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Fibrosis progression in interferon treatment-naive Chinese plasma donors with chronic hepatitis C for 20 years: a cohort study

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SUMMARY

Objectives: To evaluate the progression of fibrosis and factors influencing this in interferon (IFN) treatment-naive Chinese plasma donors infected with hepatitis C virus (HCV) for approximately 20 years.

Methods: From July 2010 to June 2011, we investigated 122 IFN treatment-naive chronic hepatitis C (CHC) patients infected by plasma donation in 1992–1995. Liver fibrosis stage and inflammation grade were evaluated by Metavir and Scheuer scoring systems, respectively.

Results: One hundred and twenty patients underwent liver biopsy. Liver biopsy was not performed in one patient with cirrhosis due to ascites, and another patient was excluded because of an invalid biopsy specimen. Cirrhosis was observed in three patients (fibrosis stage F4 in two patients revealed by biopsy, and one patient with ascites confirmed by physical and Doppler ultrasound examination). Fibrosis stages F1 and F2 were present in 55 and 50 patients, respectively. The severity of liver inflammation was independently related to moderate to severe fibrosis (F $\geq$2). Older age and male sex showed an increasing tendency for more severe fibrosis (F3/F4) in the present cohort.

Conclusions: Based on histopathology results, the progression of fibrosis in patients with CHC infected by repeated plasma donation is slow after HCV infection of approximately 20 years. Liver inflammation is closely related to the development of moderate to severe liver fibrosis.

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1. Introduction

Hepatitis C virus (HCV) infects 130–170 million people worldwide and is the leading cause of cirrhosis and hepatocellular carcinoma.[1] Understanding the natural history of chronic hepatitis C (CHC) may assist in clinical decision-making and prompt medical interventions to decrease the development of complications. However, it is difficult to investigate the natural history of CHC, because monitoring disease progression needs long-term follow-up and the disease can often be modified by antiviral treatment.

It has been suggested that different modes of HCV transmission affect the progression of liver fibrosis in CHC patients.[2,3] Early studies of CHC caused by blood transfusion showed that, after approximately 20 years of infection, 18–20% of patients progressed to cirrhosis.[4,5] For community-acquired infection, only 8% of patients progressed to overt cirrhosis after a mean 25 years of follow-up.[6] Additionally, other reports on chronic HCV infection following exposure to HCV-contaminated immunoglobulin showed a low frequency of cirrhosis (only 2%) in young women with HCV infection of 17 years.[7] Similar findings were obtained in a 20-year follow-up evaluation of East German women who also received contaminated immunoglobulin, and none of the biopsy specimens showed incomplete or complete cirrhosis in the women with chronic viremia.[8] These studies indicate that the mode of HCV transmission may play a key role in the progression of liver fibrosis in CHC patients.

Paid plasma donation was an important route of blood-borne HCV infection worldwide before the early 1990s.[9–11] One study reported an HCV infection rate of 34% in plasma donors, which was

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higher than the 8% in patients receiving HCV-contaminated whole blood in China in the early 1990s.[12] Donors donated their plasma by plasmapheresis, and the separated blood cells were infused back to the donors. This process was previously performed using recycled rather than disposable tubes, pipes, bottles, and other equipment. During the donation process, the donors were infected by HCV through sharing contaminated, recycled equipment. This mode of CHC infection differed from that of blood transfusion, by which recipients were infected by receiving HCV-contaminated whole blood from infected individuals. The progression of CHC in patients infected by plasma donation might differ from that of patients with other routes of infection. Therefore, it makes sense to explore the progression of CHC in plasma donors. Presently, the infection period for these CHC patients is nearly 20 years. Little is known about the fibrosis status in patients with CHC acquired by plasma donation. Although the progression of fibrosis in paid plasma donors has been reported previously,[13] the results have not been confirmed by liver biopsy in a large population until now.

In order to clarify the natural history of CHC patients infected by paid plasma donation, we performed a cohort study on a single-ethnicity group (Chinese Han) of CHC plasma donors who had persistent HCV infection for approximately 20 years, without interferon (IFN) therapy; liver biopsy was used to evaluate histological progression. Other clinical data were collected and factors that may influence the progression of fibrosis were also analyzed.

2. Materials and methods

2.1. Patients

We have been observing 122 CHC patients of Chinese Han ethnicity from rural villages in Dingxi City, Gansu Province, China since the early 1990s (Figure 1). This cohort of patients suffered from HCV infection through regular plasma donations with repeated blood cell re-transfusions between 1992 and 1995, which was documented in the local blood donation center. They did not have a history of any other possible route of HCV infection such as intravenous drug use. Due to the limited living conditions and no cold chain and logistics systems being established in these rural valleys, the patients had not received IFN or ribavirin treatment during the infection period. Additionally, this group of lean patients with similar lifestyles did not have a habit of alcohol abuse and presented no obesity or diabetes. The patients had no evidence of other forms of liver disease, or co-infection with hepatitis B virus or other viruses. The diagnosis of CHC was in accordance with established criteria.[14,15]

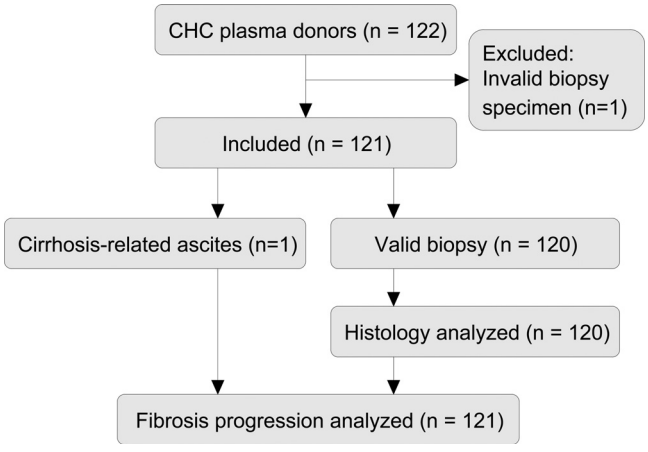

Figure 1. Flow of patient selection and study design.

From July 2010 to June 2011, all of these CHC patients received a comprehensive examination. The cubital vein blood of fasting patients was collected on the day of biopsy. Serological indicators were tested. HCV-RNA quantification was performed by real-time PCR (Daan Biological Engineering, Guangzhou, China). HCV genotypes 1b and 2, as the predominant genotypes in China, were measured using the line probe assay genotyping method. The other genotypes, which are not common in China, were not detected in this study.[16] The procedure and measurements were conducted in strict accordance with the manufacturer's protocols. The study protocol was approved by the Institutional Review Board of Beijing YouAn Hospital, Capital Medical University and was also performed in accordance with the provisions of the Declaration of Helsinki 1975 and its revision. Written informed consent was obtained from each patient.

2.2. Liver histology

The patients underwent ultrasound-guided liver biopsies. The specimens had a length of >1.5 cm and contained at least six complete portal areas. The specimens were fixed in formalin and embedded in paraffin. Pathological analysis of the liver biopsies from 120 patients was performed. For the other two patients, one did not undergo liver biopsy because of ascites and the other was excluded because of the small size of the biopsy specimen, which was unsuitable for pathological analysis. The specimens were reviewed by two senior pathologists who were unaware of the patients' clinical data.

The Metavir scoring system was used to assess fibrosis stage.[17,18] The fibrosis score was assessed on a five-point scale: F0, no fibrosis; F1, portal fibrosis without septa; F2, few septa; F3, numerous septa without cirrhosis; F4, cirrhosis. F2 or higher was considered moderate to severe fibrosis. For the evaluation of histological activity, the Scheuer scoring system (G0–4) was used.[19] Hepatic steatosis was graded based on the proportion of hepatocytes with fat on a four-point scale: grade 0, <5%; grade 1, 5–33%; grade 2, 34–66%; and grade 3, >66%.[20]

2.3. Statistical analysis

Data were expressed as the mean ± standard deviation (SD) or number (percentage). In the univariate analysis, depending on the data distribution, differences in continuous variables between two groups were analyzed using the independent-samples *t*-test or the Mann–Whitney test. Categorical variables were analyzed by Pearson Chi-square test. During the analysis of factors that may influence the presence of moderate to severe fibrosis, indicators with a significant difference in the univariate analysis were assessed by stepwise forward multivariate logistic regression. *p*-Values of entry into and removal from the regression equation were respectively set to 0.05 and 0.1. The statistical analysis was performed using IBM SPSS Statistics for Windows, version 19.0 (IBM Corp., Armonk, NY, USA); a two-sided *p*-value of <0.05 was considered statistically significant.

3. Results

3.1. Characteristics of the study cohort (n = 120)

The characteristics of the study participants who underwent liver biopsy (n = 120) are summarized in Table 1. This cohort comprised 57 male and 63 female patients, with a mean age at biopsy of 51.33 years. HCV genotype 2 (44.2%) predominated in the study cohort, followed by genotype 1b (36.7%). The rest were non-genotype 1b or 2 (19.2%). Mean serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values were 60.42 and 47.94 U/l, respectively, and were mildly elevated at

Table 1
Demographic and clinical characteristics of the patients who underwent liver biopsy (n = 120)

Variable	Value
Age at biopsy, years, mean ± SD	51.33 ± 7.33
Male sex, n (%)	57 (47.5)
BMI, kg/m^2, mean ± SD	22.34 ± 2.73
HCV genotype, n (%)	
1b	44 (36.7)
2	53 (44.2)
Unknown	23 (19.2)
Scheuer inflammation grade, n (%)	
G0	1 (0.8)
G1	16 (13.3)
G2	67 (55.8)
G3	34 (28.3)
G4	2 (1.7)
Steatosis, n (%)	
Grade 0	84 (70.0)
Grade 1	26 (21.7)
Grade 2	6 (5.0)
Grade 3	4 (3.3)
ALT, U/l, mean ± SD	60.42 ± 70.88
AST, U/l, mean ± SD	47.94 ± 44.30
Total bilirubin, μmol/l, mean ± SD	16.51 ± 7.25
Globulin, g/l, mean ± SD	29.19 ± 17.28
γ-GGT, U/l, mean ± SD	22.04 ± 16.50
Glucose, mmol/l, mean ± SD	5.26 ± 0.80
Platelet count, 10^9/l, mean ± SD	171.36 ± 53.20
Prothrombin time, s, mean ± SD	12.45 ± 9.84

SD, standard deviation; BMI, body mass index; HCV, hepatitis C virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase.

the time of biopsy. The mean values of the remaining blood indicators were within the normal range.

3.2. Liver histological status

One hundred and twenty patients had a valid liver biopsy. For evaluation of the progression of fibrosis, one cirrhosis patient who did not undergo liver biopsy due to portal hypertension-related ascites was also included (Figure 1). A total of 121 patients were thus available for the analysis of liver fibrosis. Three cirrhosis patients were identified, including two confirmed by biopsy (fibrosis stage F4) and one diagnosed by physical examination. The morbidity of cirrhosis was 2.5% (3/121) after approximately 20 years of HCV infection. In addition, one patient (0.8%) had an observed histological analysis result of F0, 55 (45.5%) patients showed stage F1, and 50 (41.3%) showed F2, which accounted for >86% of all patients. Twelve (9.9%) patients had numerous septa without cirrhosis (F3).

According to the Scheuer inflammatory activity analysis in 120 patients, G2 was found in 67 patients, which accounted for the largest proportion (55.8%). None or minimal portal inflammation (G0) and severe necroinflammation (G4) were present in only one patient (0.8%) and two (1.7%) patients, respectively. For hepatic steatosis, grade 0 and 1 were found in 84 (70.0%) and 26 patients (21.7%), respectively. Only a few patients with grade 2 (6/120, 5.0%) and grade 3 (4/120, 3.3%) were found in this cohort (Table 1).

3.3. Factors related to the severity of fibrosis

It has been confirmed that the rate of progression of fibrosis is high in patients whose initial biopsies show septal fibrosis (F ≥2), so these patients have a high probability of developing advanced cirrhosis in the ensuing decade.[21,22] In order to identify the factors that influence the development of moderate to severe fibrosis (F ≥2), confirmed by liver biopsy (n = 120), univariate and multivariate analyses of the probable variables were performed.

Liver inflammation was significantly associated with moderate to severe fibrosis on univariate analysis ($p < 0.001$). Additionally, ALT ($p < 0.01$) and AST ($p < 0.001$) showed significant differences between those with and without moderate to severe fibrosis. Other factors related to moderate to severe fibrosis included serum HCV RNA load ($p < 0.001$), globulin ($p = 0.04$), γ-glutamyl transferase ($p = 0.03$), platelet count ($p < 0.01$), and prothrombin time ($p = 0.01$) (Table 2). The significant factors listed above were analyzed by logistic regression. Finally, the multivariate analysis identified two factors that were independently related to moderate to severe fibrosis: liver inflammation (odds ratio (OR) 5.02, 95% confidence interval (CI) 2.17–11.63) and AST (OR 1.03, 95% CI 1.0–1.06).

Table 2
Univariate and multivariate analysis of factors associated with moderate to severe fibrosis (Metavir F ≥2)

Variable	F <2 (n = 56)	F ≥2 (n = 64)	p-Value[a]	OR (95% CI)	p-Value[b]
Age at liver biopsy, years, mean ± SD	50.4 ± 8.31	52.11 ± 6.32	0.28		
Sex, n (%)					
Male	23 (40.4)	34 (59.6)	0.19		
Female	33 (52.4)	30 (47.6)			
BMI, kg/m^2, mean ± SD	22.25 ± 2.41	22.42 ± 3.02	0.82		
HCV genotype, n (%)					
1b	18 (40.9)	26 (59.1)	0.75		
2	20 (37.7)	33 (62.3)			
HCV RNA load, IU/ml, n (%)					
<4 log$_{10}$	18 (90.0)	2 (10.0)	<0.001		
4–5 log$_{10}$	19 (37.3)	32 (62.7)			
6–7 log$_{10}$	19 (38.8)	30 (61.2)			
Scheuer inflammation score, mean ± SD	1.82 ± 0.61	2.47 ± 0.64	<0.001	5.02 (2.17–11.63)	<0.001
Steatosis score, mean ± SD	0.46 ± 0.81	0.38 ± 0.68	0.75		
ALT, U/l, mean ± SD	40.67 ± 25.62	77.70 ± 90.92	<0.01		
AST, U/l, mean ± SD	33.93 ± 12.92	60.21 ± 56.87	<0.001	1.03 (1.00–1.06)	0.02
Total bilirubin, μmol/l, mean ± SD	15.76 ± 6.18	17.17 ± 8.06	0.41		
Globulin, g/l, mean ± SD	29.95 ± 25.05	28.53 ± 3.94	0.04		
GGT, U/l, mean ± SD	17.16 ± 7.63	26.30 ± 20.58	0.03		
Glucose, mmol/l, mean ± SD	5.35 ± 0.87	5.18 ± 0.73	0.36		
Platelet count, 10^9/l, mean ± SD	187.18 ± 49.32	157.52 ± 52.97	<0.01		
Prothrombin time, s, mean ± SD	13.27 ± 14.41	11.73 ± 0.78	0.01		

OR, odds ratio; CI, confidence interval; SD, standard deviation; BMI, body mass index; HCV, hepatitis C virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase.

[a] p-Value was acquired by univariate analysis.

[b] p-Value was acquired by multivariate analysis.

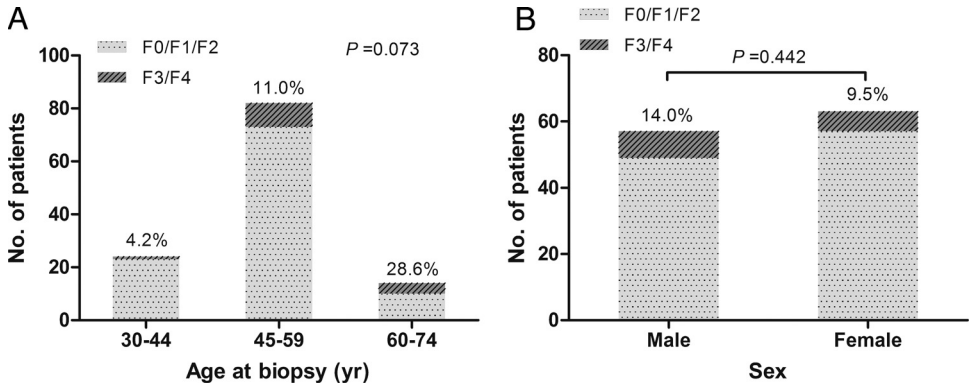

Figure 2. Occurrence of severe fibrosis (F ≥3) according to sex and age. (A) Difference in severe fibrosis in different age groups. (B) Difference in severe fibrosis between male and female patients. Percentage denotes the proportion of severe fibrosis.

No statistically significant differences in age at liver biopsy or sex ratio were found between those with and without the presence of moderate to severe fibrosis. Further analysis demonstrated an increasing trend towards the occurrence of severe fibrosis (F3/F4) with increasing age (p = 0.073) (Figure 2A). Male sex showed a tendency towards more severe fibrosis in the present cohort (Figure 2B).

4. Discussion

The principal finding of the present cohort study is that the progression of fibrosis in patients with CHC infected by repeated plasma donation is slow after HCV infection of approximately 20 years based on histopathology results. The main strength of our study is that liver biopsies were performed in a unique Chinese plasma donor cohort (single ethnicity, no alcohol addiction, obesity, or diabetes) who had never received IFN treatment after infection. Therefore we may consider this a cohort with a natural history of HCV infection, without the influence of antiviral treatment and concomitant diseases. Meanwhile, the limited sample size must be acknowledged. However, based on the gold standard method for diagnosis of liver biopsy performed in this cohort, we could clearly evaluate the progression of fibrosis and analyze the factors related to the severity of fibrosis in these paid repeat plasma donors with CHC.

According to the Metavir fibrosis score, patients with portal fibrosis accounted for the greatest proportion (F1/F2: 86.8%). Cirrhosis was found in only three patients (2.5%). Compared to a recent study on plasma donors with CHC for 12–19 years,[13] the morbidity of cirrhosis in our study was lower. We speculate that this difference is due to the different diagnostic tools used, because ultrasonography was applied to evaluate liver fibrosis in the previous study.[13] Also, the absence of a history of alcohol consumption might be another factor related to the lower morbidity from cirrhosis in the present study.

Furthermore, in CHC caused by whole blood transfusion, the morbidity of cirrhosis is up to 18–20% after approximately 20 years of infection.[4,5] The present study confirms that infection by a different mode of transmission may influence the progression of liver fibrosis in CHC patients. Patients with CHC caused by plasma donation showed a lower rate of progression to cirrhosis than those infected by blood transfusion. In a comparison to studies involving Irish[7] and East German[8] women with CHC infected by contaminated immunoglobulin after nearly 20 years of follow-up, our study showed the morbidity from cirrhosis in our cohort to approach that of the previous studies. However, due to the apparently different mode of HCV infection and the important impact of sex on the natural history of HCV infection,[2,3] there might be some discrepancy in the natural history of HCV infection between the Chinese plasma donors and Western Caucasian women. There is a need to establish the detailed mechanisms of the different modes of infection resulting in different progression of fibrosis.

Another aim of our study was to explore the possible factors associated with the presence of moderate to severe fibrosis (F ≥2). Currently, several well-accepted factors are reported to be associated with the development of fibrosis in CHC, including age at onset of infection[23] and sex.[24] For a similar infection period in the present study, the age at biopsy should have a similar meaning as the age at onset of infection. We found no significant difference in the age at biopsy or the sex proportion between patients with and without moderate to severe fibrosis. However, older age at biopsy and male sex showed a tendency towards more severe fibrosis. We speculate that the limited number of patients might have led to the present negative results.

It is worth mentioning that we found steatosis not to be associated with moderate to severe liver fibrosis (F ≥2). This finding is consistent with those of other investigations in which steatosis was demonstrated not to be related to the progression of fibrosis.[25] Accordingly, we speculate that hepatic steatosis might not be involved in the development of fibrosis in CHC patients. Additionally, the previous study demonstrated that decreased viral loads may reduce liver inflammation and further affect the progression of liver fibrosis indirectly.[26] Our study showed that patients with an HCV RNA load <4 $\log_{10}$ had a low proportion of moderate to severe liver fibrosis and we speculate that the relief of liver inflammation caused by a low virus load might play an important role in the progression of fibrosis. Although the question of whether or not liver inflammation has an effect on the progression of fibrosis remains controversial,[24,27] our results indicate that liver inflammation enhances the progression of fibrosis in CHC. For identifying moderate to severe liver fibrosis (F ≥2), the adjusted OR for liver inflammation and AST, which can non-invasively reflect changes in inflammation, reached 5.02 and 1.03, respectively, after adjusting for other factors. Therefore, we considered liver inflammation in patients with CHC related to repeated plasma donation, which was independently related to the presence of moderate to severe liver fibrosis. Thus, early inhibition of liver inflammation might delay fibrosis,[26] thus decreasing the occurrence of end-stage liver disease.

In summary, to our knowledge this is the first liver pathology analysis performed in a Chinese cohort of CHC plasma donors without IFN therapy. The progression of fibrosis in CHC patients infected by repeated plasma donation was found to be slow after HCV infection of approximately 20 years. Liver inflammation was found to be independently related to the development of moderate

to severe liver fibrosis, so prompt control of liver inflammation may postpone the progression of cirrhosis. Our study provides novel insights into the natural history of HCV infection caused by repeated plasma donation. Further experimental studies are needed to determine the underlying mechanisms responsible for these associations.

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Conflict of interest: The authors declare no conflicts of interest.

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