World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i12.1385

World J Gastroenterol 2012 March 28; 18(12): 1385-1390
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Assessment of disease progression in patients with transfusion-associated chronic hepatitis C using transient elastography

Ryota Masuzaki, Ryosuke Tateishi, Haruhiko Yoshida, Toru Arano, Koji Uchino, Kenichiro Enooku, Eriko Goto, Hayato Nakagawa, Yoshinari Asaoka, Yuji Kondo, Tadashi Goto, Hitoshi Ikeda, Shuichiro Shiina, Masao Omata, Kazuhiko Koike

Ryota Masuzaki, Ryosuke Tateishi, Haruhiko Yoshida, Toru Arano, Koji Uchino, Kenichiro Enooku, Eriko Goto, Hayato Nakagawa, Yoshinari Asaoka, Yuji Kondo, Tadashi Goto, Shuichiro Shiina, Kazuhiko Koike, Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, 1138655 Tokyo, Japan
Hitoshi Ikeda, Department of Clinical Laboratory, The University of Tokyo Hospital, 1138655 Tokyo, Japan
Masao Omata, Yamanashi Prefectural Hospital Organization, 4008506 Yamanashi, Japan
Author contributions: Masuzaki R, Tateishi R and Yoshida H designed research; Masuzaki R, Arano T, Uchino K, Enooku K, Goto E, Nakagawa H, Asaoka Y, Kondo Y and Goto T collected data; Tateishi R and Yoshida H analyzed data; Masuzaki R and Yoshida H wrote the paper; Tateishi R, Ikeda H and Shiina S reviewed the paper; Omata M and Koike K supervised the entire project.
Correspondence to: Dr. Ryota Masuzaki, Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, 113-8655 Tokyo, Japan. ryota-m@umin.net
Telephone: +81-3-38155411 Fax: +81-3-38140021
Received: May 12, 2011 Revised: August 1, 2011
Accepted: January 22, 2012
Published online: March 28, 2012

Abstract

AIM: To evaluate the relationship between liver stiffness and duration of infection in blood transfusion-associated hepatitis C virus (HCV) patients with or without hepatocellular carcinoma (HCC).

METHODS: Between December 2006 and June 2008, a total of 524 transfusion-associated HCV-RNA positive patients with or without HCC were enrolled. Liver stiffness was obtained noninvasively by using Fibroscan (Echosens, Paris, France). The date of blood transfusion was obtained by interview. Duration of infection was derived from the interval between the date of blood transfusion and the date of liver stiffness measurement (LSM). Patients were stratified into four groups based on the duration of infection (17-29 years; 30-39 years; 40-49 years; and 50-70 years). The difference in liver stiffness between patients with and without HCC was assessed in each group. Multiple linear regression analysis was used to determine the factors associated with liver stiffness.

RESULTS: A total of 524 patients underwent LSM. Eight patients were excluded because of unsuccessful measurements. Thus 516 patients were included in the current analysis (225 with HCC and 291 without). The patients were 244 men and 272 women, with a mean age of 67.8 ± 9.5 years. The median liver stiffness was 14.3 kPa (25.8 in HCC group and 7.6 in non-HCC group). The patients who developed HCC in short duration of infection were male dominant, having lower platelet count, with a history of heavier alcohol consumption, showing higher liver stiffness, and receiving blood transfusion at an old age. Liver stiffness was positively correlated with duration of infection in patients without HCC (r = 0.132, P = 0.024) but not in patients with HCC (r = -0.103, P = 0.123). Liver stiffness was significantly higher in patients with HCC than in those without in each duration group (P < 0.0001). The factors significantly associated with high liver stiffness in multiple regression were age at blood transfusion (P < 0.0001), duration of infection (P = 0.0015), and heavy alcohol consumption (P = 0.043)

CONCLUSION: Although liver stiffness gradually increases over time, HCC develops in patients with high stiffness value regardless of the duration of infection.

Key words: Transfusion-associated hepatitis C; Transient elastography; Hepatocellular carcinoma; Liver stiffness; Ultrasonography; Liver fibrosis

Peer reviewer: Sebastian Mueller, MD, PhD, Professor of Medicine, Department of Internal Medicine, Salem Medical Center, and Center for Alcohol Research, University of Heidelberg, Zeppelinstraße 11-33, Heidelberg 69121, Germany

Masuzaki R, Tateishi R, Yoshida H, Arano T, Uchino K, Enooku K, Goto E, Nakagawa H, Asaoka Y, Kondo Y, Goto T, Ikeda H, Shiina S, Omata M, Koike K. Assessment of disease progression in patients with transfusion-associated chronic hepatitis C using transient elastography. *World J Gastroenterol* 2012; 18(12): 1385-1390 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i12/1385.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i12.1385

INTRODUCTION

Hepatitis C virus (HCV) is a leading cause of chronic liver diseases, presenting serious public health problems worldwide[1,2]. HCV infection generally shows an asymptomatic onset and slow fibrosis progression, with cirrhosis developing after 20-50 years[3-7]. Progression of disease is known to depend on patients' characteristics at the onset of infection[8-12]. Infection at old age, male gender and heavy alcohol consumption are reported to be independently associated with rapid disease progression.

The onset of HCV infection can be reliably estimated in transfusion-associated chronic hepatitis C patients, in contrast to repeating injecting-drug users. In Japan, about 40% of chronic hepatitis C patients and HCV-related HCC patients have a history of blood transfusion typically in 1950s and 1960s[13], when blood supply depended on paid blood donors. Not a few of the blood donors were also injecting-drug users, mainly methamphetamine, among whom HCV spread first after the end of World War Ⅱ. Viral spread started to decline in Japan after commercial blood banks were entirely abolished in 1969[14].

Chronic hepatitis C with cirrhosis is a strong risk factor for hepatocellular carcinoma (HCC)[15,16]. It has been shown that the risk of HCC increases with the degree of liver fibrosis[17]. Until recently, however, the degree of liver fibrosis could be reliably assessed only with liver biopsy, an invasive procedure with the possibility of serious complications[18,19].

Liver stiffness, which can be noninvasively measured with transient elastography, has been recently reported to be well correlated with histologically assessed liver fibrosis stage[20]. We previously reported that liver stiffness is strongly associated with the risk of HCC[21,22]. The calculated stratum-specific likelihood ratio indicated that the post-test odds for the presence of HCC increase five-fold when liver stiffness is higher than 25 kPa and decrease to one-fifth when lower than 10 kPa. Furthermore, we have confirmed in a prospective study that liver stiffness is a significant risk factor for HCC development, together with male gender, clinical cirrhosis and serum albumin level. However, in those studies we did not fully consider the duration of HCV infection and the age at the onset of infection, which are indicated in several studies to be associated with disease progression.

The aim of this study is to evaluate the association between liver stiffness and the duration of infection in blood transfusion-associated hepatitis C patients with and without HCC, focusing on the risk of HCC development.

MATERIALS AND METHODS

Patients

This study conformed to the ethical guideline of the 1975 Helsinki Declaration and the ethical guidelines for epidemiologic research designed by Japanese Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labor and Welfare. The study design was approved by the ethics committee of the authors' institution. Between December 2006 and June 2008, a total of 1562 patients positive for HCV RNA visited the liver clinic of authors' institution. Among these patients, those with a history of receiving blood transfusion were consecutively enrolled (229 with HCC and 295 without). We excluded from the present study those patients with concomitant hepatitis B virus surface antigen positivity, patients with uncontrollable ascites, patients on interferon therapy, and patients who received multiple blood transfusions.

Transient elastography

Liver stiffness was obtained noninvasively by using Fibroscan (Echosens, Paris, France), a newly developed medical device based on elastometry. Liver stiffness measurement (LSM) was considered valid only when at least eight acquisitions were successful with a success rate of at least 60% and the ratio of inter-quartile range (IQR) to the median value was larger than 30%.

Diagnosis of hepatocellular carcinoma

In patients with HCC, the cancer was diagnosed by dynamic computed tomography (CT), where intrahepatic nodules with hyperattenuation in the arterial phase with washout in the late phase were considered as definite HCC[23,24]. Histopathological diagnosis, using ultrasound-guided biopsy samples, was also performed when required. In patients without HCC, the cancer was ruled out by ultrasonography. No HCC was detected in the subsequent six-month follow-up period among these patients.

Laboratory tests

We determined the following parameters on the day of LSM: serum albumin and total bilirubin concentrations, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, prothrombin activity and platelet count. Serogrouping of HCV was assessed by enzyme-linked immunosorbent assay (ELISA) (Immucheck F-HCV Gr Kokusai; Sysmex, Kobe, Japan)[25]. Based on the prevalence of each HCV genotype in Japan, serogroup 1 was assumed to represent genotype 1b and serogroup 2 to represent genotype 2a or 2b.

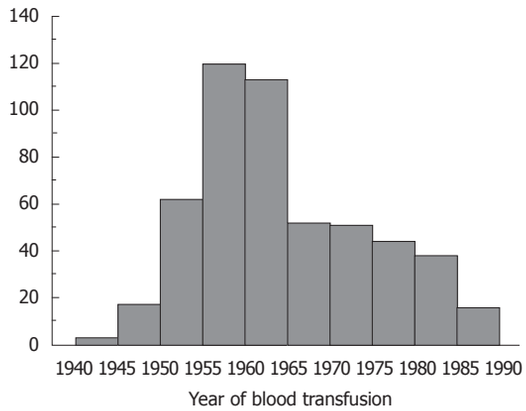

Figure 1 Frequency distribution of the year of receiving blood transfusion among the subjects. There is a peak around the year 1960.

Table 1 Characteristics of patients according to presence of hepatocellular carcinoma *n* (%)

Characteristics	HCC	Non-HCC	*P* value
Overall patients	*n* = 225	*n* = 291	
Gender (M/F)	126/99	118/173	0.0005
Age (yr)[1]	71.2 (66.1-75.7)	68.1 (58.7-72.4)	< 0.0001
Platelet count (10^9/L)[1]	95 (74-133)	161 (111-200)	< 0.0001
ALT (IU/L)[1]	48 (34-68)	42 (25-69)	0.006
Alcohol consumption > 50 g/d	51 (22.7)	28 (9.6)	< 0.0001
Liver stiffness (kPa)[1]	25.8 (17.3-37.4)	7.6 (5.6-13.9)	< 0.0001
IQR (kPa)[1]	4.0 (2.5-6.0)	1.6 (1.2-2.6)	< 0.0001
Duration (17-29 yr)	*n* = 34	*n* = 64	
Gender (M/F)	25/9	38/26	0.0028
Age (yr)[1]	73.1 (65.7-77.1)	59.7 (47.2-69.2)	0.033
Platelet count (10^9/L)[1]	95 (76-154)	180 (116-229)	< 0.0001
ALT (IU/L)[1]	51 (34-89)	42 (22-77)	0.2071
Alcohol consumption > 50 g/d	12 (35.3)	9 (14.1)	0.023
Liver stiffness (kPa)[1]	26.1 (16.8-53.3)	5.9 (4.9-12.1)	< 0.0001
Duration (30-39 yr)	*n* = 40	*n* = 59	
Gender (M/F)	16/24	23/36	0.9191
Age (yr)[1]	72.0 (65.4-76.7)	62.3 (55.7-68.6)	< 0.0001
Platelet count (10^9/L)[1]	93 (68-120)	151 (97-215)	< 0.0001
ALT (IU/L)[1]	42 (33-65)	48 (27-80)	0.7591
Alcohol consumption > 50 g/d	6 (15)	7 (11.9)	0.7641
Liver stiffness (kPa)[1]	28.7 (20.1-37.8)	7.4 (5.7-13.8)	< 0.0001
Duration (40-49 yr)	*n* = 101	*n* = 127	
Gender (M/F)	58/43	51/76	0.0113
Age (yr)[1]	69.2 (65.8-73.6)	69.9 (65.7-72.7)	0.8107
Platelet count (10^9/L)[1]	97 (67-136)	163 (112-195)	< 0.0001
ALT (IU/L)[1]	48 (34-69)	38 (23-64)	0.0080
Alcohol consumption > 50 g/d	25 (24.8)	8 (6.3)	0.0001
Liver stiffness (kPa)[1]	25.1 (17.5-37.4)	8.2 (5.8-14.1)	< 0.0001
Duration (50-70 yr)	*n* = 50	*n* = 41	
Gender (M/F)	27/23	18/23	0.4016
Age (yr)[1]	74.4 (70.0-78.1)	73.7 (66.3-79.2)	0.5658
Platelet count (10^9/L)[1]	97 (81-141)	147 (117-189)	0.0001
ALT (IU/L)[1]	52 (36-69)	46 (32-63)	0.1700
Alcohol consumption > 50 g/d	8 (16)	4 (9.8)	0.5363
Liver stiffness (kPa)[1]	16.0 (8.0-36.3)	7.9 (6.5-15.8)	< 0.0001

[1]Data are expressed as median (25th-75th percentiles). ALT: Alanine aminotransferase; IQR: Inter-quartile range; HCC: Hepatocellular carcinoma; M: Male; F: Female.

Duration of infection and liver stiffness progression

The date of blood transfusion was obtained by interview. Duration of infection was derived from the interval between the date of blood transfusion and the date of LSM. The rate of liver stiffness progression was calculated as follows: present liver stiffness-minimal stiffness value in the cohort (kPa)/interval after blood transfusion (years).

Statistical analysis

Data were expressed as median and 25th-75th percentiles in parenthesis unless otherwise indicated. The categorical variables were compared by χ^2 tests, whereas continuous variables were compared with unpaired Student's *t* test (parametric) or Mann-Whitney *U* test (non-parametric). A P value < 0.05 on two-tailed test was considered significant.

The correlation between liver stiffness and the interval from blood transfusion was assessed by Spearman's rank correlation. The duration of infection was arbitrarily stratified into 4 groups, 17-29 years; 30-39 years; 40-49 years; and 50-70 years. The difference in liver stiffness according to the presence of HCC was assessed in each group. Multiple linear regression analysis was used to determine the factors associated with liver stiffness. Processing and analysis were performed by using the S-plus Version 7 (TIBCO Software Inc., Palo Alto, CA, United States).

RESULTS

Patients' profile

A total of 524 patients underwent LSM. Eight patients were excluded because of unsuccessful measurements, mostly due to obesity (four patients had IQR/median > 30% and four had a success rate lower than 60%). Thus 516 patients were included in the current analysis (225 with HCC and 291 without). Their characteristics at the time of LSM are summarized in Table 1. The patients were 244 men and 272 women, with a mean age of 67.8 ± 9.5 years. The median liver stiffness was 14.3 kPa (25.8 in HCC group and 7.6 in non-HCC group). Figure 1 shows the frequency distribution of the year of receiving blood transfusion among the subjects. A peak is noted around the year 1960.

Characteristics of patients according to the duration of infection

Characteristics of patients were compared between patients with and without HCC in each duration of infection group (Table 1). The patients who developed HCC in short duration of infection were male dominant, having low platelet count, with a history of heavier alcohol consumption, showing higher liver stiffness, and receiving blood transfusion at an older age.

Correlation between liver stiffness and duration of infection

The correlation between liver stiffness and duration of

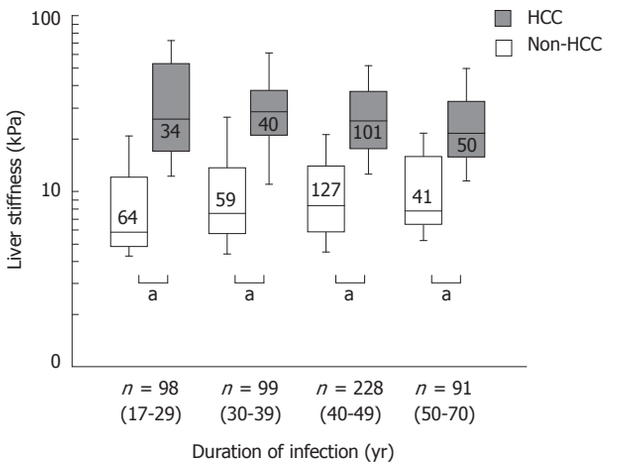

Figure 2 Duration of infection and liver stiffness. Liver stiffness was higher in patients with HCC than in patients without in each infection duration group ($^aP < 0.0001$ by Mann-Whitney *U* test).

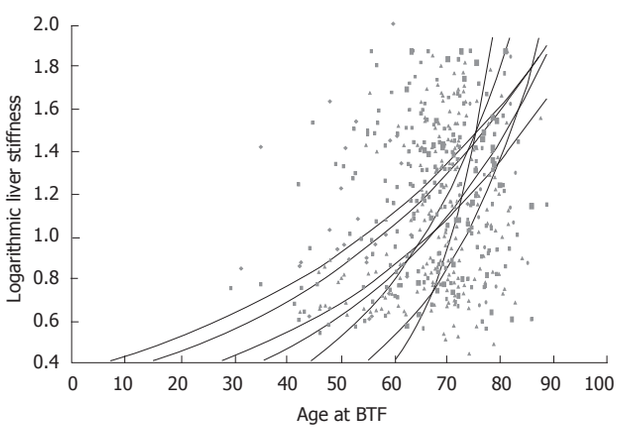

Figure 3 Age at blood transfusion and liver stiffness. Stiffness at present (each dot) and stiffness at BTF (assumed to normal value) were connected approximate logarithmic curve. Stiffness progressions become rapid in older age at BTF.

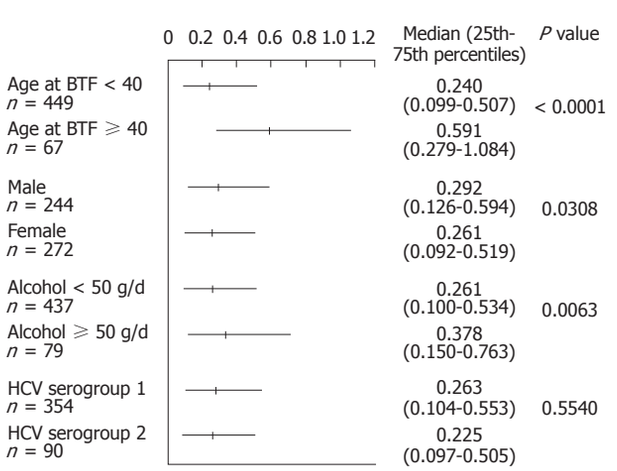

Figure 4 Liver stiffness progression rate. The progression rate is significantly higher in patients who were older than 40 at the time of blood transfusion, whose alcohol consumption is more than 50 g/d, and who are male. There is no significant difference according to hepatitis C virus (HCV) serotypes. Horizontal bar represents median value and 25th-75th percentiles.

infection was significant in patients without HCC ($r = 0.132$, $Z = 2.256$, $P = 0.024$) but not in patients with HCC ($r = -0.103$, $Z = -1.54$, $P = 0.123$). When the duration of infection was stratified into 4 groups, 17-29 years; 30-39 years; 40-49 years; and 50-70 years, liver stiffness was higher in patients with HCC than in patients without in each group ($P < 0.0001$, Figure 2).

Multiple regression analysis

The relationship between present liver stiffness and patients' characteristics, i.e., the age at blood transfusion, duration of infection, gender, and alcohol consumption (alcohol > 50 g/d) was analyzed with multiple linear regression analysis. The results showed that the age at blood transfusion was positively correlated with liver stiffness, with a coefficient of +0.336 per year for kPa, $P < 0.0001$, independently of the duration of infection (coefficient +0.272 per year for kPa, $P = 0.0015$). This suggests that fibrosis progression is more rapid when infection is acquired at older ages. Alcohol consumption was also significantly correlated with a positive coefficient (coefficient +4.183 for kPa, $P = 0.043$).

Stiffness progression and the age at blood transfusion

The progression of liver fibrosis, as represented by the increase in liver stiffness, must have been rapid in patients who have high liver stiffness in spite of short duration of HCV infection. We assumed that the liver stiffness was normal, that is, 2.9 kPa, when patients received blood transfusion. In Figure 3, the slopes represent the estimated increase rates of liver stiffness. In accordance with the results of multiple regression, the estimated increase rate was higher when patients received blood transfusion at older ages.

The progression rate of liver stiffness was assessed in subgroups according to three parameters (Figure 4). The progression rate was significantly higher in patients who were older than 40 at the time of blood transfusion ($P < 0.0001$), which is in accordance with the results of multiple regression. Heavy alcohol consumption (more than 50 g ethanol/d, $P = 0.0308$) and male gender ($P = 0.0063$) also showed significant difference by Mann-Whitney *U* test. There was no significant difference among HCV genotypes.

DISCUSSION

The natural history of chronic hepatitis C concerning liver fibrosis progression has been vigorously studied using liver biopsy specimens. The extent of liver fibrosis is usually evaluated as categorical stages. For example, METAVIR Score uses five stages, F0-F4, for fibrosis evaluation[26]. The fibrosis progression in hepatitis C patients, calculated by using paired liver biopsy, was reported to be 0.1-0.133 Unit per year[12,13]. Liver stiffness measured by transient elastography is now widely accepted as a surrogate marker of liver fibrosis[27]. Liver stiffness is expressed as a continuous variable in kPa unit. The cut-

off for cirrhosis is reportedly 13-17 kPa, and the upper limit of measurement is currently 75 kPa. Thus LSM has a wider dynamic range than histological staging, and the rate of fibrosis progression may be more accurately analyzed with LSM.

In the present study, the increase rate of liver stiffness was positively correlated with the age at blood transfusion, as shown by the steeper slopes of approximation curves when patients received BTF at older ages. The cause of this phenomenon is not clear but age-related changes in immunity may be involved. If this is the case, the increase rate is likely to become higher in the same patient with age. Indeed, each approximation curve in the figure apparently becomes steeper with age, suggesting age-related acceleration. This is to be confirmed in future longitudinal studies.

LSM is useful not only as a surrogate of liver biopsy but also as a risk indicator of HCC development. Indeed, in the present study, liver stiffness is high in patients with HCC regardless of duration of infection. The patients who developed HCC with short duration of infection received blood transfusion at an older age and were older at the time of LSM, male dominant, and showed higher liver stiffness than patients without HCC with similar duration of infection. The difference between patients with and without HCC became smaller with longer duration of infection, as the average liver stiffness in patients with HCC became lower and that in patients without HCC became higher. We speculated that patients with high liver stiffness who received blood transfusion in the early period have already died of HCC or liver failure and were eliminated from the study population. Another possibility is that HCC may develop in patients with relatively low liver stiffness when infection has lasted a long time.

In the present study, the median increase in liver stiffness was calculated as 0.275 kPa per year. Using 13.01 kPa as a cut-off for cirrhosis[28], it will take around 40 years on average to develop cirrhosis, which is consistent with previous reports based on liver biopsy[29]. Admittedly, the present study is basically cross-sectional, and prospective longitudinal LSM will be obviously superior in understanding the natural course of liver fibrosis progression. However, the estimated average increase rate of liver stiffness indicates that such studies will require repeated LSM at an interval of at least five years.

Age at viral infection, alcohol consumption, and male gender were reported to be associated with accelerated fibrosis progression[8-11]. In the present study, we performed subgroup analysis and indeed found that blood transfusion at an age older than 40, male gender, and alcohol consumption more than 50 g ethanol/d were significantly associated with rapid increase in liver stiffness. There is consensus that heavy alcohol consumption is associated with fibrosis progression[30]. Alcohol, which by itself can cause liver disease and fibrosis, may affect liver stiffness and worsen fibrosis in hepatitis C[31]. We did not find a difference in liver stiffness increase rate between HCV genotypes 1 (mostly 1b) and 2 (2a/2b), although we could not evaluate genotypes 1a, 3 or 4.

This study has some limitations. First, since this is a cross-sectional study performed after LSM became available, patients with more rapid disease progression may have died and been excluded from the study. Second, because transfusion-associated HCV infection has been virtually eliminated in Japan since 1992, we could not include patients with shorter duration of infection. Lastly, we did not perform paired LSM but assumed that liver stiffness was normal at the time of infection. Longitudinal observation is now on-going but will take several years to obtain results.

In conclusion, although liver stiffness gradually increases over time from the onset of infection in general, HCC develops in patients with high liver stiffness regardless of the duration of infection. Patients who acquired HCV infection at older ages showed higher increase rate of liver stiffness and probably more rapid disease progression.

COMMENTS

Background

Liver stiffness, which can be noninvasively measured with transient elastography, has been recently reported to be well correlated with histologically assessed liver fibrosis stage.

Research frontiers

This study evaluated the association between liver stiffness and the duration of infection in blood transfusion-associated hepatitis C patients with and without hepatocellular carcinoma (HCC), focusing on the risk of HCC development.

Innovations and breakthroughs

Liver stiffness is expressed as a continuous variable in kPa unit. The cut-off for cirrhosis is reportedly 13-17 kPa, and the upper limit of measurement is currently 75 kPa. Thus liver stiffness measurement (LSM) has a wider dynamic range than histological staging, and the rate of fibrosis progression may be more accurately analyzed with LSM.

Applications

Although liver stiffness gradually increases over time from the onset of infection in general, HCC develops in patients with high liver stiffness regardless of the duration of infection. Patients who acquired hepatitis C virus (HCV) infection at older ages showed higher increase rate of liver stiffness and probably more rapid disease progression.

Terminology

Transient elastography (Fibro-Scan®; EchoSens, Paris, France) is a rapid, reliable and well-tolerated imaging technique for the assessment of liver fibrosis by measuring liver stiffness.

Peer review

This is an interesting and timely study on liver stiffness in patients with transfusion associatated HCV. The authors show that HCC develops in patients with high liver stiffness regardless of the duration of infection. Patients who acquired HCV infection at older ages showed higher increase rate of liver stiffness. Co-exposure to alcohol is critical. The methodology is sound and the paper is well and clearly written.

REFERENCES

1 **Strader DB**, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004; **39**: 1147-1171

2 **Di Bisceglie AM**. Hepatitis C. *Lancet* 1998; **351**: 351-355

3 **Seeff LB**. The history of the "natural history" of hepatitis C (1968-2009). *Liver Int* 2009; **29** Suppl 1: 89-99

4 **Alberti A**, Chemello L, Benvegnù L. Natural history of hepatitis C. *J Hepatol* 1999; **31** Suppl 1: 17-24

5 **Kiyosawa K**, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, Furuta S, Akahane Y, Nishioka K, Purcell RH. In-

terrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. *Hepatology* 1990; **12**: 671-675
6 **Marcellin P**, Asselah T, Boyer N. Fibrosis and disease progression in hepatitis C. *Hepatology* 2002; **36**: S47-S56
7 **Seeff LB**, Hollinger FB, Alter HJ, Wright EC, Cain CM, Buskell ZJ, Ishak KG, Iber FL, Toro D, Samanta A, Koretz RL, Perrillo RP, Goodman ZD, Knodell RG, Gitnick G, Morgan TR, Schiff ER, Lasky S, Stevens C, Vlahcevic RZ, Weinshel E, Tanwandee T, Lin HJ, Barbosa L. Long-term mortality and morbidity of transfusion-associated non-A, non-B, and type C hepatitis: A National Heart, Lung, and Blood Institute collaborative study. *Hepatology* 2001; **33**: 455-463
8 **Poynard T**, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997; **349**: 825-832
9 **Matsumura H**, Moriyama M, Goto I, Tanaka N, Okubo H, Arakawa Y. Natural course of progression of liver fibrosis in Japanese patients with chronic liver disease type C--a study of 527 patients at one establishment. *J Viral Hepat* 2000; **7**: 268-275
10 **Hamada H**, Yatsuhashi H, Yano K, Daikoku M, Arisawa K, Inoue O, Koga M, Nakata K, Eguchi K, Yano M. Impact of aging on the development of hepatocellular carcinoma in patients with posttransfusion chronic hepatitis C. *Cancer* 2002; **95**: 331-339
11 **Khan KN**, Yatsuhashi H. Effect of alcohol consumption on the progression of hepatitis C virus infection and risk of hepatocellular carcinoma in Japanese patients. *Alcohol Alcohol* 2000; **35**: 286-295
12 **Minola E**, Prati D, Suter F, Maggiolo F, Caprioli F, Sonzogni A, Fraquelli M, Paggi S, Conte D. Age at infection affects the long-term outcome of transfusion-associated chronic hepatitis C. *Blood* 2002; **99**: 4588-4591
13 **Yoshizawa H**, Tanaka J, Miyakawa Y. National prevention of hepatocellular carcinoma in Japan based on epidemiology of hepatitis C virus infection in the general population. *Intervirology* 2006; **49**: 7-17
14 **Tanaka Y**, Hanada K, Mizokami M, Yeo AE, Shih JW, Gojobori T, Alter HJ. A comparison of the molecular clock of hepatitis C virus in the United States and Japan predicts that hepatocellular carcinoma incidence in the United States will increase over the next two decades. *Proc Natl Acad Sci USA* 2002; **99**: 15584-15589
15 **Masuzaki R**, Yoshida H, Tateishi R, Shiina S, Omata M. Hepatocellular carcinoma in viral hepatitis: improving standard therapy. *Best Pract Res Clin Gastroenterol* 2008; **22**: 1137-1151
16 **Ikeda K**, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Koida I, Arase Y, Fukuda M, Chayama K, Murashima N, Kumada H. Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. *J Hepatol* 1998; **28**: 930-938
17 **Yoshida H**, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, Inoue O, Yano M, Tanaka M, Fujiyama S, Nishiguchi S, Kuroki T, Imazeki F, Yokosuka O, Kinoyama S, Yamada G, Omata M. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. *Ann Intern Med* 1999; **131**: 174-181
18 **Dienstag JL**. The role of liver biopsy in chronic hepatitis C. *Hepatology* 2002; **36**: S152-S160
19 **Bedossa P**, Dargère D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003; **38**: 1449-1457
20 **Castéra L**, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, Couzigou P, De Lédinghen V. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128**: 343-350
21 **Masuzaki R**, Tateishi R, Yoshida H, Goto E, Sato T, Ohki T, Imamura J, Goto T, Kanai F, Kato N, Ikeda H, Shiina S, Kawabe T, Omata M. Prospective risk assessment for hepatocellular carcinoma development in patients with chronic hepatitis C by transient elastography. *Hepatology* 2009; **49**: 1954-1961
22 **Masuzaki R**, Tateishi R, Yoshida H, Yoshida H, Sato S, Kato N, Kanai F, Sugioka Y, Ikeda H, Shiina S, Kawabe T, Omata M. Risk assessment of hepatocellular carcinoma in chronic hepatitis C patients by transient elastography. *J Clin Gastroenterol* 2008; **42**: 839-843
23 **Makuuchi M**, Kokudo N, Arii S, Futagawa S, Kaneko S, Kawasaki S, Matsuyama Y, Okazaki M, Okita K, Omata M, Saida Y, Takayama T, Yamaoka Y. Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. *Hepatol Res* 2008; **38**: 37-51
24 **Kudo M**, Okanoue T. Management of hepatocellular carcinoma in Japan: consensus-based clinical practice manual proposed by the Japan Society of Hepatology. *Oncology* 2007; **72** Suppl 1: 2-15
25 **Moriyama M**, Matsumura H, Nirei K, Arakawa Y, Yamagami H, Ogawa M, Kaneko M, Matsuoka S, Amaki S, Tanaka N, Arakawa Y. Factors influencing treatment efficacy of 24-week combination therapy with interferon alpha-2b plus ribavirin for chronic hepatitis C. *Dig Dis Sci* 2007; **52**: 2418-2426
26 **Bedossa P**, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; **24**: 289-293
27 **Ghany MG**, Kleiner DE, Alter H, Doo E, Khokar F, Promrat K, Herion D, Park Y, Liang TJ, Hoofnagle JH. Progression of fibrosis in chronic hepatitis C. *Gastroenterology* 2003; **124**: 97-104
28 **Friedrich-Rust M**, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008; **134**: 960-974
29 **Shiratori Y**, Imazeki F, Moriyama M, Yano M, Arakawa Y, Yokosuka O, Kuroki T, Nishiguchi S, Sata M, Yamada G, Fujiyama S, Yoshida H, Omata M. Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. *Ann Intern Med* 2000; **132**: 517-524
30 **EASL International Consensus Conference on Hepatitis C**. Paris, 26-28, February 1999, Consensus Statement. European Association for the Study of the Liver. *J Hepatol* 1999; **30**: 956-961
31 **Mueller S**, Millonig G, Sarovska L, Friedrich S, Reimann FM, Pritsch M, Eisele S, Stickel F, Longerich T, Schirmacher P, Seitz HK. **Increased liver stiffness in alcoholic liver disease:** differentiating fibrosis from steatohepatitis. *World J Gastroenterol* 2010; **16**: 966-972

S- Editor Shi ZF **L- Editor** O'Neill M **E- Editor** Xiong L