Cancer Chemother Pharmacol (1992) 31 (Suppl I): S150–S156

Cancer Chemotherapy and Pharmacology

Clinical aspects and epidemiology of hepatitis B and C viruses in hepatocellular carcinoma in Japan*

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Summary. The incidence of hepatocellular carcinoma (HCC) in Japan has increased over the past two decades. Of the 379 patients with HCC treated at Shinshu University Hospital over the past 20 years, 112 underwent treatment between 1971 and 1980 and 267 were treated between 1981 and 1990. The prevalence of hepatitis B virus-associated HCC and hepatitis C virus-associated HCC was 54% and 34%, respectively, during the first decade and 31% and 60%, respectively, during the second decade. Major factors contributing to the increased incidence of HCC include an increase in the incidence of type C chronic hepatitis and an increase in the incidence of cirrhosis of the liver, which in turn are the result of blood transfusions received about 30 years ago. Donated blood testing positive for hepatitis C virus antibody is currently rejected from the blood supply. However, the occurrence of post-transfusion hepatitis with the potential to develop into HCC has not been entirely eliminated. In addition, there is an as yet unelucidated route of horizontal transmission of hepatitis C virus.

Introduction

Hepatocellular carcinoma (HCC) has long been recognized as one of the most fatal cancers in the world, especially in Japan and other parts of Asia [1, 17]. HCC is the third leading cause of death among all men suffering from cancer, and approximately 180,000 patients in Japan died of HCC in 1989. It is now generally accepted that chronic infection with hepatitis B virus (HBV) leads to HCC. Recently, the genome of a non-A, non-B hepatitis agent was cloned and designated as hepatitis C virus (HCV) [3]. This discovery led to the development of a recombinant-based immunoassay that tests for the presence of circulating HCV antibodies (anti-HCV) [16]. A relationship between HCV and HCC is suggested by measurement of anti-HCV [4, 6]. In Japan, the incidence of HCC, particularly among men, is increasing, and the main factor contributing to this increased incidence of HCC is an increase in the incidence of type C chronic hepatitis [19].

Epidemiology of HCC in Japan

According to vital statistics for Japan, the mortality of patients with HCC has increased dramatically over the last 10 years. Nishioka et al. [19] reported that the number of deaths reported annually in conjunction with HCC per 100,000 population were 9.5 in 1968–1977, 11.7 in 1978–1979, 12.8 in 1980–1981, 14.4 in 1982–1983, and 16.0 in 1984–1985. In an analysis of Osaka Cancer Registry data, Okuda et al. [20] reported that the annual incidence of primary liver cancer in men in the Osaka area, where chronic liver disease is most prevalent in Japan, was 16.3/100,000 in 1966–1968 and 34.2/100,000, a record 2-fold increase, in 1981–1982. In 1958–1959, HCC constituted 5.78% of all malignancies, but by 1982 the relative incidence had increased to 14.0%, representing more than a 2-fold increase. Recent vital statistics reported by the Japanese Ministry of Health and Welfare indicate that the mortality due to HCC in men is much higher than that in women and that HCC is the second leading cause of death in men aged between 50 and 60 years (Fig. 1). The geographical distribution by Japanese prefecture of corrected mortality rates for HCC shows a gradient, with the incidence being higher in the West than in the East (Fig. 2). Figure 3 shows the prevalence of HBsAg positivity by prefecture, and Fig. 4 illustrates the prevalence of anti-HCV among blood donors in selected regions as determined with the Ortho Diagnostic anti-HCV test system. In both cases, the prevalence is higher in the West than in the East, and both gradients parallel that of HCC.

* Presented at the Second International Symposium on Treatment of Liver Cancer. Taipei, 3–4 February 1991

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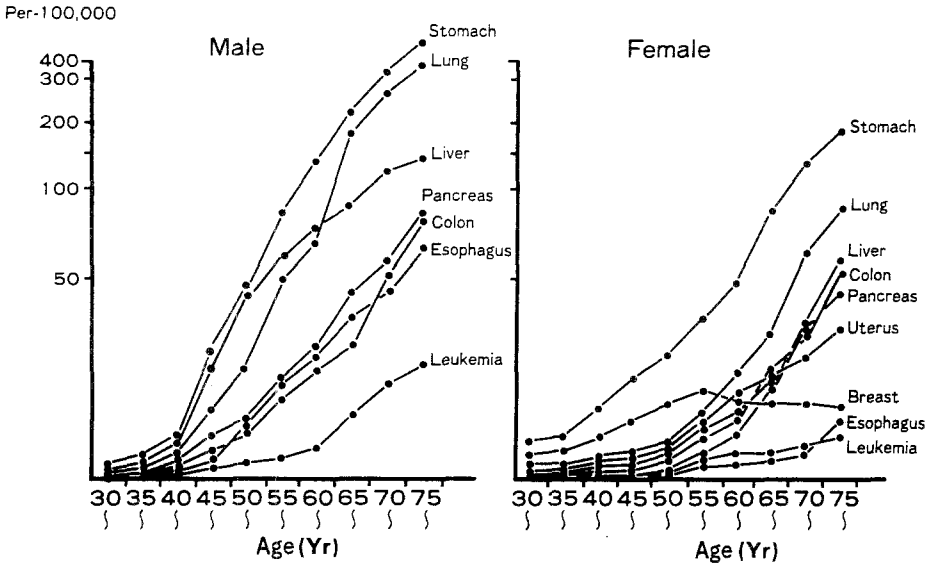

Fig. 1. Age-adjusted mortality rates for various cancers (1989 vital statistics from the Japanese Ministry of Health and Welfare)

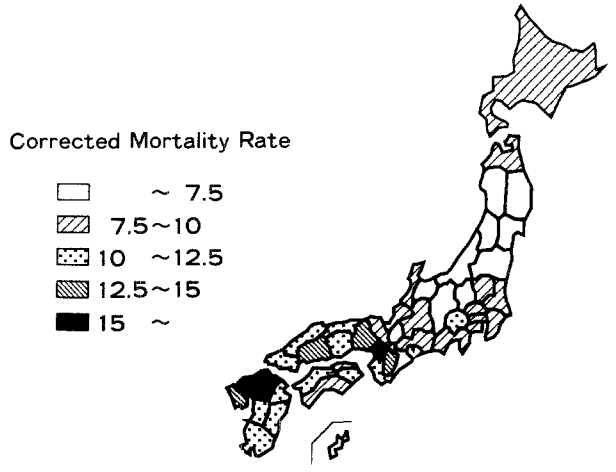

Fig. 2. Geographical distribution throughout Japan of adjusted mortality rates for HCC (vital statistics for 1985 from the Japanese Ministry of Health and Welfare)

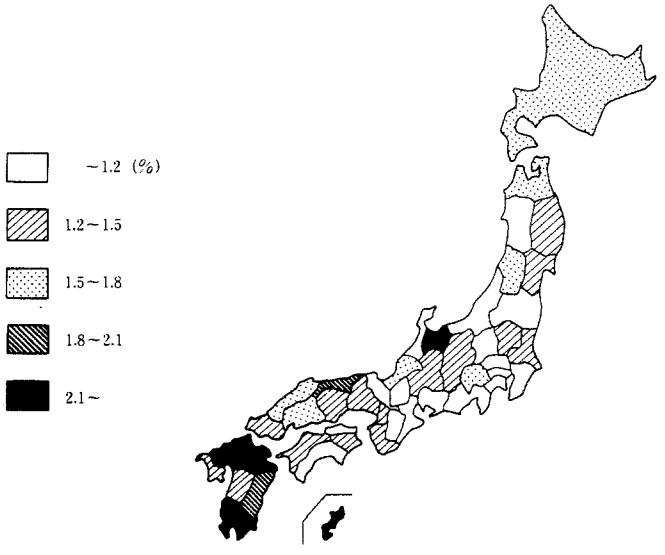

Fig. 3. Geographical distribution of the incidence of HBsAg positivity among blood donors (1985, Japan Red Cross Central Blood Center)

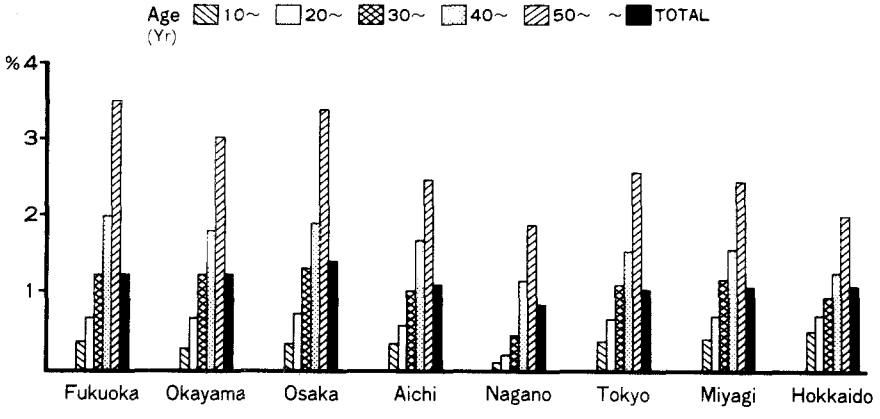

Fig. 4. Prevalence of anti-HCV positivity among blood donors in selected regions of Japan (1990, Japan Red Cross Central Blood Center)

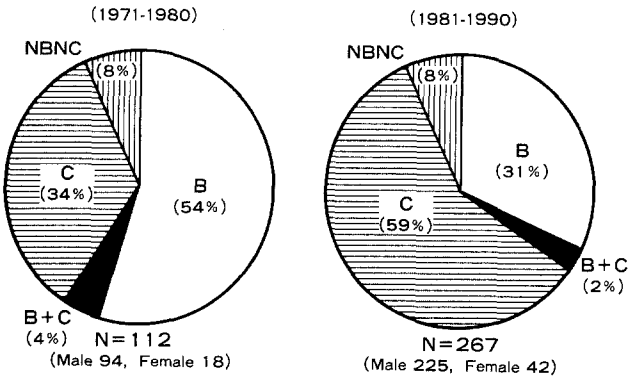

Fig. 5. Prevalence of each viral type of HCC among patients seen at Shinshu University Hospital in 1971–1980 and in 1981–1990

Changes in numbers and viral type of patients with HCC

The number of patients with HCC who were treated at Shinshu University Hospital between 1971 and 1990 are shown in Table 1. The diagnosis of HCC was histologically confirmed in liver tissue obtained by needle biopsy, surgery, or autopsy. In some patients, HCC was diagnosed using imaging techniques such as ultrasonography, computed tomography, or selective angiography. In all, 112 patients with HCC (94 men and 18 women) were treated during the period from 1971 to 1980, and 267 (225 men and 42 women) underwent treatment during the period from 1981 to 1990. The ratio of men to women was 5.2 for the early decade and 5.4 for the later decade, showing that men predominated in both decades and that this ratio was almost the same in both decades. These data indicate that the incidence of HCC has increased rapidly not only in men but also in women.

The prevalence of each type of HCC is shown in Fig. 5. In the earlier decade, 60 (54%) patients had HBsAg-positive (type B) HCC, 38 (34%) had anti-HCV-positive (type C) HCC, 5 (4%) had both HBsAg- and anti-HCV-positive (type B+C) HCC, and 9 (8%) had HBsAg- and anti-HCV-negative (other) HCC. In the later decade, the corresponding numbers were 82 (31%), 159 (59%), 4 (2%), and 22 (8%). These findings clearly indicate that the prevalence of each type of HCC changed over the course of these two decades. The main factor contributing to this phenomenon is the recent increase in the number of patients with type C HCC. This increase was also reported by Nishioka et al. [19], who analyzed 180 cases of clinically and histologically confirmed HCC at 5 hospitals in Japan and found that 25% of cases of HCC were related to HBV, whereas 57% of cases were related to HCV. Similar findings have been reported in the United States. Yu et al. [26] analyzed serum samples from 51 patients with HCC (12 blacks and 39 whites) in Los Angeles County and found that HCV positivity was a significantly greater risk factor for HCC (relative risk, 10.5) than was HBV positivity (relative risk, 7.0).

The cause of the increase in the prevalence of HCC in Japan is not clear, although one possible, if not likely, cause might be the increase in the number of patients with type C chronic hepatitis, who have a history of having received a blood transfusion 10–20 years ago [20].

Carcinogenesis of HBV

The strong epidemiological and molecular biological association between HBV and HCC suggests that HBV is causally related to this fatal cancer. The circumstantial evidence can be summarized as follows: (1) geographic parallelism between the number of HBsAg carriers and the incidence of HCC; (2) familial clustering of HBsAg carrier status, cirrhosis of the liver, and HCC; (3) a high prevalence of positive seromarkers for HBV in patients with HCC; (4) presence of HBsAg in the hepatoma cells; (5) production of HBsAg by HCC cell lines; (6) integration of HBV DNA into the hepatoma cell DNA; (7) hepadna virus leads to malignant transformation in animal models; and (8) HCC occurs more frequently among HBsAg carriers than among noncarriers in the same population.

Chisari et al. [2] recently introduced a new hypothesis to account for carcinogenesis by HBV on the basis of experiments using HBV-gene transgenic mice. They found that the accumulation of toxic quantities of HBsAg within hepatocytes resulted in severe, prolonged hepatocellular injury, which in turn led to neoplasia. Moreover, regardless of the etiology, chronic cell injury and associated inflammatory and regenerative responses further a preneoplastic condition that inevitably proceeds toward malignancy.

Our studies on HBsAg in the serum and liver of patients with type B liver disease support this hypothesis. Figure 6

Table 1. Numbers of patients with HCC of each viral type seen between 1971 and 1990 at Shinshu University Hospital

Period	Total		HBsAg(+) Anti-HCV(−)		HBsAg(+) Anti-HCV(+)		HBsAg(−) Anti-HCV(+)		HBsAg(−) Anti-HCV(−)	
	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women
1971 ↓ 1980	94	18	50	10	4	1	32	6	8	1
	112		60		5		38		9	
	(100%)		(54%)		(4%)		(34%)		(8%)	
1981 ↓ 1990	225	42	72	10	4	0	128	31	21	1
	267		82		4		159		22	
	(100%)		(31%)		(2%)		(59.0%)		(8%)	

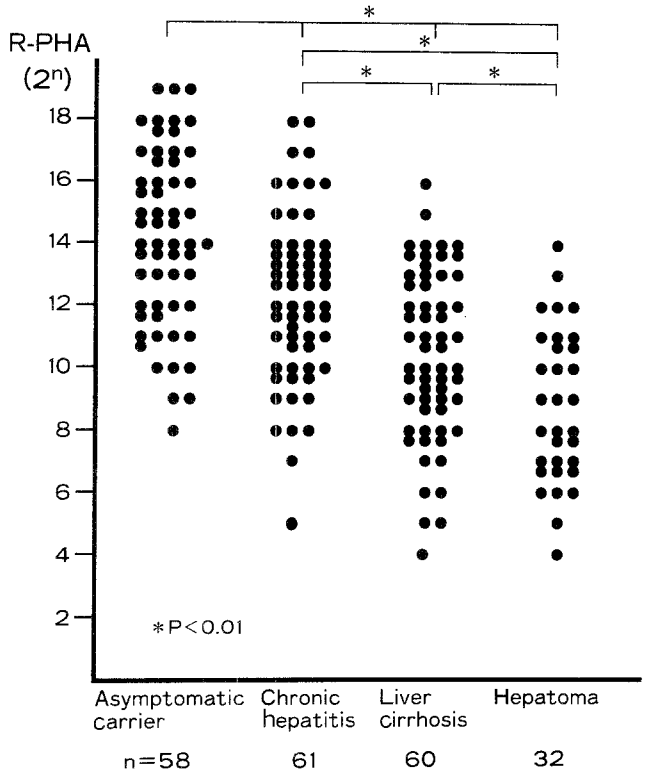

Fig. 6. Titers of HBsAg detected by reverse passive hemagglutination in sera of patients with asymptomatic HBsAg carrier status, chronic persistent and active hepatitis, cirrhosis of the liver, and HCC

Table 2. Prevalence of types of intrahepatic expression of HBsAg

Disease	Pattern of localization of HBsAg in hepatocytes[a]			
	Membranous	Mixed	Cytoplasmic	
			Diffuse	Inclusion body
Asymptomatic HBsAg carrier ($n = 25$)	21 (84%)	4 (16%)	0	0
Chronic persistent hepatitis ($n = 31$)	5 (16%)	22 (71%)	4 (13%)	0
Chronic active hepatitis ($n = 26$)	0	23 (89%)	2 (8%)	1 (3%)
Liver cirrhosis ($n = 18$)	0	0	12 (67%)	6 (33%)
HCC ($n = 13$)	0	0	4 (41%)	9 (59%)

[a] Membranous: localized mainly on membranes of hepatocytes; Mixed: localized equally on membranes and in the cytoplasm; Cytoplasmic: Diffuse, localized mainly diffusely in the cytoplasm; Inclusion body, localized in the cytoplasm like an inclusion body

shows the titers of HBsAg measured in the sera of patients with asymptomatic HBsAg carrier status, chronic hepatitis, cirrhosis of the liver, and HCC. The HBsAg titer declines with the development of liver disease and is at its lowest in HCC. Figure 7 shows the various types of intrahepatic expression of HBsAg detected by an immunofluorescent technique in type B liver disease. The intrahepatic HBsAg is localized mainly on the cell membranes of hepatocytes in asymptomatic carriers, diffusely in the cytoplasm and on the cell membranes of hepatocytes in chronic active hepatitis, and densely in the cytoplasm in cirrhosis of the liver, and it appears to be similar to an inclusion body in the cytoplasm in noncancerous areas in HCC (Table 2). We have previously observed changes in the expression of HBsAg and of hepatitis B core antigen (HBcAg) in the liver during the transition from asymptomatic carrier status to chronic hepatitis [13] as well as during the transition from the HBeAg-positive phase to the anti-HBe-positive phase of chronic hepatitis [8] and have found that the expression of HBsAg in the liver changes from membranous to cytoplasmic localization in both cases.

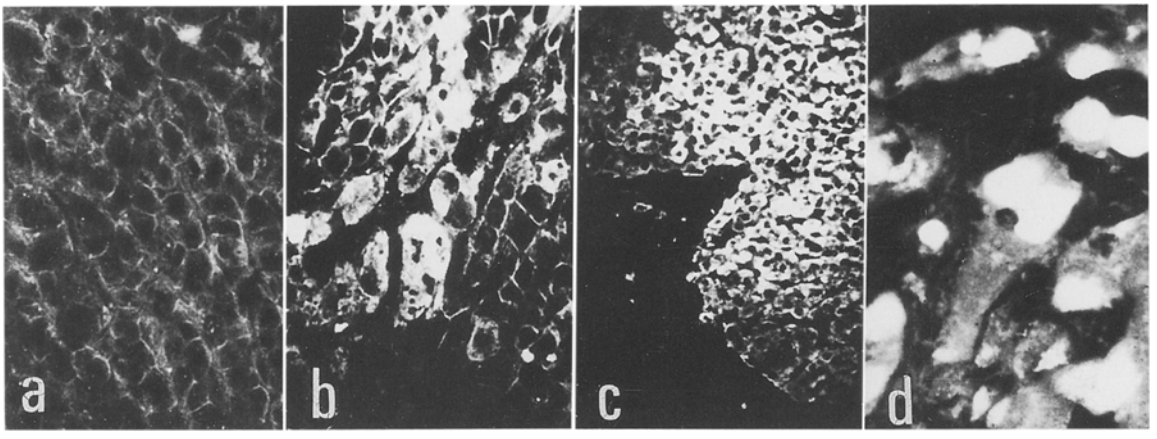

Fig. 7 a–d. Intrahepatic expression of HBsAg detected by the immunofluorescent method in patients with **a** asymptomatic HBsAg carrier status, **b** chronic persistent and active hepatitis, **c** cirrhosis of the liver, and **d** HCC (in noncancerous liver tissue)

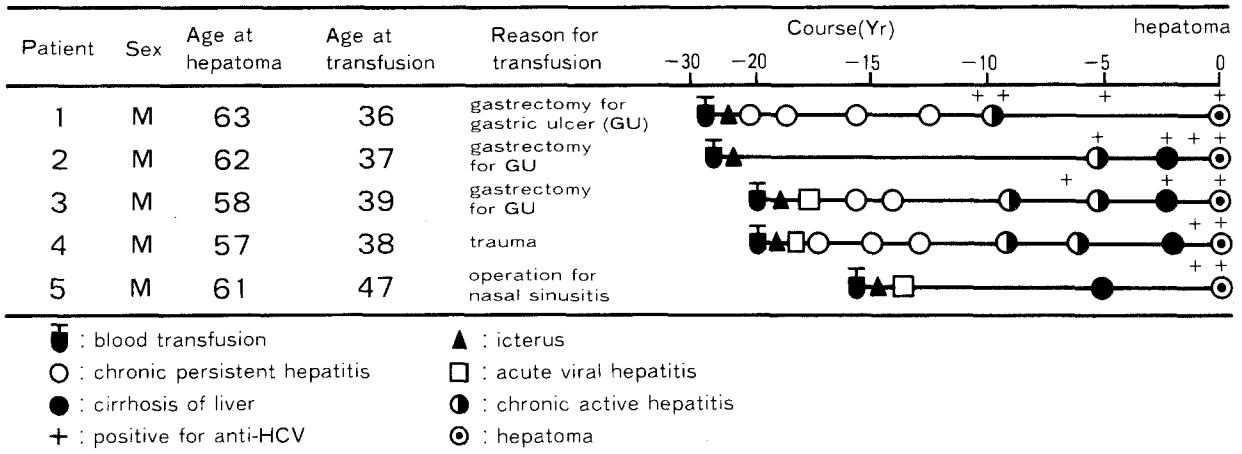

Fig. 8. Clinical course and anti-HCV status in five patients who developed HCC after non-A, non-B posttransfusion hepatitis

These results indicate that the secretion of HBsAg into the blood by hepatocytes is greatest in asymptomatic carriers, relatively copious in chronic hepatitis, of low grade in cirrhosis, and lowest in HCC. Thus, HBsAg accumulates most densely in the noncancerous area of the liver in HCC. These phenomena are compatible with the hypothesis of Chisari et al..

The role of the X gene in the causation of HCC is an area of recent interest. The X protein, the product of the X gene, appears to be expressed in the liver of HBV carriers, and an antibody specific for the X protein is present in the serum of some HBV-infected individuals, especially those with HCC. Moriarty et al. [18] evaluated 254 serum samples and reported that antibody to the X protein was detected in 6% of asymptomatic carriers, in 13% of chronic hepatitis patients, in 16% of those with cirrhosis, and in 73% of those with HCC but was not detected in any healthy control. Recently, Takada et al. and Koike [23] demonstrated that the X protein has a function of transactivating some viral and cellular factors. Furthermore, this function depends on the sequence "LGGCRHK (Leu-Gly-Gly-Cys-Arg-His-Lys)," which resembles the serine protease inhibitor's having the functions of stimulating cell division and promoting cellular oncogene [24].

Interrelationship of blood transfusions and type C HCC

Evidence has accumulated for an etiological association between infection with blood-borne non-A, non-B hepatitis virus and HCC. The most powerful evidence for such an association came from the three reports described below.

Resnick et al. [22] reported on a 59-year-old woman who developed non-A, non-B hepatitis 5 weeks after receiving a blood transfusion and developed HCC 7 years later. We reported on a 57 year-old nonalcoholic man who developed HCC at 18 years after the onset of chronic non-A, non-B hepatitis [12]. Acute hepatitis occurred approximately 2 months after the patient had been given a blood transfusion during surgery for a bleeding gastric ulcer. Five liver biopsies obtained during the 5th through 15th years of his illness were negative for HBsAg and HBcAg as determined by immunoperoxidase and immunofluorescence methods. The histological diagnoses reached on the basis of the five liver biopsies were, in order, unresolved viral hepatitis, chronic persistent hepatitis, chronic active hepatitis, chronic active hepatitis with bridging necrosis, and postnecrotic cirrhosis. Gilliam et al. [9] reported the case of a 63-year-old man who developed chronic non-A, non-B hepatitis 5 weeks after receiving a transfusion and developed HCC 9 years later.

Other evidence includes the frequent tendency for patients to develop chronic hepatitis after posttransfusion hepatitis and the high prevalence of a history of blood transfusion among patients with non-A, non-B chronic liver disease [5].

Recently, we demonstrated close correlations between blood transfusion; non-A, non-B hepatitis; and HCC by analyzing anti-HCV in serial serum samples from 21 patients with transfusion-associated HCC [14]. Anti-HCV was present in each serial sample available for testing, including samples obtained up to 14 years before the diagnosis of HCC. Figure 8 shows the clinical course and the results of serial assays for anti-HCV in five representative patients who could be followed from the onset of posttransfusion hepatitis to the development of HCC. We demonstrated that the mean intervals from the date of transfusion to the dates of diagnosis of anti-HCV-positive chronic hepatitis, cirrhosis of the liver, and HCC were about 10, 20, and 30 years, respectively. These data suggest a slow, sequential progression from acute hepatitis C virus-related non-A, non-B hepatitis through chronic hepatitis and cirrhosis to HCC, and they support a causal association between hepatitis C virus and HCC.

Causes of transmission of HCV: history of blood transfusion

As mentioned above, in Japan, the main factors contributing to the increase in the number of patients with HCC are increases in the incidence of both type C chronic hepatitis

Table 3. Prevalence of a history of blood transfusion in patients with type C chronic liver disease seen at Shinshu University Hospital in 1971–1980 and in 1981–1990

Period	Chronic hepatitis		Cirrhosis of the liver		HCC	
	Number	Prevalence	Number	Prevalence	Number	Prevalence (%)
1971–1980	183	42%	70	21%	53	13%
1981–1990	210	45%	150	41%	140	39%

and cirrhosis. Table 3 shows the prevalence of a history of blood transfusion in patients with type C chronic liver disease seen in the earlier and later decades. In the earlier decade, the prevalence of a history of blood transfusion was 42%, 21%, and 13% in chronic hepatitis, cirrhosis, and HCC, respectively, and in the later decade it was 45%, 41%, and 39%, respectively. These findings indicate that a history of blood transfusion is a major factor contributing to the increase in the incidence of type C chronic liver disease, including HCC, in Japan.

Many patients who had previously undergone surgery for diseases such as pulmonary tuberculosis, kidney tuberculosis, peptic ulcer, and myoma uteri had received transfusions [11]. After the establishment of the blood-bank system and before the current volunteer blood-donation system came into practice, as many as 80% of surgical patients who had received blood transfusions developed posttransfusion hepatitis. The incidence of non-A, non-B posttransfusion hepatitis remained very high (around 10%), even after the practice of rejecting HBsAg-positive blood had been instituted. However, the majority of patients with type C chronic liver disease have no history of blood transfusion. Yu et al. [26] reported that 67% of patients with HCC had never received a transfusion. Thus, the existence of another route of HCV infection should be considered. Watanabe et al. [25] found no anti-HCV-positive children among 200 Japanese school children, and we also reported a low incidence (2%) of anti-HCV positivity in sons and/or daughters who were born to anti-HCV-positive mothers with chronic liver disease [15]. The possibility of maternal transmission of HCV to offspring remains a mother of controversy.

With regard to the sexual transmission of HCV, in our study of Japanese families [15], anti-HCV was present in 9% of subjects whose spouses had type C chronic liver disease, a rate similar to that reported from Italy [4]. However, we cannot know whether or not those anti-HCV-positive subjects had been anti-HCV-positive or had developed liver disease before marriage. Everhart et al. [7] reported that none of the sexual partners of anti-HCV-positive patients with chronic hepatitis were positive for anti-HCV. Thus, there is no evidence indicating the existence of sexual transmission. The presence of HCV has been reported in a few patients with a history of intravenous drug abuse or tattooing. This suggests the existence of some route of horizontal transmission of HCV, although it is difficult to speculate upon a specific route of infection in patients who have no history of blood transfusion, intravenous drug abuse, or tattooing.

Although anti-HCV-positive blood as well as other surrogate-marker-positive blood is presently eliminated from the donated blood supply, the occurrence of posttransfusion hepatitis has not completely disappeared [10, 21]. To prevent the occurrence of HCC, complete elimination of HCV-containing blood from the donated blood supply is urgently needed. Furthermore, the elucidation of as yet unknown route(s) of horizontal transmission of the hepatitis-causing viruses is a subject requiring further study.

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