Prognostic factors for progression of liver structural lesions in chronic hepatitis C patients
Rapid Communication

Prognostic factors for progression of liver structural lesions in chronic hepatitis C patients

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Abstract

AIM: To evaluate the epidemiological, clinical, laboratory and histological variables capable of predicting the progression of hepatic structural disturbances in chronic hepatitis C patients during the time interval between two liver biopsies.

METHODS: Clinical charts of 112 chronic hepatitis C patients were retrospectively analyzed, whereas liver biopsies were revised. Immunohistochemical detection of interferon receptor was based on the Envision-Peroxidase System.

RESULTS: In the multivariate analysis, the variables in the age at first biopsy, ALT levels, presence of lymphoid aggregates and siderosis were the determinants of the best model for predicting the severity of the disease. The direct progression rate of hepatic structural lesions was significantly higher in untreated patients, intermediate in treated non-responders and lower in treated responders to antiviral therapy (non-treated vs responders, 0.22 ± 0.50 vs -0.15 ± 0.46, P = 0.0053). Immuno-expression of interferon receptor is not a relevant factor.

CONCLUSION: The best predictors of the progression of fibrosis are age at the first liver biopsy, extent of ALT elevation, inflammation at liver histology and hepatic siderosis. Antiviral treatment is effective in preventing the progression of liver structural lesions in chronic hepatitis C patients.

Key words: Hepatitis C; Histology; Fibrosis; Interferons; Disease progression

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INTRODUCTION

Studies on hepatitis C virus (HCV) in Brazil indicate a moderate prevalence in the country as a whole. The prevalence of HCV infection in the city of São Paulo[1] was estimated at 1.4%, ranging from 0.7% to 2.12% according to age, geographical region and socioeconomic characteristics. The most prevalent genotype in North America, comprising approximately 60% of all cases of HCV, is genotype 1, followed by genotypes 2 and 3. In Brazil, genotype 1, subtype 1b is also the most prevalent except in the south of the country where there are more cases of infection by genotype 3[2-4].

Hepatic cirrhosis and hepatocellular carcinoma (HCC) are consequences of chronic hepatitis C. It is estimated that HCC will develop in 1%-4% of patients per year in the first five years following the onset of hepatic cirrhosis[5]. The mean interval from the time of infection to onset of cirrhosis is approximately 30 years, but cirrhosis may occur within a range of 10-50 years[5]. In the majority of the patients, progression of the disease involves fibrosis, its extension in the hepatic tissue being the determinant of more severe clinical events in patients with HCV[6]. Once cirrhosis is established, it represents an irreversible condition, and strategies to avoid the progression of fibrosis are essential in order to avoid progressive liver dysfunction[7]. During the chronic infection, 30% of the patients will evolve asymptptomatically with no significant fibrosis or evidence of serious hepatic dysfunction even in the presence of persistently high enzyme levels[8]. In another 30% of cases, the persistently elevated aminotransferase levels will result in fibrogenesis and progressive liver dysfunction. In
10% cases, the clinical course is variable.

Previous studies have identified some factors associated with the worst evolution of chronic hepatitis C, such as age > 40 years at the time of infection and alcohol intake > 50 g/d, as independent factors for the worst prognosis.[9] Controversial reports on elevated alanine aminotransferase (ALT) levels as a predictive factor for increased histological deterioration have been published in the literature; however, increased histological deterioration has been described as related to apoptosis in patients with chronic hepatitis C and normal ALT.[10]

Hepatic fibrogenesis is a result of the action of several aggressive factors on the liver. Various cells are involved in this process, including Kupffer cells (liver macrophages), sinusoidal endothelial cells, hepatocytes and stellate cells (Ito cells or lipocytes). Hepatic stellate cells play a key role in the development of fibrosis and are the major source of extracellular matrix. There is approximately one such cell for every six hepatocytes.[7] Chronic hepatitis C is defined as the continuous hepatic aggression associated with the elevation of aminotransferases or positive viral markers for periods longer than 6 months. Various consensus has concluded that treatment modality should usually be based on structural alterations and hepatic necroinflammatory activity status.[11]. The purpose of a biopsy in the pretreatment phase is different in those patients with moderate inflammation and an advanced level of fibrosis for whom treatment would be indicated, and in those with mild inflammation and an absence or minimal portal fibrosis in whom treatment would be of little use. The French Cooperative Group METAIVIR[12] proposed a scoring system to distinguish fibrosis of the portal spaces from that associated with the lobular-central vein, and graded the stage of portal fibrosis on a five-point scale, consisting of 2 different grades in the septum and 3 grades in the central lobul ar veins according to the amount of fibrosis and its association with perisinusoidal fibrosis. According to the METAIVIR scoring system, patients with fibrosis stages F2, F3 and F4 should be considered for antiviral therapy. The objective of studying serial biopsies is to evaluate the progression of liver damage in chronic HCV. Several parameters have been used in the staging of hepatic disease at the tissue level. The ideal staging system should evaluate each histological component separately such as piecemeal necrosis, confluent necrosis, lobular activity and portal inflammation. Among the patients treated with IFN in various regimens and with various drug combinations, a significant number failed to respond to treatment, while of those who did respond to treatment, some experienced a relapse. Several virological variables such as HCV genotypes 2 and 3 and low HCV RNA load, as well as other variables related to the host such as young age, short disease duration and absence of cirrhosis, have already been cited as being directly related to a higher chance of responding to IFN. Data in the literature on the ability of IFN receptors to predict response rates are sparse. Yatsuhashi et al.[13] studied 55 patients with chronic HCV infection treated with IFN-α for 16 wk at a dose of 6 million units/day in the first two weeks followed by 6 million units three times per week up to the end of treatment. The patients not responding to the treatment were those with lower hepatic IFN receptor expression.

The aim of this study was to evaluate the epidemiological, clinical, laboratory and histological variables that may be predictive of the progression of fibrosis in chronic hepatitis C patients during the interval between two liver biopsies.

MATERIALS AND METHODS

Patients

Epidemiological, clinical, laboratory and histological data were retrospectively analyzed from 112 patients with chronic hepatitis C, receiving care at the Hepatology Clinic of the Department of Gastroenterology, Hospital das Clínicas, University of São Paulo School of Medicine (São Paulo, Brazil). The study was approved by the institute’s Internal Review Board and was carried out in accordance with the Helsinki Declaration. The patients had been subjected to two liver biopsies at two different periods of time. The first biopsy was performed between March 1992 and January 2002. The patients received or did not receive treatment with interferon alpha-2 and ribavirin for 6-12 mo according to the genotype. Patients with non-genotype 1 HCV were treated for 6 mo following the first biopsy, while those with genotype 1 or when genotype was unknown at the time of the initial biopsy received treatment for a year. The patients who remained HCV RNA negative for 6 mo or longer after the treatment were considered responders.

Inclusion criteria: Chronic hepatitis C, defined by the presence for at least 6 mo of serum anti-HCV antibodies, as confirmed by ELISA (Enzyme-Linked Immunosorbent Assay) and HCV RNA, evaluated using qualitative and quantitative methods in a reverse transcription polymerase chain reaction assay; adults over 18 years of age whose medical charts were available at the time of the first liver biopsy; no HCV treatment prior to the first biopsy.

Exclusion criteria: History of prior HCV treatment, hepatitis B virus (HBV) infection; HIV infection or AIDS; chronic renal failure; patients with transplants; cancer patients or those in use of any immunosuppressive drugs; alcohol use (> 20 g/d); autoimmune and metabolic diseases of the liver; schistosomiasis mansoni; abnormalities in serum alpha-fetoprotein levels suggesting primary hepatic neoplasia; and use of any hepatotoxic drug.

Histopathological evaluation

Fragments of liver tissue were obtained by percutaneous needle biopsy before and after therapy in the treated group and on two different occasions according to clinical indication during follow-up in the untreated group. In all biopsies, sections were stained with hematoxylin and eosin (H&E), Masson’s trichrome, Perl’s Prussian blue and reticulin stain, and re-evaluated blindly by a single pathologist. Image analysis was done with Image Pro-Plus 4.5.1 software (Media Cybernetics, Bethesda, MD, USA). By evaluating the natural history of chronic hepatitis C infection in the interval between the two biopsies, it was possible to predict whether evolution would be mild or
severe. Severe histopathological disease was defined as necroinflammatory activity $\geq 2$ (A $\geq 2$) and/or fibrosis $\geq 2$ (F $\geq 2$) in accordance with the classification defined by POYNARD et al\textsuperscript{[6]}.

To predict the evolution of hepatic structural disturbances in the interval between the two biopsies, the presence or none histological findings of ductal lesion, lymphoid aggregates, steatosis and siderosis were assessed in the first biopsy.

Necroinflammatory activity was graded by integrating the intensity of piecemeal and lobular necrosis as proposed by the METAVIR group\textsuperscript{[3]}. A0: No histologic necroinflammatory activity; A1: Minimal histologic necroinflammatory activity; A2: Moderate histologic necroinflammatory activity; and A3: Severe histologic necroinflammatory activity.

The levels of structural changes were defined following the scoring system by the METAVIR group\textsuperscript{[3]}. F0: No fibrosis; F1: Portal fibrosis without septa; F2: Portal fibrosis with few septa; F3: Numerous septa without cirrhosis; and F4: Cirrhosis.

The progression of hepatic architectural disturbances was assessed according to the METAVIR scoring system\textsuperscript{[3,14]}. In particular, the estimated (indirect) progression of fibrosis was calculated from the ratio between the stage of fibrosis at the first biopsy and the time after infection, i.e. the estimated duration of the infection in years, expressed in METAVIR units of fibrosis per year (METAVIR FU/year). In this model, it is assumed that at the time of infection the patient had no hepatic fibrosis (F0) and from that day on, the progression of fibrosis was constant. In those patients in whom the time of infection was unknown, it was impossible to calculate the indirect progression of fibrosis. In order to evaluate these cases correctly, we excluded patients with any other possible epidemiological causes of fibrosis (see exclusion criteria). The direct progression of fibrosis over time was defined as the difference in the stages of fibrosis between the first and the second biopsy, i.e. progression during the interval of years between the two biopsies, with the final result expressed in fibrosis units per year.

**IFN receptor immunohistochemical detection**

Whenever there was sufficient remaining tissue available from the paraffin block, two histological slides for each patient were submitted to immunohistochemical detection of interferon receptor, using a monoclonal antibody (clone ANOCK 4866; Otsuka Pharmaceutical, Tokushima, Japan) which was kindly provided by Dr. Michitami Yano of the Institute for Clinical Research, Nagasaki Chuo National Hospital, Japan. As previously standardized, antibody dilution was 1:100 followed by the dextran polymer-peroxidase Envision System (Dako, Carpinteria, CA). The results were expressed as positive or negative, and a reaction was considered positive when hepatocytes showed cytoplasmic reactivity.

**Serum biochemical analysis**

Laboratory biochemical tests were performed by standard methods using automated techniques (Modular P800; Roche Diagnostics; Indianapolis, IN, USA).

**Statistical analysis**

Descriptive analysis was carried out for both continuous and qualitative variables, obtaining means or medians and frequencies or percentages, respectively. The results were then correlated with the treatment. For analysis of the prognostic factors related to the progression of the mild or severe forms of the disease throughout its natural history at the time of the first biopsy, univariate and multivariate methods were applied. In the univariate analysis, Student's $t$ test for independent samples was used in the evaluation of continuous variables, and the Kruskal-Wallis test was applied when the variables were nominal or continuous with non-normal distribution. Pearson's $\chi^2$ test or Fisher's exact test were also used in the evaluation of proportions between the categorical variables. Multivariate techniques were applied to evaluate whether significant variables in the univariate analysis were able to predict the mild or severe forms of hepatic disease. Logistics regression with forward selection using the likelihood ratio test was used to identify significant variables. Significance was established at $P \leq 0.05$.

**RESULTS**

The general features of the patients in this study are described in Table 1. Table 2 shows the results of the univariate analysis in which age at first biopsy, duration of the infection, ALT levels, albumin, prothrombin activity, lymphoid aggregates and siderosis were identified as potential candidates for the multivariate analysis.

The variables were studied in various models and the final model was obtained through progressive comparison using the likelihood ratio test to identify the most adequate and stable model capable of distinguishing between progression to mild or severe liver disease. The variables including age at first biopsy, ALT levels, lymphoid aggregates and siderosis, were determinants of the best model for predicting the severity of the disease (Table 3).

The indirect progression to hepatic fibrosis was evaluated in all patients. Patients with more severe forms of the disease had a significantly higher mean progression rate than those with mild disease ($P < 0.0001$) (Table 4).

Regarding the direct progression of hepatic fibrosis, a total of 112 patients underwent two liver biopsies. Following the first biopsy, 79 of these patients were treated, while 33 received no treatment. The direct progression of hepatic fibrosis was different (0.2184 ± 0.4987) in the groups of untreated patients, treated non-responders and treated responders ($P = 0.01$) as seen in Table 5. Untreated patients had higher progression rates contrasting to lowest rates in those who responded to antiviral treatment ($0.1459$ ± 0.4584). It is important to acknowledge that even the non-responders to the anti-viral treatment were benefited, showing herein intermediate rates of progression of liver structural disturbances (0.0382 ± 0.3661). There was a clear reduction in the progression of fibrosis in those treated patients. Compared with the untreated patients, treated non-responders and treated responder groups, the
relationship between direct progression of hepatic fibrosis (according to the METAVIR scoring system) and IFN receptor-positivity (by immunohistochemistry) was not predictive of response to treatment. Although necroinflammatory activity at the first biopsy was not a determinant of the direct progression of fibrosis, a good correlation was observed between progression of necroinflammatory activity at the first biopsy was not a determinant of response to treatment. Although necroinflammatory activity at the first biopsy was not a determinant of the direct progression of fibrosis, a good correlation was observed between progression of necroinflammatory activity (defined as the difference in activity between the first and the second biopsy) and direct progression of fibrosis ($P = 0.03$).

### DISCUSSION

According to the model described by Poynard et al\(^1\), the annual rate of fibrosis progression can only be calculated in those patients with a clear duration of infection prior to the first liver biopsy (natural history). Therefore, the uni- and multivariate analysis could only be performed in 93 of the 112 patients. Since none of these patients had been treated previously, excluding other causes of fibrosis, fibrosis was assumed to have been absent at the time of initial infection. Poynard et al\(^1\) analyzed 2235 patients with...
chronic hepatitis C and found a mean fibrosis progression rate of 0.133 FU/year, similar to the mean rate found in this study (0.106 FU/year). On the other hand, in 2003, Ghany et al. reported a fibrosis progression rate of 0.44 FU/year. Considering the difficulty in identifying other fibrosis markers, various clinical, epidemiological, laboratory and histological variables have been evaluated to define their relevance in the natural history of chronic hepatitis C[17]. Using uni- and multivariate analysis, Poynard et al. reported that age at first liver biopsy was higher in those patients who progressed to severe disease (P < 0.001; OR:1.09), mean age at first biopsy being 53.4 ± 10.6 years for patients with severe disease and 43.8 ± 10.9 years for patients with mild disease. Previous studies have described the duration of infection and the occurrence of fibrosis as relevant factors associated with progressive liver dysfunction. Verbaan et al. described fibrosis (rather than histological activity) as a factor related to the duration of infection; however, in the present study, no difference was found in the duration of infection between the groups with severe and mild disease.

On the other hand, the mean age of the patient at infection was significantly different between the two groups (P < 0.004). The univariate analysis showed the mean age at infection of 30.2 ± 13.4 years in the group that progressed to severe disease, was almost 10 years higher than those with mild disease. Poynard et al. observed a worse evolution of hepatitis C in patients infected after the age of 40 years. It is possible that in the present study, the age at first biopsy was statistically significant as an independent predictor of a more severe disease because of the older age at infection rather than the duration of the infection. In addition, most of these patients were under 40 years of age. In our series, age at first biopsy was a significant variable in the logistic model. Tassopoulos et al. also found that patients with mild chronic hepatitis were younger than those with moderate to severe diseases, with mean ages of 41 and 45 years, respectively.

Although there was a tendency towards significance, elevated levels of alanino aminotransferase (ALT) and aspartate aminotransferase (AST) were not found to be predictive of the evolution to mild or severe disease. Mathurin et al. evaluated 204 untreated patients prospectively, and found slower fibrosis progression in those with normal ALT. Koda et al. developed the FibroIndex, which is derived from the platelet count, AST and gamma globulin measurements. The authors concluded that this is a simple and reliable index for predicting significant fibrosis in chronic hepatitis C. In our study, elevated GGT failed to identify patients with severe disease at the first biopsy. Mathurin et al. described significantly elevated GGT in patients with abnormal ALT levels, which was in turn correlated with higher fibrosis scores.

Platelet count was not predictive of the severity of liver disease. Our results contradict the data reported by Poynard et al. showing the age of the patient and platelet count as being independent factors for progression to severe liver disease. Moreover, Ghany et al. described platelet count as a factor for differentiating early from advanced stages of liver disease. The lack of statistical significance for platelet count as a predictor in the present study may be due to the inclusion of cases of fibrosis grade 2 (F2) in the severe group in contrast to the findings of previous studies reporting a correlation between platelet count and advanced liver disease with fibrosis 3 (F3) and 4 (F4). Ohta et al. developed a simple surrogate index consisting of platelet count and albumin level, which reflect the histological fibrosis stage of patients with chronic hepatitis C.

Steatosis was not related to severe disease in 93 patients of this study. Some authors reported a higher frequency of steatosis in patients with genotype 3 HCV[24,25]. Westin et al. studied 98 treatment-naive patients and described a higher fibrosis progression rate in those with steatosis and genotype 3 HCV. Wyatt et al. reported that steatosis is strongly associated with fibrosis and tends to increase over time, but is reduced in patients developing cirrhosis.

Other histological variables such as interferon receptors failed to correlate with the evolution of the disease. Interferon receptor expression is associated with a higher response to interferon treatment[13]; however, no analysis has been carried out taking the natural history of the disease into consideration. Lower receptor expression would be expected in the group with worse evolution. The lack of significance may be due to the qualitative method used, and further studies are necessary since patients with liver cirrhosis are known to be less responsive to interferon treatment[28].

The presence of siderosis at the first biopsy was correlated with the evolution of liver disease, with an odds ratio of 9.54 in the univariate and 6.48 in the multivariate analysis. This variable has been reported by some authors to be associated with higher fibrosis progression[29].

The presence of lymphoid aggregates had an odds ratio of 3.97 for evolution of disease in the univariate analysis. After the logistic regression model, this factor persisted as a significant factor, with an odds ratio of 4.83. Similar results have also been observed by Delladetsima et al. Univariate analysis showed that the time interval between the two liver biopsies was similar in the three groups: untreated patients, non-responders and responders to treatment. The direct fibrosis progression rate was higher in the untreated group followed by the non-responder and responder groups. However, when the mean fibrosis progression rates of the untreated and non-responder groups were compared, no significant difference was found between the two groups. The direct fibrosis progression rates were 0.0382 and 0.2184 FU/year, respectively (P < 0.15). Sobesky et al. previously found a higher direct fibrosis progression rate in untreated patients and a lower progression rate in patients who responded to treatment, while the progression rate was slightly lower in patients who did not respond to treatment. Similarly, Poynard et al. reported a lower progression rate of fibrosis in treated patients, which was correlated with virological response and duration of treatment. In addition, an improvement at histology was described in patients who responded to treatment.

Interferon receptor expression is associated with a greater response to interferon treatment[13]. The presence
of receptors would increase the probability of response to both endogenous and synthetic interferon, resulting in reduced progression of fibrosis. However, this was not observed in this study. The lack of correlation between interferon receptor expression and response to treatment may be explained by the qualitative method used in this study in contrast to the quantitative methods used in other studies.\[10\]

Elevated levels of ALT and progression of liver fibrosis has been previously described\[30,34\]. However, in this study, elevated ALT levels were not predictive of a higher direct progression rate of fibrosis, probably due to the effect of treatment since the patients with higher ALT values were in the treated group.

Histological variables failed to correlate with the direct progression of fibrosis. Even siderosis, an independent factor for indirect fibrosis progression in the multivariate analysis, failed to show any association with direct fibrosis progression.

Although steatosis was not associated with fibrosis progression in this study, Westin et al.\[30\] gave a higher direct fibrosis progression rate in patients with genotype 3 HCV and steatosis.

The patients with deteriorated necroinflammatory activity at histology had a mean direct fibrosis progression rate of 0.1883 FU/year, while patients with histological improvement had a rate of 0.0751 FU/year. These results indicate a good correlation between direct fibrosis progression rate and histological activity because fibrosis might be a result of the necroinflammatory activity.

In conclusion, among untreated chronic hepatitis C patients (natural history), the mean fibrosis progression rate was 0.036 ± 0.06 METAVIR units per year in those with mild disease and 0.17 ± 0.14 in those with severe disease. The best predictors of fibrosis progression are: age at first liver biopsy, the extent of ALT elevations, inflammation at liver histology and hepatic siderosis. The other factors, such as interferon receptor expression, are not significantly associated. When the histological progression rate is evaluated between 2 liver biopsies, the progression of fibrosis in METAVIR units per year is higher in non-treated patients and lower in those patients who responded to treatment. The worst histological evolution is correlated with the highest progression rate of fibrosis. No laboratory or histological variable is able to predict the evolution of fibrosis between two liver biopsies.

**COMMENTS**

**Background**

Chronic hepatitis C virus (HCV) infection may progress to cirrhosis over an average of 30 years. Once cirrhosis is established, it represents an irreversible condition, and strategies to avoid the progression of fibrosis are essential in order to avoid progressive liver dysfunction. The extent of inflammation, neovascular formation and fibrosis are debated as possible determinants of more severe clinical course of HCV.

**Research frontiers**

Hepatitis C virus infection is often referred to as “the Silent Epidemic”, because the progression to symptomatic liver disease may take decades. The clinical profiles in chronic hepatitis C are highly heterogeneous in terms of severity and progression rates towards end stage complications. Therefore staging and prognostic assessment in the individual case would be a valuable strategy to identify patients at higher risk of progression.

**Innovations and breakthroughs**

This study identified age at first liver biopsy, extent of ALT elevation, inflammation at liver histology and hepatic siderosis as the best predictors of the progression of fibrosis. Antiviral treatment was effective in preventing progression of liver structural lesions in patients with chronic hepatitis C.

**Applications**

The mean interval from time of hepatitis C virus infection to onset of cirrhosis is approximately 30 years, but cirrhosis may occur within a range of 10-50 years. The results of our study contribute to a better understanding of the predictors of progression of fibrosis in chronic hepatitis C and will help identify patients at higher risk of rapid progression of the disease.

**Terminology**

The progression of hepatic structural disturbances was assessed according to the METAVIR scoring system. The direct progression of fibrosis over time was defined as the difference in the stages of fibrosis between the first and the second biopsy, i.e. progression during the interval of years between the two biopsies, with the final result expressed in fibrosis units per year (Table 5).

**Peer review**

The scientific conclusions are reliable and valuable for practical medicine. The references are appropriate, relevant and updated. The study is of particular interest to the practical medicine.

**REFERENCES**

6. Myers RP, Hilsden RJ, Lee SS. Historical features are poor predictors of liver fibrosis in Canadian patients with chronic hepatitis C. J Viral Hepat 2001; 8: 249-255
12. Intraobserver and interobserver variations in liver biopsy

www.wjgnet.com


14 Poynard T. Interferon alpha in hepatitis C: a cytokine for reducing fibrosis progression. *Eur Cytokine Netw* 1997; 8: 319-320


17 Albanis E, Friedman SL. Diagnosis of hepatic fibrosis in patients with chronic hepatitis C. *Clin Liver Dis* 2006; 10: 821-833


28 Wright TL. Treatment of patients with hepatitis C and cirrhosis. *Hepatology* 2002; 36: S185-S194


33 Rumi MG, De Filippi F, Donato MF, Del Ninno E, Colombo M. Progressive hepatic fibrosis in healthy carriers of hepatitis C virus with a transaminase breakthrough. *J Viral Hepat* 2002; 9: 71-74


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