Title page

Type of manuscript: Article

Title: Prediction of hepatic fibrosis in patients coinfected with human immunodeficiency virus and hepatitis C virus based on genetic markers

Running head: CRS and liver fibrosis progression

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Meetings at which parts of the data were presented:

Some parts have been presented in the 20th Conference on Retroviruses and Opportunistic Infections (CROI): Fernández-Rodríguez A; Berenguer J, Jiménez-Sousa MA, Guzmán-Fulgencio M, Micheloud D, López JC, Álvarez E, Bellón JM, Miralles P, Resino S. The cirrhosis risk score (CRS) implication in fibrosis prediction among human immunodeficiency virus/hepatitis C virus coinfected patients. 20th Conference on Retroviruses and Opportunistic Infections (CROI 2013). Atlanta, Georgia, USA. March 3-6, 2013.

Potential conflicts of interest:

The authors do not have any commercial or other association that might pose a conflict of interest.

Funding/Support:

This work has been supported by grants given by Fondo de Investigacion de Sanidad en España (FIS) [Spanish Health Founds for Research] [grant numbers PI08/0738, PI11/00245; PI08/0928, and PI11/01556], Red Española de Investigación en SIDA (RIS) [AIDS Research Network] [grant numbers RD12/0017/0024 and RD12/0017/0004] and "Fundación para la Investigación y la Prevención del Sida en España" (FIPSE) [grant number 361020/10].

AFR, MGF, PGB, and MAJS are supported by "Instituto de Salud Carlos III" [grant numbers UIPY-1377/08, CM09/00031, FI12/00036, and CM10/00105, respectively].

ABSTRACT

Objective: To assess the ability of the cirrhosis risk score (CRS) to predict liver fibrosis progression in HIV/HCV coinfected patients.

Design: Retrospective follow-up study.

Methods: Based on a minimum follow-up time of 10 years with HCV infection, 190 HIV/HCV coinfected patients were classified according to their METAVIR score: i) 25 non-progressor patients who did not develop fibrosis (F0); and ii) 165 progressor patients who developed fibrosis (F≥1). Seven polymorphisms of CRS signature and *IL28B* genotype were performed using the GoldenGate® assay. The CRS signature was calculated by Naïve Bayes formula as previously described.

Results: Non-progressors had CRS values significantly lower than progressors (0.61 versus 0.67; p=0.043). Among the progressors, we observed similar CRS values through all the fibrosis stages (F1/F2/F3/F4). The percentage of patients with CRS>0.70 (high-risk of developing fibrosis) was higher in progressors than in non-progressors; but the percentages with values between 0.50-0.70 (intermediate risk) and <0.50 (low risk) were quite similar for each of the fibrosis stages (p=0.047). The area under the receiver operating characteristic curve (AUROC) of CRS for discriminating non-progressor versus progressor was 0.625 (p=0.043). When clinical variables were considered (age at HCV infection, IDU, gender, *IL28B* and HCV genotype), the AUROC of CRS improved up to 0.739 (p<0.001).

Conclusion: CRS itself seems not to be a good marker for identifying HIV/HCV coinfected patients who are at high risk of developing liver fibrosis. However, CRS score coupled with clinical factors might help to distinguish between non-progressors and progressors patients.

Key-words: AIDS; Chronic hepatitis C; Genetic polymorphisms; Liver fibrosis; Predictive genetic markers

INTRODUCTION

Chronic hepatitis C (CHC) is one of the leading causes of end-stage liver disease, hepatocellular carcinoma and liver transplantation worldwide [1,2]. Liver fibrosis in CHC is believed to be progressive and largely irreversible, although the progression rate is highly variable and difficult to predict. Some individuals experience fast fibrosis progression with rapid development of end-stage liver disease (ESLD), whereas other individuals experience a slow progression, which makes the development of liver decompensation very unlikely [3]. This variability in fibrosis progression is probably due to multifactorial interactions between viral and host factors such as age at hepatitis C virus (HCV) infection, gender, daily alcohol intake, intravenous drug use (IDU), obesity, metabolic syndrome, HCV genotype [4-6], and coinfection by other viral pathogens such as human immunodeficiency virus (HIV), which is common among HCV-infected patients [7] and increases the rates of fibrosis progression and ESLD [8,9]. Unfortunately, the determinants of liver fibrosis progression in CHC are largely unknown and the current methods for predicting progression are not sufficiently accurate to identify which patients will progress to fibrosis/cirrhosis [3].

There is increasing evidence that host genetic factors may play an important role in fibrosis progression in CHC [10]. In 2007, a genome-wide association study performed in HCV mono-infected patients showed that the combination of seven single nucleotide polymorphisms (SNPs) (rs62522600, rs4986791, rs886277, rs2290351, rs4290029, rs17740066, rs2878771) in a cirrhosis risk score (CRS) was able to predict progression to advanced fibrosis/cirrhosis [11]. Most of the genes where the seven SNPs are located are known (*AZIN1, TLR4, TRPM5, AP3S2, STXBP5L, and AQP2*), except for rs4290029, which is located in an intergenic region downstream of *DEGS1*. However, to date, only one polymorphism, located in *TLR4 gene,* has been functionally evaluated [12,13]. The genetic signature represented by the seven polymorphisms seems to represent the best available tool for the genetic prediction of liver fibrosis in HCV mono-infected patients so far [14-16]. However, the usefulness of CRS for predicting fibrosis progression in HIV/HCV coinfected patients remains unknown. Moreover, CRS has not been studied together with other factors associated with fibrosis such as HCV genotype, which has showed to affect the fibrosis progression rates in HIV/HCV coinfected patients [17]. Another variable affecting liver fibrosis is the *IL28B* gene, which is similarly known to affect fibrosis progression in both HCV mono-infected [18] and HIV/HCV coinfected patients [19,20].

The most common HCV treatment in HCV/HIV coinfected patients is still a combination of pegylated-interferon alpha plus ribavirin (pegIFNα/RBV) [21]; which has a low rate response, a high cost, and numerous side effects [22-25]. However, the newer directly acting agents (DAAs) for HCV treatment have vastly improved the efficacy over current pegIFNα/RBV therapy, although these new DAAs are still costly[26]. For this reason, it is desirable to identify patients who urgently need HCV treatment , or conversely those who do not need to be treated [27]. Thus, an accurate assessment of the risk of fibrosis development may be helpful in determining, depending on the risk of the patients, the urgent need of HCV treatment or may be helpful to identify those not needing to be treated.

The aim of our study was to assess the ability of CRS to predict hepatic fibrosis progression in HIV/HCV coinfected patients.

METHODS

Patients

We carried out a retrospective study on HIV/HCV coinfected patients that underwent a liver biopsy at Hospital Gregorio Marañón (Madrid, Spain) between September 2000 and November 2008. All patients were of European ancestry.

Liver biopsies were performed on patients who were potential candidates for anti-HCV therapy and had not received previous interferon therapy. Selection criteria for the study were: no clinical evidence of hepatic decompensation, detectable HCV RNA by polymerase chain reaction (PCR), negative hepatitis B surface antigen, CD4+ lymphocyte count higher than 200 cells/μL, stable antiretroviral therapy or no need for antiretroviral therapy. Patients with active opportunistic infections, active drug addiction or unknown date of infection were excluded. Thus, from our cohort of 361 HIV/HCV coinfected patients with liver biopsy data, 205 patients had a DNA sample collected and available CRS data, but only 190 out of 205 patients had an estimate for HCV infection date.

The study was conducted in accordance with the Declaration of Helsinki. All patients gave their written consent for the liver biopsy and genetic testing, and the Institutional Ethics Committee approved the study.

Clinical and laboratory data

On the date of the liver biopsy, the following information was obtained from medical records: age, gender, HIV transmission category, weight, height, alcohol intake (consumption of more than 50 g of alcohol per day for at least 12 months was considered as a high intake), Centers for Disease Control (CDC) clinical category, nadir $CD4^+$ T-cell count, current CD4+ T-cell count, plasma HIV viral load, antiretroviral therapy (if any), HCV genotype, and plasma HCV viral load.

The duration of HCV infection for patients with a history of intravenous drug use (IDU) was estimated starting from the first year they shared needles and other injection paraphernalia, which are the most relevant risk practices for HCV transmission [28]. For non-IDU patients, we only included those patients for which the initiation of their HCV infection could be determined with certainty.

Cirrhosis risk score and IL28B genotyping

Genomic DNA was extracted from peripheral blood by using Qiagen columns (QIAamp DNA Blood Midi/Maxi; Qiagen, Hilden, Germany). Genotyping was performed by the Spanish National Genotyping Center (CeGen; http://www.cegen.org/) using GoldenGate® assay with VeraCode® Technology (Illumina Inc. San Diego, CA, USA).

The CRS signature was performed by genotyping seven SNPs [11,15]: rs62522600 (*AZIN1*), rs4986791 (*TLR4*), rs886277 (*TRPM5*), rs2290351 (*AP3S2*), rs4290029 (downstream of *DEGS1*), rs17740066 (*STXBP5L*), and rs2878771 (*AQP2*). From these SNPs, we calculated the CRS values using a Naïve Bayes formula previously described [11]. The CRS value varied from 0 to 1 with a higher CRS associated with a greater risk of developing fibrosis. Two categorical cut-off points for different levels of risk have also been described in CHC patients: low risk (<0.50) versus high risk (>0.70) [11].

The rs12980275 polymorphism near *IL28B* was genotyped in a previous study [20].

Liver fibrosis evaluation

Liver biopsies were performed as previously described [29], and liver fibrosis was estimated according to Metavir score 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.

Clinical outcome

HIV/HCV coinfected patients were classified into two groups according to fibrosis stage developed after a minimum follow-up time of 10 years with HCV infection: i) non-progressors: patients with F0 from the liver biopsy; and ii) progressors: patients with F1 to F4.

Statistical methods

The statistical analysis was performed by Statistical Package for the Social Sciences (SPSS) 15.0 (SPSS INC, Chicago, IL, USA). Categorical data and proportions were analyzed by using the chi-squared test or Fisher's exact test. Mann-Whitney U test was used to compare data among independent groups. All p-values were two-tailed. Statistical significance was defined as $p<0.05$.

We performed both univariate and multivariate logistic regression analyses to investigate the association among CRS values and fibrosis stage. In each multiple logistic regression analysis, we included CRS ("Enter" algorithm) and the most significant covariables selected by the "Stepwise" algorithm. The covariables analyzed by the "Stepwise" algorithm were CHC clinical factors (age at HCV infection, gender, alcohol intake, IDU, HCV genotype, and *IL28B* genotype) and HIV clinical factors (nadir CD4+, AIDS, time on cART). Thus, each logistic regression was always adjusted for significant covariates associated with the outcome variable.

Later, we analyzed the diagnostic performance of CRS for predicting fibrosis progression. We also analyzed whether the predictive accuracy could be improved by accounting for the most important clinical factors that can be determined in the first contact between clinician and patient (age at HCV infection, gender, IDU, HCV genotype, and *IL28B* genotype (rs12980275)). Thereafter, several indexes were built in order to express the likelihood of developing fibrosis as a probability ranging from 0 to 1, through a logistic probability function [30]. The area under the receiver operating characteristic curve (AUROC) was obtained to evaluate the predictive accuracy. Criteria for levels of accuracy were as follows: 0.90–1 = excellent, 0.80–0.90 =good, 0.70–0.80 =fair and 0.60–0.70 = poor. The diagnostic performance of CRS was evaluated according to the two cut-offs previously described by Huang et al. in order to identify patients with low risk (CRS<0.50) and high risk (CRS>0.70) of developing fibrosis [11]. The sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and percentage of patients correctly classified (accuracy) were calculated for each cut-off point.

RESULTS

Patient characteristics

The clinical characteristics of the 190 HIV/HCV coinfected patients are shown in **Table 1**. There were 25 non-progressor patients with a median time of HCV infection of 25 years approximately [percentile 25th (P25th); percentile 75th (P75th): 17.1; 27.5)], and 165 progressor patients with a median time of HCV infection of 21.3 years (P25th; P75th: 17.3; 24). A history of injection drug use was significantly less frequent among non-progressors than among progressors. Of note, non-progressors had a longer duration of HCV infection than progressors, suggesting a high stability in the non-progression of liver fibrosis. The median time on cART was also significantly longer in non-progressor than in progressors.

Table 1. Main clinical characteristics of 190 HIV/HCV coinfected patients involved in the study.

	Non-progressors (F=0)	**Progressors (F≥1)**
No.	25	165
Epidemiological history		
Gender (male)	16 (64%)	126 (76.4%)
Age at biopsy (years)	43.2 (38.5; 46.6)	39.6 (37.7; 43.8)
Injection drug use	19 (76.0%)	159 (96.4%) (*)
Age at HCV infection (years)	20 (18.1; 20.5)	19.1 (16.6; 22.1)
Years since HCV infection	25.0 (17.1; 27.5)	21.3 (17.3; 24.0)
High alcohol intake	12 (48.0%)	97 (58.7%)
Antiretroviral therapy		
cART	22 (88%)	137 (83.0%)
Time on cART (years)	8.0 (6.7; 9.2)	4.3 (2.5; 6.1) (*)
HIV markers		
CDC category C	4 (16.0%)	48 (29.1%)
Nadir CD4+ T-cells/uL	153 (111; 281)	185 (77; 320)
Nadir CD4+ <200 cells/uL	14 (56.0%)	85 (51.5%)
HCV genotypes (no.=186)		
1	14 (58.3%)	93 (57.4%)
2	–	4 (2.5%)
3	3 (12.3%)	38 (23.5%)
4	7 (29.2%)	27 (16.7%)
Metavir fibrosis stage		
No fibrosis (F0)	25 (100%)	–
Portal fibrosis (F1)	–	71 (43%)
Periportal fibrosis (F2)	–	52 (31.5%)
Advanced fibrosis (F3)	–	24 (14.5%)
Cirrhosis (F4)	–	18 (10.9%)
CRS	0.61 (0.41 – 0.62)	0.67 (0.43 – 0.77) (*)
<0.5	11 (44%)	44 (26.7%)
0.5 – 0.7	10 (40%)	41 (24.8%)
>0.7	4 (16%)	80 (48.5%) (*)
rs12980275 *IL28B*		
AG/GG	11 (44%)	82 (49.7%)
AA	14 (56%)	83 (50.4%)

Values were expressed as absolute count (percentage) and median (percentile 25; percentile 75).
(*), Significant differences between Non-progressors versus Progressors group (p<0.05).

Abbreviations: HCV, Hepatitis C virus; HIV, Human immunodeficiency virus; CDC, Centers for Disease Control and Prevention; CRS, cirrhosis risk score (<0.5, low-risk; 0.5 – 0.7, intermediate; >0.7, high-risk).

CRS and fibrosis progression

CRS values were significantly lower in non-progressors [median 0.61 (P25th; P75th: 0.41; 0.62)] than in progressors [median 0.67 (P25th; P75th: 0.43; 0.77)]; *P*=0.043. However, among progressors, we observed similar CRS values for all fibrosis stages (F1/F2/F3/F4) (**Fig. 1A**). Understandably, the proportion of subjects with CRS>0.70 was higher among progressors than among non-progressors. However, the proportion of patients with CRS values between 0.50-0.70 (intermediate risk) and <0.50 (low risk) was similar for each of the fibrosis stages (p=0.047) (**Fig. 1B**). Moreover, a higher proportion of CRS values above 0.70 was also observed among F≥1 patients who were female, had an age at HCV infection above 18 years, HCV genotype 1/4, or rs12980275 AA genotype (**Fig. 2**).

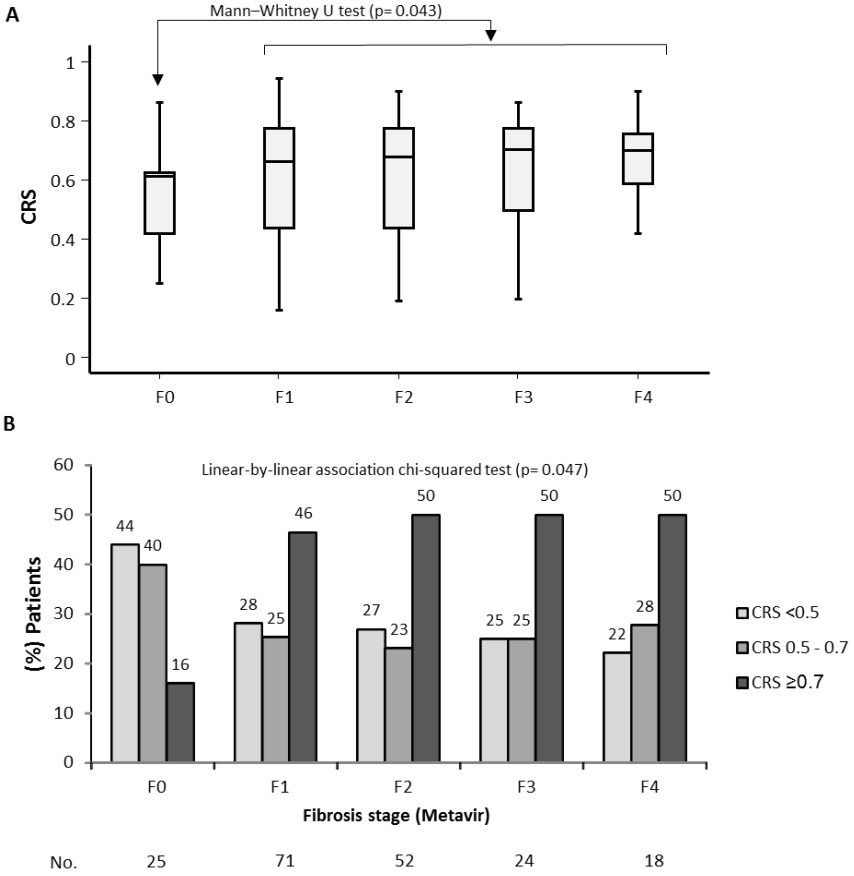


Figure 1. Cirrhosis risk score (CRS) values stratified by fibrosis stage. A) Median (25th percentile; 75th percentile); B) Percentage of HIV/HCV coinfected patients for each CRS cut-off.

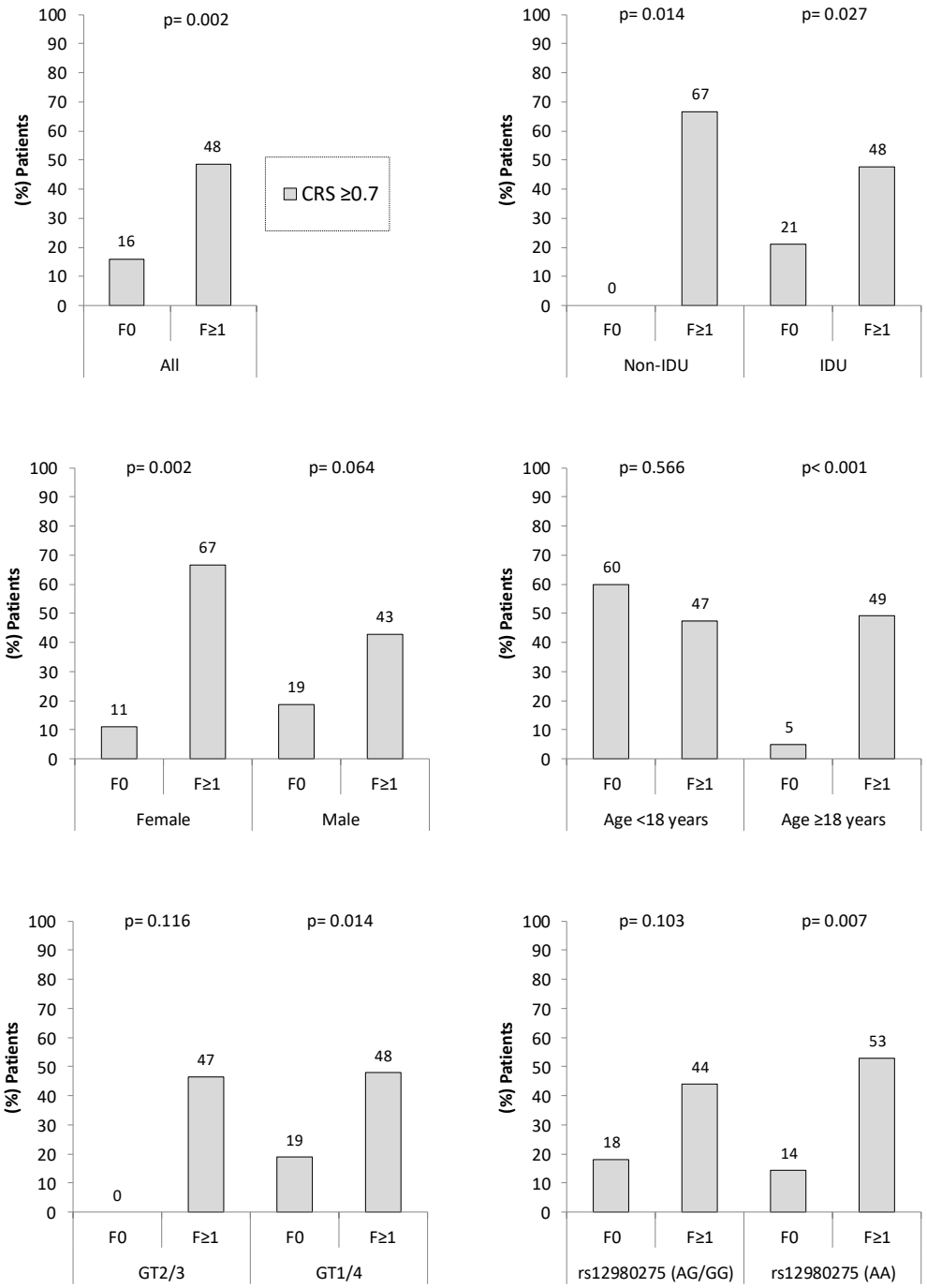


Figure 2. Percentage of patients with CRS>0.70 (high risk of developing liver fibrosis) according to different clinical variables. Statistical significance was calculated using the Chi-square test.

Overall, logistic regression analyses showed that CRS was significantly associated with liver fibrosis progression (**Table 2**). For all patients, a value of CRS>0.70 corresponded with a higher likelihood of fibrosis progression (adjusted OR (aOR)=9.20; p=0.002). Likewise, for every ten points of CRS value, a higher likelihood of developing fibrosis was detected (aOR=1.46; p=0.008) (**Table 2**). Logistic regression analysis also showed a strong association of CRS values and CRS>0.70 with liver fibrosis progression among females, patients who acquired HCV infection after turning 18, rs12980275 AA genotype, HCV genotype 1/4, and IDU (**Table 2**).

Table 2. Summary of odds ratio (OR) for liver fibrosis progression (F0 versus F≥1) among HIV/HCV coinfected patients according to CRS.

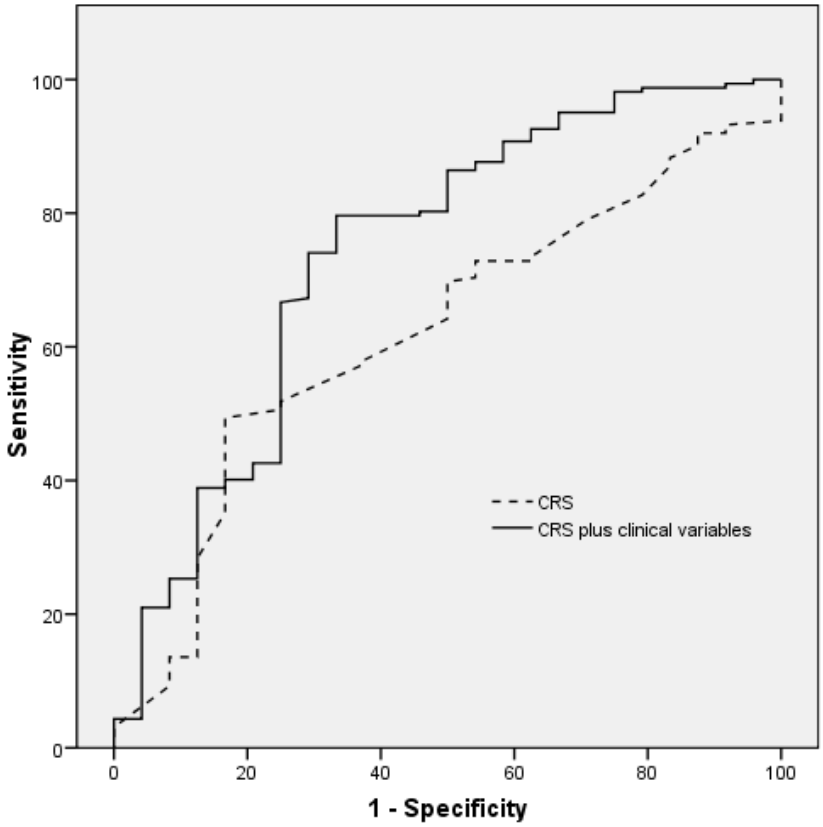
		Unadjusted		Adjusted (**)	
		OR (95%CI)	**p**	**OR (95%CI)**	**p**
All patients	CRS (x10) **(*)**	1.21 (0.98 – 1.49)	0.070	1.46 (1.10 – 1.94)	**0.008**
	>0.7	4.04 (1.62 – 15.02)	**0.005**	9.20 (2.33 – 36.35))	**0.002**
Gender	**Male (n=142)**				
	CRS (x10) **(*)**	1.12 (0.87 – 1.44)	0.368	1.32 (0.95 – 1.84)	0.099
	>0.7	3.25 (0.88 – 11.97)	0.076	4.41 (0.97 – 20.12)	0.055
	Female (n=48)				
	CRS (x10) **(*)**	1.65 (1.07 – 2.54)	**0.023**	2.29 (1.19 – 4.15)	**0.012**
	>0.7	16.0 (1.80 – 141.9)	**0.013**	19.86 (1.89 – 208.7)	**0.013**
Age at HCV infection	**<18 years (n=62)**				
	CRS (x10) **(*)**	0.81 (0.46 – 1.41)	0.454	0.79 (0.44 – 1.42)	0.439
	>0.7	0.60 (0.09 – 3.86)	0.591	0.51 (0.07 – 3.64)	0.499
	≥18 years(n=128)				
	CRS (x10) **(*)**	1.32 (1.04 – 1.69)	**0.021**	2.33 (1.37 – 3.96)	**0.002**
	>0.7	18.30 (2.36 – 141.6)	**0.005**	36.84 (3.62 – 375.3)	**0.002**
rs12980275 *IL28B*	**AG/GG (n=93)**				
	CRS (x10) **(*)**	1.12 (0.83 – 1.51)	0.463	1.20 (0.87 – 1.65)	0.263
	>0.7	3.52 (0.71 – 17.32)	0.121	4.87 (0.86 – 27.38)	0.072
	AA (n=97)				
	CRS (x10) **(*)**	1.33 (0.98 – 1.79)	0.060	1.59 (0.84 – 3.04)	0.154
	>0.7	6.77 (1.42 – 32.14)	**0.016**	10.69 (1.27 – 89.75)	**0.029**
HCV genotype	**GT1/4 (n=140)**				
	CRS (x10) **(*)**	1.21 (0.95 – 1.54)	0.123	1.43 (1.05 – 1.96)	**0.022**
	>0.7	3.91 (1.24 – 12.30)	**0.020**	7.52 (1.95 – 29.07)	**0.003**
	GT2/3 (n=46)				
	CRS (x10) **(*)**	1.35 (0.81 – 2.26)	0.247	6.51 (0.30 – 139.4)	0.231
	>0.7	NA	-	NA	-
IDU	**Non-IDU (n=12)**				
	CRS (x10) **(*)**	2.45 (0.81 – 7.43)	0.113	NA	-
	>0.7	NA	-	NA	-
	IDU (n=178)				
	CRS (x10) **(*)**	1.20 (0.95 – 1.51)	0.115	1.40 (1.05 – 1.87)	**0.022**
	>0.7	3.43 (1.09 – 10.80)	**0.035**	4.99 (1.37 – 18.08)	**0.014**

Abbreviations: HCV, hepatitis C virus; GT, HCV genotype; IDU, intravenous drug use, CRS, cirrhosis risk score (x10, for every tenth of CRS value; >0.7, high-risk; OR, odds ratio; 95% CI, 95% of confidence interval, p, significant value.
(*), indicates an increase of 0.10 of the CRS score.
(**), this test was adjusted by the most relevant covariables, as appropriate, such as CHC clinical factors (age at HCV infection, gender, alcohol intake, IDU, HCV genotype, and *IL28B* genotype) and HIV clinical factors (nadir CD4+, AIDS, time on cART).

Predictive performance of CRS

The AUROC of CRS for discriminating between non-progressors and progressors was low but significant (0.625; p=0.043) (**Fig. 3**). In this setting, the predictive performance of CRS>0.70 had values of 48.5 Se (95%CI: 41 - 56.1), 84 Sp (95%CI: 65.3 - 93.6), 95.2 PPV (95%CI: 88.4 - 98.1), 19.8

NPV (95%CI: 13.3 - 28.4), and 53.2 accuracy (95%CI: 46.1 - 60.1) for identifying patients with risk of fibrosis progression.



	AUROC (95% CI)	p-value
CRS	0.625 (0.517 – 0.734)	0.043
CRS plus clinical variables	0.739 (0.621 – 0.857)	<0.001

Figure 3. The area under the receiver operating characteristic curve (AUROC) for the predictive value of CRS alone, and in combination with clinical variables.

In order to improve the predictive value of CRS, we analyzed the CRS in combination with clinical factors,

$$\Pr[(F \geq 1)] = 1/[1+e]^{(-(-1.621 + 3.069 * (CRS) + 1.978 * (IDU) - 1.274 * (GT1/4) + 0.616 * (Gender\ (male)) + 0.048 * (Age\ at\ HCV\ infection) - 0.675 * (rs12980275\ AA)))}$$

This worked clearly for improving it with an AUROC of 0.739 (p<0.001) (**Fig. 3**). The predictive performance of CRS above 0.70 in combination with clinical variables had values of 95.1 Se (95%CI: 90.6 - 97.5), 29.2 Sp (95%CI: 14.9 - 49.2), 90.1 PPV (95%CI: 84.7 - 93.7), 46.7 NPV (95%CI: 24.8 - 69.9), and 86.6 accuracy (95%CI: 80.9 - 90.7) for identifying patients with risk of fibrosis progression.

DISCUSSION

We showed for the first time that there was a relationship between CRS and liver fibrosis progression in HIV/HCV coinfected patients. CRS helped to discriminate between non-progressor (F0) and progressor patients (F1/F2/F3/F4), something of interest for therapeutic decision-making in clinical practice. Moreover, using CRS together with clinical factors improved the performance for discriminating between non-progressors and progressors, and its cut-off >0.70 had an acceptable predictive performance for discriminating between non-progressors and progressors. However, CRS had low performance for predicting advanced fibrosis/cirrhosis (data not shown), similarly as in the report of Huang et al. [11]

The association between CRS and fibrosis progression was first established in a cross-sectional study comparing a control group of HCV mono-infected patients without fibrosis (F0) with a case group with fibrosis/cirrhosis (F3/F4) [11]. Since then, three studies have validated the predictive value of CRS in patients infected with HCV [14-16], and another in recipients of liver transplantation for HCV infection [31]. Conversely, some contradictory results have been identified such as the study of Grünhage *et al* [32], where no association between any of the seven SNPs and inflammation of fibrosis was found in a regression model. However, the role of CRS in predicting liver disease in HCV patients with HIV coinfection, which clearly accelerates fibrosis progression and development of ESLD [33], has not been explored so far. Our study found a weak association of CRS with the risk of developing liver fibrosis in HIV/HCV coinfected patients; and the CRS score had an AUROC for predicting fibrosis progression of only 0.625, which is considered poor. Therefore, CRS score seems to be less useful in HIV/HCV co-infected patients than in HCV mono-infected patients. Due to the low accuracy, it is unlikely that CRS may be helpful in aiding clinical decision making about liver fibrosis development. However, the combination of CRS with clinical factors clearly improved the performance for discriminating between progressor and non-progressor patients. Many environmental cofactors and common comorbidities are known to affect the course of CHC in HIV/HCV coinfected patients [8,9], and these effects may hinder any underlying genetic predisposition affecting disease progression.

CHC is a slow disease in most cases and takes a long time to progress to advanced fibrosis [3,8]. Subgroups of patients from non-progressors to rapid progressors have been clearly described in the literature [34,35]. CHC progression, like many other complex diseases, probably involves a large number of genes, which makes difficult to define the relative contribution of each one. However, the importance of the CRS score lies in the combination of the effect of seven polymorphisms, which have been proved to be associated with liver fibrosis progression [11]. This genetic signature is made from the contribution of each single gene into a single score, which allow us to infer the probability of liver fibrosis development for each patient. Hence, the CRS remains invariable throughout the duration of HCV infection, and its determination would be necessary only once in a lifetime, unlike other non-invasive markers of liver fibrosis such as aspartate aminotransferase/platelet ratio (APRI) [36] or FIB4 score [37]. These widely used fibrosis tests are mainly based on evaluation of serum biomarkers, which might fluctuate during concurrent illnesses or disease stage, and they identify patients with significant fibrosis only at a particular moment in time. However, genetic markers are robust and invariable between different clinical settings.

Our results suggest that CRS was associated with liver fibrosis progression in patients who were female, acquired HCV infection after turning 18, had HCV genotype 1/4, or carry the *IL28B* genotype shown to be favorable for HCV therapy response (rs12980275 AA).

A link between the favorable *IL28B* genotype and increased odds of liver disease severity has been reported for both HCV mono-infected patients [38,39] and for HIV/HCV coinfected patients [19,20]. The *IL28B* gene encodes IFN-λ3, a type III interferon cytokine with antiviral activity against HCV in the liver, via an innate immunity pathway and involving expression of inflammatory cytokines [40,41]. In addition, *IL28B* is able to modulate adaptive immune responses, promote the Th1 immune pathway, increase T regulatory cells and increase CD8 T-cell cytotoxicity and memory responses [40]. Thus, it is reasonable to assume that *IL28B* could also have an influence on liver fibrosis progression in CHC. However, this controversial association and the possible mechanisms involved are still unknown.

With respect to HCV genotype, which does not appear to exert any influence on the progression of liver disease, however, some reports have identified an increased rate of fibrosis progression in both HCV mono-infected patients and HIV/HCV coinfected patients infected with HCV genotype 3 [17,42,43]. In our study, we observed that CRS is able to predict fibrosis progression in patients with HCV genotype 1/4. However, no significant results were obtained for HCV genotype 2/3, which may be due to the reduced number of HCV genotype 2/3 patients that do not have liver fibrosis (data not shown).

Another factor that is associated with a higher likelihood of progression to fibrosis is age at the time of HCV infection. The chronicity rate in HCV infection appears to be lower in younger individuals, which has been widely reported in follow-up studies with children with post-transfusion or vertical transmission and patients younger than 20 [44]. Our results indicate a high predictive power of CRS in patients who were older than 18 when they acquired HCV, but not for those who were younger.

Similarly, fibrosis progression seems to be lower in women, particularly in younger women [44]. Upon examining these variables, CRS seems to better predict fibrosis progression in females in our cohort. Therefore, in addition to a lower fibrosis progression in females, those that do finally develop a fibrosis stage are more likely genetically predisposed to it. Regarding males, CRS showed a reduced predictive value, which is probably due to confounding variables for different epidemiological history such as a higher alcohol intake and a greater likelihood of being IDUs than females.

The AUROC for predicting the risk of fibrosis progression reported by Huang et al. [11] was 0.75, a value high enough to conclude that the CRS is a useful tool for identifying CHC patients with HCV mono-infection that are at high risk for developing fibrosis. In our study, the performance of the CRS alone was low indicating that the genetic signature alone is not sufficient for predicting liver fibrosis among HIV/HCV coinfected individuals, perhaps because HIV infection markedly influences fibrosis progression in CHC. However, the combination of the CRS score with readily available clinical parameters (age at HCV infection, IDU, gender, *IL28B* genotype, and HCV genotype) improved the diagnostic ability of CRS to identify likely progressors (AUROC 0.73). Thus, those patients with a cutoff value above 0.70 have significant odds of developing liver fibrosis. However, CRS was unable to correctly classify HCV/HIV coinfected patients with values less than 0.7. Then, the logistic function with CRS plus clinical factors may be of help for decision-making in clinical management of HIV/HCV coinfected patients, by identifying those patients who do not need to be treated. Unfortunately, CRS plus clinical factors are unable to distinguish between who will develop advanced fibrosis or cirrhosis (F3/F4) from those who will have low or mild fibrosis (F0/F1)."

Some clarifications need to be made in order to properly interpret our results. First, this is a cross-sectional study and the strategy for monitoring of our cohort was not designed to develop a model for predicting different stages of fibrosis progression. Ideally, the design would be longitudinal with serial biopsies from all patients, but only a small percentage of patients had repeated biopsies prior to any HCV therapy, and based on current practice the time interval between the two biopsies is generally short (3-5 years). Second, the patients selected for our study met a set of criteria for starting HCV treatment (eg, low alcohol abuse, high CD4 cell counts, controlled HIV replication, and good treatment adherence), and it is possible that this may have introduced some selection bias. In addition, since the IDU patients may well be more likely to die from IDU-related causes, the non-progressors may be enriched for those not injecting drugs; and, therefore, have a longer follow up.

In conclusion, the CRS itself seems not to be a good marker for identifying HIV/HCV coinfected patients who are at high risk of developing liver fibrosis. However CRS score coupled with clinical factors (age at HCV infection, IDU, gender, IL28B and HCV genotype) might help to distinguish between non-progressors and progressors patients.

ACKNOWLEDGEMENTS

The authors wish to thank the Spanish National Genotyping Center (CeGen) for providing the genotyping services (http://www.cegen.org). We also acknowledge the patients in this study for their participation.

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