone, almost half of the patients could not be classified according to the possibility of presenting or not significant fibrosis. Thus, using a second test to improve discrimination would enhance the accuracy of these results in order to diagnose significant fibrosis. In the present study, the use of first generation tests combined with FibroMeter<sup>Virus3G</sup> demonstrated to be a lower cost strategy since it reduced

FibroMeter<sup>Virus3G</sup> requirement, without loss of accuracy, eliminating the requirement for liver biopsy procedure.

When analyzed as a class classification test, FibroMeter<sup>Virus3G</sup> presented an overall accuracy of 86% similar to the rate of 87% described in FibroMeter<sup>Virus3G</sup> original report<sup>[21]</sup>. The AUROC for significant fibrosis was 0.85, comparable to previous reports ranging from 0.84 to 0.86<sup>[16,17,21]</sup>. Analysing under a practical point of view, and considering significant fibrosis as the criteria to treat or not the patient, we presented important results that may help hepatologists on clinical decision-making. We found a cut-off of 0.61 as the best numeric value to discriminate significant fibrosis for FibroMeter<sup>Virus3G</sup>, which is close to the value displayed in the manufacturer's bar graph of FibroMeter<sup>Virus3G</sup> report of 0.63, representing the transition from F1 to F2 METAVIR stage<sup>[24]</sup>. The cut-off of 0.61 presented similar performance in comparison to manufacturer's cut-off of 0.63 in the French validation cohort.

FibroMeter<sup>Virus3G</sup> applied as a numeric score also enables its association to other scores. Sebastiani *et al.*<sup>[25]</sup> provided a sequential algorithm for fibrosis evaluation (SAFE) biopsy combining APRI and Fibrotest, another biomarker based on fibrosis class classification, resulting in a 46% reduction of liver biopsy requirement to identify significant fibrosis. In a more recent study performed with 1785 CHC patients, Boursier *et al.*<sup>[26]</sup> reported that the diagnosis of significant fibrosis using SAFE still required liver biopsy in 64% of the cases. To our knowledge, to date the stepwise combination of APRI or Forns with FibroMeter<sup>Virus3G</sup> has never been evaluated. The sequential algorithm of either APRI or Forns combined with FibroMeter<sup>Virus3G</sup> represents a lower cost method with similar accuracy when compared to the isolated use of FibroMeter<sup>Virus3G</sup> test. This represents an advantage when reducing the number of unclassified APRI and Forns patients in the grey zone, without the need for liver biopsy. This is a useful and alternative approach in countries with less financial resources, considering the easy applicability and low cost of APRI and Forns for significant fibrosis. This procedure may represent a more comprehensive proposal to apply these non-invasive tests in the clinical setting.

Some limitations may be discussed in this study. The prevalence of significant fibrosis in our population was higher than the prevalence reported in a meta-analysis including more than 30000 CHC patients which showed a rate of 48% of significant fibrosis histologically assessed<sup>[27]</sup>. Our prevalence is greatly influenced by the fact that this study was carried out in a tertiary-care setting. Another limitation is that derivation and validation populations came from different racial ethnic backgrounds. Nevertheless, most patients included in the derivation population were Caucasians and both populations shared similar characteristics regarding laboratorial results and distribution of significant fibrosis.

The diagnostic accuracy of the APRI and FibroMeter<sup>Virus3G</sup> combination in validation population was 69%. A decrease in diagnostic accuracy is usually expected in the validation population when compared to the derivation

population; however some points need to be emphasized. The PPV of the algorithm APRI and FibroMeter<sup>Virus3G</sup> in the validation population was high (87%). Consequently the algorithm enabled the selection of a subset of patients where very few false positive results were found. In other words, this algorithm allowed treatment to be given to those patients who really required antiviral drugs. The low sensitivity (57%) remains a limitation, since a considerable number of patients who need to be treated will not be correctly identified by the algorithm. Nevertheless, when analysing the subgroup of false negative patients in the validation population, we observed that the majority (69%) were represented by F2 and only 8% were cirrhotic. In the whole validation population, most of the F0 patients (100%), the F1 patients (88%) and the F4 (85%) were well classified by the algorithm, as well as two thirds of the F3. Therefore, the algorithm identifies the more severe patients (F3 and F4) while most of the misclassification concerns the F2 stage. In the derivation population, even though the accuracy of the algorithm was found to be better, the worst result was also observed in F2 stage. And lastly, even when considering a gold standard such as liver biopsy, there is a considerable misclassification of F2 patients<sup>[28]</sup>. In low income countries, where therapy is offered only to patients with advanced fibrosis, a close follow-up is required until these untreated patients fulfil the criteria for direct antiviral therapy. We may consider following the "missed" patients by reapplying the algorithm to better identify when patients change their fibrosis stage and require treatment<sup>[29]</sup>. Since most misclassified patients present F2 fibrosis stage, there is sufficient time before they become cirrhotic.

Our findings demonstrated that the association of a more robust non-invasive marker of fibrosis such as FibroMeter<sup>Virus3G</sup> and first generation tests may represent a useful alternative for fibrosis staging in CHC without loss of accuracy and without the need for liver biopsy. This might be an attractive approach mainly in limited resource countries.

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## COMMENTS

### Background

Despite recent advances in chronic hepatitis C therapy, diagnosis of significant fibrosis still represents a challenge when defining which patients should have priority in treatment, mainly in resource limited countries. Although liver biopsy has been classically considered the standard tool to evaluate fibrosis, it presents well-known inconveniences and limitations which make its use controversial amongst various authors. Thus, the development and improvement of alternative methods to identify candidates for an early treatment or intensive fibrosis monitoring is still recommended. First generation non-invasive tests such as aminotransferase-to-platelet ratio index (APRI), FIB4 and Forns are free of charge, easily accessible and well validated for chronic hepatitis C, however are limited when classifying all patients. Therefore, in order to increase assessment availability for patients

with significant fibrosis, the authors evaluated the performance of a stepwise combination of first generation tests of fibrosis - APRI and Forns - followed by FibroMeter<sup>Virus3G</sup>, a more robust test, whenever results remained unclassified after first generation tests, always using liver biopsy as reference. This proposed combination allows costs to be optimized with no loss of accuracy and no need of liver biopsy, thus representing a favorable economic approach in resource limited areas.

### Research frontiers

This study introduces alternative approaches to evaluate significant fibrosis in chronic hepatitis C using a stepwise algorithm with first generation tests and FibroMeter<sup>Virus3G</sup> both to improve clinical decision-making and reduce costs. The authors considered this topic of great interest for clinicians and hepatologists in the daily practice management of chronic hepatitis C.

### Innovations and breakthroughs

The present study demonstrated that the association of a more robust non-invasive marker of fibrosis such as FibroMeter<sup>Virus3G</sup> and first generation tests such as APRI and Forns may represent a useful alternative for fibrosis staging in chronic hepatitis C. This might be an attractive non-invasive approach to evaluate liver fibrosis and to optimize the access to potent but expensive direct-acting antiviral agents. To our knowledge, to date the stepwise combination of APRI or Forns with FibroMeter<sup>Virus3G</sup> has never been evaluated.

### Applications

The sequential algorithm of either APRI or Forns combined with FibroMeter<sup>Virus3G</sup> represents an alternative approach to recognize and prioritize patients with chronic hepatitis C to antiviral therapy. It reduces the number of unclassified APRI and Forns patients allocated in the grey zone and reduces the total FibroMeter<sup>Virus3G</sup> requirement in 50%, representing an alternative approach in countries with less financial resources, without loss of accuracy, eliminating the requirement for liver biopsy procedure. This procedure may represent a more comprehensive proposal to apply these non-invasive tests in the clinical setting.

### Terminology

FibroMeter<sup>Virus3G</sup> is a non-invasive test to evaluate liver fibrosis in chronic hepatitis C represented by a patented formula including the biologic parameters prothrombin index, AST, ALT, Urea, GGT, alpha-2-macroglobulin and platelets.

### Peer-review

It is a carefully planned study, it takes in to consideration the Liver biopsy and the Fibrometer Virus2 and Virus3 and makes a head to head comparison of the three, as to discover the safety profile and the accuracy when it comes to clinical use especially for the group patients with cirrhosis and to evaluate the change in fibrosis stage in pts with cirrhosis that are unable to undergo liver biopsy with a non-invasive procedure. The Fibre meter requires more wide use in the clinical setting as to prove its self as a reliable and non-invasive method of estimating the fibrosis stage.

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