Jpn. J. Cancer Res. 85, 485–490, May 1994

Cumulative Risk of Hepatocellular Carcinoma in Hepatitis C Virus Carriers: Statistical Estimations from Cross-sectional Data

Hideo Tanaka,[1] Tomohiko Hiyama,[1] Hideaki Tsukuma,[1] Isaburo Fujimoto,[1] Hajime Yamano,[2] Yasuto Okubo[2] and Akira Kitada[2]

[1]Department of Field Research, Center for Adult Diseases, Osaka, 1-3-3 Nakamichi, Higashinari-ku, Osaka 537 and [2]Osaka Red Cross Blood Center, 2-4-43 Morinomiya, Jotou-ku, Osaka 536

The probability of developing hepatocellular carcinoma (HCC) among hepatitis C virus (HCV) carriers during their life-time is unknown. This paper addresses the estimation of the cumulative risk of HCC among HCV carriers using cross-sectional data. Age-specific prevalences of HCV carriers among the general population were estimated according to 5-year age group, based on the data of 2nd-generation anti-HCV assay in blood donors resident in Osaka (33,226 males and 29,054 females). Seropositivity of anti-HCV among 422 HCC cases, and the Osaka Cancer Registry data on HCC were used in the estimations of 5-year age-specific incidence rates of HCV-linked HCC. Using these data, the cumulative risk, i.e., the probability of contracting HCC within the following 15 years in 50-year-old HCV carriers, was estimated as 28% for males and 6% for females.

Key words: Hepatitis C virus — Hepatocellular carcinoma — Hepatitis C virus carrier — Cumulative risk — Epidemiology

Since hepatitis C virus (HCV) was cloned and a serologic assay for anti-HCV became available, several case-control studies[1–7] and clinical follow-up studies[8, 9] have revealed that HCV is closely associated with an increased risk of hepatocellular carcinoma (HCC). A positive correlation was also demonstrated between the prevalence of anti-HCV and the incidence rate of primary liver cancer (PLC) in Osaka, Japan.[10] These findings suggest that chronic HCV infection has an important role in the development of primary liver cancer. It is unclear, however, what proportion of HCV carriers will develop HCC during their lifetime.

Osaka is one of the highest incidence areas for PLC in the world,[11] and about 70% of it is thought to be associated with HCV infection.[12] Therefore, estimating the risk of HCC in HCV carriers within the Osaka area is very important from the viewpoint of prevention of HCC. In order to evaluate exactly or directly the risk of HCC in HCV carriers, a long-term follow-up study of HCV carriers should be conducted. To our knowledge, however, no such study has been reported. This study was therefore designed to estimate the cumulative risk of developing HCC in HCV carriers based on estimated age-specific incidence rates among HCV carriers in Osaka, Japan.

SUBJECTS AND METHODS

In order to estimate the cumulative risk of HCC among HCV carriers, we calculated the age-specific incidence rates of HCC among HCV carriers by gender. Denominators were obtained from the prevalences of HCV-RNA in voluntary blood donors, and numerators were obtained from the incidence rates of HCV-RNA positive HCC.

Prevalence of HCV-RNA in voluntary blood donors Prevalences of HCV-RNA in voluntary blood donors were estimated from the screening data of anti-HCV in voluntary blood donors and its validity. They are given by;

$$A \times B \times 1/C \qquad (X)$$

A: prevalence of anti-HCV in blood donors; B: prevalence of HCV-RNA among anti-HCV-positive blood donors; C: prevalence of anti-HCV among HCV-RNA-positive blood donors.

We used the screening data in blood donors 45–64 years of age who donated blood to the Osaka Red Cross Blood Center voluntarily between February and October, 1992. All of the blood units were tested for anti-HCV by passive hemagglutination assay (PHA, 2nd generation, Dainabot Co. Ltd., Tokyo). The assay is based on a combination of structural (core) and nonstructural (NS3–NS4) recombinant HCV proteins (pHCV-34, C100-3, and pHCV-31 antigens).[13] The blood samples from repeat donors in the above period were eligible only if they were initial specimens. The prevalence of anti-HCV was calculated according to 5-year age group and sex ("A"). "B" and "C" were obtained from the report of the Japanese Red Cross Non-A, Non-B Hepatitis Research Group.[14] They were reported as 81% and 99%, respectively, determined by the nested double polymerase chain reaction.

Thus, for example, prevalence of HCV-RNA in male blood donors aged 55–59 is estimated as follows:

$$419/6{,}090 \times 0.81 \times 1/0.99 \times (100\%) = 5.63\% \text{ (see Table I)}$$

Incidence rates of HCV-RNA-positive HCC We estimated the rates as follows:

$$\text{incidence rate of HCC} \times \text{prevalence of HCV-RNA positivity among HCC patients} = (D \times E) \times (F \times G \times 1/H) \quad (Y)$$

D: incidence rate of PLC; E: proportion of HCC incident cases among PLC incident cases; F: prevalence of anti-HCV among HCC patients; G: prevalence of HCV-RNA among anti-HCV-positive HCC patients; H: prevalence of anti-HCV among HCV-RNA-positive HCC patients.

Five-year age specific average annual incidence rate of PLC in 1987–89 ("D") was obtained from the Osaka Cancer Registry.[15] "E" was estimated as 96% using the incident cases classified into ICD-9; 1550 in the Osaka Cancer Registry in 1988.[16]

To calculate 5-year age specific prevalence of anti-HCV among HCC patients ("F"), we collected data on the seropositivities of anti-HCV and HBsAg in the 422 HCC patients (347 males, 75 females) who were treated in six teaching hospitals in the Osaka area (Center for Adult Diseases, Osaka; Osaka University Hospital; Osaka Prefectural Hospital; Osaka National Hospital; Osaka Kouseinenkin Hospital and Hyougo Prefectural Nishinomiya Hospital) in 1990–91. All the patients were diagnosed histologically and/or by a combination of angiography, computed tomography and ultrasonography. Anti-HCV was tested by enzyme-linked immunosorbent assay (ELISA), which employed the C100-3 HCV protein. When we calculated the prevalence of anti-HCV among HCC patients, the cases which were both HBsAg- and anti-HCV-positive were excluded from both numerator and denominator because of uncertainty as to whether they were linked with HBV or HCV.

"G" and "H" were calculated from the data reported by Hayashi *et al.*[17] who examined the presence of anti-HCV (ELISA C100-3 assay) and HCV-RNA in 96 Japanese HCC patients. We determined "G" to be 79% (46/58) and "H" to be 79% (46/58).

For example, the estimated incidence rate of HCV-RNA-positive HCC in males aged 55–59 is calculated as follows:

$$(212.7 \times 0.96) \times (0.764 \times 0.79 \times 1/0.79) = 156.0 \quad \text{(see Tables II and III)}$$

Estimation of the cumulative risk of HCC among HCV carriers Cumulative risk is the risk for an individual to develop the disease in question during a certain age period if no other cause of death intervenes. If the instantaneous incidence rate at an age t is given by $I(t)$, then the cumulative risk between ages t_1 and t_2 is given by:

$$1 - \exp\left(-\int_{t_1}^{t_2} I(t)\,dt\right) \quad \text{(see ref. 18)}$$

The expression inside the exponential is closely approximated by the sum of the age-specific incidence rates for each year of age between t_1 and t_2. We used "$I(t)$" from 5-year age specific incidence rates of HCC among HCV carriers. It is given by:

$$\text{(the number of incident cases of HCV-RNA-positive HCC per 100,000 population)/(the number of HCV-RNA positives per 100,000 population)} \times 100{,}000 \quad (Z)$$

Here, the denominator is given by expression (X), and the numerator is obtained from expression (Y).

RESULTS

Since blood donors were limited to individuals 16–64 years old, and the number of HCC cases aged 44 or under obtained from the six teaching hospitals was small, incidence rates were calculated for individuals aged 45 to 64. Table I presents the prevalence of anti-HCV (PHA) and estimated prevalence of HCV-RNA in voluntary blood donors within Osaka Prefecture. The estimated prevalence increased with age from 1.90% in the 45–49 age group to 7.99% in the 60–64 age group among males, and from 2.35% to 6.49% among females in the same age groups. Table II indicates the prevalences of anti-HCV (ELISA C100-3 assay) and HCV-RNA positives among HCC patients in the Osaka area. In both genders, the prevalence tended to be higher with increased age. Five-year age-specific average annual incidence rates of PLC, HCC and those of HCV-RNA-positive HCC are presented in Table III.

Table I. Prevalence of Anti-HCV[a] and HCV-RNA in Voluntary Blood Donors by Sex and Age

Age	No. of blood donors	Anti-HCV+	Anti-HCV+ (%)	HCV-RNA+ (%)
Male				
45–49	12,894	299	2.32	1.90
50–54	10,803	300	2.78	2.27
55–59	6,090	419	6.88	5.63
60–64	2,627	256	9.74	7.97
Female				
45–49	9,866	283	2.87	2.35
50–54	9,860	378	3.83	3.14
55–59	6,436	410	6.37	5.21
60–64	2,892	229	7.92	6.48

a) Second-generation PHA assay.

Table II. Prevalence of Anti-HCV[a] and HCV-RNA among HCC Patients in Six Teaching Hospitals by Sex and Age

Age	No. of HCC	Anti-HCV+	HBsAg+ Anti-HCV+	Anti-HCV+ (%)	HCV-RNA+ HCC (%)
Male					
45–49	26	10	2	33.3	33.3
50–54	44	18	3	36.6	36.6
55–59	127	98	4	76.4	76.4
60–64	150	124	4	82.2	82.2
Female					
45–49	2	0	0	—	—
50–54	5	3	0	60.0	60.0
55–59	22	16	0	72.7	72.7
60–64	46	38	4	81.0	81.0

a) First-generation ELISA assay using C100-3 peptide.
Prevalence of anti-HCV and HCV-RNA among HCC patients was considered to be the same because the positive predictive value and the sensitivity of anti-HCV were the same (see "Subjects and Methods").

Table III. Incidence Rates of PLC, HCC and of HCV-RNA Positive HCC in Osaka, 1987–89 (per 100,000)

Age	Male			Female		
	PLC	HCC	HCV-RNA+ HCC	PLC	HCC	HCV-RNA+ HCC
45–49	27.0	25.9	8.6	5.0	4.8	—
50–54	85.7	82.3	30.1	9.8	9.4	5.6
55–59	212.7	204.2	156.0	27.3	26.2	19.1
60–64	260.0	249.6	205.2	52.8	50.7	41.1

Fig. 1 shows the estimated 5-year age-specific incidence rates of HCC among HCV carriers, given by expression (Z). They were over 1,000 among male HCV carriers aged 50 years or more; on the other hand they were less than 1,000 for all ages (45–64) among female HCV carriers. The incidence rate increased with age to 55–59 years among male carriers. We could not find a peak in female HCV carriers aged up to 64 years.

Using the estimated 5-year specific incidence rates of HCC among HCV carriers, cumulative risk of HCC among HCV carriers was calculated (Table IV). The risk from age 50 to 64 among male carriers was 28%, which was about 5 times higher than that among female HCV carriers (6%).

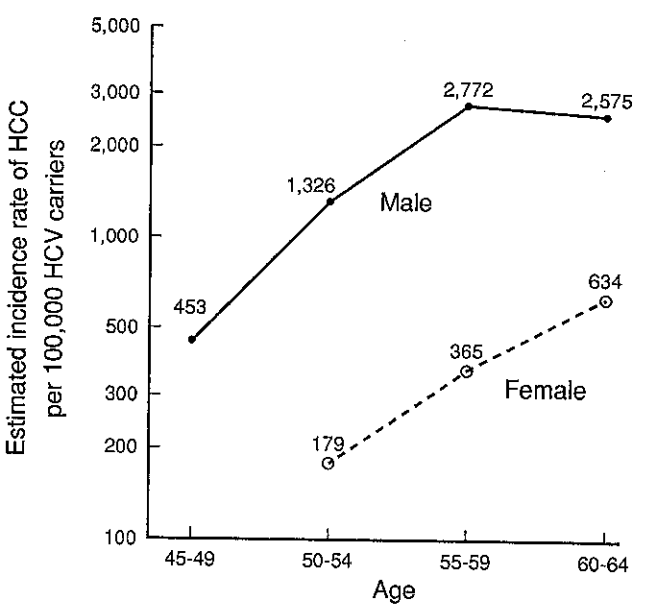

Fig. 1. Five-year age-specific incidence rates of HCC among HCV carriers by sex.

DISCUSSION

It has been reported that in some cases of posttransfusion non-A, non-B hepatitis (PTNANBH)(most of them were thought to have been caused by HCV infection), patients developed HCC 20–40 years after they were transfused.[9, 19–21] As there are considerable numbers of

Table IV. Cumulative Risk of HCC among HCV Carriers

Age	Male	Female
45–64	30%	—
50–64	28%	6%

HCV carriers who do not have a history of blood transfusion, it is supposed that not only the HCV carriers who were infected by blood transfusion but also those who were infected from other sources may possibly contract HCC. Estimating the cumulative risk of HCC in HCV carriers is very important for public health, especially in Japan, where there are quite large numbers of HCV carriers.[22]

The present study suggests that 28% of 50-year-old male HCV carriers in Osaka will contract HCC by age 64 if no other cause of death intervenes, and indicates that the risk is approximately 5 times greater in male HCV carriers than in female HCV carriers. Tsukuma *et al.*[8] reported in a clinical follow-up study that the 5-year cumulative risk of HCC was 13.3% for 301 patients with anti-HCV-positive chronic hepatitis. Using cross-sectional data in Japan, the cumulative risk of HCC among HBsAg carriers was estimated as 20% for males and 5.6% for females.[23] Although we cannot directly compare these findings with our results, they may suggest the possible extent of the cumulative risk among HCV carriers. We should next consider the limitations in the present estimation.

It is assumed in the present estimation that HCV is not eradicated naturally during the HCV carrier's life. We do not have definite evidence on this issue. However, it was reported that HCV-RNA remained positive in hepatitis C patients even after the biochemical resolution.[24, 25] This finding suggests that many HCV carriers will continue to have persistent infection, regardless of the status of liver inflammation.

We estimated the prevalence of HCV carriers in a general population using the data from voluntary blood donors. Because blood donors are likely to be more healthy and to have fewer symptoms or diseases than subjects who are randomly selected, there is a possibility of underestimating the prevalence of HCV carriers, which might lead to overestimating the incidence of HCC among HCV carriers. Further studies, using a large number of subjects collected randomly from the general population, are required.

Various factors which might be related to the development of HCC among HCV carriers, such as the subjects' age at the time of transmission, potential source of exposure to HCV, genotype of HCV and serum HCV-RNA levels, were not taken into consideration in this study. If the distributions of such factors were different among birth cohorts, the cumulative risk might have been modified by these factors.

A study of PTNANBH cases (most of which are thought to have occurred by HCV infection) conducted in the United States[26] has shown that there was some mortality from liver diseases and HCC (19/568 and 1/568 respectively, average 18-year follow-up). The authors pointed out that the observation period might be too short to detect an increase in mortality and that the death certificates they used to determine the cause of death were less than ideal. In addition, we suppose that the rather advanced age at the time of transfusion (49.1 ± 12.6 years) and poor prognosis of the subjects (51% died in the follow-up period) might have influenced the cumulative mortality of HCC in this report. Moreover, differences in HCV genotype[27] and potential environmental factors which might influence the development of HCC might produce different risks of HCC in the two countries.

The present study suggests that there is a different risk of developing HCC among male and female HCV carriers. It has been reported that serum testosterone level,[28] and parity number[29–32] are positively related to the risk of HCC. There is evidence of a positive association between oral contraceptive use and primary liver cancer.[29, 33–36] These findings suggest that hormonal difference influences the occurrence of HCC, and may cause the difference in the development of HCC among male and female HCV carriers. Differences in alcohol consumption and smoking habits, which are possible risk factors of HCC,[37–40] may partly explain the discrepant risk of HCC between males and females in Japan.

Although there were some methodological limitations as mentioned above, we estimated the cumulative risk of developing HCC in HCV carriers using cross-sectional data of the Japanese population. Gender appears to be an important factor for HCC in HCV carriers. To obtain more exact information, a prospective study observing a large number of HCV carriers is needed.

ACKNOWLEDGMENTS

This study was supported in part by a Grant-in-Aid for Cancer Research from the Japanese Ministry of Health and Welfare. The authors wish to express their gratitude to Drs. Takenobu Kamata, Norio Hayashi, Tohoru Kashiwagi, Yoshitake Shinshi, Youji Shimizu, and Manabu Masuzawa for providing access to clinical data on HCC patients. Thanks are also due to Ms. Mikiko Kiwata and the staff of the Osaka Red Cross Blood Center for their technical assistance.

(Received January 10, 1994/Accepted February 9, 1994)

REFERENCES

1) Yu, M. C., Tong, M. J., Coursaget, P., Ross, R. K., Govindarajan, S. and Henderson, B. E. Prevalence of hepatitis B and C viral markers in white and black patients with hepatocellular carcinoma in the United States. *J. Natl. Cancer Inst.*, **82,** 1038–1041 (1990).
2) Tanaka, K., Hirohata, T., Koga, S., Sugimachi, K., Kanematsu, K., Ohryohji, K., Nawata, H., Ishibashi, H., Maeda, Y., Kiyokawa, H., Tokunaga, K. and Irita, Y. Hepatitis C and hepatitis B in the etiology of hepatocellular carcinoma in the Japanese population. *Cancer Res.*, **51,** 2842–2847 (1991).
3) Yu, M.-W., You, S.-L., Chang, A.-S., Lu, S.-N., Liaw, Y.-F. and Chen, C.-J. Association between hepatitis C virus antibodies and hepatocellular carcinoma in Taiwan. *Cancer Res.*, **51,** 5621–5625 (1991).
4) Kaklamani, E., Trichopoulos, D., Tzonou, A., Zavitsanos, X., Koumantaki, Y., Hatzakis, A., Hsieh, C. C. and Hatziyannis, S. Hepatitis B and C viruses and their interaction in the origin of hepatocellular carcinoma. *J. Am. Med. Assoc.*, **265,** 1974–1976 (1991).
5) Chuang, W.-L., Chang, W.-U., Lu, S.-N., Su, W.-P., Lin, Z.-Y., Chen, S.-C., Hsieh, M.-Y., Wang, L.-Y., You, S.-L. and Chen, C.-J. The role of hepatitis B and C viruses in hepatocellular carcinoma in a hepatitis endemic area. *Cancer,* **69,** 2052–2054 (1992).
6) Zavitsanos, X., Hatzakis, A., Kaklamani, E., Tzonou, A., Toupadaki, N., Broeksma, C., Chrispeels, J., Troonen, H., Hadzyannis, S., Hsieh, C.-C., Alter, H. and Trichopoulos, D. Association between hepatitis C virus and hepatocellular carcinoma using assays based on structural and nonstructural hepatitis C virus peptides. *Cancer Res.*, **52,** 5364–5367 (1992).
7) Simonetti, G. G., Camma, C., Fioretto, F., Cottone, M., Rapicetta, M., Marino, M., Firentino, G., Craxi, A., Ciccaglione, A., Giuseppeti, R., Stroffolini, T. and Pagliaro, L. Hepatitis C virus infection as a risk factor for hepatocellular carcinoma in patients with cirrhosis. *Ann. Intern. Med.*, **116,** 97–102 (1992).
8) Tsukuma, H., Hiyama, T., Tanaka, S., Nakao, M., Yabuuchi, T., Kitamura, T., Nakanishi, K., Fujimoto, I., Inoue, A., Yamazaki, H. and Kawashima, T. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N. Engl. J. Med.*, **328,** 1797–1801 (1993).
9) Kiyosawa, K., Sodeyama, T., Tanaka, E., Gibo, Y., Yoshizawa, K., Nakano, Y., Furuta, S., Akahane, Y., Nishioka, K., Purcell, R. H. and Alter, H. J. 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).
10) Tanaka, H., Hiyama, T., Okubo, Y., Kitada, A. and Fujimoto, I. Primary liver cancer incidence rates related to hepatitis C virus infection: a correlational study in Osaka. *Cancer Causes Control,* **5,** 61–65 (1994).
11) Parkin, D. M., Muir, C. S., Whelan, S. L., Gao, Y.-T., Ferlay, J. and Powell, J. "Cancer Incidence in Five Continents Volume VI," IARC Scientific Publications No. 120 (1992). International Agency for Research on Cancer, Lyon.
12) Hiyama, T., Tsukuma, H., Fujimoto, I. and Pyong, S.-J. Comparison of liver cancer occurrence among Koreans in Korea and Koreans and Japanese in Osaka. *Proc. Korea-Japan Liver Cancer Symp.*, pp. 27–35 (1992).
13) Iino, S., Koike, K., Yasuda, K., Hino, K., Tsumakami, S., Suzuki, H., Akahane, Y., Kiyosawa, K., Tanaka, E., Yosizawa, H., Nakanishi, T., Tamura, T., Sata, M., Watoji, S., Nishioka, K. and Watanabe, J. Second-generation assay for antibody to hepatitis C virus by passive hemagglutination. *Prog. Med.*, **7,** 1911–1921 (1991) (in Japanese).
14) Watanabe, J., Matsumoto, C., Fujimura, K., Shimada, T., Yoshizawa, H., Okamoto, H., Iizuka, H., Tango, T., Ikeda, H., Endo, N., Mazda, T., Nojiri, T., Aoyama, K., Kanemitsu, K., Yamano, H., Mizui, M., Yokoishi, F., Tokunaga, K. and Nishioka, K. Predictive value of screening tests for persistent hepatitis C virus infection evidenced by viremia. *Vox Sang.*, **65,** 199–203 (1993).
15) Fujimoto, I., Hanai, A., Oshima, A., Hiyama, T., Tsukuma, H., Murakami, R., Sobue, T., Tanaka, H. and Ajiki, W. Trend in incidence and mortality rates for selected primary sites in Osaka. *In* "Cancer Incidence and Mortality in Osaka 1963–89," ed. I. Fujimoto, pp. 21–29 (1993). Osaka Foundation for Prevention of Cancer and Circulatory Diseases, Osaka.
16) "Annual Report of Osaka Cancer Registry No. 50" (1991). Osaka Prefectural Health Department, Osaka (in Japanese).
17) Hayashi, N., Takehara, T., Hagiwara, H., Fusamoto, H. and Kamata, T. Clinical importance of hepatitis C virus in Japanese hepatocellular carcinoma. *Saishin Igaku,* **47,** 1459–1465 (1992) (in Japanese).
18) Day, N. E. Cumulative rate and cumulative risk. *In* "Cancer Incidence in Five Continents, Volume IV," ed. J. Waterhouse, C. Muir, K. Shanmugaratnam and J. Powell, IARC Scientific Publications No. 42, pp. 443–445 (1982). International Agency for Research on Cancer, Lyon.
19) Resnick, R. H., Stone, K. and Antonioli, D. Primary hepatocellular carcinoma following non-A, non-B post-transfusion hepatitis. *Dig. Dis. Sci.*, **28,** 908–911 (1983).
20) Kiyosawa, K., Akahane, Y., Nagata, A. and Furuta, S. Hepatocellular carcinoma after non-A, non-B posttransfusion hepatitis. *Am. J. Gastroenterol.*, **79,** 777–781 (1984).
21) Cohen, E. B., Gang, D. L. and Zeldis, J. B. Primary hepatocellular carcinoma following nonspecific non-B hepatitis with DNA negative for HBV DNA. *Dig. Dis. Sci.*, **32,** 1428–1430 (1987).
22) Yano, M., Yatsuhashi, H., Inoue, O. and Koga, M. Epidemiology of hepatitis C virus in Japan: role in chronic liver disease and hepatocellular carcinoma. *J. Gastro-*

enterol. Hepatol. (Suppl.), **1**, 31–35 (1991).
23) Tsukuma, H., Fujimoto, I., Oshima, A. and The Liver Cancer Study Group of Japan. Estimation of risk for hepatocellular carcinoma among HBsAg carriers and heavy drinkers in Japan. *Acta Hepatol. Jpn.*, **27**, 1281–1289 (1986).
24) Alter, M. J., Margolis, H. S., Krawczynski, K., Judson, F. N., Mares, A., Alexander, W. J., Hu, P. Y., Miller, J. K., Gerber, M. A., Sampliner, R. E., Meeks, E. L. and Beach, M. J., for The Sentinel Counties Chronic Non-A, Non-B Hepatitis Study Team. The natural history of community-acquired hepatitis C in the United States. *N. Engl. J. Med.*, **327**, 1899–1905 (1992).
25) Lau, J. Y. N., Mizokami, M., Ohno, T., Diamond, D. A., Kniffen, J. and Davis, G. L. Discrepancy between biochemical and virological responses to interferon-α in chronic hepatitis C. *Lancet*, **342**, 1208–1209 (1993).
26) Seeff, L. B., Buskell-Bales, Z., Wright, E. C., Durako, S. J., Alter, H. J., Iber, F. L., Hollinger, F. B., Gitnick, G., Knodell, R. G., Perrillo, R. P., Stevens, C. E., Hollingsworth, C. G. and The National Heart, Lung and Blood Institute Study Group. Long-term mortality after transfusion-associated non-A, non-B hepatitis. *N. Engl. J. Med.*, **327**, 1906–1911 (1992).
27) Okamoto, H., Kurai, K., Okada, S., Yamamoto, K., Lizuka, H., Tanaka, T., Fukuda, S., Tsuda, F. and Mishiro, S. Full-lenght of a hepatitis C virus genome having poor homology to reported isolates: comparative study of four distinct genotypes. *Virology*, **188**, 331–341 (1992).
28) Yu, M.-W. and Chen, C.-J. Elevated serum testosterone levels and risk of hepatocellular carcinoma. *Cancer Res.*, **53**, 790–794 (1993).
29) Trichopoulos, D. Etiology of primary liver cancer and the role of steroidal hormones. *Cancer Causes Control*, **3**, 3–5 (1992).
30) Stanford, J. L., Thomas, D. B. and WHO Collaborative Study of Neoplasias and Steroid Contraceptives. Reproductive factors in the etiology of hepatocellular carcinoma. *Cancer Causes Control*, **3**, 37–42 (1992).
31) Tzonou, A., Zavitsanos, X., Hsieh, C.-C. and Trichopoulos, D. Liveborn children and risk of hepatocellular carcinoma. *Cancer Causes Control*, **3**, 171–174 (1992).
32) La Vecchia, C., Negri, E., Franceschi, S. and D'Avanzo, B. Reproductive factors and the risk of hepatocellular carcinoma in women. *Int. J. Cancer*, **52**, 351–354 (1992).
33) Henderson, B. E., Preston-Martin, S., Edmondson, H. A., Peters, R. L. and Pike, M. C. Hepatocellular carcinoma and oral contraceptives. *Br. J. Cancer*, **48**, 437–440 (1983).
34) La Vecchia, C., Negri, E., Franceschi, S. and Parazzini, F. Oral contraceptives and primary liver cancer. *Br. J. Cancer*, **59**, 460–461 (1989).
35) Hsing, W. A., Hoover, R. N., McLaughlin, J. K., Co-Chien, H. T., Wacholder, S., Blot, W. J. and Fraumeni, J. F., Jr. Oral contraceptives and primary liver cancer among young women. *Cancer Causes Control*, **3**, 43–48 (1992).
36) Tavani, A., Negri, E., Parazzini, F., Franceschi, S. and La Vecchia, C. Female hormone utilization and risk of hepatocellular carcinoma. *Br. J. Cancer*, **67**, 635–637 (1993).
37) Tsukuma, H., Hiyama, T., Oshima, A., Sobue, T., Fujimoto, I., Kasugai, H., Kojima, J., Sasaki, Y., Imaoka, S., Horiuchi, N. and Okuda, S. A case-control study of hepatocellular carcinoma in Osaka, Japan. *Int. J. Cancer*, **45**, 231–236 (1990).
38) Adami, H. O., Hsing, A. W., McLaughlin, J. K., Trichopoulos, D., Hacker, D., Ekbom, A. and Persson, I. Alcoholism and liver cirrhosis in the etiology of primary liver cancer. *Int. J. Cancer*, **51**, 898–902 (1992).
39) Trichopoulos, D., MacMahon, B., Spparos, L. and Merikas, G. Smoking and hepatitis-B-negative primary hepatocellular carcinoma. *J. Natl. Cancer Inst.*, **65**, 111–114 (1980).
40) Lam, K.-C., Yu, M.-C., Leung, J. W. C. and Henderson, B. E. Hepatitis B virus and cigarette smoking; risk factors for hepatocellular carcinoma in Hong Kong. *Cancer Res.*, **42**, 5246–5248 (1982).