Grading and Staging Chronic Hepatitis C and Its Relation to Genotypes and Epidemiological Factors in Brazilian Blood Donors

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Progression of chronic hepatitis C is known to be associated with some factors, but influence of HCV genotypes is still controversial. Association between HCV genotypes and other risk factors was examined to determine which factors are associated with progression of infection. One hundred consecutive anti-HCV positive volunteer blood donors were evaluated for several risk factors, examined for HCV genotypes, and submitted to hepatic biopsy and biochemical exams.HCV genotyping were carried out in 89 patients and hepatic biopsy in 78. Transmission routes were found to be illicit intravenous drug use (26%), Gluconergan® use in a non-safe manner (48%) and blood transfusion (15%). HCV genotype was 1 in 45%, 3 in 40%, and it was not associated with the stage of fibrosis or with inflammatory activity. There was no significant association of factors related to infection, chronic alcohol use, or duration of illness, with progression of the lesion. There was a significant association of aminotransferase levels and the fibrosis stage. Univariate analysis showed that the age at contamination, patient's age, GT-gamma, and aminotransferase levels over three times the upper normal limits, were associated with fibrosis stages 2 to 4. Multivariate analysis detected age (odds ratio=1.19), and GT-gamma (odds ratio=2.02) as independent factors.

Key Words: Hepatitis C, fibrosis, risk factors.

Hepatitis C virus infection (HCV) has a worldwide distribution, with a prevalence of about 3%. Almost 85% of infected individuals fail to achieve clearance of the virus and become chronically infected [1]. Almost 20% of them will develop hepatic cirrhosis within about 20 years. Six percent will culminate in terminal hepatic disease, and 4% will develop hepatocellular carcinoma (HCC) [2-5], but which of the patients will have a non-favorable evolution cannot be predicted. Although, of a few factors associated with HCV infection, such as the male sex, age at which the contamination occurred, duration of the disease and use of alcohol, is known to accelerate the progression of hepatic disease [5-7], the influence of HCV genotypes on disease progression is still controversial [6] .

There are geographical variations in the distribution of the different HCV genotypes, and some of them are related to specific infection routes [6]. The importance of characterizing the HCV genotype is due to its potential association with variations in the evolution of hepatic disease, which is not totally clarified [6,8,9]; moreover the HCV genotype is a strong predictor of sustained response to therapy [6,8].

Hepatic biopsy is the gold-standard exam to estimate the severity of tissue damage in chronic hepatitis and to determine histological activity; the decision to treat or not the patient is based on these data, and tissue damage severity seems to be predictive for the future development of fibrosis [10]. However, it has been noted that fibrosis staging and inflammatory activity grading are not always related. The progression of fibrosis culminates in the disarrangement of the hepatic architecture in an individually-variable period

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of time [11]. Current knowledge on chronic HCV hepatitis has increased with new concepts of fibrosis rate progression [12]. The estimation of fibrosis progression and knowledge concerning associated factors in chronic hepatitis C is extremely important for understanding its natural history.

The aim of our study was to investigate the association of fibrosis stage and inflammatory activity with HCV genotypes and other risk factors in blood donors in the Botucatu region, state of São Paulo, Brazil.

Material and Methods

One hundred volunteer blood donors referred to the Botucatu University Hospital from 1997 to 1999 who tested positive for anti-HCV were studied. The exclusion criteria were: presence of hepatitis B virus surface antigen (AgHBs) and presence of antibodies for the human immunodeficiency virus (HIV).

All the individuals were informed about the study, and they signed a consent form. The Botucatu School of Medicine (UNESP) Ethical Research Committee approved this study.

The anti-HCV (EIA II or III, Abbott, Chicago) positive patients were submitted to a clinical and laboratorial protocol. The first was composed of a questionnaire with clinical and epidemiological data (sex, age at biopsy, routes of contamination, age at infection, consumption of alcohol, and estimated duration of infection, defined as the time elapsed between the presumed date of infection and the date of biopsy). The following biochemical exams were included in the laboratorial protocol: ALT and AST determination (number of times above upper limit of normal: 1, 1.1-2.9 and $\geq$3), gamma-GT (number of times above normal level), protein electrophoresis, bilirrubins, coagulation and hematological exams. A sample of 10mL of blood was collected in a tube with separating gel, to obtain serum, which was stored at -80°C for determination of HCV RNA by PCR, and for posterior HCV genotyping.

The patients were submitted to a percutaneous hepatic biopsy, when clinically indicated, with a Menghini needle. The biopsy fragments were submitted to conventional histological procedures.

Genotype determination

The extraction of HCV RNA from each sample was carried out with TRIZOL LS (Gibco). The synthesis of viral cDNA was followed by its amplification. HCV genotyping was carried out for each sample by a reverse hybridization technique, using INNO-Lipa II (Innogenetics, NV, Belgium) [13].

Histopathologic evaluation

A hepatic biopsy was performed in 78 patients. Histological sections were submitted to a routine staining technique, and the samples were considered adequate for analysis when at least eight portal areas were seen. The criteria used for the chronic hepatitis classification included staging of fibrosis and grading of inflammatory activity [14,15]. The stage of fibrosis was evaluated as the following: 0= no fibrosis, 1= portal fibrosis without septa, 2= few septa, 3= numerous septa delineating nodules without cirrhosis, 4= cirrhosis.

The grading of activity was also performed by taking into account the inflammatory activities in the portal tract, and in the periportal and lobular regions: 0= no histological activity, 1= minimal lesion, 2= mild activity, 3= moderate activity, 4= severe activity.

Statistical analysis

The Chi-square test and the Exact Fisher Test were used to check for an association between fibrosis quantification and HCV risk factors, including genotype. The significance level of the test was considered as 5%. The same analysis was made to evaluate inflammatory activity and risk factor associations.

Univariate and multivariate regression logistic analysis was performed to analyze variables that might have influenced the severity of HCV infection. After univariate analysis, a stepwise logistic regression analysis was performed to determine independent

association of some variables with the presence of more advanced fibrosis stages (2, 3 or 4). All the factors for which the P-value on univariate analysis was less than 0.05 were entered into the model (confidence interval (CI=95%).

The software used was the SAS System for Windows (Statistical Analysis System), version 6.12. SAS Institute Inc., 1989-1996, NC, USA.

Results

Eighty-nine out of 100 HCV-positive patients were evaluated (75 men and 14 women; mean age 37.5 ± 7.37). The remaining 11 were excluded for not having HCV genotyping, due to the following reasons: extraction of the HCV RNA was negative in eight patients, and amplification of the c-DNA was not achieved in three others.

At least one risk factor for infection by HCV was detected in most of the individuals (87%). Most of these (48% of the total) reported the use of Gluconergan® (a medication composed of glucose, vitamin C and 2-amino-5 guanidinovaleric acid chloride, classified as an energetic drug) administered with shared needles, 26% reported use of illegal intravenous drugs (IVDU) in the past and 15 % had received blood transfusions. The use of inhaled cocaine (UIC) was mentioned by 44% of the individuals and 20% had tattoos (Table 1). Thirty-three percent of them mentioned promiscuous sexual behavior in the past and 22% presented a history of sexually-transmitted diseases.

The duration of infection was evaluated in 82 patients; it was below 11 years in 18 (22%), between 11 and 20 years in 48(59%), and above 20 years in 16 patients (20%). The mean age at infection was 21.1 ± 6.7 years (range: 1-41). Many of the patients (45%) reported greater than 80 grams daily alcohol consumption (Table 1).

Among the 78 patients submitted to hepatic biopsy, 38% presented fibrosis grades 2, 3 and 4, while 63% presented grades 0 and 1 (table 2). The histological necro-inflammatory activity is presented in Table 2.

The HCV genotypes had the following distributions: 45% for genotype 1; 5.7% for genotype 2 and 40% for genotype 3. Nine percent presented mixed infection (Table 2).

The association between the severity of hepatic lesion (stage of fibrosis) and aminotransferase levels was significant. The higher the AST level, the more advanced the stage of fibrosis found (P=0.01). Analogous results were obtained for ALT (P=0.002). We could not find an association with gender (P=0.770), past IVDU (P=0.602), use of Gluconergan® (P=0.129) and previous blood transfusions (P=0.923). We also did not observe association between the severity of the infection and the use of alcohol (P=0.991). There was no significant association between HCV genotypes and the stage of fibrosis (P=0.241).

There was no significant association between the HCV genotype and the routes of transmissions of the disease, or even with the necro-inflammatory activities in the portal, peri-portal and lobular compartments.

The univariate analysis revealed that the donors with AST and ALT activities three times above the upper limit of normality (ULN), presented 6.9 (CI-95% = 1.525; 31.515) and 7.0 (CI-95% = 1.309; 37.889) times greater risk, respectively, of presenting stages 2, 3 or 4 of fibrosis. It was also found that the individuals with age at contamination over 20 had 2.9 (CI-95% = 1.081; 7.955) times more chance of having a greater stage of fibrosis. The other variables were age (Odds=1.173 – CI-95% = 1.072; 1.284) and gamma-GT (Odds=1.672 – CI-95% = 1.057; 2.643), (Figure 1).

In the multivariate analysis, age and gamma-GT were the factors that influenced the staging of the disease, with 1.19 (CI-95% = 1.079; 1.329) and 2.02 (CI-95% = 1.215; 3.378) times greater chance, respectively, of having stages 2, 3 or 4 of fibrosis (Figure 1).

Discussion

The profile of the patients consists of candidates for blood donation, being predominantly young males. Shortly after the identification of HCV, many

Table 1. Characteristics of the study population

Study design	
Type	Prospective
Patients Consecutive blood donors with hepatitis C	
Inclusion criteria	
Number who met criteria	89
Mean age in years	37.5±7.37 (20 – 56)
Men	75 (84.3%)
Women	14 (15.7%)
Transfusion	13 (14.9%)
Intravenous drug use (cocaine)	23 (25.8%)
Intravenous drug use (Gluconergan)	41 (48.2%)
Surgery	7 (7.8%)
Inhalatory drug use	39 (43.8%)
Tattoo	18 (20.2%)
Promiscuous sexual activity	30 (33.7%)
Unknown source	3 (3.3%)
Age at infection	
≤ 20	43 (52.4%)
21 – 40	38 (46.3%)
> 40	1 (1.2%)
Daily alcohol consumption (g)	
0	17 (20%)
<40	21 (24.7%)
40 – 80	9 (10.6%)
>80	38 (44.7%)
Duration of infection in years	
0-10	18 (22%)
11-20	48 (58.5%)
>20	16 (19.5%)

epidemiological studies failed to relate it to a specific source of contamination in at least 40% of the cases [16, 17]. More recently, Khan et al. [18], analyzing 455 patients with HCV infection in Australia, observed that it was not possible to identify the form of contamination in most of the patients not born on that continent. In studies carried out in Brazil, no risk factor could be established in 30% to 40% of the cases [19,20].

In our study, 7 out of the 11 patients who did not report previous use of illegal intravenous drugs, unsafe injections or blood transfusion, reported previous surgeries, and one indicated frequent use of injections with glass syringes. So, we could not find any known risk factor related to HCV infection in only three cases (3.4%).

In the USA and Australia, after the blood banks were controlled, the use of illegal injected drugs became the most frequent means of virus contamination [18,21]. In our group of patients, 26% admitted the use of illegal drugs.

During the last 20 years, a medication classified as an energetic drug (Gluconergan®) has been frequently

Table 2. Results of exams of the study population

AST number of times above upper limit of normal		
Mean (range)	1.825±1.65	(0.4 – 7.6)
ALT number of times above upper limit of normal		
Mean (range)	3.251±2.726	(0.38 – 12.8)
Gama-GT number of times above upper limit of normal		
Mean (range)	1.637±2.138	(0.15 – 13.9)
Stage of fibrosis (n=78)		
No fibrosis (F0)	5	(6.4%)
Portal fibrosis (F1)	44	(56.4%)
Few septa (F2)	14	(17.9%)
Many septa (F3)	08	(10.3%)
Cirrhosis (F4)	07	(9.0%)
Histological activity (n=78)	Periportal / Lobular	
None (A0)	17 (21.8%) / 20	(25.6%)
Mild (A1)	29 (37.2%) / 31	(39.8%)
Moderate (A2 –A3)	32 (41.0%) / 27	(34.6%)
Severe (A4)	0 (0%) / 0	(0%)
Genotype (n=89)		
1a	20	(22.5%)
1b	20	(22.5%)
2	5	(5.7%)
3a	36	(40.4%)
Mixed	8	(8.9%)

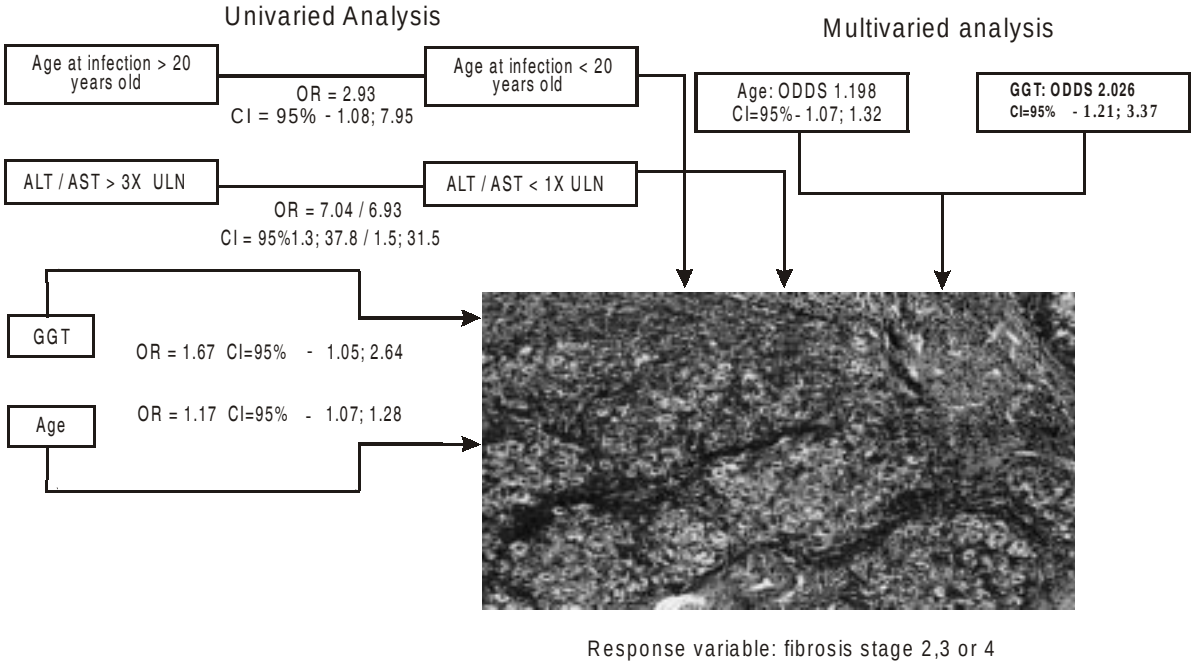
used in Brazil. It is intravenously administered, and reports of needle sharing or reusing of needles are common. In our study, 41 patients (48%) reported use of this energetic drug, and 21 of them presented it as the only risk factor involving HCV transmission. Recently, it has been described that in some countries there are habits associated with HCV infection. In Italy, Maio et al. [22] showed previous reuse of glass syringes among families or neighbors as a risk factor for HCV infection. In Japan, acupuncture carried out by non-professionals was also related to this infection [23].

In our patients the prevalence of a first transfusion before 1993 (when tests for HCV in blood banks began) was 15%. Murphy [21] detected it in 17% of the cases. In Brazil, studies have shown a prevalence varying from 13 to 38% for this risk factor [19,20].

North-American studies indicate sexual transmission of HCV virus at a percentage of 6 to 20% [1,21]. Our patients who indicated promiscuous sexual behavior always presented another associated risk factor (Table 1). Seven patients of this study reported contact with spouses who had hepatitis C. Six of them reported other risk factors, such as drug use. In the case of the seventh patient, the spouse presented frequent bleeding as a consequence of cirrhosis and glomerulonephritis by HCV.

Saracco et al. [24] found an association of non-transfusional transmission and low levels of ALT with genotype 1; they reported elderly patients and a history of blood transfusion for genotype 2 and younger patients and a history of drug abuse for genotype 3. Martinot-Pegnoux et al. [6] reported an association of

Figure 1. Based on univariate analysis, patients with ALT and AST activity three times above the ULN, had 7.0 and 6.9 times greater risk, respectively, of presenting stages 2, 3 or 4 of fibrosis, and patients with age at contamination over 20, had 2.9 times greater chance of having a higher stage of fibrosis. The other variables were age (OR=1.17) and GGT (OR=1.67). In the multivarate analysis, age and GGT were independent factors associated with advanced stages of the disease (2, 3 or 4 of fibrosis).



Abbreviations: AST, aspartate transaminase; ALT, alanine transaminase; GGT, gammaglutamiltranspeptidase; ULN, upper limit of normality; OR, Odds Ratio; CI, confidence intervals.

genotype 1b with older age at contamination and a longer period of disease. We found no association between HCV genotypes and the means of HCV transmission. There was a trend towards significant differences (P=0.056) in the distribution of patients according to age at contamination and the genotypes, with a predominance of type 2 in patients over 20 years of age. There were no associations between the genotypes and the necro-inflammatory activity in the portal, peri-portal and intralobular areas.

The worst consequence of HCV infection is the progression of fibrosis, leading to an unbalance of the hepatic architecture; consequently, fibrosis is an important indicator of the state of the hepatic disease. Analyzing the associations of the severity of the hepatic lesion with gender, routes of transmission and alcohol consumption, we did not find significant correlations. Ramalho et al. [27] found an association between sporadic means of contamination and advanced fibrosis. Gordon et al. [28] associated the evolution of hepatic disease with transmission by transfusion. Many of the patients in this study presented an alcohol consumption of over 80 grams a day at a specific phase of their lives (Table 1). However, we did not observe any association with the evolution of the disease in these patients, in disagreement with Niederau et al. [29] and Bellentanni et al. [7].

Matsumura et al. [9] found a greater rate of progression of fibrosis in patients who received blood transfusions at 30 or more years of age. Martinot-Pegnoux [6] reported an independent relation, based on multivariate analysis, of a more advanced age at

exposition to the HCV and of a longer duration of the infection with cirrhosis (P<0.001). We did not find associations between the evolution of the disease and the age of patients during exposition and duration of the infection. Most of our patients were young at the time of contamination; all but one was below 40 years. Poynard et al. [12] described a greater difference in the evolution of fibrosis in patients over 40. However, we found that in patients more than 20 years old at infection the chances of stages fibrosis 2, 3 or 4 were 2.9 times higher (P=0.03) than in younger patients (univariate analysis).

We did not find an association between the genotypes of HCV and the evolution of fibrosis. A significant association was found between elevated activity of transaminases and the progression of fibrosis (P=0.010 and P=0.002, for AST and ALT, respectively).

Finally, based on multivariate analysis, we concluded that age (odds ratio=1.19) and gamma-GT (odds ratio=2.02) were the independent variables that most influenced the severity of infection by HCV.

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