Natural history of liver fibrosis progression in patients with chronic hepatitis C

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Summary

Background Our aim was to assess the natural history of liver fibrosis progression in hepatitis C and the factors associated with this progression.

Methods We recruited 2235 patients from the Observatoire de l’Hépatite C (OBSVIRC) population, the Cohorte Hépatite C Pitié-Salpêtrière (DOSVIRC) population, and the original METAVIR population. All the patients had a biopsy sample compatible with chronic hepatitis C as assessed by the METAVIR scoring system (grades the stage of fibrosis on a five-point scale, F0=no fibrosis, F4=cirrhosis, and histological activity on a four-point scale, A0=no activity, A3=severe activity). No patient had received interferon treatment before the liver biopsy sample was obtained. We assessed the effect of nine factors on fibrosis progression: age at biopsy; estimated duration of infection; sex; age at infection; alcohol consumption; hepatitis C virus C (HCV) genotype; HCV viraemia; cause of infection; and histological activity grade.

We defined fibrosis progression per year as the ratio between the duration of infection for progression to cirrhosis was 30 years (28–32), ranging from 13 years in men infected after the age of 40 to 42 years in women who did not drink alcohol and were infected before the age of 40. Without treatment, 377 (33%) patients had an expected median time to cirrhosis of less than 20 years, and 356 (31%) will never progress to cirrhosis or will not progress for at least 50 years.

Interpretation The host factors of ageing, alcohol consumption, and male sex have a stronger association with fibrosis progression than virological factors in HCV infection.

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Introduction

Chronic hepatitis C has a prevalence of at least 1% worldwide.1 Mortality associated with chronic hepatitis C results mainly from the development of liver fibrosis and the subsequent occurrence of cirrhosis, with complications such as hepatocellular carcinoma.2 However, most studies of the natural history of chronic hepatitis C defined liver lesions according to so-called activity grades—ie, classification of necrosis and inflammation without identification of fibrosis stages. In such studies patients were classified as having chronic persistent or active hepatitis, but the development of fibrosis itself was not assessed.3,4 These activity grades have been used for cost-effective analysis5 and investigations of the severity of the disease.2 Some studies suggest that activity and fibrosis should be separated, because the association between these two pathological features is not known in chronic hepatitis C.3,4 The time course of earlier fibrosis stages and the factors associated with the rate of fibrosis progression are not known. In theory, the best way to investigate fibrosis in chronic hepatitis C would be to prospectively follow a large representative sample of patients from infection to death, with repeated liver biopsies and no treatment. But this type of study is unethical and unfeasible. Studies that have investigated biopsy samples were retrospective and included few and selected patients.6–12

Our cross-sectional study was designed to describe the natural history of liver fibrosis progression and to identify risk factors in a large group of patients who had undergone a single liver biopsy and a standard assessment of fibrosis stage. This standard assessment classifies liver fibrosis into five stages (F0, F1, F2, F3, F4) and has been validated by the METAVIR group.13,14 Although histological assessment of liver fibrosis is more commonly done in patients for whom the duration of infection is known, its validity has been checked by indirect estimates of age at biopsy and in two smaller-scale longitudinal studies in which repeated biopsy samples were obtained.6–12

Methods

We recruited patients from three populations: the Observatoire de l’Hépatite C (OBSVIRC) population,20 the Cohorte Hépatite C Pitié-Salpêtrière (DOSVIRC) population, and the original population from the METAVIR scoring-system validation study.13 The characteristics of the three populations are shown in table 1. All these patients had chronic hepatitis C with at least a second-generation ELISA test positive for the presence of hepatitis C virus (HCV) antibodies and a liver biopsy sample compatible with chronic hepatitis C, as assessed by the METAVIR scoring system.

Patients were enrolled in our study if they met the following inclusion criteria: no previous interferon treatment before the liver biopsy, no other liver disease (autoimmune, primary biliary cirrhosis), the absence of hepatocellular carcinoma, known date of biopsy and date of birth, and an interpretable liver biopsy sample with fibrosis graded according to the METAVIR scoring system. Patients in the DOSVIRC population who also belonged to the OBSVIRC multicentre population were analysed only once and excluded from the multicentre population.

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We used a two-step process to identify risk factors associated with fibrosis progression; this approach was adapted to ensure that the maximum number of patients were recruited and that all nine confounding factors were identified. The first step analysed the associations between the nine risk factors. The second step analysed the association between the stage of fibrosis and the risk factors. For this analysis the following qualitative factors were compared by χ² test: sex; cause of infection (use of intravenous drugs, transfusion, admission to hospital with surgery, unknown); daily alcohol consumption (none, <50 g, >50 g); and HCV genotype (1a, 1b, 2, 3, 4, or 5, or 6). We compared the following quantitative factors by analysis of variance and Kruskal Wallis analysis: age at biopsy, estimated duration of infection, age at infection, and HCV quantification. Multivariate logistic regression analysis: age at biopsy, estimated duration of infection, age at infection, and HCV quantification. Multivariate logistic regression analysis: age at biopsy, estimated duration of infection, age at infection, and HCV quantification. Multivariate logistic regression analysis: age at biopsy, estimated duration of infection, age at infection, and HCV quantification.

We defined fibrosis progression per year as the ratio between the stage of fibrosis in METAVIR units and the estimated duration of infection in years. For example, for a patient with fibrosis stage 2 and an 8-year duration of infection, the fibrosis progression rate was 0.25 fibrosis units per year.

Liver biopsy samples of more than 10 mm in length were fixed, paraffin-embedded, and stained with haematoxylin-eosin safran and Masson's trichrome, or picrosirius red for collagen. For each liver biopsy sample, a stage of fibrosis and a grade of activity was done to analyse the independent association of each factor with fibrosis progression; this approach was adapted to ensure that the maximum number of patients were recruited and that all nine confounding factors were identified. The first step analysed the associations between the nine risk factors. The second step analysed the association between the stage of fibrosis and the risk factors. For this analysis the following qualitative factors were compared by χ² test: sex; cause of infection (use of intravenous drugs, transfusion, admission to hospital with surgery, unknown); daily alcohol consumption (none, <50 g, >50 g); and HCV genotype (1a, 1b, 2, 3, 4, or 5, or 6). We compared the following quantitative factors by analysis of variance and Kruskal Wallis analysis: age at biopsy, estimated duration of infection, age at infection, and HCV quantification. Multivariate logistic regression analysis: age at biopsy, estimated duration of infection, age at infection, and HCV quantification. Multivariate logistic regression analysis: age at biopsy, estimated duration of infection, age at infection, and HCV quantification. Multivariate logistic regression analysis: age at biopsy, estimated duration of infection, age at infection, and HCV quantification.

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Liver biopsy samples of more than 10 mm in length were fixed, paraffin-embedded, and stained with haematoxylin-eosin safran and Masson’s trichrome, or picrosirius red for collagen. For each liver biopsy sample, a stage of fibrosis and a grade of activity was established. The stage of fibrosis was assessed on a five-point scale: 0=no fibrosis, 1=portal fibrosis without septa, 2=few septa, 3=numerous septa without cirrhosis, 4=cirrhosis. These categories have been shown to be highly reproducible among pathologists (κ=0.80). Activity was graded according to the intensity of necroinflammatory lesions: A0=no histological activity, A1=mild activity, A2=moderate activity, A3=severe activity.
The first validation method compared the estimated rate of fibrosis progression calculated from duration of infection with that calculated from the mean and median ages of patients for the different METAVIR grades of fibrosis. This approach was used because the difference in time between the patients’ mean and median ages at different stages of fibrosis was also an estimate of the mean and median rate of fibrosis progression. For example, if the mean age for F4 was 58 years and the mean age for F0 was 38 years, the mean rate of fibrosis progression was calculated as the difference between the scores at two consecutive biopsies divided by the time in years between these two biopsies. This first estimate was done in 1157 patients for whom the duration of infection was known so that we were able to compare in the same patients the rate of fibrosis progression calculated from duration of infection with that calculated indirectly by age at biopsy. The second estimate was done in 1078 patients for whom the duration of infection was not known. This group of patients was representative of the whole population and, thus, we avoided bias that would result from the exclusion of the subgroup of patients for whom the duration of infection was unknown. We also did a sensitivity analysis based on only those patients who had undergone transfusion and for whom the time to acquisition of infection was probably more reliable than in patients with a history of intravenous drug use or surgery.

The second validation method compared the estimated rate of fibrosis progression with the rate observed in paired liver biopsy samples (170 samples) from 70 patients at Pitié-Salpêtrière Hospital, Paris, who had not received treatment for chronic hepatitis C and who did not have cirrhosis at the first biopsy. For these patients, the rate of fibrosis progression was calculated as the difference between the scores at two consecutive biopsies divided by the time in years between these two biopsies. These patients had the same characteristics as those in the cross-sectional study of single liver biopsy (data not shown).

The third validation method compared the estimated rate of fibrosis progression with the observed rate in the control groups of randomised clinical trials of interferon in chronic hepatitis C. In three of these randomised trials, fibrosis progression (assessed by the Knodell scoring system from 0 to 4) after 15, 16, 17, and 21 months of follow-up was compared with initial biopsies of 58 patients who had not received any treatment. The progression of fibrosis, as assessed by the METAVIR score, was transformed into fibrosis METAVIR units by linear regression in 500 patients in whom these two fibrosis-scoring systems had been used.

We defined patients according to the risk of fibrosis progression by a combination of these independent factors and by arbitrary clinical criteria. A low-risk patient, or a very slow fibroser, was defined as a patient with a duration of infection of longer than 20 years and a biopsy without septa fibrosis (F0 or F1). A high-risk patient, or a very rapid fibroser, was defined as a patient who developed cirrhosis (F4) before the age of 50 years.

We used three methods for our validation analysis. First, we compared the estimated rate of fibrosis progression calculated from duration of infection with that calculated from the mean and median ages of patients for the different METAVIR grades of fibrosis. This approach was used because the difference in time between the patients’ mean and median ages at different stages of fibrosis was also an estimate of the mean and median rate of fibrosis progression. For example, if the mean age for F4 was 58 years and the mean age for F0 was 38 years, the mean rate of fibrosis progression was calculated as the difference between the scores at two consecutive biopsies divided by the time in years between these two biopsies. These patients had the same characteristics as those in the cross-sectional study of single liver biopsy (data not shown).

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**Fibrosis stage**

- >=50 g alcohol daily
- 0-49 g alcohol daily

**Figure 3** : Association between stage of fibrosis and age at biopsy or duration of infection by alcohol consumption

The rate increased by 31% between patients aged 31-40 years and those aged 21-40 years, by 45% between patients aged 41-50 years and 31-40 years, and by 67% between patients who were older than 50 and those aged 41-50 years. In the 18 patients infected with HCV before the age of 10 years, the median rate of fibrosis progression per year was only 0.059 (0.030-0.087, range 0-0.141).

After adjustment for estimated duration of infection, the grade for stage of fibrosis was significantly higher in patients infected with HCV at 40 years or older than in those infected at younger ages (p=0.001, figure 6).

The two models for the multivariate analysis fitted well. The first model, based on 1570 patients, identified the following risk factors: age at biopsy (odds ratio=1.08 [1.07-1.09], p<0.0001), male sex (2.08 [1.59-2.71], p<0.0001), and alcohol consumption (2.36 [1.62-3.45], p=0.0001). The second model, based on 1038 patients, identified age at infection (1.07 [1.06-1.08], p<0.0001), estimated duration of infection (1.09 [1.07-1.11], p<0.0001), male sex (2.66 [1.90-3.72], p<0.0001), and alcohol consumption (1.49 [1.18-3.03], p=0.008) as the only independent risk factors. Both models allowed the correct classification of 807 (78%) patients into those with severe fibrosis (F3 or F4) and those without severe fibrosis (F0, F1, F2). These models explained only 17% (r²) of the fibrosis variability. There was no interaction between these factors, except for the second model, which showed a weak interaction between age at infection and alcohol consumption (p=0.047).

Male sex was associated with a younger age at infection.
All patients with duration 0·133 (0·125 to 0·143) 30 (28–32)

Except for genotypes, there was a significant difference between medians for all classifications (p<0·05).

N/A = not available because sample size was too small. All values are median (95% CI).

Identification of risk of fibrosis progression

After retrospective classification of patients according to the three independent factors identified in regression analysis, the median rate of fibrosis progression per year ranged from 0·095 in women who were not heavy drinkers and were infected at 40 years or younger (median expected cirrhosis in 42 years), to a median rate of 0·301 in men who were infected after the age of 40 years (median expected cirrhosis in 13 years).

We identified 68 patients as very slow fibrosers (low risk) who did not have septal fibrosis after 20 years of infection—ie, 5·9% of the 1157 patients for whom the duration of infection was known. The median rate of fibrosis progression was 0·040 in these patients versus 0·143 in patients who were not slow fibrosers (p<0·0001). Comparison of the patients who were very slow fibrosers with those who were not showed that these patients were more likely to be women (69% vs 43%), and to have a history of transfusion (69% vs 52%, p=0·01), that they had a younger median age at infection (24 vs 27 years, p=0·01, including six patients infected before 10 years of age [9% vs 1%, p<0·001]), and were often non-drinkers (71% vs 57%, p=0·04). Genotype distributions did not differ between patients who were very slow fibrosers and those who were not (type 3: 13% vs 16%; type 1b: 48% vs 45%).

We identified 102 very rapid fibrosers who developed cirrhosis before the age of 50 years (4·6% of the 2235 patients). Their mean age was 40 years (range 21–49). The median rate of fibrosis progression per year was 0·364 in rapid fibrosers versus 0·125 in patients who were not very rapid fibrosers (p<0·0001). Comparison of rapid fibrosers with those who were not showed that a higher proportion were men (76% vs 57%, p=0·0002) who were also heavy drinkers (30% vs 10%, p<0·0001). Genotype distributions were similar in patients who were very rapid fibrosers and those who were not (type 1b 50% vs 57%, p=0·0002) who were also heavy drinkers (30% vs 10%, p<0·0001).
In patients for whom the duration of infection was known (0.133), was similar to the progression rates estimated from differences in age in the same population (0.146), in a different population (0.154), or in paired biopsy samples (0.183). The means were also similar to the rates of progression observed in paired biopsy samples from patients in the control groups of the randomised trials (0.252 vs 0.231).

In 612 patients with a history of transfusion and for whom the duration of infection was known, the estimated rate of progression per year was 0.143, which is similar to the overall estimate. The analyses of risk factors gave identical results (data not shown).

In 1157 patients for whom the duration of infection was known, the difference between fibrosis scores divided by the difference between median ages at biopsy was 0.173 for 139 patients with grade F4 and 153 patients with grade F0 (ie, 4:450), and 0.133 for 157 patients with grade F3 and 451 patients with grade F1 (ie, 2:450). The weighted median was 0.146.

In 1078 patients for whom the duration of infection was not known, the difference between fibrosis scores divided by the difference between median ages at biopsy was 0.173 for 261 patients with grade F4 and 74 patients with grade F0 (ie, 4:450), and 0.143 for 183 patients with grade F3 and 355 patients with grade F1 (ie, 2:450). The weighted median was 0.154.

In the three randomised trials, after transformation in METAVIR units, the fibrosis scores varied from 1.88 to 2.09 in 15 months, that is 0.168 per year in one trial (36 patients); from 1.59 to 2.12 in 21 months, that is 0.303 per year in the second trial (15 patients); and from 2.38 to 2.91 in 16 months, that is 0.398 per year in the third trial (seven patients). The weighted mean was 0.231.

Discussion

An understanding of the natural history of hepatitis C is important for making rational decisions about public health, be they screening or therapeutic. Because there are no predictive surrogate markers of fibrosis, only studies with liver biopsy samples are useful. There have been only a few studies of the time period required for the development of fibrosis. In those mostly retrospective studies, the number of patients was small, biopsy samples were assessed by non-validated methods without distinguishing between fibrosis score and activity scores, and in some studies only patients contaminated by transfusion were included without...
The main limitation of our study was the small number of paired biopsy samples (170 biopsy samples in 70 patients) compared with the 2235 single biopsy samples. It is difficult to use cross-sectional observational data to estimate longitudinal parameters. However, estimates in patients with one biopsy sample, whose duration of infection was known, were similar to two estimates calculated by the differences between ages and to those estimates observed in the paired biopsy samples.

The second limitation was the unknown variability of our estimates of the duration of infection. In the absence of prospective follow-up of patients from infection date to biopsy, our estimate of the duration of infection relied on the history of patients, which takes the day of the first transfusion or of first intravenous drug use as the date of infection. Duration of infection was known in only 52% of the overall population; however, in two populations, OBSVIRC (known in 62%) and DOSVIRC (known in 75%), efforts were made to prospectively select patients for whom the duration of infection was known. Furthermore, sensitivity analyses based on only those patients who had undergone transfusions and for whom the time of acquisition of infection was probably more reliable, gave exactly the same results.

The main advantages of our study were that it gathered together a large number of untreated patients and liver biopsy samples were interpreted by the same validated method (METAVIR). We also included patients who had been infected by different sources. In addition, different populations were used without selection—ie, we did not include only patients from randomised trials, patients who had repeated liver biopsies, patients for whom the duration of infection was known, or patients who did not consume alcohol. Indeed, the three different cohorts used in our study should reduce the risk of selection bias. For example, in some OBSVIRC centres patients with concentrations of aminotransferase within the normal range underwent biopsy, whereas in the METAVIR cohort patients were selected for early trials of interferon and had more advanced liver disease than in the two other cohorts.

Our first major finding is that chronic hepatitis C is a progressive fibrotic disease and not an inflammatory hepatitis. From portal tract enlargement (stage 1) to cirrhosis (stage 4), stage of liver fibrosis was almost linear according to time. By contrast, activity grades were not as linearly correlated as fibrosis stages. Thus, clinically relevant progression of chronic hepatitis C would be better estimated by the fibrosis stage than by the grade of histological activity. Our study was not designed to assess activity grades as predictors of the rate of fibrosis progression. We believe that longitudinal studies are needed to assess the independent predictive value of activity grades for the prediction of fibrosis progression, especially in the early stages of infection.

The rate of fibrosis progression was not normally distributed (median 0·133 lower than the mean 0·252), with an asymmetrical distribution. This finding suggests the presence of at least three populations: rapid fibrosers, intermediate fibrosers, and slow fibrosers. Therefore, the expressions of a mean (or median) rate of fibrosis progression per year and of a mean expected time to cirrhosis does not mean that the progression to cirrhosis is universal and inevitable. Based on the median rate of fibrosis progression and without treatment, the median expected time to cirrhosis was 30 years; 33% of patients had an expected median time to cirrhosis of less than 20 years and 31% will never progress to cirrhosis or will not progress for at least 50 years.

Our second major finding is the identification of risk factors associated specifically with fibrosis progression. Age at infection was the main risk factor for fibrosis. We found that the rate of fibrosis progression was low in individuals younger than 20 years, intermediate in those aged 21–40 years, increased in those aged 40–50 years, and highest in those older than 50 years. Although we do not know why age is a risk factor, it may be that the host defence mechanisms against HCV is weaker in older people. The extremely low rate of fibrosis progression in the 18 patients who were younger than 10 years of age at infection must be confirmed in studies of children infected by their mother or from transfusion at a very young age.

Alcohol consumption was a difficult factor to assess, and the risk of variability is great. Information in one population (METAVIR) was not recorded by a standard questionnaire and was not taken into account in our study. For the other two populations, alcohol consumption was recorded by the same method based on the mean number of glasses per day. Daily alcohol consumption of more than 50 g was associated with an increased rate of fibrosis progression. Alcohol consumption was recorded by the same method based on the mean number of glasses per day. Daily alcohol consumption of more than 50 g was associated with an increased rate of fibrosis progression. Alcohol consumption was associated with a higher viraemia. The findings indicate that all studies of hepatitis C should include alcohol consumption as a potential risk factor. The recommendation to limit alcohol consumption as much as possible is mandatory in patients with hepatitis C.

Male sex was associated with fibrosis independently of the age at infection and of alcohol consumption. We were unable to explain this association.

Virus risk factors were assessed in 376 (17%) patients for the genotype and in 173 (8%) for quantification. However, most of these patients (323) were consecutively included in this single centre population in which 53% of patients had been genotyped. These patients did not differ from patients for whom the genotype was not known (data not shown). There was no association between genotypes and progression of the fibrosis. Genotype 1b was not correlated with an increased rate of progression and genotype 3 was
not correlated with a reduced progression rate. Even after age at infection, sex, and alcohol consumption had been taken into account, no genotype had any independent prognostic value. Because of the small sample size of patients with non-1b genotype, small differences in rates of fibrosis progression may have been missed. Kobayashi and colleagues, reported a weak association between fibrosis progression and genotype 1, but this association disappeared in multivariate analysis. This association may simply have resulted from confounding variables such as duration of infection, alcohol consumption, and age at infection. In our study, because of the small sample size and because viraemia was assessed by comparison with biopsy samples for 50% of patients, the absence of an association between viraemia and fibrosis progression should be interpreted with caution.

There may also have been some variability associated with cause of infection, because it was impossible to be sure that this factor was responsible for infection, and the date of infection was uncertain if the patient had several episodes of transfusion or intravenous drug use. However, the epidemiology of chronic hepatitis C in patients with a transfusion history was similar to that of patients who had been admitted to hospital and undergone surgery and to patients not deemed to be at risk of infection (so-called sporadic infection). Age at infection was particularly homogenous for patients with a history of intravenous drug use.

Our findings could explain conflicting findings on the incidence of cirrhosis observed in different cohorts, such as the low incidence of cirrhosis observed in the Irish anti-D study which included women infected before the age of 40.

References