

Occurrence and Recurrence of Hepatocellular Carcinoma Were Not Rare Events during Phlebotomy in Older Hepatitis C Virus-Infected Patients

Tatsuo Kanda Shingo Nakamoto Shin Yasui Masato Nakamura
Tatsuo Miyamura Shuang Wu Xia Jiang Makoto Arai Fumio Imazeki
Osamu Yokosuka

Department of Gastroenterology and Nephrology, Graduate School of Medicine,
Chiba University, Chiba, Japan

Key Words

Adverse events · Hepatitis C virus · Hepatocellular carcinoma · Nonalcoholic fatty liver disease · Phlebotomy

Abstract

The use of phlebotomy is relatively common for 'difficult-to-treat by antiviral therapies' hepatitis C virus (HCV)-infected patients and for certain patients having chronic liver diseases with an iron overload of the liver. In the present study, we retrospectively analyzed patients treated with phlebotomy and their adverse events. We observed the occurrence and recurrence of hepatocellular carcinoma, and the appearance of ascites in some patients infected with HCV as well as the reduction of serum ferritin and alanine aminotransferase levels. Severe adverse events necessitating a cessation of phlebotomy occurred independently of α -fetoprotein (≥ 10 ng/ml) in patients infected with HCV according to multivariate logistic regression analysis. These findings may serve as a basis for phlebotomy especially in older patients with chronic hepatitis C.

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Tatsuo Kanda, MD, PhD
Department of Gastroenterology and Nephrology
Graduate School of Medicine, Chiba University
1-8-1 Inohana, Chuo-ku, Chiba 260-8670 (Japan)
E-Mail kandat-cib@umin.ac.jp

Introduction

In patients with chronic liver diseases such as chronic hepatitis C, hereditary hemochromatosis and nonalcoholic steatohepatitis, iron depletion by phlebotomy improves serum alanine aminotransferase (ALT) levels [1–4]. Nonresponder patients still exist, although antiviral treatments for hepatitis C virus (HCV)-infected patients have been developed, and these new therapies are currently available [5–8]. Several treatments have proven to reduce serum ALT levels, such as phlebotomy, ursodeoxycholic acid and Stronger Neo-Minophagen C, a glycyrrhizin [9]. These treatments might be effective in HCV-infected patients without achieving a sustained virological response [9].

Iron overload is one of the abnormal laboratory values in patients with chronic hepatitis C, showing elevation of the serum ferritin level [10]. About 30–40% of patients with chronic HCV infection have elevated serum iron, transferrin saturation and heightened ferritin levels [11]. Hepcidin is a 25-amino-acid peptide that is synthesized in the liver and modulates iron homeostasis in the body [11, 12]. Hepcidin is a type II acute phase protein whose upregulation systemically suspends iron absorption from intestinal enterocytes and iron recycling from macrophages [11]. Patients infected with HCV have reduced serum hepcidin levels, which are correlated with worse necroinflammation and fibrosis of the liver [12, 13]. Thus, hepcidin may play a role in the pathogenesis of iron overload in patients with chronic hepatitis C [14].

It was reported that serum ferritin is an independent predictor of histological severity and advanced fibrosis in patients with nonalcoholic fatty liver disease (NAFLD) [15]. Patients with alcoholic liver disease frequently exhibit increased body iron stores, as reflected by higher serum ferritin levels and hepatic iron concentration [16]. It is unknown whether iron depletion by phlebotomy is a useful therapy, although phlebotomy improves serum ferritin levels [17, 18].

In Japan, the average age of patients infected with HCV is becoming increasingly higher. Many HCV-infected older people did not respond to peginterferon plus ribavirin therapies or could not undergo these treatments either with or without direct-acting antivirals against HCV due to certain medical conditions [19, 20]. Chronic hepatitis C patients with advanced liver fibrosis are at risk for hepatocellular carcinoma (HCC). Older age as well as advanced liver fibrosis is one of the risk factors associated with HCC [20, 21]. We have recently had an increased number of opportunities to perform phlebotomy in patients with HCV or NAFLD. In the present study, we retrospectively analyzed patients undergoing phlebotomy and their adverse events. Occurrence and recurrence of HCC, and the appearance of ascites in some of the HCV-infected patients as well as the reduction of serum ferritin and ALT levels were observed. We also found that severe adverse events, requiring a cessation of phlebotomy, occurred independently of α -fetoprotein (AFP, ≥ 10 ng/ml) in patients infected with HCV according to multivariate logistic regression analysis. These findings might serve as a basis for phlebotomy especially in older patients infected with HCV.

Patients and Methods

Patients

We retrospectively analyzed 26 patients with chronic liver disease who had undergone phlebotomy for at least 6 months at the Chiba University Hospital. Patients were eligible if they met the following inclusion criteria: (1) age ≥ 20 years, (2) no pregnancy, (3) no severe heart disease, (4) no abnormal hemoglobinemia, (5) no chronic renal disease, (6) no hepatic

failure, and (7) no current intravenous drug abuse. This analysis was approved by the ethics committee of the Chiba University School of Medicine, and informed consent was obtained from all patients. In this study, phlebotomy of 200–400 ml was performed every 1 or 2 months at the outpatient clinic of the Chiba University Hospital. Clinical and laboratory assessments were also performed each time. Abdominal ultrasound was performed at least once every 6 months. Adverse events were noted by verbal inquiry, physical examinations and laboratory tests. HCV RNA was measured by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan), with levels ranging from 1.2 to 7.8 log IU/ml [20]. Serum aspartate aminotransferase (AST), ALT, ferritin, hemoglobin and other laboratory tests were carried out by standard methods.

Definition of Severe Adverse Events

Severe adverse events were defined as events requiring a cessation of phlebotomy.

Diagnosis of Hepatic Cirrhosis

Patients were diagnosed as having cirrhosis mainly by ultrasound examination. Six patients infected with HCV and 2 patients without HCV were diagnosed with cirrhosis using mainly liver biopsy.

Diagnosis of HCC

Patients were subsequently followed up using an HCC surveillance program based on biochemical and US evaluation every 3–6 months as previously described [20]. An intrahepatic nodule was considered to be HCC on computed tomography or magnetic resonance imaging when both the hypervascular nodule in the arterial phase and the hypovascular nodule in the late phase were shown [20].

Statistical Analysis

Data were expressed as mean \pm SD. Differences were evaluated by Student's t test or χ^2 . $p < 0.05$ was considered statistically significant. Variables with $p < 0.05$ at univariate analysis were retained for multiple logistic regression analysis. Statistical analysis was performed using the Excelstatistics program for Windows, version 7 (SSRI, Tokyo, Japan).

Results

Patient Characteristics

The characteristics of all 26 patients are shown in table 1. Mean age was 63.9 years. Of a total of 21 patients infected with HCV, 20 were monoinfected with HCV and 1 was coinfecting with hepatitis B virus and HCV. In the HCV group, 15 and 6 were infected with HCV genotype 1 and genotype 2, respectively; 15 of the 21 HCV-positive patients had received interferon treatment, and 5 of the 21 patients had received treatment for HCC. Of all 26 patients, 13 patients were diagnosed with cirrhosis. Of the 5 patients with NAFLD, 2 each showed fibrosis staging of F2 and F4, respectively. These 4 patients and 1 other patient had nonalcoholic steatohepatitis and simple steatosis, respectively. The body mass index and the albumin level of patients with NAFLD tended to be higher than those of patients infected with HCV.

Effects of Phlebotomy on Laboratory Data of All 26 Patients

In all 26 patients, AST, ALT, hemoglobin and ferritin were significantly reduced by phlebotomy (fig. 1). Among the other parameters, γ -glutamyltransferase and AFP levels tended to be reduced by phlebotomy (from 88.8 ± 95.7 to 56.1 ± 56.8 , $p = 0.140$, and from 8.6 ± 6.9 to 6.5 ± 4.0 , $p = 0.185$, respectively). These results indicated that phlebotomy was effective in providing an improvement in AST and ALT levels as has been shown in previous reports [1–4].

Effects of Phlebotomy on Laboratory Data in a Total of 21 Patients Infected with HCV

Next, we compared the data of patients infected with HCV before and after phlebotomy. In the 21 HCV-infected individuals, ALT, hemoglobin and ferritin levels were significantly reduced by phlebotomy, although the HCV RNA level did not change (table 2). Among the other parameters, AST and AFP levels tended to be reduced by phlebotomy. Although the study population was very small (data not shown), among the 5 patients with NAFLD, only AST and ferritin levels, respectively, tended to be reduced before and after phlebotomy (from 56.2 ± 24.8 to 36.2 ± 15.2 , $p = 0.162$, and from 248 ± 219 to 64.3 ± 52.4 , $p = 0.105$).

Severe Adverse Events during Phlebotomy

We observed severe adverse events in 9 patients. Of interest, these 9 patients were all infected with HCV (table 3). In 6 of these 9 patients, the background liver was cirrhotic. One and 3 had occurrence and recurrence of HCC, respectively, and phlebotomy in the other 5 patients was stopped due to angina pectoris in 1, ascites in 1, pleural tuberculosis in 1, nausea in 1, and dizziness in 1. Univariate analysis showed that lower albumin contributed to the occurrence of severe adverse events (table 4). Factors associated with severe adverse events by univariate analysis were again analyzed by multivariate logistic regression analysis. Severe adverse events occurred independently of AFP (≥ 10 ng/ml) in patients infected with HCV (table 5).

Discussion

The observation that phlebotomy improves ALT levels has now been confirmed in many studies [1–4], even though iron reduction does not improve the rate of sustained response among previous nonresponders [22]. The present study revealed the possibility that even during phlebotomy, severe adverse events including the occurrence or recurrence of HCC could occur in patients infected with HCV even if their ALT and ferritin levels were improved. Among the patients with severe adverse events in this study, all except 1 were more than 50 years of age (table 3).

Kato et al. [23] also performed phlebotomy with low-iron diet therapy in 35 HCV-infected patients, with a mean age of 61 years, and they observed HCC occurrence in 4/35 (11.4%) during a 6-year period. They found that the average serum ferritin level independently affected the hepatocarcinogenesis rate and the relative risk of HCC in the patients treated with iron depletion therapy. Iron overload is one of the risk factors for the development of HCC in patients infected with chronic HCV infection [24]. Although the study population was small, the present study also showed that chronic phlebotomy treatment is not always useful for the prevention of HCC and that further studies are needed. We cannot exclude the possibility that most of the current study patients infected with HCV might have been patients ‘difficult-to-treat’ by antiviral therapies such as peginterferon plus ribavirin

[5]. Of course, among these patients, elderly patients and/or patients with advanced liver fibrosis might be susceptible to HCC [20, 21, 25].

In Japan, patients infected with HCV are getting increasingly older, and they are older than those in other countries [19, 26]. Matsuo et al. [26] reported that HCC developed more frequently in blood donors in whom chronic hepatitis had been diagnosed at age ≥ 60 years than ≤ 59 years. In the era of direct-acting antivirals against HCV [27, 28], our current study strongly suggests that HCV-infected patients >50 years might be treated with antivirals as soon as possible. If certain treatments such as phlebotomy were temporarily selected, attention should be paid to the potential occurrence or recurrence of HCC or the occurrence of other complications during these treatments. In the present study, in 4 (30.7%) patients of 13 cirrhotic patients, phlebotomy was stopped due to the occurrence or recurrence of HCC.

In conclusion, the occurrence or recurrence of HCC or hepatic failure with ascites was observed in certain patients infected with HCV in addition to the reduction of serum ferritin and ALT levels during phlebotomy. Severe adverse events, which required phlebotomy to be stopped, occurred independently of AFP (≥ 10 ng/ml) in patients infected with HCV according to multivariate logistic regression analysis. The findings of this study will provide a guideline as a basis for phlebotomy treatments, especially for older patients with chronic hepatitis C. If possible, older patients infected with HCV should also be treated with antiviral agents.

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Table 1. Baseline characteristics of total patients in this study

Factors	Total patients (n = 26)	HCV group (n = 21)	NAFLD group (n = 5)	p values ¹ (HCV vs. NAFLD)
Age, years	63.9±9.3	64.6±8.7	61±12.4	n.s.
Males/females, n	14/12	13/8	1/4	n.s.
Body mass index	24.4±3.9	23.6±3.4	26.9±4.5	0.078
Cirrhosis (+/-)	13/13	11/10	2/3	n.s.
Past history of HCC (+/-)	5/21	5/16	0/5	n.s.
AST, IU/l	62.7±31.0	64.2±32.6	56.2±24.8	n.s.
ALT, IU/l	77.3±51.8	75.3±47.5	86±73.4	n.s.
γ-GTP, IU/l	88.8±95.7	94.4±104	66.4±47.0	n.s.
Total bilirubin, mg/dl	1.0±0.53	0.98±0.47	1.2±0.78	n.s.
Albumin, g/dl	4.2±0.39	4.2±0.41	4.5±0.13	0.123
Hemoglobin, g/dl	13.8±1.5	13.7±1.5	14.4±1.2	n.s.
Platelets, ×10 ⁴ /μl	13.4±4.6	12.2±4.0	18.3±3.8	0.00500
AFP, ng/ml	8.6±6.9	9.7±7.3	4.3±1.6	0.118
Ferritin, ng/ml	213±176	205±169	248±219	n.s.
Total volume of phlebotomy (<2.4/≥2.4 liters)	17/9	12/9	5/0	0.197

Data are expressed as mean ± SD. γ-GTP = γ-Glutamyltransferase; n.s. = not statistically significant.

¹ p values between the HCV group and the NAFLD group are indicated by Student's t test or χ² test.

Table 2. Changes in laboratory data before and after phlebotomy in a total of 21 HCV-positive patients

Factors	Before phlebotomy (n = 21)	After phlebotomy (n = 21)	p values ¹
HCV RNA, log IU/ml	5.89±1.58	5.87±1.65	n.s.
AST, IU/l	64.2±32.6	49.5±25.1	0.109
ALT, IU/l	75.3±47.5	48.3±28.2	0.0307
γ-GTP, IU/l	94.4±104	60.9±62.3	n.s.
Total bilirubin, mg/dl	0.98±0.47	0.83±0.47	n.s.
Albumin, g/dl	4.2±0.41	4.0±0.48	n.s.
Hemoglobin, g/dl	13.7±1.5	12.7±1.4	0.0398
Platelets, ×10 ⁴ /μl	12.2±4.0	13.0±4.4	n.s.
AFP, ng/ml	9.7±7.3	7.1±4.2	0.164
Ferritin, ng/ml	205±169	38.9±32.5	0.000236

Data are expressed as mean ± SD. γ-GTP = γ-Glutamyltransferase; n.s. = not statistically significant.

¹ p values between before and after phlebotomy are indicated by Student's t test or χ² test.

Table 3. Nine patients infected with HCV no longer received phlebotomy due to severe adverse events

Age, years	Gender	Background	Phlebotomy (total volume/duration), l/months	Severe adverse events	Hemoglobin (before phlebotomy), g/dl	Hemoglobin (after phlebotomy), g/dl
69	Male	Cirrhosis	<2.4/12	Angina pectoris	16	14
79	Male	Cirrhosis	<2.4/6	HCC	13.8	12.4
62	Male	Cirrhosis, post-HCC therapy	<2.4/10	HCC recurrence	10.6	12.5
54	Male	Cirrhosis, post-HCC therapy	≥2.4/26	HCC recurrence	12.3	12.5
65	Male	Cirrhosis, post-HCC therapy	<2.4/6	HCC recurrence	13.7	12.1
67	Female	Chronic hepatitis	<2.4/6	Nausea	14.2	12
73	Male	Chronic hepatitis, type B + C	≥2.4/93	Pleural tuberculosis	13.3	13
48	Female	Cirrhosis	<2.4/16	Ascites	13	11.1
79	Male	Chronic hepatitis	≥2.4/24	Dizziness	15.5	11.1

Table 4. Comparison of baseline characteristics of HCV-infected patients with and without severe adverse events by univariate analysis

Factors	With severe adverse events (n = 9)	Without severe adverse events (n = 12)	p values ¹
Age, years	66.2±10.4	63.4±7.3	n.s.
Males/females	7/2	6/6	n.s.
Body mass index	24.4±4.7	23.1±2.6	n.s.
Cirrhosis (+/-)	6/3	5/7	n.s.
Past history of HCC (+/-)	3/6	2/10	n.s.
AST, IU/l	74.2±38.8	56.8±26.3	n.s.
ALT, IU/l	83.6±57.0	69.0±40.4	n.s.
γ-GTP, IU/l	140±137	56.5±46.2	0.0622
Total bilirubin, mg/dl	1.0±0.59	0.94±0.39	n.s.
Albumin, g/dl	3.9±0.48	4.4±0.23	0.00499
Hemoglobin, g/dl	13.6±1.6	13.8±1.5	n.s.
Platelets, ×10 ⁴ /μl	11.5±3.5	12.7±4.3	n.s.
AFP, ng/ml	12.9±8.8	7.2±5.0	0.0749
Ferritin, ng/ml	276±208	152±116	0.0973
Total volume of phlebotomy (<2.4/≥2.4 liters)	6/3	6/6	n.s.

Data are expressed as mean ± SD. γ-GTP = γ-Glutamyltransferase; n.s. = not statistically significant.

¹ p values between the HCV group and the NAFLD group are indicated by Student's t test or χ² test.

Table 5. Factors associated with severe adverse events among patients infected with HCV by multivariate analysis

Factor	Category	Odds ratio	95% CI	p value
AFP	<10/≥10	10.0	1.28–78.1	0.0281

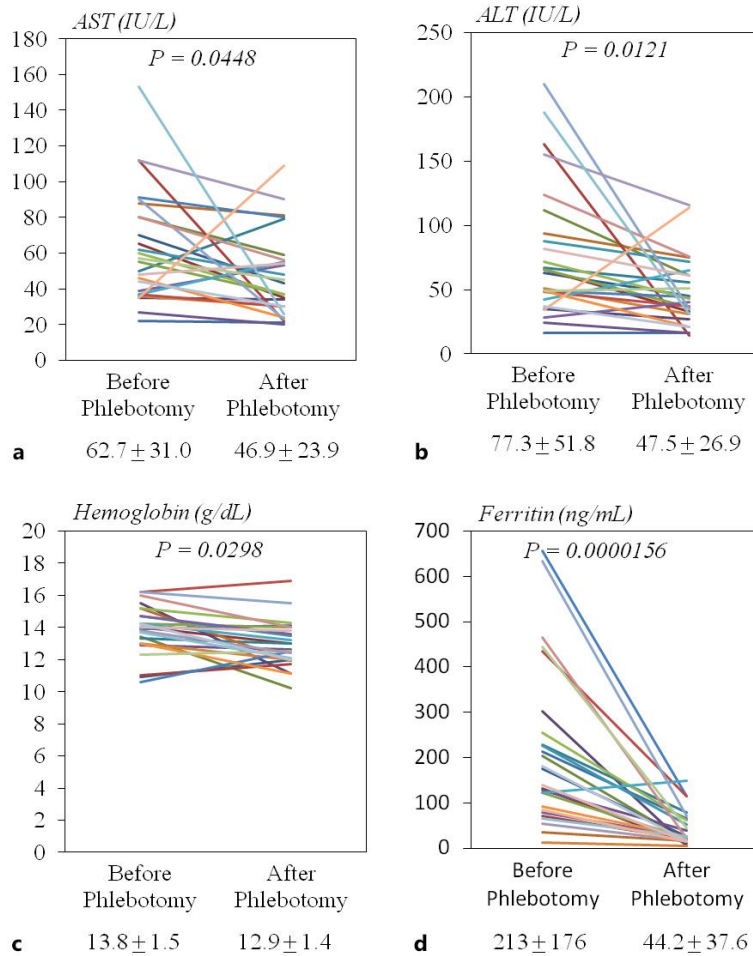


Fig. 1. Changes in AST (**a**), ALT (**b**), hemoglobin (**c**) and ferritin (**d**) before and after phlebotomy in all the 26 patients. Values below the x-axis represent mean \pm SD.