Disease progression in Chinese patients with hepatitis C virus RNA-positive infection via blood transfusion

YAN-FENG PAN[1], YAN ZHENG[1], TAO QIN[2], LEI FENG[3], QIAN ZHANG[1], XIAO-GONG PING[1], YAN-TING PAN[4], XIAO-PING WANG[1], LI BAI[1] and HUA-HUA LI[1]

[1]Department of Infection, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450000; [2]Department of Hepatobiliary Pancreatic Surgery, Henan Provincial People's Hospital, Zhengzhou, Henan 450003; [3]Department of Infection, The First Hospital Affiliated to Henan University, Kaifeng, Henan 475000; [4]Department of Surgery, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450000, P.R. China

Received August 4, 2015; Accepted August 23, 2016

DOI: 10.3892/etm.2016.3792

Abstract. The majority of patients with hepatitis C virus (HCV) in China were infected via blood transfusion prior to the year 1996. In this systematic retrospective cohort study, disease progression in 804 consecutive patients with transfusion-acquired HCV is investigated. In addition, the occurrence of compensated cirrhosis, decompensated cirrhosis and hepatocellular carcinoma (HCC) is analyzed among these patients, along with the risk factors for disease progression. Patients with cirrhosis or HCC were classified as the serious development group (SD group) and the remaining patients with chronic hepatitis were classified as the hepatitis group (H group). Significant differences were found between the two groups in age at the time of infection, duration of infection and age at the time of observation. SD group patients were significantly older at the time of transfusion (33.73 vs. 23.56 years; P<0.001), with a significantly longer mean duration of HCV infection (21.88 vs. 21.15 years; P=0.029) compared with that in the H group. Male gender and age at the time of transfusion were significant risk factors for HCC (OR=2.48, P=0.031 and OR=1.07, P=0.002, respectively). Age was a significant risk factor for disease progression in older Chinese patients with transfusion-acquired HCV, and there were significant differences in the prevalence of compensated cirrhosis, decompensated cirrhosis and HCC between the age groups (P<0.001), suggesting that more patients with HCV may develop cirrhosis or HCC in their third and fourth decades of infection. Results of the present study will be helpful for predicting disease progression in Chinese patients with HCV infected via blood transfusion.

Correspondence to: Dr Yan-Feng Pan, Department of Infection, The First Affiliated Hospital of Zhengzhou University, 1 East Jianshe Road, Zhengzhou, Henan 450000, P.R. China
E-mail: yanfengyf9@sina.com

Key words: blood transfusion, hepatitis C, cirrhosis, hepatocellular carcinoma, risk factors

Introduction

The hepatitis C virus (HCV) is considered to be the primary cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC) (1), and has a worldwide incidence of 3% (2-4). Based on China's National Seroprevalence Survey (years 1992-1995), China has a slightly higher incidence of ~3.2% (5). Diagnosis of HCV is based on the detection of anti-HCV antibodies and HCV RNA in the serum or liver (6,7).

Approximately 50-85% of patients with HCV develop a chronic infection with persistent viremia (8). A previous study demonstrated that, at 20 years after infection, 10-20% of these patients develop cirrhosis and 1-3% develop HCC (9). However, patients who achieved a sustained viral response (SVR) after treatment showed low rates of disease progression and end-stage liver disease at 35 years after infection (10).

The HCV genotype is thought to serve a role in determining the disease course and prognosis of hepatitis C, and has been shown to be associated with the sensitivity and specificity of diagnostic tests for hepatitis C (11). HCV genotype 1 is the most prevalent worldwide accounting for 83.4 million cases, which represents 46.2% of all HCV cases (12). The HCV 1b genotype has been found in 31.3% of chronic hepatitis cases, 50% of liver cirrhosis cases and 57.1% of HCC cases (13). Genotype affects the HCV treatment outcome. Pegylated interferon α (PEG IFNα) administered once weekly in combination with ribavirin was effective in 85% of patients with HCV infected with genotypes 2 or 3, but only in 45% of patients infected with genotypes 1 and 4 (14). Genotype 1 patients who are treated with telapravir and boceprevir in combination with PEG IFNα were shown to have increased SVR compared with patients treated with PEG IFNα alone (15,16). Transfusion of blood and blood products represents the primary transmission route for HCV in China. The wide-spread implementation of standardized HCV detection techniques in 1993 resulted in a significant decrease in the number of new infections via blood transfusions (17). However, since most transfusion-acquired HCV infections in China occurred between the years 1990 and 1996, individuals infected during that period have been infected with HCV for ~20 years, and progression of fibrosis puts them at risk of HCC (18).

Although the natural history of HCV infection and HCV infection routes have been extensively studied, there is limited information describing the association between the progression of HCV infection and the HCV infection route. Since blood transfusion remains an important mode of HCV infection, it is important to understand the progression of the disease in patients with HCV infected via blood transfusion. The molecular mechanisms underlying the progression of chronic HCV infection to cirrhosis and HCC remain largely unclear. Based on the clinical experience at the First Affiliated Hospital of Zhengzhou University (Zhengzhou, China_, a 20-year duration of HCV infection marks an important time point at which to evaluate disease progression. However, disease progression in patients with HCV has not been studied systematically in patients who were infected with HCV via transfusions twenty years earlier. In this systematic, retrospective cohort study, the progression of disease in patients who were infected with HCV through blood transfusions, and who did not receive antivirus therapy, is investigated.

Materials and methods

Study participants. The present study was a systematic retrospective analysis of 804 consecutive patients with chronic HCV infection who were admitted to the First Affiliated Hospital of Zhengzhou University between January 2011 and December 2013. Inclusion criteria were as follows: i) Patients infected with HCV via blood transfusion prior to 1996, and who did not receive antivirus treatment; ii) patients positive for anti-HCV antibodies and for HCV viral load. Routine screening of blood products for HCV using techniques and kits with better specificity and sensitivity was implemented in China in 1996. Therefore, the year 1996 was established as the cut-off point for transfusion history. Patients who had a history of blood transfusion prior to 1996, who were positive for HCV RNA and who had no other risk factors for HCV, were diagnosed with transfusion-associated hepatitis C. Factors such as drug abuse, hemodialysis and sexual exposure were excluded prior to concluding that HCV infection occurred via blood transfusion. Reasons for transfusion included bleeding associated with childbirth, ectopic pregnancy, surgery (breast, lung and thyroid cancer or benign lesions), trauma or bleeding disorders. No age-related issues were noted. The quantity of blood transfused was not recorded. Among HCV patients screened for inclusion in the present study, 43.7% had no history of blood transfusion. Exclusion criteria, which were established to rule out other possible causes of HCV infection, were as follows: i) Patients with other potential causes of liver disease concurrent with HCV (such as hepatitis B surface antigen-positive patients, patients with hepatitis A, hepatitis E, drug-induced liver injury and EB virus infection); ii) excess alcohol consumption (i.e., consuming >50 g of alcohol per day); iii) infection with human immunodeficiency virus (HIV) or presence of autoimmune or metabolic liver disease. In addition, patients who had received multiple transfusions were excluded. Demographic and clinical characteristics, including age, gender, time of infection and alcohol consumption, were collected from patient records. In addition, information regarding known risk factors, such as intravenous drug use, travel history, acupuncture, sexual habits and incarceration, was obtained from patient medical records. When the information was not available in the medical records, a telephone follow-up was performed. Body mass index data were not collected.

The HCV genotype was identified in 53.6% of the enrolled study patients. For the purpose of comparative analysis, patients with cirrhosis or HCC were classified as the serious development group (SD group) and the remaining patients with chronic hepatitis were classified as the hepatitis group (H group). Patients in the SD group were subdivided further into those with compensated cirrhosis, decompensated cirrhosis and HCC.

Ethical considerations. The internal review board of the First Affiliated Hospital of Zhengzhou University reviewed and approved the study protocol. Patients' records were reviewed retrospectively without patient identity so informed consent was waived for this study.

Laboratory and clinical evaluation of HCV infection. Serum samples were collected from the patients and the presence of anti-HCV antibodies was determined using commercially a available third generation ELISA kit (cat. no. 1707-12; Diagnostic Automation, Inc., Calabasas, CA, USA) according to the manufacturer's instructions. Seroreactive samples were tested for HCV RNA by quantitative polymerase chain reaction (PCR) using an ABI-7500 Fast Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The detection range of HCV RNA was >500 IU/ml, and since HCV RNA values did not show normal distribution and there was a large skewing of the data, logarithmic transformations were performed.

Diagnosis of cirrhosis was based on imaging indicators, such as irregular liver shape, presence of liver parenchyma nodules, presence of widened portal vein, splenomegaly or hypersplenism, or liver biopsy results which showed the formation of false lobules. Diagnosis of decompensated cirrhosis was based on the presence of cirrhosis, bleeding in the esophageal varices, ascites and hepatic encephalopathy. The diagnosis of HCC was based on pathological, histological and clinical criteria, and included evaluation of clinical manifestations, serum AFP levels and imaging features of HCC obtained through dynamic contrast-enhanced computed tomography or magnetic resonance imaging (MRI), which was performed in all patients. Among the typical imaging features noted were heterogeneous enhancement of the space-occupying lesion in the liver during the arterial phase of Multi-Detector-Row Computed Tomography and (or) dynamic contrast-enhanced MRI, which disappears during the venous phase or lag phase. Twenty-three patients had follow-up pathological confirmation of HCC diagnosis. Diagnosis of cirrhosis included the following: i) Indicators based on imaging, such as irregular liver shape, liver parenchyma nodule, widening of portal vein diameter and splenomegaly; ii) hypersplenism; and iii) liver biopsies revealing formation of false lobules. A total of 33 patients had biopsy results and the remaining patients were diagnosed based on the first and second criteria described above.

HCV genotyping. Direct sequencing of products generated by nested reverse transcription (RT)-PCR was performed using

the VERSANT HCV Genotype Assay (Siemens Healthcare Diagnostics, Tarrytown, NY, USA), according to the manufacturer's instructions. Viral RNA was extracted using TRIzol (Thermo Fisher Scientific, Inc.) and the first-round amplification was performed by RT-PCR under the following cycling conditions: 42˚C for 30 min; denaturation at 94˚C for 3 min; followed by 25 cycles of 94˚C for 10 sec, 55˚C for 20 sec and 72˚C for 30 sec. The product from the first round amplification (1 μl) was used as the template for the second-round PCR amplification under the following cycling conditions: 94˚C for 5 min; followed by 25 cycles of 94˚C for 10 sec, 55˚C for 20 sec and 72˚C for 30 sec. After digestion of the excess dNTPs and primers in the reaction mix with shrimp alkaline phosphatase (SAP), the PCR product was sequenced using the ABI 3130 DNA Sequencer (DIAN Medical Diagnostic Center Co., Ltd., Hangzhou, China), and the data were analyzed using genotyping software (www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi) of the National Center for Biotechnology Information.

Statistical analysis. Continuous variables are presented as the mean ± standard deviations. Independent *t* test was performed to compare the differences between two groups. One-way analysis of variance with Bonferroni post-hoc test was performed to compare the differences between more than two groups. Categorical variables are presented as counts and percentages, with chi-square tests or Fisher's exact tests for group comparison, appropriately. Logistic regression models were performed to detect the risk factors for patients with serious, progressive hepatitis disease or with HCC. Factors with P<0.05 in univariate analyses were included in the multivariate model. P<0.05 was considered to indicate a statistically significant difference. The statistical analyses were performed using SAS version 9.2 software (SAS Inc., Cary, NC, USA).

Results

Demographic and clinical characteristics of patients in the chronic hepatitis and serious development groups. Of the 804 subjects included in this study, 564 (70.15%) were included in the H group and 240 (29.85%) were included in the SD group. The study population comprised 333 males and 471 females with a mean age of 47.96 years. Fourteen subjects were infected with HCV prior to the year 1980; 179 subjects were infected during the years 1981-1990, and 607 subjects were infected during 1990 and 1996. Data for the year of HCV infection were missing in 4 cases. A total of 431 patients had diagnostic information for genotype. Genotype analysis showed that 301 subjects had genotype 1b, 124 subjects had genotype 2a, 4 subjects had genotype 1a, 1 subject had genotype 2b and 1 subject had genotype 6a. Genotype information was not available for 373 patients. The mean age for blood transfusion was 26.6 years, the mean duration of HCV infection was 21.38 years and the mean value of log HCV RNA was 14.9 (Table I).

The mean age of patients in the SD group was significantly higher compared with that of patients in the H group at the time of observation in this study (55.61 vs. 44.71 years; P<0.001). Patients in the SD group had a significantly higher mean age at the time of blood transfusion compared with patients in the H group (33.73 vs. 23.56 years, P<0.001). The mean duration of HCV infection was significantly longer in the SD group compared with that in the H group (21.88 vs. 21.15 years; P=0.029). The mean log HCV RNA value was significantly higher in the H group compared with that in the SD group (15.12 vs. 14.37; P<0.001). No significant differences were noted between the H and SD groups with regard to gender, HCV infected year, HCV RNA levels and genotypes (P>0.05). In addition, there was no significant difference between the two groups with regard to the incidence of diabetes and hypertension, which were the major co-morbidities in these patients. All study patients were screened for HBV. However, co-infection was rare, and only ~1% of patients were co-infected with HBV (Table I).

The mean platelet count (PLT), WBC count and albumin (ALB) levels of SD group patients were significantly lower in the SD group compared with those in the H group (PLT, 59.37 vs. 143.86, respectively, P=0.001; WBC, 4.16 vs. 4.77, respectively, P=0.004; ALB, 35.06 vs. 42.14, respectively, P<0.001). The mean prothrombin time (PT), aspartate transaminase (AST), γ-glutamyl transferase (GGT) and total bilirubin (TB) of SD group patients were significantly higher compared with those of H group patients (PT, 12.74 vs. 9.89, P<0.001; AST, 72.2 vs. 40.42, P<0.001; GGT, 63.00 vs. 43.00, P=0.002; TB, 24.32 vs. 13.25, P<0.001) (Table I).

Risk factors in the serious development group. Risk factors associated with the SD group are shown in Table II. Univariate logistic regression analysis revealed that age at blood transfusion, duration of HCV infection and log HCV RNA values were all significant risk factors in the SD group compared with the H group (P<0.001, P=0.02 and P<0.001, respectively). There was a significant increase in risk with every year's increase in age (OR=1.09, P<0.001) (data not shown), with every year's increase in age at the time of blood transfusion (OR=1.09, P<0.001) and with every year's increase in the duration of HCV infection (OR=1.05, P=0.02). However, the log HCV RNA value significantly decreased with disease progression (OR=0.87, P<0.001) (Table II).

Factors with P<0.05 in univariate analyses, including age at the time of blood transfusion and duration of HCV infection, were included in the multivariate logistic regression model. log HCV RNA values were excluded since log HCV RNA is a time-dependent variable. After adjusting for duration of HCV infection and log HCV RNA values, the risk of progression in SD group patients increased with every year's increase in age at the time of blood transfusion (OR=1.1, P<0.001). After adjusting for age at the time of blood transfusion and log HCV RNA values, the risk of progression in SD group patients increased with every year's increase in the duration of HCV infection (OR=1.09, P<0.001) (Table II).

Demographic and clinical characteristics of the SD group. The distribution of patients in the SD group is presented in Table III. Of the 240 patients in the SD group, 157 had compensated cirrhosis, 54 had decompensated cirrhosis and 29 had HCC. The mean age and age at the time of blood transfusion were both significantly higher in the HCC group compared with the compensated cirrhosis and decompensated cirrhosis groups (age, 61.34 vs. 54.89 and 54.61, P≤0.013; age

Table I. Demographic and clinical characteristics of patients by group.

	Total (n=804)	H group (n=564)	SD group (n=240)	P-value
Gender				0.141
Male	333 (41.42%)	243 (43.09%)	90 (37.5%)	
Female	471 (58.58%)	321 (56.91%)	150 (62.5%)	
Age (years)	47.96±12.42	44.71±11.81	55.61±10.3	<0.001[a]
Age at blood transfusion, years	26.6±12.25	23.56±11.62	33.73±10.66	<0.001[a]
Comorbidity				
Diabetes	15 (1.87%)	12 (2.13%)	3 (1.25%)	0.527
Hypertension	10 (1.24%)	8 (1.42%)	2 (0.83%)	0.482
HCV combined HBV	10 (1.24%)	3 (0.53 %)	7 (2.92%)	0.942
HCV infected year				0.08
≤1980	14 (1.75%)	7 (1.25%)	7 (2.93%)	
1981-1990	179 (22.38%)	118 (21.03%)	61 (25.52%)	
>1990	607 (75.88%)	436 (77.72%)	171 (71.55%)	
HCV infected duration, years	21.38±4.03	21.15±3.76	21.88±4.56	0.029[a]
HCV RNA, $x10^7$	2.75±27	3.40±32	1.19±3.23	0.106
log HCV RNA	14.9±2.29	15.12±2.28	14.37±2.24	<0.001[a]
Genotype[b]				0.555
Type 1b	301 (69.84%)	240 (70.80%)	61 (66.30%)	
Type 2a	124 (28.77%)	95 (28.02%)	29 (31.52%)	
Type 1a	4 (0.93%)	2 (0.59%)	2 (2.17%)	
Type 2b	1 (0.23%)	1 (0.29%)	0 (0%)	
Type 6a	1 (0.23%)	1 (0.29%)	0 (0%)	
Platelets, $10^3/\mu l$	-	143.86±58.90	59.37±35.47	0.001
WBC, $10^3/\mu l$	-	4.77±1.81	4.16±2.25	0.004
Prothrombin time, sec	-	9.89±1.32	12.74±2.80	<0.001[a]
Albumin, g/l	-	42.14±5.04	35.06±5.97	<0.001[a]
Alanine transaminase, U/l	-	48.11±60.68	51.88±41.79	0.245
Aspartate, U/l	-	40.42±34.58	72.20±53.02	<0.001[a]
γ-glutamyl transferase, U/l	-	43.00±47.08	63.00±49.52	0.002
Total bilirubin, mg/l	-	13.25±10.26	24.32±29.09	<0.001[a]

[a]Significant differences between groups; [b]use of Fisher's exact test. Continuous variables are presented as means and standard deviations, with independent *t* test for group comparisons. Categorical variables are presented as counts and percentages, with chi-square tests or Fisher's exact test for group comparisons. There were 4 missing values in HCV infected year, and 374 missing values in genotype. Patients with cirrhosis or HCC are classified as the serious development group (SD group) and the remaining patients with chronic hepatitis are classified as the hepatitis group (H group). H group, HCV, hepatitis C virus; HBV, hepatitis B virus; WBC, white blood cell.

at blood transfusion, 39.59 vs. 32.68 and 33.63, P≤0.043). No significant differences were found between groups in gender, year of HCV infection, duration of HCV infection, HCV RNA levels, log HCV RNA values and genotype studies (P>0.05; Table III).

Risk factors for HCC in the SD group. Risk factors for HCC in SD patients are shown in Table IV. Univariate logistic regression analysis showed that male gender and age at the time of blood transfusion were significant risk factors for HCC among SD patients with progressive disease (OR=2.35, P<0.037; and OR=1.07, P=0.002, respectively) (Table IV).

Male gender and age at transfusion were included in multivariate logistic regression analysis. Since there was a high correlation between variables of age at the time of observation and age at the time of blood transfusion, these variables were separated into two models. Multivariate analysis showed that males had a higher risk of developing HCC than females (OR=2.48, P=0.031); after adjusting for gender, the risk of HCC increased with every year's increase in age at the time of blood transfusion (OR=1.07, P=0.002) (Table IV).

Prevalence of hepatitis and progressive disease within different age groups. Fig. 1 depicts the prevalence of hepatitis (H group patients) and disease progression (SD group patients) in the different age groups (40-49, 50-59 and 60-69 years). Prevalence was highest in H group patients at 40-49 years old and SD group patients at 50-59 years old. A significant

Table II. Risk factors for the serious development group.

	Univariate		Multivariate	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Gender (ref = female)	0.79 (0.58-1.08)	0.142		
Age at blood transfusion	1.09 (1.07-1.10)	<0.001[a]	1.1 (1.08-1.11)	<0.001[a]
HCV infected duration	1.05 (1.01-1.08)	0.02[a]	1.09 (1.05-1.14)	<0.001[a]
log HCV RNA	0.87 (0.81-0.93)	<0.001[a]		
Genotype (ref = type 2)	0.92 (0.57-1.49)	0.746		

[a]Significant risk genotypes were analyzed as Type 1 vs. Type 2. Logistic regression was performed for risk factor comparisons between both groups; factors with P<0.05 in univariate analyses were included in the multivariate model. Log HCV RNA value was excluded from the multivariate analysis since it was a time-dependent variable. The significant effects in the multivariate model are shown in table. OR, odds ration; CI, confidence interval; HCV, hepatitis C virus.

Table III. Characteristics of patients in the serious development group by disease type and progression.

	Compensated cirrhosis (n=157)	Decompensated cirrhosis (n=54)	HCC (n=29)	P-value
Gender				0.105
Male	54 (34.39%)	20 (37.04%)	16 (55.17%)	
Female	103 (65.61%)	34 (62.96%)	13 (44.83%)	
Age, years	54.89±10.19	54.61±9.76	61.34±10.34[a,b]	0.005
Age at blood transfusion, years	32.68±10.5	33.63±10.35	39.59±10.55[a,b]	0.005
HCV infected year[c]				0.593
≤1980	7 (4.49%)	0 (0%)	0 (0%)	
1981-1990	40 (25.64%)	14 (25.93%)	7 (24.14%)	
>1990	109 (69.87%)	40 (74.07%)	22 (75.86%)	
HCV infection duration, years	22.22±5.07	20.98±3.29	21.76±3.36	0.226
HCV RNA, $x10^7$	1.13±2.64	1.69±5.14	0.65±1.27	0.355
log HCV RNA	14.51±2.2	14.13±2.31	14.06±2.33	0.428
Genotype[c]				0.934
Type 1	55 (69.62%)	10 (66.67%)	7 (77.78%)	
Type 2	24 (30.38%)	5 (33.33%)	2 (22.22%)	

[a]Significant differences compared with the compensated cirrhosis group; [b]significant differences compared with the decompensated cirrhosis group; [c]use of Fisher's exact test. Continuous variables are presented as means and standard deviations, with one-way analysis of variance and Bonferroni post hoc test for group comparisons. Categorical variables are presented as counts and percentages, with chi-square tests or Fisher's exact test for group comparisons. There was 1 missing value in HCV infected year, and 137 missing values in genotype. HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

difference was found in age between patients in the H and SD groups (P<0.001; Fig. 1).

Prevalence of compensated cirrhosis, decompensated cirrhosis and HCC within different age groups. Fig. 2 depicts the prevalence of compensated cirrhosis, decompensated cirrhosis and HCC in the different age groups (40-49, 50-59 and 60-69 years). The highest prevalence of both compensated and decompensated cirrhosis was seen in the 50-59 year-old age group and the highest prevalence of HCC was seen in the 60-69 year-old age group. Significant differences were found between different age groups in the prevalence of compensated cirrhosis, decompensated cirrhosis and HCC (P<0.001; Fig. 2).

Discussion

The detection of HCV antibodies prior to blood transfusion has been mandatory in China since 1993, and routine HCV screening using highly specific and sensitive kits has been implemented since 1996. It is therefore likely that patients who received transfusion prior to this time period, and who were HCV positive, had transfusion-associated HCV. The present study investigated disease progression in patients with blood

Table IV. Risk factors for hepatocellular carcinoma in the serious development group.

	Univariate		Multivariate	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Gender (ref = female)	2.35 (1.05-5.24)	0.037[a]	2.48 (1.09-5.65)	0.031[a]
Age at blood transfusion	1.07 (1.02-1.11)	0.002[a]	1.07 (1.02-1.11)	0.002[a]
HCV infected year	0.98 (0.89-1.07)	0.631		
log HCV RNA	0.92 (0.77-1.09)	0.322		
Genotype (ref = type 2)	1.53 (0.3-7.9)	0.613		

[a]Significant risk factors. Logistic regression analysis was performed for risk factor comparisons between patients with hepatocellular carcinoma and those with compensated cirrhosis; factors with P<0.05 in univariate analyses were included in the multivariate model. The significant effects in the multivariate model are shown in the table. Genotypes were analyzed in Type 1 vs. Type 2. OR, odds ratio; CI, confidence interval; HCV, hepatitis C virus.

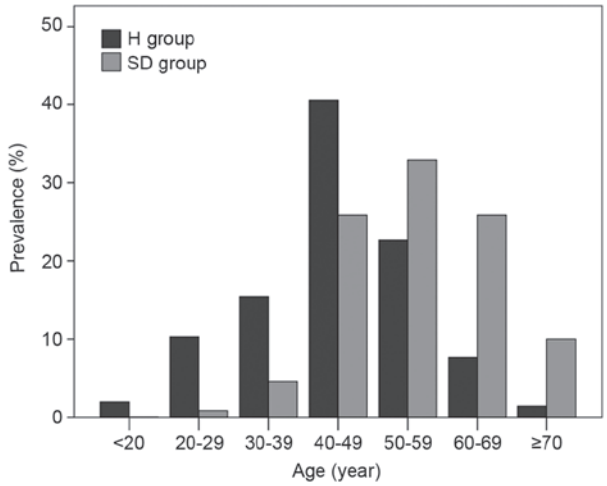

Figure 1. Prevalence of patients in the H and SD groups within different age groups. H group, hepatitis group; SD group, serious development group.

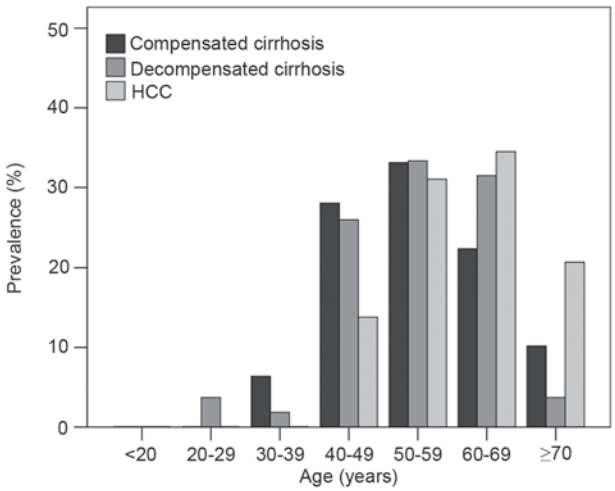

Figure 2. Prevalence of patients with compensated cirrhosis, decompensated cirrhosis and HCC in different age groups. HCC, hepatocellular carcinoma.

transfusion-acquired HCV who had not received antivirus therapy. Significant differences were identified in the age at the time of transfusion-acquired HCV infection, duration of HCV infection, age at the time of observation and HCV RNA load at the time of observation between those with disease progression to fibrosis and HCC (SD group) and those with chronic hepatitis without disease progression (H group). The two groups did not differ significantly in HCV genotype. Male gender and age at the time of transfusion were significant risk factors for HCC in these patients. Patients in the SD group who had developed liver cirrhosis and HCC were significantly older at the time of transfusion and had a longer mean duration of HCV infection compared to patients in the H group without fibrosis or HCC. Based on clinical experience and results of the present study, it can be proposed that a 20-year duration of HCV infection marks a critical time point at which to evaluate disease progression in patients with transfusion-acquired HCV.

Despite enormous progress in the treatment of HCV over the past two decades, almost a third of treated non-responders who fail to achieve SVR are at risk for disease progression (19). It has been suggested that the mode of HCV acquisition does not significantly impact the outcome of the disease (20). Nevertheless, a number of studies have investigated risk factors for disease progression in HCV patients, regardless of mode of transmission, and results have varied. Age at the time of infection, alcohol consumption and gender were shown to be primary risk factors of disease progression, and the median duration of infection associated with progression to cirrhosis was 30 years (21). However, a number of clinical trials investigating the incidence of cirrhosis have ignored the duration of infection, transmission route, alcohol consumption, and other associated factors, such as HIV co-infection in patients with HCV (22-25). In addition, numerous clinical trials failed to perform continuous, long-term observation of disease progression, resulting in significant differences in the rate of disease progression between various clinical trials (22-25). One report showed that progression of mild fibrosis occurred over a median interval of 52.5 months, while another reported that progression of fibrosis occurred in patients with mild chronic HCV within 5-10 years after infection and was associated with age, alcohol consumption and inflammatory activity (26,27). Although children with chronic HCV showed no significant histologic progression of disease at 5 years after infection, almost a third of the children showed increased severity of

fibrosis (28). A long-term study of HCV infections acquired at birth and followed for 35 years showed a slow disease progression and mild outcome (29). Results from another long-term study showed that cirrhosis in transfusion-acquired HCV patients was significantly associated with age at the time of infection and disease activity (30).

The progression to cirrhosis is often clinically silent, and some patients are not diagnosed with HCV until they present with complications of end-stage liver disease or HCC (31). Approximately 10-20% of patients developed cirrhosis and 1-3% of them developed HCC after 20 years of HCV infection (9). In the current study, based on clinical observations, patients with HCV gradually progressed to severe liver disease after ~20 years of HCV infection, and the progression seemed more rapid after 20 years, suggesting that 20 years is an important time point for evaluation. Since the majority of cases of HCV infection in China today were transfusion-acquired more than twenty years earlier, it can be suggested that patients with a 20-year duration of HCV infection are at a critical time point in terms of disease progression and must be evaluated for signs of progression.

It was previously reported that more than half of chronic patients with HCV had a history of transfusion, with a mean interval of 10 years, 21.2 years and 29 years between the time of transfusion and the time of diagnosis of chronic HCV, cirrhosis and HCC, respectively (32). Another study showed that 15.3% of patients with post-transfusion chronic HCV died from liver failure or HCC (33). These data suggested a slow, sequential progression of HCV infection acquired following transfusion. The lack of large-scale screening for hepatitis C in China's blood product industry prior to 1993 resulted in a large number of transfusion-related HCV infections.

Results of the present study indicated that patients in the SD group with progressive disease were significantly older at the time of observation compared with patients in the H group with chronic hepatitis and no obvious progression. In addition patients in the SD group were significantly older at the time of blood transfusion and had a longer mean duration of HCV infection compared with patients in the H group. In this study, duration of infection signified the time between the onset of HCV infection and the time at which the case was closed. Age at the time of transfusion, duration of infection and log HCV RNA values were significant risk factors for serious development. The present study showed that a majority of HCV-positive patients with HCC had also been diagnosed with cirrhosis. This is in contrast with HBV-related HCC, in which cirrhosis is present in a majority of cases (73-85%), but HBV infection is known to progress to HCC in the absence of cirrhosis (34).

Data from the present study showed that male gender and age at the time of blood transfusion were significant risk factors for HCC in patients with transfusion-acquired HCV. However, other studies have suggested that females may be at higher risk for HCC. Approximately 62.5% of asymptomatic patients with post-transfusion HCV who underwent biopsy for cirrhosis were female, and the median duration of infection was 21.5 years (35). However, that study reported a low progression to cirrhosis (20%), possibly because subjects were middle-aged, asymptomatic and infected at a young age. In contrast, a recent study in Italy by Zavaglia *et al* (36) of 248 previously transfused patients with HCV infection agreed with the data in the current study and showed that age at transfusion and male gender were independent predictors of HCC development; in fact, age at transfusion was shown to affect the risk for decompensation. In the present study, although gender was not a risk factor for the progression of liver disease, among those who showed the development of progressive disease (SD group), males had a higher risk of progression to HCC compared with females. This suggested that sex hormones may serve a role in HCV-related HCC. This finding of gender disparity is similar to results of previous studies (37,38). The Shimizu and Ito (39) study has suggested that estrogens protect against oxidative stress in liver injury and hepatic fibrosis.

Importantly, the present study demonstrated that patients with cirrhosis and HCC had significantly lower HCV RNA titers compared with patients with chronic hepatitis. This may possibly be due to hepatocyte damage induced by HCV replication and the immune response, resulting in necrosis of a large number of liver cells and a subsequent decrease in viral replication. A previous study indicated that HCV RNA in serum tended to increase with the progression of histopathological changes in the liver (40), while other data have demonstrated that patients with chronic active hepatitis had significantly higher HCV RNA titers compared with patients with chronic persistent hepatitis and those with cirrhosis or HCC (41). Although the data in the current study demonstrated a correlation between HCV RNA titers in patients with chronic hepatitis, cirrhosis and HCC, the HCV RNA titers reported in this study represent the titers at the time of patient enrollment, and not over the entire course of the disease.

A number of studies have investigated the role of HCV genotype in determining the severity and outcome of disease in HCV-infected patients (42). Genotype 1b was shown to be more prevalent among patients with decompensated cirrhosis and HCC (2,11,43). In addition, HCV genotype 1b was shown to influence the risk of HCC in patients with cirrhosis (44-46). However, other studies concluded that there was no significant association between HCV genotype and occurrence of cirrhosis (47,48). In the present study, although not statistically significant, a higher number of patients with HCC had genotype 1, compared with genotype 2, and genotype was not a significant risk factor for HCV disease progression in the selected geographic area. In future studies, the aim is to investigate further whether HCV genotypes serve a role in the pathogenicity of HCV disease in China.

Results of the present study are limited by several factors. First, this was a retrospective study, which precludes inferring direct causation. The retrospective nature of the study also precluded access to direct evidence of HCV-positive donor status. Secondly, the study focused on the progression of HCV infection and risk factors associated with transfusion-acquired disease, and did not investigate other risk factors for progression, such as obesity, insulin resistance, organ transplantation and tobacco smoking. In addition, the study did not collect data on the number of patients with HIV-positive status, although all enrolled patients were HIV negative. Additionally, almost half the patients were not genotyped, either due to economic considerations, or because their physicians did not recommend it (possibly because the majority of Chinese patients are known

to have genotype 1b) (49). Samples which were genotyped were grouped into the SD or H groups based on disease severity. The number of samples in the two groups was therefore not the same, and it is not possible to make a definitive conclusion regarding the influence of genotypes on outcomes. In addition, it is acknowledged that a study based on anti-HCV and HCV-RNA seropositivity may introduce bias regarding higher progression rates. Future large multicenter prospective cohort studies are necessary to confirm the findings of the present study and to further define the role of HCV genotypes in the outcomes of Chinese patients with transfusion-acquired HCV infection.

In conclusion, Chinese patients with HCV aged >50 years, who were infected for more than 20 years with transfusion-acquired HCV, showed significant disease progression. There was a positive correlation between the duration of HCV infection and the possibility of progressing to chronic hepatitis. Male gender and age at the time of transfusion were significant risk factors for HCC development. The exponential progression of transfusion-acquired HCV makes this disease a serious challenge for infected patients in the third and fourth decades after infection. Although the present study did not demonstrate a significant correlation between genotype and disease progression, the results will be particularly helpful for clinical nursing staff and physicians to predict the progression of transfusion-acquired HCV infection based on patients' gender and age at the time of transfusion-acquired infection and duration of infection.

References

1. Alter MJ: Epidemiology of hepatitis C virus infection. World J Gastroenterol 13: 2436-2441, 2007.
2. Irshad M, Mankotia DS and Irshad K: An insight into the diagnosis and pathogenesis of hepatitis C virus infection. World J Gastroenterol 19: 7896-7909, 2013.
3. Lauer GM and Walker BD: Hepatitis C virus infection. N Engl J Med 345: 41-52, 2001.
4. Pybus OG, Barnes E, Taggart R, Lemey P, Markov PV, Rasachak B, Syhavong B, Phetsouvanah R, Sheridan I, Humphreys IS, *et al*: Genetic history of hepatitis C virus in East Asia. J Virol 83: 1071-1082, 2009.
5. Xia GLLC, Cao HL, Bi SL, Zhan MY, Su CA, Nan JH and Qi XQ: Prevalence of hepatitis B and C virus infections in the general Chinese population. Results from a nationwide cross-sectional seroepidemiologic study of hepatitis A, B, C, D and E virus infections in China. Int Hepatol Commun 5: 62-73, 1996.
6. Kato N, Yokosuka O, Hosoda K, Ito Y, Ohto M and Omata M: Detection of hepatitis C virus RNA in acute non-A, non-B hepatitis as an early diagnostic tool. Biochem Biophys Res Commun 192: 800-807, 1993.
7. Yuki N, Hayashi N, Ohkawa K, Hagiwara H, Oshita M, Katayama K, Sasaki Y, Kasahara A, Fusamoto H and Kamada T: The significance of immunoglobulin M antibody response to hepatitis C virus core protein in patients with chronic hepatitis C. Hepatology 22: 402-406, 1995.
8. TCLDA: IDaPDB: Prevention and treatment of hepatitis C. Chin J Hepatol 12: 194-198, 2004.
9. Ding JYX and Zhang HM: Analysis on testing of elderly patients with hepatitis C Lab Med Clin 17: 1474-1475, 2009.
10. Wiese M, Fischer J, Lobermann M, Göbel U, Grüngreiff K, Güthoff W, Kullig U, Richter F, Schiefke I, Tenckhoff H, *et al*; East German HCV Study Group: Evaluation of liver disease progression in the German hepatitis C virus (1b)-contaminated anti-D cohort at 35 years after infection. Hepatology 59: 49-57, 2014.
11. Zein NN: Clinical significance of hepatitis C virus genotypes. Clin Microbiol Rev 13: 223-235, 2000.
12. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG and Barnes E: Global distribution and prevalence of hepatitis C virus genotypes. Hepatology 61: 77-87, 2015.
13. Utama A, Budiarto BR, Monasari D, Octavia TI, Chandra IS, Gani RA, Hasan I, Sanityoso A, Miskad US, Yusuf I, *et al*: Hepatitis C virus genotype in blood donors and associated liver disease in Indonesia. Intervirology 51: 410-416, 2008.
14. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL Jr, Häussinger D, Diago M, Carosi G, Dhumeaux D, *et al*: Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 347: 975-982, 2002.
15. Kieffer TL, Sarrazin C, Miller JS, Welker MW, Forestier N, Reesink HW, Kwong AD and Zeuzem S: Telaprevir and pegylated interferon-alpha-2a inhibit wild-type and resistant genotype 1 hepatitis C virus replication in patients. Hepatology 46: 631-639, 2007.
16. Kieffer TL, Kwong AD and Picchio GR: Viral resistance to specifically targeted antiviral therapies for hepatitis C (STAT-Cs). J Antimicrob Chemother 65: 202-212, 2010.
17. Allain JP, Thomas I and Sauleda S: Nucleic acid testing for emerging viral infections. Transfus Med 12: 275-283, 2002.
18. Xu GG, Wu SM, Zhou XQ, Zhang QB, Kang LY, Zhou XM, Jiang Y, Qi X and Ren XJ: Clinical characteristics of 638 patients infected with hepatitis C virus by post blood transfusion, in Shanghai. Zhonghua Liu Xing Bing Xue Za Zhi 32: 388-391, 2011 (In Chinese).
19. Dabbouseh NM and Jensen DM: Future therapies for chronic hepatitis C. Nat Rev Gastroenterol Hepatol 10: 268-276, 2013.
20. Rerksuppaphol S, Hardikar W and Dore GJ: Long-term outcome of vertically acquired and post-transfusion hepatitis C infection in children. J Gastroenterol Hepatol 19: 1357-1362, 2004.
21. Poynard T, Bedossa P and Opolon P: Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR and DOSVIRC groups. Lancet 349: 825-832, 1997.
22. Conjeevaram HS, Fried MW, Jeffers LJ, Terrault NA, Wiley-Lucas TE, Afdhal N, Brown RS, Belle SH, Hoofnagle JH, Kleiner DE, *et al*: Peginterferon and ribavirin treatment in African American and Caucasian American patients with hepatitis C genotype 1. Gastroenterology 131: 470-477, 2006.
23. Ferenci P, Fried MW, Shiffman ML, Smith CI, Marinos G, Goncales FL Jr, Häussinger D, Diago M, Carosi G, Dhumeaux D, *et al*: Predicting sustained virological responses in chronic hepatitis C patients treated with peginterferon alfa-2a (40 KD)/ribavirin. J Hepatol 43: 425-433, 2005.
24. Jensen DM, Marcellin P, Freilich B, Andreone P, Di Bisceglie A, Brandão-Mello CE, Reddy KR, Craxi A, Martin AO, Teuber G, *et al*: Re-treatment of patients with chronic hepatitis C who do not respond to peginterferon-alpha2b: A randomized trial. Ann Intern Med 150: 528-540, 2009.
25. Shiffman ML, Di Bisceglie AM, Lindsay KL, Morishima C, Wright EC, Everson GT, Lok AS, Morgan TR, Bonkovsky HL, Lee WM, *et al*; Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis Trial Group.: Peginterferon alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. Gastroenterology 126: 1015-1023; discussion 1947, 2004.
26. Alberti A, Benvegnù L, Boccato S, Ferrari A and Sebastiani G: Natural history of initially mild chronic hepatitis C. Dig Liver Dis 36: 646-654, 2004.
27. Boccato S, Pistis R, Noventa F, Guido M, Benvegnù L and Alberti A: Fibrosis progression in initially mild chronic hepatitis C. J Viral Hepat 13: 297-302, 2006.
28. Mohan P, Barton BA, Narkewicz MR, Molleston JP, Gonzalez-Peralta RP, Rosenthal P, Murray KF, Haber B, Schwarz KB and Goodman ZD: Evaluating progression of liver disease from repeat liver biopsies in children with chronic hepatitis C: A retrospective study. Hepatology 58: 1580-1586, 2013.
29. Casiraghi MA, De Paschale M, Romanò L, Biffi R, Assi A, Binelli G and Zanetti AR: Long-term outcome (35 years) of hepatitis C after acquisition of infection through mini transfusions of blood given at birth. Hepatology 39: 90-96, 2004.
30. Minola E, Prati D, Suter F, Maggiolo F, Caprioli F, Sonzogni A, Fraquelli M, Paggi S and Conte D: Age at infection affects the long-term outcome of transfusion-associated chronic hepatitis C. Blood 99: 4588-4591, 2002.
31. Chen SL and Morgan TR: The natural history of hepatitis C virus (HCV) infection. Int J Med Sci 3: 47-52, 2006.

32. Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, Furuta S, Akahane Y, Nishioka K, Purcell RH, *et al*: Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: Analysis by detection of antibody to hepatitis C virus. Hepatology 12: 671-675, 1990.
33. Tong MJ, el-Farra NS, Reikes AR and Co RL: Clinical outcomes after transfusion-associated hepatitis C. N Engl J Med 332: 1463-1466, 1995.
34. Salhab M and Canelo R: An overview of evidence-based management of hepatocellular carcinoma: A meta-analysis. J Cancer Res Ther 7: 463-475, 2011.
35. Reggiardo MV, Fay F, Tanno M, Garcia-Camacho G, Bottaso O, Ferretti S, Godoy A, Guerrita C, Paez M, Tanno F, *et al*: Natural history of hepatitis C virus infection in a cohort of asymptomatic post-transfused subjects. Ann Hepatol 11: 658-666, 2012.
36. Zavaglia C, Silini E, Mangia A, Airoldi A, Piazzolla V, Vangeli M, Stigliano R, Foschi A, Mazzarelli C and Tinelli C: Prognostic factors of hepatic decompensation and hepatocellular carcinoma in patients with transfusion-acquired HCV infection. Liver Int 34: e308-e316, 2014.
37. Akiyama T, Mizuta T, Kawazoe S, Eguchi Y, Kawaguchi Y, Takahashi H, Ozaki I and Fujimoto K: Body mass index is associated with age-at-onset of HCV-infected hepatocellular carcinoma patients. World J Gastroenterol 17: 914-921, 2011.
38. Farinati F, Sergio A, Giacomin A, Di Nolfo MA, Del Poggio P, Benvegnu L, Rapaccini G, Zoli M, Borzio F, Giannini EG, *et al*; Italian Liver Cancer group: Is female sex a significant favorable prognostic factor in hepatocellular carcinoma? Eur J Gastroenterol Hepatol 21: 1212-1218, 2009.
39. Shimizu I and Ito S: Protection of estrogens against the progression of chronic liver disease. Hepatol Res 37: 239-247, 2007.
40. Kato N, Yokosuka O, Hosoda K, Ito Y, Ohto M and Omata M: Quantification of hepatitis C virus by competitive reverse transcription-polymerase chain reaction: Increase of the virus in advanced liver disease. Hepatology 18: 16-20, 1993.
41. Mita E, Hayashi N, Kanazawa Y, Hagiwara H, Ueda K, Kasahara A, Fusamoto H and Kamada T: Hepatitis C virus genotype and RNA titer in the progression of type C chronic liver disease. J Hepatol 21: 468-473, 1994.
42. Ripoli M and Pazienza V: Impact of HCV genetic differences on pathobiology of disease. Expert Rev Anti Infect Ther 9: 747-759, 2011.
43. Silini E, Bono F, Cividini A, Cerino A, Bruno S, Rossi S, Belloni G, Brugnetti B, Civardi E, Salvaneschi L, *et al*: Differential distribution of hepatitis C virus genotypes in patients with and without liver function abnormalities. Hepatology 21: 285-290, 1995.
44. Kobayashi M, Tanaka E, Sodeyama T, Urushihara A, Matsumoto A and Kiyosawa K: The natural course of chronic hepatitis C: A comparison between patients with genotypes 1 and 2 hepatitis C viruses. Hepatology 23: 695-699, 1996.
45. Silini E, Bottelli R, Asti M, Bruno S, Candusso ME, Brambilla S, Bono F, Iamoni G, Tinelli C, Mondelli MU and Ideo G: Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: A case-control study. Gastroenterology 111: 199-205, 1996.
46. Takada A, Tsutsumi M, Zhang SC, Okanoue T, Matsushima T, Fujiyama S and Komatsu M: Relationship between hepatocellular carcinoma and subtypes of hepatitis C virus: A nationwide analysis. J Gastroenterol Hepatol 11: 166-169, 1996.
47. Dusheiko G, Schmilovitz-Weiss H, Brown D, McOmish F, Yap PL, Sherlock S, McIntyre N and Simmonds P: Hepatitis C virus genotypes: An investigation of type-specific differences in geographic origin and disease. Hepatology 19: 13-18, 1994.
48. Yamada M, Kakumu S, Yoshioka K, Higashi Y, Tanaka K, Ishikawa T and Takayanagi M: Hepatitis C virus genotypes are not responsible for development of serious liver disease. Dig Dis Sci 39: 234-239, 1994.
49. Dong ZX, Zhou HJ, Wang JH, Xiang XG, Zhuang Y, Guo SM, Gui HL, Zhao GD, Tang WL, Wang H and Xie Q: Distribution of hepatitis C virus genotypes in Chinese patients with chronic hepatitis C: Correlation with patients' characteristics and clinical parameters. J Dig Dis 13: 564-570, 2012.