ANNALS OF HEPATOLOGY

Volume **4** *Number* **2** *April-June* **2005**

Artículo:

Prediction of the hepatitis C viremia using immunoassay data and clinical expertise

Annals of Hepatology 2005; 4(2): April-June: 107-114

Annals of Hepatology

Original Article

Prediction of the hepatitis C viremia using immunoassay data and clinical expertise

Erwin Chiquete;[1] Laura V. Sánchez;[1] Montserrat Maldonado;[1] Daniel Quezada;[1] Arturo Panduro[1]

Abstract

Detection of anti-hepatitis C virus (anti-HCV) antibodies may yield a high frequency of false-positive results in people at low risk. To date, no clinical rule had been developed to predict viremia in HCV-seropositive patients. Therefore, we aimed to generate a prediction rule on the basis of clinical and serologic data, which can be used in outpatient care. We selected 114 seropositive patients without antiviral treatment or hepatitis B coinfection. Subsequently we identified independent predictors of the hepatitis C viremia by logistic regression and selected the quantitative value of the screening test for anti-HCV antibodies with the best performance in detecting viremia. Then, we combined clinical and serologic data to generate different prediction rules. Ratio of immunoassay signal strength of the sample to cut-off (S/CO) >15 had accuracy, positive predictive value (PPV) and positive likelihood ratio (LR+) of 84%, 83%, and 3.7; respectively. The rule compounded of the antecedent of blood transfusion before 1993 and S/CO >15 performed the best in prediction of viremia in all patients, with accuracy, PPV and LR+ of 71%, 88%, and 5.6; respectively. In the group of asymptomatic patients this rule improved in efficacy of prediction, with accuracy, PPV and LR+ of 79%, 91% and 12.8; respectively. In conclusion, a clinical rule is better than S/CO alone in prediction of the hepatitis C viremia. In a patient that meet the rule the probability of having viremia is high, therefore, it can be indicated directly an assay for viral load instead of other supplemental tests, thus, saving time and economic resources.

[1] Department of Molecular Biology in Medicine, Hospital Civil de Guadalajara "Fray Antonio Alcalde" México.

Address for correspondence:
Arturo Panduro.
Servicio de Biología Molecular en Medicina, Hospital Civil de Guadalajara "Fray Antonio Alcalde", Hospital 278, Guadalajara Jalisco Mexico, Postal Code 44280. Phone: 52-3614-7743, Fax: 52-3614-7743, E-mail: apanduro@prodigy.net.mx.

Abbreviations: HCV: hepatitis C virus; NAT: nucleic acid amplification testing; CDC: The Centers for Disease Control and Prevention; RIBA: recombinant immunoblot assay; PCR: polymerase chain reaction; S/CO: immunoassay signal strength of the sample to cut-off; OR: odds ratio; CI: confidence intervals; PPV: positive predictive value; NPV: negative predictive value, LR+: positive likelihood ratio; LR-: negative likelihood ratio.

Manuscript received and accepted: 11 and 26 March, 2005.

Key words: Decision making, hepatitis C, liver, practice guideline, serology, test.

According to World Health Organization estimations, approximately 3% of the world population may be infected with the hepatitis C virus (HCV).[1] HCV is transmitted primarily by exposure to infected blood. Major risk factors associated are illicit intravenous drug abuse and transfusion of blood products before the establishment of blood bank screening for HCV, remaining a number of cases without an identifiable risk factor.[1-3] However, prevalence of HCV infection and associated risk factors vary by geographic region.[1] These possible differences in personal antecedents, along with the wide clinical spectrum that can be seen in HCV-infected patients have contributed to make the diagnosis of this infection mainly on the basis of laboratory tests. However, most assays often behave differently among different subsets of patients, generating a high probability to identify a positive case in later stages of florid disease and a low chance in early, mild stages.[4,5] Hence, to analyze a patient from the result of a laboratory test only, may imply leaving out important clinical data, as current tests do not distinguish the full clinical spectrum of patients with HCV infection.

The diagnosis of this infection basically relies in two approaches: detection of anti-HCV antibodies and of HCV RNA (i.e., test for viremia) with different nucleic acid amplification testing (NAT) techniques.[5,6] Evolution of screening assays for detection of anti-HCV antibodies has resulted in generation of much more sensitive tests. However, they may yield a considerably high frequency of false-positive results in people at low risk[5,6] or in other non-related diseases as malaria and autoimmune hepatitis.[7,8] The Centers for Disease Control and Prevention (CDC) has urged that a person with reactivity to a screening test for anti-HCV antibodies should have a more specific serologic test (e.g., recombinant immunoblot assay [RIBA]) or a NAT (either qualitative or quantitative), to be considered infected with HCV.[5] However, after being confirmed as infected a patient needs a quantitative NAT to assess the viral load for treatment planning and further monitoring.[5,6,9] All these tests that must be practiced to initiate a health counseling and management have an important economic impact. To address this issue, CDC has also recommended using the quantitative value of the screening tests for anti-HCV antibodies to evaluate the amount of supplemental testing that needs to be per-

formed while improving the reliability of reported test results.[5] Rational use of diagnostic tests leads to optimal patient care and saves unnecessary expenses and concerns. However, as testing for HCV infection has become increasingly complex, some health care professionals could lack complete knowledge about the interpretation of the screening test results and regarding which supplemental tests are needed.[5]

To add knowledge on the diagnosis of HCV infection and on the estimation of the patients that should be tested directly for viral load after a positive screening test, we aimed to generate a prediction rule on the basis of clinical and serologic data, which can be used in outpatient care, with a simple calculation.

Patients and methods

This cross-sectional, descriptive study was performed from March 2003 through May 2004 at the Department of Molecular Biology in Medicine of the Hospital Civil de Guadalajara, which is an academic reference facility for molecular research on viral hepatitis of both rural and urban population from the West of Mexico.

Design

We first selected ambulatory seropositive patients with or without overt liver decompensation, excluding those with factors that may affect the presence of HCV RNA or anti-HCV antibodies in serum (i.e., with past or current anti-HCV treatment, or with hepatitis B or human immunodeficiency virus coinfection). Subsequently, we identified independent predictors of the hepatitis C viremia and selected the quantitative value of the screening test for anti-HCV antibodies with the best characteristics in distinguishing patients who do have HCV-infection. Then, we combined clinical and serologic data to generate several rules for prediction of the presence of HCV RNA in serum.

Study population

A primary group of 171 consecutive screening-test-reactive patients was eligible for the study. Ambulatory patients were referred from blood bank and infectology, gastroenterology, general internal medicine, pediatrics and hematology departments, to confirm HCV infection. We considered the next inclusion criteria: (1) patients reactive to a screening test for anti-HCV antibodies in at least two occasions and seronegative to anti human immunodeficiency virus antibodies in assays performed at least 6 months prior to arrival to our service; and (2) outpatients with or without clinical manifestations of liver decompensation. Patients were excluded if a new anti-HCV assay performed in our service tested negative or if co-infection with the hepatitis B virus was identified by a home-made qualitative nested polymerase chain reaction (PCR), as described elsewhere;[10] which was practiced per duplicate in our service systematically in all cases. Thus, after applying selection criteria a final group of 114 patients was analyzed.

A standardized structured questionnaire was used to collect data from the patient regarding demography, relevant antecedents and risk factors. The study population was first divided in two groups. Patients without clinical evidence of liver failure composed the asymptomatic group. Patients with clinically overt liver decompensation composed the decompensated liver disease group. Overt liver decompensation was specifically considered to occur in a patient if jaundice, ascitis or collateral superficial veins were present. Also, antecedent of a diagnosis or previous hospitalization for liver decompensation (e.g., upper gastrointestinal bleeding, hepatic encephalopathy, and other complications) ≥ 1 month prior to the arrival to our service were considered as being part of the liver failure syndrome.

Detection of anti-HCV antibodies

An automated third generation microparticle enzyme immunoassay (MEIA, IMx HCV Version 3.0 Abbott Diagnostics, Chicago, IL, USA) was used to assess the presence of anti-HCV antibodies in sera samples stored at -70ºC. Immunoassay signal strength (fluorescence) of the sample to cut-off rate (S/CO) is the numeric value of the test. This is a semi-quantitative assay in which S/CO ratio is directly proportional to anti-HCV antibodies levels.[11] S/CO ratio >1 is considered as positive, according to directions of the manufacturer.

Detection of the HCV RNA in serum

A home-made qualitative nested reverse-transcription polymerase chain reaction (RT-PCR) was used to detect HCV RNA in all sera samples, first stored at -70ºC. Total RNA was extracted from each serum without pooling, using QIAamp Viral RNA Mini Kit (QIAGEN, Chatsworth, CA) as indicated the manufacturer. Then, RT was carried out to obtain complementary DNA (cDNA) using M-MLV RT kit (MMLV, GIBCO/BRL). PCR amplification of cDNA and later a nested-PCR were performed with two pairs of primers that hybridize in a segment of the 5' non-coding region of the HCV genome, as is described elsewhere.[12] Nested RT-PCR was performed by duplicate in all samples for confirmation and the products of the reaction were analyzed in gel electrophoresis. If the second assay resulted different from the first, then a third nested RT-PCR round was performed and two matching results out of three were required for a positive or negative adjudication. Qualitative nested RT-PCR is considered here as the gold standard for diagnosis of viremia. Absence of viremia does not rule out HCV infection,[13-16] nonetheless

the presence of HCV RNA in serum is considered here as the confirmation of cases, since viremia is the main feature considered regarding decision to offer antiviral treatment.[5,6] Confirmed cases were referred to an external party to undergo liver biopsy, if the patient accepted this procedure. Some patients had liver biopsy prior to presentation to our service.

The internal Committee of Ethics of our hospital approved the present study. No informed consent was required, except for the liver biopsy.

Statistical analysis

Demographic data are reported as simple frequencies. Age and S/CO ratios are presented and analyzed as medians with minimum and maximum, as these variables followed a non-normal distribution. Fisher exact test was used for nominal variables in univariate analyses. Student *t* test was used to compare continuous, normally distributed variables. Mann-Whitney *U* test was performed when an ordinal or scale non-parametric variable was distributed between two groups. Spearman rho was used to test ranked correlations. To find independent predictors for the presence of HCV RNA in serum, a multivariate analysis was constructed by forward stepwise logistic regression. Adjusted odds ratios (OR) with 95% confidence intervals (CI) are provided, and as the frequency of the hepatitis C viremia was >10%, corrected OR were calculated for categorical predictors to gain precision in the estimation of the actual relative risk, in a sample with a high frequency of an statistical outcome as follows:[17] Corrected OR = Multivariate OR / [(1 – Incidence of the outcome in the nonexposed group) + (Incidence of the outcome in the nonexposed group X multivariate OR)]. Thus, corrected OR are taken as the approximation of the true relative risk obtained from logistic regression analysis. The fitness of the model was evaluated by using the Hosmer-Lemeshow goodness-of-fit test, which was considered as reliable if p was > 0.2; and results are provided in the respective table.

After identification of independent predictors of viremia, the breaking point of S/CO ratio with the best performance in detecting cases with viremia was selected. To analyze the diagnostic dynamics of different S/CO ratio breaking points, the cumulative proportion of cases with viremia was plotted against S/CO ratio values. Afterwards, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+) and negative likelihood ratio (LR-) were calculated. Since S/CO ratio breaking points are categorical variables, the area under the receiver operator characteristic curve was not estimated; instead, accuracy was calculated as follows: Accuracy = (True positives + True negatives) / Total number of persons. For all parameters of the diagnostic appraisal, 95% CI are provided. For the clinical prediction rules to be created, S/CO ratio breaking point with the largest PPV and LR+ was selected to be combined with the clinical data that independently predicted viremia in logistic regression. Performance of the clinical prediction rules was also appraised calculating sensitivity, specificity, accuracy, PPV, NPV, LR+ and LR-. Since of primary interest in clinical practice is to know the probability that a patient with a particular test result actually has the target disorder, we selected predictors on the basis of their performance in detecting cases with viremia (e.g., PPV and LR+) and not on the basis of the probability that a patient with viremia has a particular test result (i.e., sensitivity).

Statistical comparisons or interactions with $p < 0.05$ were regarded as significant. All p values reported are two-sided. SPSS v12.0 statistical package was used for comparisons and interactions. Appraisal of test results and clinical rules was done using EBMcal for Palm OS v1.1a.

Results

Patients

We analyzed 114 patients (*Table I*). There were 69 (60.5%) women and 45 (39.5%) men, with median age of 52 (range 11 to 82) and 40 (range 10 to 73) years, respectively ($p = 0.007$). Possibly due to referral behavior, the population studied was compounded of a large participation of the female gender, with a female-to-male ratio of 1.5. Thirty-four (29.8%) patients composed the group with decompensated liver disease and 80 (70.2%) patients the asymptomatic group. Age of the patients with decompensated liver disease was higher than of the asymptomatic patients with a greater proportion of patients aged ≥50 years (70.6% *vs* 30%, respectively; $p < 0.001$). However, mean values of liver enzymes and major demographic features were similar between both groups (*Table I*).

Hepatitis C viremia and liver disease

Overall, there were 65 (57%) patients with hepatitis C viremia. The group with decompensated liver disease had 28 (82%) of 34 patients with viremia, compared with 37 (46%) of 80 in the group of asymptomatic patients ($p < 0.001$) (*Table II*). Of the patients with viremia, 26 (40%) subsequently underwent liver biopsy, all of them with some degree of fibrosis (including cirrhosis) in their histologic assessment. However, no correlation was found between the degree of liver damage and S/CO ratio or the number of patients with viremia. Noteworthy, six RT-PCR negative patients had results of biopsy in their note of referral; in four of them steatosis or mild fibrosis were reported and the other two were completely normal.

Factors related with the presence of hepatitis C viremia

Transfusion of blood products before 1993 and any past surgical procedure were the risk factors related with

Table I. Characteristics and risk factors of the 114 patients analyzed.

Variable	Overall	Group		p value*
		Decompensated liver disease	Asymptomatic	
Gender				
Male, n (%)	45 (39.5)	9 (26.5)	36 (45)	0.06
Female, n (%)	69 (60.5)	25 (73.5)	44 (55)	0.06
Age in years				
Median (range)	46.5 (10-82)	55.5 (21-71)	42 (10-82)	< 0.001
School education				
More than six years, n (%)	58 (50.9)	17 (50)	41 (51.2)	0.90
Liver enzymes				
ALT, mean (SD)	48.8 (49.6)	52.7 (22.2)	47.7 (43.3)	0.74
AST, mean (SD)	43.3 (31.5)	58.2 (37.2)	39.4 (29.1)	0.11
GGT, mean (SD)	54.3 (35.7)	79.5 (48.8)	48 (32.7)	0.29
Risk factors for the HCV infection				
Blood transfusion, n (%)†	57 (50)	20 (58.8)	37 (46.3)	0.21
Surgical procedures, n (%)‡	81 (71.1)	28 (82.4)	53 (66.3)	0.08
Hemodialysis, n (%)	3 (2.6)	2 (5.9)	1 (1.3)	0.16
Infected sex partner, n (%)	2 (1.8)	1 (2.9)	1 (1.3)	0.52
More than ten sex partners, n (%)§	11 (9.6)	2 (5.9)	9 (11.3)	0.37
Contact with prostitutes, n (%)	16 (14)	3 (8.8)	13 (16.3)	0.29
Use of any illicit drug, n (%)	11 (9.6)	3 (8.8)	8 (10)	0.84

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase.
* p value for the decompensated liver disease group versus the asymptomatic group. Fisher exact test was used for nominal variables, Student *t* test for parametrical continuous variables (liver enzymes) and Mann-Whitney *U* test for non-parametrical continuous variables (age).
† Transfusion of blood products before 1993.
‡ Any surgical procedure before the first screening test for HCV infection resulted positive.
§ Antecedent of more than ten sex partners in life.

Table II. Univariate analysis on factors related with the presence of the hepatitis C viremia.

Risk factors for HCV infection	Gender		p value*	Viremia		p value†
	Male	Female		Positive	Negative	
Decompensated liver disease, n (%)‡	9 (20)	25 (36.2)	0.06	28 (43.1)	6 (12.2)	< 0.001
Blood transfusion, n (%)§	17 (37.8)	40 (58)	0.03	40 (61.5)	17 (34.7)	0.005
Surgical procedures, n (%)‖	31 (68.9)	50 (72.5)	0.68	52 (80)	29 (59.2)	0.01
Hemodialysis, n (%)	3 (6.7)	0 (0)	0.06	3 (4.6)	0 (0)	0.13
Infected sex partner, n (%)	1 (2.2)	1 (1.4)	0.99	2 (3.1)	0 (0)	0.21
More than ten sex partners, n (%)¶	11 (24.4)	0 (0)	< 0.001	6 (9.2)	5 (10.2)	0.86
Previous contact with prostitutes, n (%)	16 (35.6)	0 (0)	< 0.001	12 (18.5)	4 (8.2)	0.12
Use of any illicit drug, n (%)	11 (24.4)	0 (0)	< 0.001	5 (7.7)	6 (12.2)	0.84

HCV, hepatitis C virus.
* p value for differences between men and women; Fisher exact test.
† p value for differences between viremia positive and negative groups; Fisher exact test.
‡ Decompensated liver disease is not a risk factor for HCV infection and actually is a consequence of it; however, this variable underwent univariate analysis for prediction of the hepatitis C viremia.
§ Transfusion of blood products before 1993.
‖ Any surgical procedure before the first serologic test for HCV infection resulted positive.
¶ Antecedent of more than ten sex partners in life.

a greater proportion of cases with viremia in univariate analysis (*Table II*), with 50 (44%) patients having both. Factors related with sexual activities and illicit drugs were more common in men than in women. There were no patients that declared past or current illicit intravenous drug abuse. There were 21 (18%) patients without an identifiable risk factor for HCV infection, while 64 (56%) had more than one. The number of risk factors slightly correlated with the number of patients with viremia (Spearman's rho = 0.289, p = 0.002). After multivariate analysis, the presence of decompensated liver disease and transfusion of blood products before 1993 were identified as independent predictors of the hepatitis C viremia (*Table III*).

Test for anti-HCV antibodies and its S/CO value

All the patients had a minimum of three serologic tests for anti-HCV antibodies: at least two performed prior to arrival to our department and one performed in our laboratory, of which results were taken for the statistical analysis. Overall, median S/CO ratio was 36.4 (range 1 to

Table III. Analysis on factors predicting the hepatitis C viremia: Binary logistic regression model.

Variable	Regression Coefficient	SE	OR (95% CI)	Corrected OR (95% CI)	p value
Decompensated liver disease					
0 = Absent					
1 = Present	1.655	0.515	5.23 (1.91-14.36)	1.77 (1.35-2.00)	0.001
Blood transfusion*					
0 = Absent					
1 = Present	1.061	0.416	2.90 (1.28-6.52)	1.58 (1.14-1.91)	0.01

CI, confidence interval; OR, odds ratio; SE, standard error.
Hosmer-Lemeshow test for goodness of fit in final step of the regression model: χ^2 3.22, 2 df, p = 0.211.
* Transfusion of blood products before 1993.

146.53). Patients with liver decompensation had higher S/CO ratios than asymptomatic patients (median 44.7 *vs* 25.2, respectively; p = 0.002) (*Figure 1*), but this difference did not remain after considering only patients with viremia from both groups (p = 0.65). The group of asymptomatic patients was very heterogenic with respect of their S/CO ratio, whereas dividing the cohort as having or not viremia resulted in more homogeneous groups than on the basis of clinical manifestations and complications of the liver disease (*Figure 1*). Thus, patients with viremia had higher S/CO ratios than those without viremia (median 46.4 *vs* 1.9, respectively; p < 0.001) (*Figure 1*), and number of patients with viremia directly correlated with S/CO ratios (Spearman's rho = 0.613, p < 0.001).

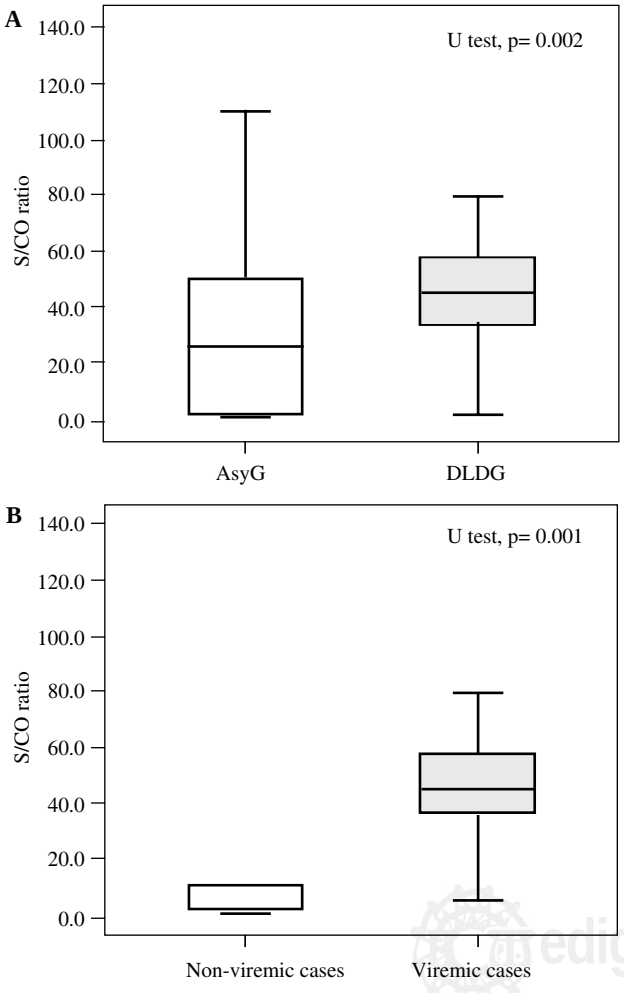

Figure 1. Box plot showing median and intercuartilar range with error bars of the asymptomatic group (AsyG) and the decompensated liver disease group (DLDG) with respect to S/CO ratio (A). Box plot showing median and intercuartilar range with error bars of non-viremic and viremic cases with respect to S/CO ratio (B).

Development of the clinical prediction rules for the hepatitis C viremia

The plot for diagnostic dynamics of different S/CO ratio breaking points had a sigmoid distribution with S/CO value of 15 having a sensitivity of 90% and the largest LR+ for any S/CO ratio breaking point alone (*Figure 2*). Hence, we selected S/CO value >15 to be combined with the clinical independent predictors identified in logistic regression to generate different prediction rules. The clinical rules derived were three (*Table IV*): (1) S/CO ratio >15 and the antecedent of transfusion before 1993. (2) S/CO ratio >15 and the presence of decompensated liver disease. (3) S/CO ratio >15, the antecedent of transfusion and decompensated liver disease. The first diagnostic cluster derived had the best performance in predicting the presence of HCV RNA in serum, and also performed well in excluding viremia when these two characteristics were absent (*Table IV*). In general, PPV and LR+ were better in the clinical prediction rules than for any S/CO ratio breaking point alone (*Table IV*). By analyzing only the 80 asymptomatic patients of our study group, we found that the clinical prediction rule of S/CO ratio >15 and the antecedent of blood transfusion improved in efficacy of the prediction of viremia, whereas this same rule applied to the patients with decompensated liver disease had a poor power in distinguishing patients with or without viremia (*Table IV*).

Discussion

A good diagnostic test is one that improves our suspicion about a particular diagnosis over the suspicion based on the general prevalence of a disease (i.e., pre-test prob-

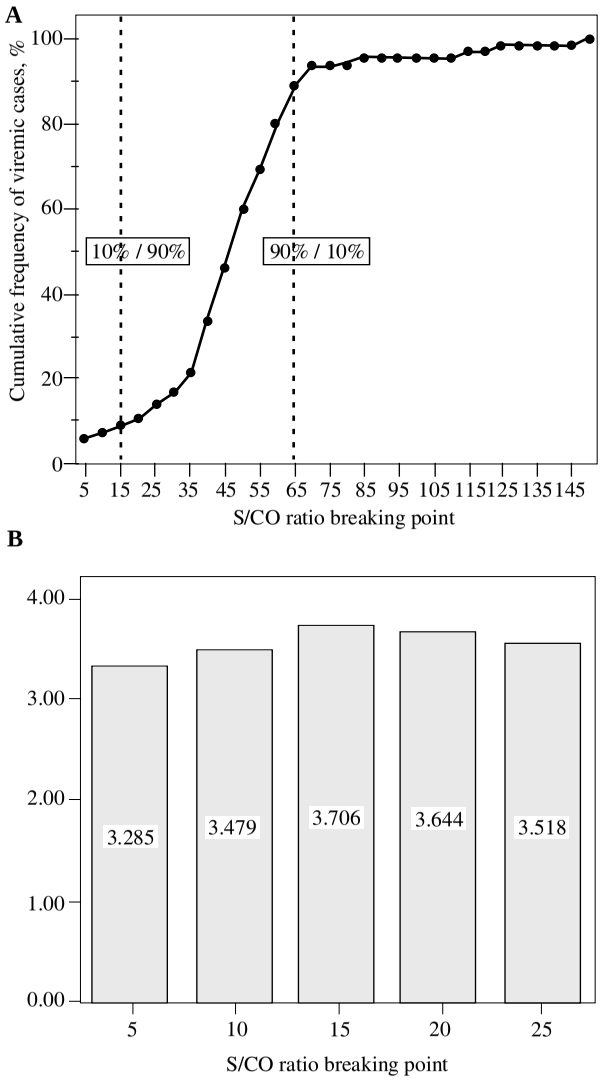

Figure 2. Cumulative proportion of patients with viremia as a function of S/CO ratio (A), and positive likelihood ratios (LR+) for prediction of viremia (B).

ability). Regarding this, clinical prediction rules have been created to help clinicians in the estimation of the probability that a particular patient has an event.[4] To date, no clinical prediction rule had been developed to predict viremia in HCV seropositive patients with different characteristics.

In the present study, patients were first analyzed on the basis of clinical features. Age was higher in those with liver decompensation than in asymptomatic patients, possibly due to the chronic nature of HCV infection and its consequences. The proportion of patients with viremia was higher in the group with decompensated liver disease than in asymptomatic patients, and also had higher S/CO ratios. However, after considering only patients with viremia, the difference between these groups with respect to S/CO ratio was not significant, which suggest that S/CO value is more related with the presence of HCV RNA in

Table IV. Diagnostic appraisal of different S/CO ratio breaking points and clinical rules in predicting the hepatitis C viremia.

Predictor	Sens (95% CI)	Spec (95% CI)	Accu (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)
S/CO ratio breaking points							
5	0.94 (0.85-0.98)	0.71 (0.58-0.82)	0.84 (0.77-0.91)	0.81 (0.71-0.88)	0.90 (0.76-0.96)	3.28 (2.10-5.14)	0.09 (0.03-0.23)
10	0.92 (0.83-0.97)	0.73 (0.60-0.84)	0.84 (0.77-0.91)	0.82 (0.72-0.89)	0.80 (0.76-0.96)	3.48 (2.17-5.57)	0.10 (0.04-0.25)
15	0.90 (0.81-0.96)	0.75 (0.61-0.85)	0.84 (0.77-0.91)	0.83 (0.73-0.90)	0.86 (0.73-0.93)	3.71 (2.25-6.10)	0.12 (0.06-0.27)
20	0.89 (0.79-0.95)	0.75 (0.62-0.85)	0.83 (0.76-0.90)	0.83 (0.72-0.90)	0.84 (0.71-0.92)	3.64 (2.21-6.00)	0.14 (0.07-0.29)
25	0.86 (0.76-0.92)	0.75 (0.62-0.85)	0.82 (0.75-0.89)	0.82 (0.72-0.90)	0.80 (0.67-0.89)	3.52 (2.13-5.81)	0.18 (0.10-0.37)
Clinical prediction rules							
S/CO >15 + Transf (All)	0.57 (0.45-0.68)	0.90 (0.78-0.96)	0.71 (0.63-0.79)	0.88 (0.75-0.95)	0.61 (0.50-0.72)	5.58 (2.37-13.15)	0.48 (0.36-0.64)
S/CO >15 + DLD (All)	0.38 (0.28-0.51)	0.90 (0.78-0.96)	0.60 (0.51-0.69)	0.83 (0.66-0.93)	0.52 (0.42-0.63)	3.77 (1.55-9.14)	0.68 (0.55-0.85)
S/CO >15 + Transf + DLD (All)	0.23 (0.15-0.35)	0.94 (0.84-0.98)	0.53 (0.44-0.62)	0.83 (0.61-0.94)	0.48 (0.38-0.58)	3.77 (1.16-12.30)	0.82 (0.70-0.95)
S/CO >15 + Transf (AsyG)	0.59 (0.44-0.74)	0.95 (0.85-0.99)	0.79 (0.70-0.87)	0.91 (0.74-0.98)	0.73 (0.60-0.83)	12.78 (3.22-50.8)	0.42 (0.29-0.63)
S/CO >15 + Transf (DLDG)	0.54 (0.36-0.71)	0.50 (0.19-0.81)	0.53 (0.40-0.66)	0.83 (0.61-0.94)	0.19 (0.07-0.43)	1.07 (0.45-2.56)	0.93 (0.39-2.27)

Accu, Accuracy; All, the 114 patients included; AsyG, asymptomatic group; CI, confidence interval; DLD, decompensated liver disease; DLDG, decompensated liver disease group; LR+, likelihood ratio for prediction of positive cases; LR-, likelihood ratio for prediction of negative cases; NPV, negative predictive value; PPV, positive predictive value; S/CO, sample to cut-off; Transf, blood products transfusion before 1993.

serum than with severity of the liver disease. S/CO value with the best performance in detecting cases with viremia was 15, as it had the largest LR+ combined with a good PPV. Other studies have addressed the relationship between quantitative data of the screening test and the presence of HCV RNA in blood or reactivity to RIBA, in different clinical settings.[5,18-21] These reports have demonstrated that sensitivity, specificity and predictive values of screening tests behave different in certain subgroups of patients, and that S/CO value can predict viremia, as higher S/CO values are present in persons with HCV RNA in serum.[5,18,20,22] Based on these facts, we derived three clinical rules for prediction of the hepatitis C viremia, applied to asymptomatic persons or patients with decompensated liver disease. The rule compounded by S/CO ratio >15 and the antecedent of transfusion of blood products before 1993 (the year in which blood-bank screening test for anti-HCV antibodies was systematically practiced in Mexico)[23] performed the best in predicting viremia, especially among asymptomatic patients. Thus, applying the evidence in clinical practice we could have that pre-test probability (prevalence) of having viremia in general population of Mexico is about 1%;[24] but if we have an asymptomatic patient with the antecedent of blood transfusion before 1993 and S/CO ratio >15, the probability of having viremia will be 91%, with about 13 times as likely that these two factors are found in a patient with viremia than someone without this condition. This clinical rule was better than any S/CO value alone in predicting viremia. However, we must take into account with precaution the population at risk from whose the results of a study regarding prediction rules are obtained. This same rule was not better than S/CO value alone when applied to patients with decompensated liver disease. Thus, other diagnostic clusters should be analyzed in populations with different prevalence of HCV infection and different risk factors (e.g., intravenous drug abuse, which was not found in our study population, as the use of illicit intravenous drugs is very infrequent in Mexico).[25,26]

Apparently we sacrifice sensitivity by using clinical prediction rules rather than S/CO ratio alone; however, these rules are not for screening of suspicious cases of having HCV infection, but to be used in prediction of the presence of HCV in serum after a positive screening test. In assays for screening we are more worried about sensitivity and not losing a true positive case; after a screening procedure that tested positive we have more concern regarding specificity and PPV, and therefore not to include a person without the disease in the group of diseased people. However, we are not proposing a new way to diagnose HCV infection on the basis of a screening test and clinical data. A confirmatory test should not be skipped, but the rational use of a rule for prediction of viremia may: (1) minimize the uncertainty of inter-assay variation of the screening test for anti-HCV antibodies, which may oscillate between a reactive and non reactive result;[5,9] (2) allow the detection of a laboratory mistake when viremia is expected (e.g., high S/CO value); (3) help with the interpretation of an inconclusive result of a quantitative NAT (i.e., when the viral load is below the detection limit of the assay or when actually there is no viremia); (4) help in decision to perform directly a quantitative NAT for assessment of viral load after a screening test, instead of a RIBA or a qualitative NAT; and thus to save time and economic resources; and (5) may increase confidence regarding patient's health and may reduce emotional harm. A positive result to anti-HCV antibodies with a negative NAT may cause some concern; however, even though the possibility of an occult HCV infection in liver without viremia exist,[13-16] information regarding long-term consequences of this state suggests a good outcome for seropositive patients without viremia;[27-29] an issue that, however, deserves more study. In the present report, seropositive patients without viremia who underwent liver biopsy did not have a substantially abnormal histologic pattern.

In conclusion, a clinical rule is better than the screening test alone in prediction of the hepatitis C viremia. In a patient that meet the prediction rule the probability of having HCV infection is high, therefore, it can be indicated directly an assay for viral load instead of other supplemental tests; and thus be treated opportunely. Clinical context dictates the importance that has to be attributed to a positive screening test for anti-HCV antibodies. Further studies are needed to validate the models in an external population and to assess the benefit on economic issues and quality of care of using a rule for prediction of viremia in different populations.

Acknowledgments

We thank to Dr. Jorge Segura, Dr. Miguel A. Jiménez, Dr. Guadalupe Leyva, Dr. Ángeles Quintero and Dr. Benjamín Cárdenas for their clinical support and kind attention to this work.

References

1. Memom MI, Memom MA. Hepatitis C: an epidemiological review. *J Viral Hepat* 2002; 9: 84-100.
2. Conry-Cantilena C, VanRaden M, Gibble J, Melpolder J, Shakil AO, Viladomiu L, Cheung L, et al. Routs of infection, viremia, and liver disease in blood donors found to have hepatitis C virus infection. *N Engl J Med* 1996; 334: 1691-1696.
3. Poynard T, Yuen MF, Ratziu V, Lai CL. Viral hepatitis C. *Lancet* 2003; 362: 2095-3000.
4. Sackett DL, Straus SE, Richardson WS, Rosenberg W, Haynes RB (eds). Diagnosis and screening. In: *Evidence-based medicine: How to teach and practice EBM*. 2nd ed. Edinburgh: Churchill-Livingstone; 2000: 67-93.
5. Alter MJ, Kuhnert WL, Finelli L; Centers for Disease Control and Prevention. Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. Centers for Disease Control and Prevention. *MMWR* Recomm Rep 2003 Feb 7; 52 (RR-3): 1-13.

6. Dal Molin G, Tiribelli C, Campello C. A rational use of laboratory tests in the diagnosis and management of hepatitis C virus infection. *Ann Hepatol* 2003; 2: 76-83.
7. McFarlane IG, Smith HM, Johnson PJ, Bray GP, Vergani D, Williams R. Hepatitis C virus antibodies in chronic active hepatitis: Pathogenetic factor or false-positive result? *Lancet* 1990; 335: 754-757.
8. Aceti A, Taliani G, Bac C, Sebastiani A. Anti-HCV false positivity in malaria. *Lancet* 1990; 336: 1442-1443.
9. Schirm J, van Loon AM, Valentine-Thon E, Klapper PE, Reid J, Cleator GM. External quality assessment program for qualitative and quantitative detection of hepatitis C virus RNA in diagnostic virology. *J Clin Microbiol* 2002; 40: 2973-2980.
10. Sánchez LV, Maldonado M, Bastidas-Ramírez BE, Norder H, Panduro A. Genotypes and S-gene variability of Mexican hepatitis B virus strains. *J Med Virol* 2002; 68: 22-34.
11. Kiely P, Kay D, Parker S, Piscitelli L. The significance of third-generation HCV RIBA-indeterminate, RNA-negative results in voluntary blood donors screened with sequential third-generation immunoassays. *Transfusion* 2004; 44: 349-358.
12. Rivas-Estilla AM, Sánchez LV, Matsui O, Campollo O, Armendáriz-Borunda Juan, Segura-Ortega JE, Panduro A, et al. Identification of hepatitis C virus (HCV) genotypes in infected patients from the west of Mexico. *Hepatology Research* 1998; 12: 121-130.
13. Navas S, Castillo I, Carreño V. Detection of plus and minus HCV RNA in normal liver of anti-HCV-positive patients. *Lancet* 1993; 341: 904-905.
14. Haydon GH, Jarvis LM, Blair CS, Simmonds P, Harrison DJ, Simpson KJ, Hayes PC. Clinical significance of intrahepatic hepatitis C virus levels in patients with chronic HCV infection. *Gut* 1998; 42: 570-575.
15. Dries V, von Roth I, Müller M, Gerken G, Schirmacher P, Odenthal M, Bartenschlager R, et al. Detection of hepatitis C virus in paraffin-embedded liver biopsies of patients negative for viral RNA in serum. *Hepatology* 1999; 29: 223-229.
16. Castillo I, Pardo M, Bartolomé J, Ortiz-Movilla N, Rodriguez-Inigo E, de Lucas S, Salas C, et al. Occult hepatitis C virus infection in patients in whom the etiology of persistently abnormal results of liver-function tests is unknown. *J Infect Dis* 2004; 189: 7-14.
17. Zhang J, Yu KF. What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcomes. *JAMA* 1998; 280: 1690-1691.
18. Sookoian S, Castaño G. Evaluation of a third generation anti-HCV assay in predicting viremia in patients with positive HCV antibodies. *Ann Hepatol* 2002; 4: 179-178.
19. Dufour DR, Talastas M, Fernandez MDA, Harris B. Chemiluminescence assay improves specificity of hepatitis C antibody detection. *Clin Chem* 2003; 49: 940-944.
20. Bossi V, Galli C. Quantitative signal of anti-HCV by an automated assay predicts viremia in a population at high prevalence of hepatitis C virus infection. *J Clin Virol* 2004; 30: 45-49.
21. Polywka S, Schröter M, Feucht HH, Zöllner B, Laufs R. Relevance in reactivity in commercially available hepatitis C virus antibody assay. *J Clin Microbiol* 2001; 39: 1665-1668.
22. Lu SN, Tung HD, Chen TM, Lee CM, Wang JH, Hung CH, Chen CH, et al. Is it possible to diagnose acute hepatitis C virus (HCV) infection by a rising anti-HCV titre rather than by seroconversion? *J Viral Hepat* 2004; 11: 563-570.
23. Marín-López A. Bancos de Sangre. *Rev Gastroenterol Mex* 2002; 67: S11-S12.
24. Méndez-Sánchez N, Aguilar-Ramírez JR, Reyes A, et al. Etiology of liver cirrhosis in Mexico. *Ann Hepatol* 2004; 3: 30-33.
25. CONADICT. Statistics on drug abuse patterns, Mexico. 2001. Available at *http://www.conadic.gob.mx/* Accessed August 24, 2004.
26. Vivas-Arceo C, Benavides SA, De Jesus Trujillo J, Panduro A, Rivas-Estilla AM. Hepatitis C virus: prevalence and routes of infection among blood donors of West Mexico. *Hepatol Res* 2003; 25: 115-123.
27. Thomas DL, Astemborski J, Rai RM, Anania FA, Schaeffer M, Galai N, Nolt K, et al. The natural history of hepatitis C virus infection. Host, viral, and environmental factors. *JAMA* 2000; 284: 450-456.
28. Jara P, Resti M, Hierro L, Giacchino R, Barbera C, Zancan L, Crivellaro C, et al. Chronic hepatitis C virus infection in childhood: Clinical Patterns and evolution in 224 white children. *Clin Infect Dis* 2003; 36: 275-280.
29. Casiraghi MA, De Paschale M, Romano L, Biffi R, Assi A, Binelli G, Zanetti AR. Long-term outcome (35 years) of hepatitis C after acquisition of infection through mini transfusions of blood given at birth. *Hepatology* 2004; 39: 90-96.