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Miguel Lino de Magalhães

Biochemical scores comparison with TE (fibroscan) in HCV cirrhotic patients treated with DAA

Comparação entre scores bioquímicos e TE (fibroscan) em doentes cirróticos infetados com VHC tratados com DAA

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Projeto de Opção do 6º ano - DECLARAÇÃO DE INTEGRIDADE

Eu, Miguel Lino de Magalhães, abaixo assinado, nº mecanográfico 201206531, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

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DESIGNAÇÃO DA ÁREA DO PROJECTO

Doenças Infeciosas

TÍTULO DISSERTAÇÃO/MONOGRAFIA (riscar o que não interessa)

Biochemical scores comparison with TE (fibroscan) in HCV cirrhotic patients treated with DAA

ORIENTADOR

Maria de Lurdes Campos dos Santos

COORIENTADOR (se aplicável)

ASSINALE APENAS UMA DAS OPÇÕES:

É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTE TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☒
É AUTORIZADA A REPRODUÇÃO PARCIAL DESTE TRABALHO (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
DE ACORDO COM A LEGISLAÇÃO EM VIGOR, (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) NÃO É PERMITIDA A REPRODUÇÃO DE QUALQUER PARTE DESTE TRABALHO.	☐

Faculdade de Medicina da Universidade do Porto, 21/03/2018

Assinatura conforme cartão de identificação: Miguel Lino de Magalhães

Dedicatória

Porque nada se faz sozinho e todo o apoio dado é precioso e requer valorização gostava de dedicar esta Tese de Mestrado a todas a pessoas que me apoiaram desde a origem da ideia para dissertação até aos que tiveram papel ativo no delineamento e concretização do projeto, com especial menção à Doutora Maria de Lurdes Campos dos Santos.

No final, apesar do trabalho escrito ser o sumo que se tira do projeto, há mais para ser recordado e apreciado, nomeadamente as inúmeras dificuldades e contratempos que muitas vezes foram as circunstâncias mais dependentes de apoio e compreensão, como a busca de inspiração, de vontade de trabalhar e a capacidade de organizar o tempo e a atenção. Nesta secção mesmo as mais pequenas ajudas, os pequenos empurrões, ajudaram a desencravar bloqueios críticos e a impulsionar a viagem para além do porto definido na partida, e estas ajudas vão desde as dicas para o trabalho até as palavras de encorajamento e a paciência para ouvir o incessante lamurio de quem tem muito para fazer e por vezes se sente sem rumo e sem noções se está a produzir um bom trabalho ou não. Por todo o apoio, e porque sem qualquer um dos apoios que tive o trabalho se teria tornado mais difícil, gostaria de dedicar este trabalho aos meus pais, Ana Paula Martins da Silva Lino e António da Conceição Magalhães, ao meu irmão, Sérgio Lino Magalhães, e aos meus amigos e colegas de curso, que, por valorizar todos os seus esforços, me vejo sem espaço para lhes dar a honra de uma menção individual adequada neste pequeno texto.

Abstract

Background: The infection by the hepatitis C virus (HCV) is one of the main causes of chronic liver disease worldwide, with elevated risk for development of cirrhosis and hepatocellular carcinoma. The implementation of Direct Acting Antiviral (DAA) therapy provided potential treatment access to every infected patient with achievement of Sustained Virological Responses (SVR) in over 90% of patients and improvement of clinical outcomes and liver fibrosis.

Methods: This retrospective study enrolled 64 monoinfected HCV patients with advanced fibrosis who achieved SVR after treatment with DAA therapy beginning from January 2015 to June 2016. The aim of this study is to evaluate the sensibility and specificity of the ALT/AST ratio (AAR), Aspartate aminotransferase to platelet ratio index (APRI) and the FIB-4 score against TE (fibroscan) in those patients at baseline and at the 24 weeks post-treatment follow-up.

Results: At the baseline checkpoint, all scores were similar: FIB-4 had AUC 0.784, 79% sensitivity and 82 % specificity. At the post-treatment checkpoint: FIB-4 had AUC 0.809, 82% sensitivity and 70% specificity; APRI had AUC 0.841, 64% sensitivity and 90% specificity; AAR was not statistically significant.

Conclusion: The FIB-4 and APRI scores proved to be acceptable alternatives for the FibroScan to evaluate the changes in the extension of liver fibrosis in post treatment follow up. And the AAR failed possibly due to confounding. However, bigger studies are needed to confirm this hypothesis.

Keywords: HCV infection, cirrhosis, DAA therapy, SVR, sensibility and specificity, AAR, APRI, FIB-4

Introduction

Hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease worldwide, with nearly 180 million people living with the infection. [1, 2] Once infected, over 80% of the individuals develop a chronic infection that has a silent evolution, sometimes during decades, that in the end may lead to cirrhosis, portal hypertension, hepatic decompensation, and the development of hepatocellular carcinoma (HCC). It's estimated that chronic HCV infection accounts for 350 000 deaths per year, as well as for 27% of all cases of cirrhosis and 25% of hepatocellular carcinoma. [1, 2]

The primary goal of HCV therapy is to cure the infection, that is, to achieve Sustained Virological Response (SVR), defined as undetectable HCV RNA at 12 or 24 weeks after treatment completion. Since their appearance, the direct-acting antiviral (DAAs) targeting HCV have transformed the treatment of HCV infection, providing the achievement of SVR in over 90% of the patients. [2-4] Furthermore, DAAs therapy has proven itself effective on all treatment-naïve and treatment-experienced patients with compensated or advanced liver disease related to HCV, regardless their liver fibrosis stage. Therefore, it is recommended for everyone with HCV infection, with the exception of those with contraindications to the treatment or low life-expectancy regardless of HCV cure. [5, 6]

During the progression of HCV infection the cumulative development of cirrhosis can be as big as 15% at every 5-year of follow-up and this cirrhotic state is the main responsible for increasing the risk of liver failure, HCC and liver related death, with an annual risk of 2.9%, 3.2% and 2.7%, respectively. [7, 8] And although the SVR in HCV infection can prolong overall survival and, according to recent data, in patients with cirrhosis, potentially promotes the regression of liver inflammation and fibrosis, the risk of HCC, complications of cirrhosis and all-cause mortality are not entirely eliminated. So, in cirrhotic patients who achieve an SVR is very important to evaluate the regression or not of the cirrhosis to evaluate prognosis, although all cirrhotic patients should have a follow up and the screen of HCC, even if they cure HCV infection. [6, 8-10]

Given this, it's of the most importance to test easier, cheaper and more available methods to see the impact in regression of chronic liver disease in treated HCV patients for better surveillance and more accurate expectancies regarding the overall quality of live and disease-free state.

Liver biopsy, despite remaining the gold standard for the assessment of liver fibrosis, has a number of limitations, namely in underdeveloped countries. To overcome these limitations were created the noninvasive tests, which are based on two approaches: analysis of scores set on the quantification of blood tests, and the mechanical measurement of the liver fitness. [2, 11, 12] Some of these scores are the ALT/AST ratio, the APRI score and the FIB-4, which have been widely tested for liver fibrosis analysis on HCV infected patients. Moreover, these tests have several advantages, namely their high applicability, good inter-laboratory reproducibility and their widespread availability. [13-18]

The purpose of this study was to determine the sensibility and specificity of these biochemical scores, against the TE (FibroScan), when evaluating the changes of liver fibrosis after SVR in HCV infected cirrhotic patients treated with DAA therapy.

Methods

Study population

This retrospective study was approved by the Ethics Committee for Health of Hospital de São João and it was carried out in S. João Hospital Center, Porto. It was based on patients treated with sofosbuvir combined with NS5A inhibitors ledipasvir or daclatasvir with or without ribavirin, and, for a selected group of patients, sofosbuvir combined with ribavirin.

For the study were only selected the patients who began treatment from 1 of January 2015 to 31 of June 2016, had, at baseline, advanced fibrosis or cirrhosis, diagnosed by TE (FibroScan) measurement (F3 and F4 category), and achieved SVR at the end of treatment. This cohort includes only HIV and HBV negative patients. This accounted for 97 patients. The following variables were collected from these patients: sociodemographic characteristics (age, gender, smoking and drinking habits, other chronic illnesses and chronic medication), VDRL test result, HCV regarding variables (RNA at baseline, 4, 12 and 24 weeks, HCV genotype and IL28 presence), TE (FibroScan) evaluation and biochemical analysis (liver function panel and complete hemogram). From the original 97 patients 33 did not have all the necessary information for the variables under investigation (**Figure1**), therefore the study was conducted on 64 patients.

Study design

At the beginning of treatment all 64 patients were tested for HCV RNA by PCR, complete hemogram, liver function and alfafeto-protein analysis, as well as an abdominal ultrasound to characterize hepatic structure and exclude focal lesions and also a TE (FibroScan) measurement. Moreover, during the study several biomarkers were done including HCV RNA by PCR at week 4, 12 and in the end of treatment (12 or 24 weeks), and then at 12 weeks and 24 weeks after treatment; monitorization with hemogram and liver function analysis at week 4, 8, 12 and at the end of treatment; and a TE (FibroScan) measurement was taken 12/24 weeks after the end of treatment.

Two checkpoints were determined for analysis and comparison: the baseline checkpoint with an TE (FibroScan) measurement and biochemical analysis with hemogram and liver function markers; and a post 12/24 weeks after the end of treatment checkpoint with a biochemical analysis identical to the baseline plus a TE (FibroScan) measurement.

In the baseline checkpoint, performed with 64 patients, the sensibility and specificity of three biochemical scores (AAR, FIB-4 and APRI) were tested against the TE (FibroScan) in its capability to evaluate the liver-stiffness extension. There were 22 patients with significant fibrosis but not cirrhosis (F3) and 42 with cirrhosis (F4). In the after-treatment checkpoint, which was the main objective of this study, the sensibility and specificity of the same three biochemical scores (AAR, FIB-4 and APRI) against the TE (FibroScan) were evaluated in its capability of predicting the regression of liver fibrosis on the SVR HCV infected patients.

Liver stiffness measurement

Liver stiffness was measured by Transient elastography, FibroScan, and used as gold-standard for the purpose of this study. The quality of the FibroScan as reference for liver stiffness determination and as a viable alternative to the liver biopsy has been proven in multiple studies.[11, 12, 19]

TE (FibroScan) was performed according to the manufacturer's instructions. Patients were investigated under fasting conditions. TE result was considered valid if three criteria were fulfilled: (a) at least 10 valid shots; (b) a success rate above 60%; and (c) an interquartile range reflecting the variability of measurements less than 30% of the median LS value. LSM less than 8.5 kPa was considered as fibrosis stage F1, less than 9.5 kPa as fibrosis stage F2, less than 12.5 kPa as stage F3, and measurements equal to or over 12.5 kPa as fibrosis stage F4 by Metavir. [20]

Biochemical scores

For this study three tests were selected for being cheap, having high applicability, good inter-laboratory reproducibility and widespread availability: the ALT/AST ratio (AAR), the Aspartate aminotransferase to platelet ratio index (APRI) and the FIB-4 score. These tests have been widely tested in many studies and their diagnostic accuracy for severe fibrosis and cirrhosis, as well as its use for the follow-up of progression of liver fibrosis has proven to be a suitable alternative for liver biopsy and TE. [13-18] AAR with 21% (0.09–0.40) sensitivity, 77% (0.68–0.84) specificity and a diagnostic accuracy of 66%; APRI with 73% (0.57-0.89) sensitivity, 84% (0.75-0,93) specificity and diagnostic accuracy of 72%; FIB-4 with 61% (0.38-0.74) sensitivity, 90% (0.81-0.98) specificity and diagnostic accuracy of 68%.[11]

Statistical analysis

The compared variables included demographic characteristics, aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transaminase (GGT), creatinine, hemoglobin, platelet count, International normalized ratio of prothrombin time of blood coagulation (INR), albumin levels and Hepatitis C Virus RNA.

Absolute frequencies and percentages were used to describe categorical variables while continuous variables were described using medians and interquartile range. The $\chi 2$ test was used for categorical variables and the Mann-Whitney U test was used to compare continuous variables.

The performance of each test was evaluated for the initial diagnosis of liver cirrhosis and as a tool for diagnosis of liver stiffness regression in previously cirrhotic patients. FibroScan® test was used as the reference test. The area under the receiver operating characteristic (ROC) curve was used as an estimate of the overall accuracy of each test.

All statistical analysis was performed using SPSS® version 24 (IBM Corp., Armonk, New York, USA), using 0.05 as the level of significance.

Results

Participants

A total of 64 patients, who were treated according to guidelines and achieved SVR, were included in this cohort: 42 (65.6%) had cirrhosis at baseline and 22 (34.4%) had severe fibrosis (Metavir F3). Overall 18 (29.5%) were male and 46 (70.5%) were female, and the median age was 54 years. For more information see **Figure 1**. Between the two groups, cirrhotic and non-cirrhotic, there was an age difference of plus 5 years on the cirrhotic patients, although this was not a statistically significant difference. The proportion of male patients was higher amongst the cirrhotic group.

Clinical characteristics

The two groups were separated according to FibroScan evaluation (F4 and F3) and between them there is a statistically significant difference (p value < 0.05) in five of the collected variables: AST, creatinine, platelet count, INR and albumin levels (see **table 1.**); there is no statically significant difference in the HCV RNA levels between the two groups. The difference is also reflected on the median values for the AAR, APRI and FIB-4 between both groups, cirrhotic groups with 0.86 (0.42), 1.80 (2.38) and 3.1 (3.7), respectively; and non-cirrhotic group with 0.68 (0.26), 0.71 (1.10) and 1.5 (1.0), respectively.

Baseline checkpoint

At baseline the score with best area under the curve (AUC) was FIB-4 with 0.784 (SE 0.058) and sensitivity and specificity, of 79% and 82%, respectively. Both the APRI and the AAR scores showed very close results, with AUC 0.722 (SE 0.066) and 0.733 (SE 0.070) respectively; sensitivity of 76% and 79%, respectively; and specificity of 68% for both scores. (see **table 2.** and **figure 2.**)

Post-treatment checkpoint

After treatment the performance of the FIB-4 score, compared with the FibroScan, resulted in AUC 0.809 (SE 0.068), 82% sensitivity and 70% specificity; and the APRI score resulted in AUC 0.841 (SE 0.059), 64% sensitivity and 90% specificity. The score that performed poorly was the AAR with AUC 0.627 (SE 0.089), sensitivity of 55% and specificity of 75%. (see **table 3.** and **figure 3.**)

Discussion

Hepatitis C, if not treated, will progress with build-up of liver fibrosis, and increasing incidence of liver and non-liver disease related adverse manifestations, that ultimately account for 350.000 deaths per year. [2, 21] Focusing on the liver related manifestations, the most common are portal hypertension (esophageal varices), hepatic decompensation, thrombosis of the hepatic and portal vein and development of HCC. [2, 21, 22] This increased risk of liver related morbidity and mortality is highly associated with the presence of cirrhosis, as for patients with minimal fibrosis have a low risk of development of complications, [2] and significant progression to liver failure and death was shown to be augmented in cirrhotic patients who fail treatment. [23]

With the new DAAs treatment SVR is achieved in over 90% of the patients, [2-4] regardless their degree of liver fibrosis, compensated or advanced chronic liver disease. This allowed us to treat patients who were not eligible for treatment before. The SVR state not only indicates cure of the HCV infection but also correlates with improving of liver parameters and clinical outcomes. [5, 6]

An observational prospective cohort study (Dolmazashvili, E., et al.) with 304 patients, in different liver-stiffness stages at baseline, was performed to evaluate the changes in the liver fibrosis, measured by FibroScan, after DAA treatment. Through this study they concluded that fibrosis can be reversed after SVR is achieved, which can explain the patients clinical improvement. However not all patients present fibrosis reversion and some may not have any changes in liver-stiffness. [24] Furthermore, the idea that DAA therapy, either through improvement of liver inflammation and fibrosis or HCV eradication, changes the clinical outcomes, even in cirrhotic patients, is represented in several studies that show a decrease of liver associated morbidity, mortality [6, 7, 9], liver adverse events and incidence of HCC, after SVR is achieved. [25-30] Although the studies for outcomes after DAA therapy only contemplate short and medium term outcomes, the improvement of clinical perspective in the long term in SVR after Interferon based therapy is well documented. [31, 32] It can be inferred that the same will happen in the future with patients who were cured with DAA therapy.

Since liver related mortality and morbidity have a great correlation with the extent of liver fibrosis, the accurate diagnosis of the liver fibrosis severity is of the most importance for a better management and surveillance. This accurate diagnosis should be performed by liver biopsy, which has many limitations namely in underdeveloped countries, and next by TE analysis (FibroScan), which also does not have widespread availability. Because of these limitations Biochemical scores gain importance in the follow-up of liver fibrosis in post-treatment patients. However, there are no studies that specifically evaluate the performance of the scores in the context of post-treatment follow-up. For this reason, the aim of this study was to test the diagnostic performance of the AAR, APRI and FIB-4 scores when evaluating the changes of liver fibrosis after SVR in HCV infected cirrhotic patients treated with DAA therapy (using as reference for cut-off values the Gokcan, H., et al. study results [16])

At baseline, the biochemical scores were tested against the FibroScan. Our results, were compatible with most of the published bibliography, though not exhibiting such strong correlation as some of the big cohort studies [11, 13-18, 20]. All scores demonstrated good correlation with the FibroScan, with the FIB-4 being the one with the best performance (AUC: 0.784; 79% sensitivity; 82% specificity) and the APRI and AAR showing similar diagnostic power (see Table2 and Figure2).

Given the good correlation at baseline the same process was performed with the patients at the second checkpoint, 24 weeks post-treatment. Both the APRI and the FIB-4 score performed well with AUC of 0.841 and 0.809, respectively, with the difference that the FIB-4 score had higher sensitivity, with 82%, (APRI 64%) but worse specificity, with 70% (APRI 90%). These results show that the decrease in liver inflammation and fibrosis affect these biochemical scores in a similar way it affects the FibroScan, with the FIB-4 being more sensitive to small changes in liver-stiffness values and the changes in APRI scores having a closer correlation with the changes in liver-stiffness values.

However, the AAR score did not perform well in the follow-up of fibrosis reversal, as it did not achieve statistically significant diagnostic power. This may be because it is the score with fewer variables and so it is more prone to confounding.

This retrospective cohort study presented a number of limitations that increase the risk of some bias. A primary one being the restricted number of patients enrolled, due to the strictness of the inclusion criteria. And a secondary one being the high prevalence of Dyslipidemia and Alcohol consumption amongst the Portuguese population [33], which are known liver pro-inflammatory conditions that worsen the liver recovery even after SVR achievement. [34] A clearer vision on the performance of biochemical scores could possibly be attained with a greater cohort so as to stratify performance through risk and confounders groups. There is a lack of studies regarding the relation of the improvement of liver fibrosis severity and clinical outcomes either on a short or a long term, and we believe this to be an important issue to better follow cured patients and to maximize resources and budgets.

In conclusion, the FIB-4 and APRI scores have proven to be acceptable alternatives for the FibroScan to evaluate the changes in the extension of liver fibrosis in post-treatment follow up of cured patients. However, bigger studies are needed to confirm this hypothesis.

Acknowledgments

There is no such thing as a work done single-handed.

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Figure 1. Selection fluxogram

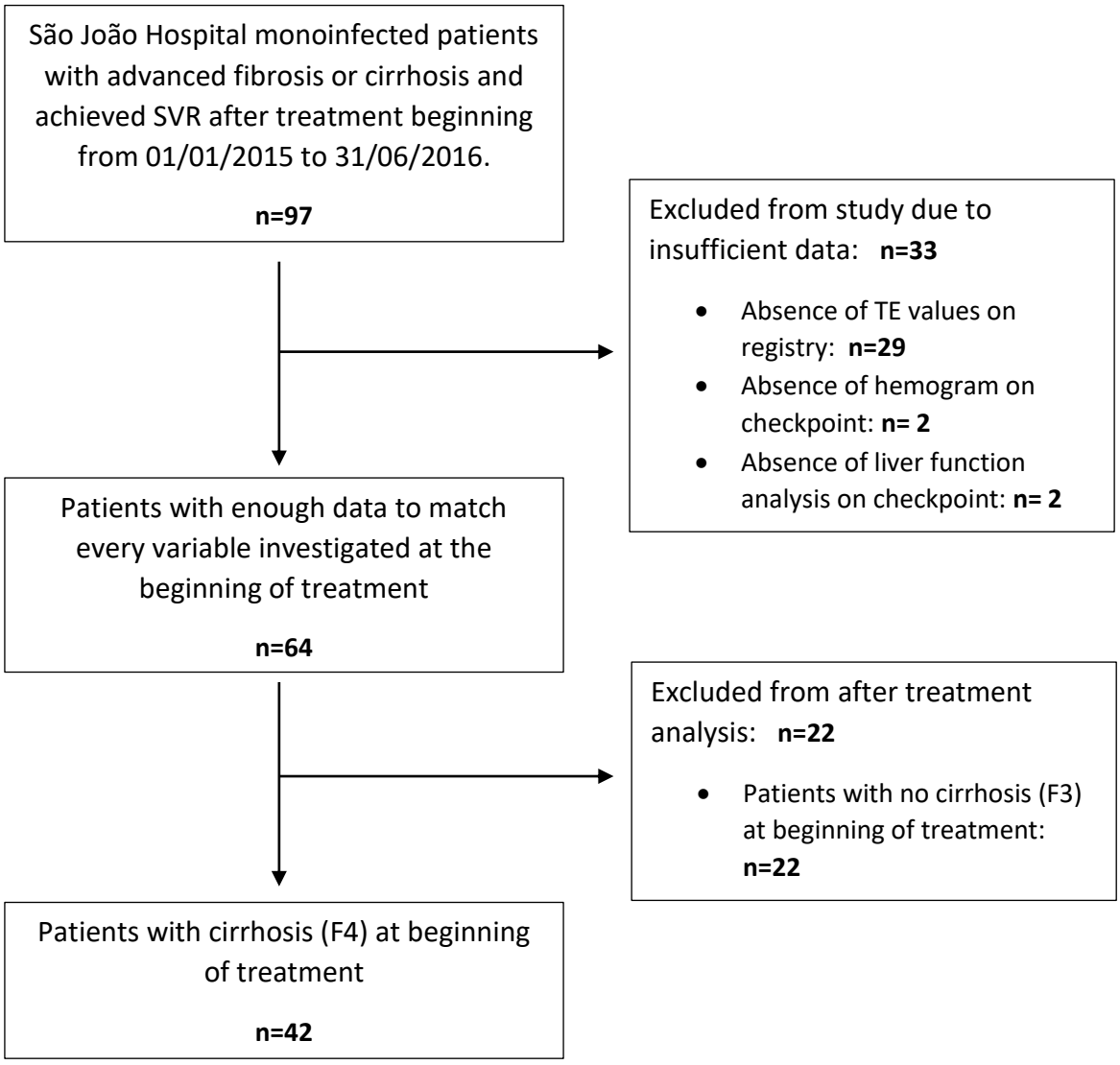


Table 1. Baseline demographic and clinical characteristics of all the subjects

	Whole sample (n=64)	Cirrhotic patients (n=42)	Non-cirrhotic patients (n=22)	*P* value
Demographic characteristics				
Age (years) median (IQR)	54 (16)	56 (17)	51 (17)	0.079†
Gender n (%)				0.200*
Male	18 (29.5%)	14 (33.3%)	4 (18.2%)	
Female	46 (70.5%)	28 (66.7%)	18 (81.8%)	
Clinical characteristics				
AST (IU/L) median (IQR)	74 (83)	84 (83)	52 (49)	**0.025†**
ALT (IU/L) median (IQR)	94 (95)	96 (82)	69 (102)	0.783†
GGT median (IQR)	91 (124)	91 (94)	95 (188)	0.994†
Creatinine median (IQR)	0.75 (0.17)	0.69 (0.15)	0.80 (0.14)	**0.009†**
Haemoglobin (g/dL) median (IQR)	15.3 (2.4)	15.0 (2.65)	15.8 (2.3)	0.309†
Platelet count (/mm^3) median (IQR)	161 (87)	133 (81.8)	187 (61)	**<0.001†**
INR median (IQR)	1.01 (0.14)	1.04 (0.20)	1.00 (0.05)	**0.031†**
Albumin (g/dL) median (IQR)	41.0 (6.1)	40.0 (6.6)	42.4 (4.2)	**0.031†**
HCV RNA(IU/mL) median (IQR)	2.06x10^6 (3.08x10^6)	2.06x10^6 (2.64x10^6)	2.08x10^6 (5.60x10^6)	0.239†
AAR median (IQR)		0.86 (0.42)	0.68 (0.26)	**0.002†**
APRI median (IQR)		1.80 (2.38)	0.71 (1.10)	**0.004†**
FIB-4 median (IQR)		3.1 (3.7)	1.5 (1.0)	**<0.001†**

Abbreviations: AAR – ALT/AST ratio; ALT – alanine transaminase; APRI – Aspartate aminotransferase to platelet ratio index; AST – aspartate transaminase; GGT – gamma-glutamyl transaminase, INR – International normalized ratio of prothrombin time of blood coagulation

* By $\chi 2$ test.

† By Mann-Whitney U test.

Table 2. Performance of non-invasive tests in the initial diagnosis of liver cirrhosis compared to FibroScan®

	AUC (SE)	*P* value	Cutoff values	Sensitivity (95%CI)	Specificity (95%CI)
AAR	0.733 (0.070)	**0.002**	>0.74	79 (63-90)	68 (45-86)
APRI	0.722 (0.066)	**0.004**	>0.93	76 (61-88)	68 (45 -86)
FIB-4	0.784 (0.058)	**<0.001**	>1.97	79 (63-90)	82 (60-95)

Abbreviations: AAR – ALT/AST ratio; ALT – alanine transaminase; APRI – Aspartate aminotransferase to platelet ratio index; AST – aspartate transaminase; AUC – Area Under the Curve; CI – Confidence Interval; SE – Standard Error.

Figure 2. Receiver operating characteristic curves comparing the performance of non-invasive tests in the initial diagnosis of liver cirrhosis compared to FibroScan

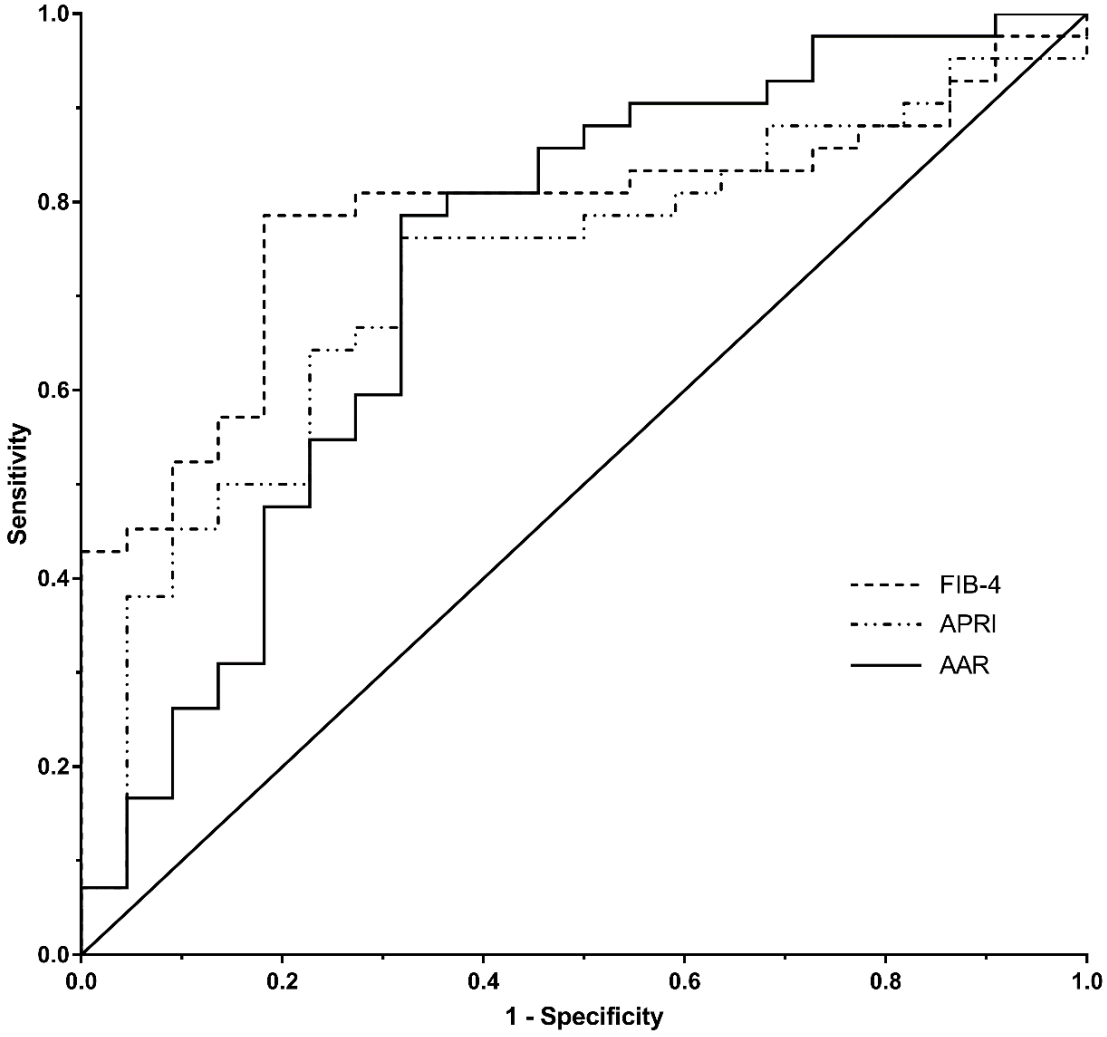
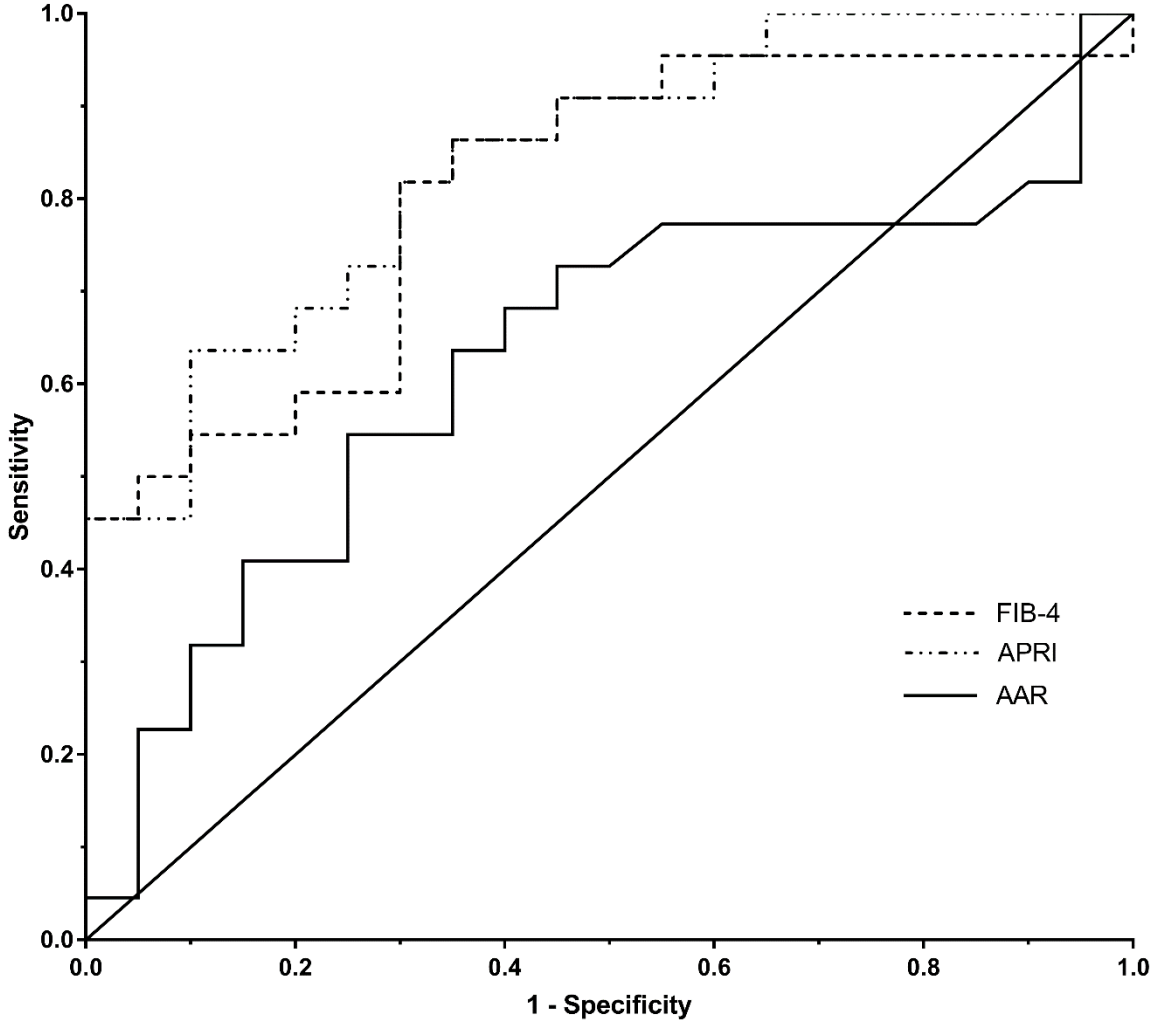


Table 3. Performance of non-invasive tests in the evaluation of liver fibrosis reversal compared to Fibroscan®

	AUC (SE)	P value	Cutoff values	Sensitivity (95%CI)	Specificity (95%CI)
AAR	0.627 (0.089)	0.158	>1,209	55 (32-76)	75 (51-91)
APRI	0.841 (0.059)	**<0.001**	>0,6448	**64 (41-83)**	**90 (68-99)**
FIB-4	0.809 (0.068)	**0.001**	>1,786	**82 (60-95)**	**70 (46-88)**

Abbreviations: AAR – ALT/AST ratio; ALT – alanine transaminase; APRI – Aspartate aminotransferase to platelet ratio index; AST – aspartate transaminase; AUC – Area Under the Curve; CI – Confidence Interval; SE – Standard Error.

Figure 3. Receiver operating characteristic curves comparing the performance of non-invasive tests in the evaluation of liver fibrosis reversal compared to FibroScan®



Anexos

Normas da revista "Porto Biomedical Journal"

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