death were a little lower than those observed. [Correction added on 11 October 2013, after first online publication: 'In females, by remarkable contrast, predicted numbers of death were a little higher than those observed' has been corrected to '. . . lower than observed'.] Thus, predicted HCC deaths in 2010 are 7093.1, corresponding to 84.7% of the 8374 observed. [Correction added on 11 October 2013, after first online publication: 'Thus, predicted HCC deaths in 2010 are 7093.1, corresponding to 118.1% of the 8374 observed' has been corrected to '. . . corresponding to 84.7% of the 8374 observed'.]

Predicted and observed numbers of sex- and age-specific HCC deaths in 2010, as well as differences between them, are given in Table 2. In males, observed HCC deaths were lower than those predicted through all age groups; observed HCC deaths accounted for 55.6–90.9% of those predicted. Likewise, in females aged 64 years or less, observed HCC deaths were lower than those predicted, and accounted for 21.1–99.4%. In females aged 65 years or more, on the contrary, observed HCC deaths were higher than those predicted, and corresponded to 115.1–132.7%.

Table 2 Comparison between predicted and observed number of deaths due to hepatocellular carcinoma in 2010

Sex and age	No. of deaths		
	Predicted	Observed	Ratio†
Male			
20–24	3.6	2	55.6%
25–29	7.5	5	66.7%
30–34	22.0	20	90.9%
35–39	64.5	40	62.0%
40–44	128.3	100	77.9%
45–49	261.4	229	87.6%
50–54	705.6	549	77.8%
55–59	1 984.4	1 258	63.4%
60–64	4 127.8	2 462	59.6%
65–69	4 266.0	2 993	70.2%
70–74	4 762.5	3 665	77.0%
75–79	6 677.7	4 752	71.2%
80–84	3 872.0	3 369	87.0%
Total	26 883.4	19 444	72.3%
Female			
20–24	2.9	1	34.5%
25–29	6.0	4	66.7%
30–34	17.8	6	33.7%
35–39	40.4	15	37.1%
40–44	71.1	15	21.1%
45–49	81.1	41	50.6%
50–54	153.8	80	52.0%
55–59	297.7	220	73.9%
60–64	599.3	596	99.4%
65–69	843.9	971	115.1%
70–74	1 312.3	1 606	122.4%
75–79	1 873.5	2 439	130.2%
80–84	1 793.4	2 380	132.7%
Total	7 093.1	8 374	118.1%

†Ratio = observed/predicted.

DISCUSSION

WE ANALYZED HCC mortality data by the APC model. Based on HCC deaths registered during 1940 through 2010 in Japan,[1] approximately 30 000 people died of HCC annually. According to hepatitis virus carrier rates among the first-time blood donors,[14] the peak frequency of hepatitis B surface antigen (HBsAg) was demonstrated by the 1941–1945 birth cohort, and the peak frequency of antibody to HCV by the 1931–1935 birth cohort. Hence, 1931–1935 birth years have the highest birth cohort effect in males with the APC model.

The national project for preventing mother-to-baby transmission of HBV was implemented in 1986 in Japan. As a result, the HBsAg positive rate among birth cohorts born after 1986 is extremely low at 0.04%.[15] In this study, birth cohorts born after 1986 were not subject to analysis and, therefore, we cannot evaluate the effect of the national immunoprophylaxis project by the APC model. However, we can reasonably expect that the birth cohort effect by the project will manifest itself in future analysis.

We applied the APC model to HCC mortality rate, and it reproduced the observed rate faithfully. However, in males, the observed mortality in 2010 is lower than the predicted mortality in 2010, which were calculated using data until 1990. This discrepancy would be a reflection of the introduction of new antiviral treatments and progress in surgical techniques since the 1990s, as well as the promotion of hepatitis virus screening and construction of clinical network between hospitals and clinics in each prefecture. On the contrary, in females, the observed number of HCC deaths in 2010 was a little higher than the predicted. The observed mortality of HCC would have increased because the female life expectancy was prolonged, and decompensated cirrhosis did not become the cause of death with progress of the treatment. In addition, it would reflect a lower response to interferon therapies

in women than men aged more than 50 years.[16] Furthermore, women may have fewer chances for receiving antiviral therapies than men. Also, we have demonstrated that the cumulative incidence of HCC increased with age of over 60 years in women, which is 10 years later than in men by the Markov model.[17] Another possibility is that effects of some factors, such as obesity, might have impacted especially women aged 65 years or more; they cannot be predicted by data before 1990 in Japan.

Comparison of HCC deaths predicted by the APC model with those observed demonstrates, for the first time, the impact of medical treatments for hepatitis and HCC in Japan and medical as well as control policies implemented by the Japanese government, including screening for HBV and HCV infections.[6,7] At the same time, the APC model is found to be limited in the application to predict HCC mortality in Japan since 2000.

The APC model examines mortality by three factors, and there are identification problems, such as "birth cohort = period – age". Thus, some methods have been invented to improve the application of the APC model to mortality data; effects of these methods are not in agreement, however. We employed the special structure in the birth cohort factor (Barrett's technique).[12] We accomplished a unique solution for each effect, but it may or may not be valid under another assumption.

Several limitations exist in this study. First, six time changes in the ICD codes might have influenced some effects, especially the period effect in the APC model. Second, we could not adjust confounding factors in applying the APC model, such as carrier rates of HBV and HCV infections; complete data on them are not available all through the studied period 1940–2010. Third, during this period, the difference of diagnostic ability might have influenced the analytic results obtained by the APC model. Finally, we must evaluate and discuss the results, keeping in mind the assumption of the birth cohort effect.

In conclusion, while the APC model is useful for reproducing observed HCC deaths, it would not be able to predict the mortality or incidence of the disease that can be influenced by medical intervention and prophylactic policies. In these regards, the present study does not only verify a high performance of the APC model in estimating HCC mortality, but also demonstrates the limitation of it in the application to disease that can be prevented by treatment or screening that keeps improving with time.

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APPENDIX I

Identification problem

THE AGE–PERIOD–COHORT (APC) model is constructed by three parts: (i) age factor; (ii) period factor; and (iii) birth cohort factor. However, three factors are not independent (birth cohort = period − age), so it has an "identification problem" in the methodology. For instance, let μ, A_i, P_j and C_k be one solution of the APC model, then

$$\mu^* = \mu,\ A_i^* = A_i - t(2i - I + 1)/2,\ P_j^* = P_j - t(2j - J + 1)/2,\ C_k^* = C_k - t(2k - K + 1)/2$$

is also the solution of the APC model for any number t. Thus, we cannot get a proper solution without some conditions.

There are several methods which overcome the identification problem. For example, Nakamura[18] proposed a Bayesian APC model, which assumes that the successive parameters should change gradually. Meanwhile, effects in each factor can have mathematically separate linear trend and curvature components. Based on this, Tango[19] suggested estimating only the calculable part that they designated "curvature components". On the other hand, Ohtaki *et al.*[20] or Kamo *et al.*[21] suggested an interaction model, which contains the age factor, period factor and interaction of the age and period factor, instead of the birth cohort factor.

APPENDIX II

Criteria of goodness of fit

USUALLY, DETERMINATION COEFFICIENT R^2 is used for simple and multivariate regression analysis because of assumption of variance. We used modified determination coefficient as below:[13]

$$R^2_{COR} = \frac{\left(\sum (r_{ij} - \bar{r})(\hat{r}_{ij} - \bar{\hat{r}})\right)^2}{\sum (r_{ij} - \bar{r})^2 \sum (\hat{r}_{ij} - \bar{\hat{r}})^2}$$

as criteria of goodness of fit, where r_{ij} means mortality, symbol "hat" means estimator and symbol "bar" means average. R^2_{COR} has a similar character with R^2 such as $0 \le R^2_{COR} \le 1$.

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Original Article

Estimating numbers of persons with persistent hepatitis B virus infection transmitted vertically and horizontally in the birth cohort during 1950–1985 in Japan

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Aim: We estimated numbers of persons, born between 1950 and 1985 in Japan, who were persistently infected with hepatitis B virus (HBV) through vertical and horizontal infections.

Methods: HBV carrier rates with vertical and horizontal infections were computed using sex- and age-specific prevalence rates of hepatitis B surface antigen (HBsAg) and hepatitis B e-antigen (HBeAg) by mathematical model. Probabilities of vertical HBV transmission in babies born to carrier mothers with and without HBeAg were presumed to be 90% and 10%, respectively.

Results: HBV carrier rates with vertical infection stayed contrast at approximately 0.3% in birth cohorts through 36 years (1950–1985), both in men and women. By a remarkable constant, HBV carrier rates with horizontal infection decreased steadily from 1.43% to 0.10% in men and from 0.95% to 0.03% in women. The estimated total number of HBV carriers born between 1950 and 1985 was 522 500 (355 488–693 606). Of them, the numbers of HBV carriers with vertical and horizontal infections were 197 574 (149 505–288 709) and 324 926 (205 983–404 896); they accounted for 37.81% and 62.19%, respectively, with a ratio of 1:1.64. The ratio between vertical and horizontal infections was 1:2.20 in men and 1:1.06 in women.

Conclusion: Vertical HBV infection had stayed constant until immunoprophylaxis of mother-to-baby transmission was implemented in 1986 in Japan. In contrast, horizontal HBV infection decreased over years. The decrease would be due to many factors, including improved socioeconomic environments, advanced medical maneuvers and equipment, and careful vaccination procedures.

Key words: hepatitis B e-antigen, hepatitis B virus carrier, horizontal infection, newborns, vertical infection

INTRODUCTION

THERE ARE AN estimated 350 million people infected persistently with hepatitis B virus (HBV) in the world.[1] Of them, the majority (75%) are living in Asia, and approximately 25% die of serious long-term complications of HBV infections, such as decompensated cirrhosis and hepatocellular carcinoma (HCC).[2]

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Persistent HBV infection is mainly established by vertical transmission from carrier mothers or horizontal transmission during their infancy. It is clinically and epidemiologically relevant to examine numbers of HBV carriers with vertical and horizontal transmissions, and the ratio between them, because different strategies are required to prevent each of them.

Hepatitis B e-antigen (HBeAg) in the serum of carrier mothers is a useful marker for a high possibility of vertical transmission. Persistent HBV infection is established in 90% of babies born to carrier mothers with HBeAg,[3–6] while only in 10% of babies born to those without HBeAg.[7] Thus, it is possible to estimate the number of vertical HBV infection in babies born to a given cohort of mothers in whom prevalence rates of hepatitis B surface antigen (HBsAg) and HBeAg are known.

Vertical HBV transmission can be prevented by passive and active immunoprophylaxis of babies born to carrier mothers with hepatitis B immunoglobulin and vaccine. Since 1986, the national immunoprophylaxis program was implemented in babies born to HBeAg positive carrier mothers in Japan. It is efficient in preventing mother-to-baby transmission, except in babies who have been infected with HBV *in utero*.[8]

In this study, numbers of vertical and horizontal HBV infection were estimated for men and women who were born during the 36 years between 1950 and 1985, before immunoprophylaxis was started in Japan.

METHODS

Study cohorts

FROM THE VITAL Statistics of Japan,[9] the following data were obtained for the Japanese born during 1950–1985: (i) the number of births by sex; (ii) the number of deliveries by mothers in 5-year age groups; (iii) sex ratio of newborns; and (iv) the mortality rate. From the census in Japan, the number of subpopulation stratified by sex and age at 2005 was obtained.[10]

HBV markers in study cohorts

Hepatitis B surface antigen positive rates stratified by sex and birth year were obtained for the first-time blood donors during 1995–2000 in Japan,[11] and from the fact sheet on HBV by the National Institute of Infectious Diseases.[7] HBeAg positive rates among HBsAg positive women grouped by 10 years were reported by Sasaki *et al.*[12] The study design conformed to the 1975 Declaration of Helsinki.

Assumptions

We assumed that age-specific HBV carrier rates in mothers who were born before 1930 to be the same as those in the birth group from 1931 to 1935, which is the oldest birth group among studied subjects.[11] The possibility of HBV transmission to her baby was assumed to be 90% for a carrier mother with HBeAg, and 10% for a carrier mother without HBeAg.[7]

Estimation

Estimation of the numbers of HBV carriers with vertical infection in birth groups notched by 1 year from 1950 to 1985

1 The number of babies born to HBV carrier mothers in 1-year notched birth year j (j = 1950, 51, 52, . . . , 85:36 points) was estimated by the equation: $\Sigma_i (N_{ji} * S_{ji})$: numbers of births to mothers in the age group "i", N_{ji} (i = 1 for 15–19 years old [y.o.], i = 2 for 20–24 y.o., . . . , i = 7 for 45–49 y.o.) (Fig. 1) and HBV carrier rate in the corresponding age group of mothers, S_{ji} (i = 1 for birth in [j − 19] − [j − 15], i = 2 for birth in [j − 24] − [j − 20], . . . , i = 7 for birth in [j − 49] − [j − 45]).

2 The numbers of babies whose mothers were positive and negative for HBeAg were estimated using a group-specific HBeAg positive rate E_i (Fig. 1), and HBeAg negative rate, $1 - E_i$, by the respective equations: $\Sigma_i N_{ji} * S_{ji} * E_i$ and $\Sigma_i N_{ji} * S_{ji} * (1 - E_i)$.

3 The number of HBV carrier babies with vertical infection (CV_j) was estimated by the formula: $CV_j = 0.1 * \Sigma_i N_{ji} * S_{ji} * (1 - E_i) + 0.9 * \Sigma_i N_{ji} * S_{ji} * E_i$.

4 Using the sex ratio (G_j), the numbers of HBV carriers with vertical infection (CV^M_j) for men and (CV^F_j) for women were calculated in birth groups notched by 1 year, by the respective equations: $CV^M_j = G_j / (1 + G_j) * CV_j$ and $CV^F_j = 1 / (1 + G_j) * CV_j$.

5 Finally, rates of HBV carriers with vertical infection in men (BS^MV_j) and women (BS^FV_j), respectively, to total number of birth in men (N^M_j) and women (N^F_j) were estimated in birth groups notched by 1 year by respective equations: $(BS^MV_j) = CV^M_j / N^M_j$ and $(BS^FV_j) = CV^F_j / N^F_j$ with 95% confidence interval (CI).

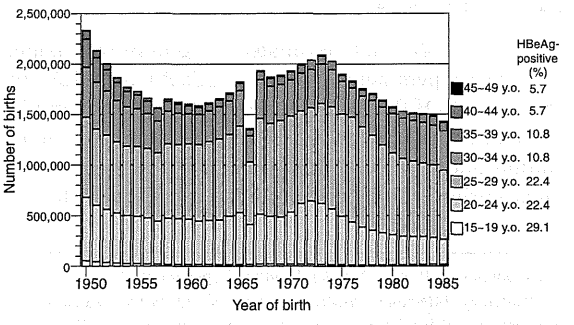

Figure 1 Number of births from mothers in 5-year age groups shifting during 1950–1985. Each component in bar graphs indicates number of live births stratified by the age of mother (15–19, 20–24, . . . , 45–49 years old). Age-specific hepatitis B e-antigen positive rates among hepatitis B virus carrier women are shown on the right.

Estimation of the number of HBV carriers with horizontal infection in birth groups notched by 1 year from 1950 to 1985

At first, number of HBV carriers was calculated by multiplying the number of births (N^M_j and N^F_j) by the HBV carrier rate in men and women in 1-year notched birth year j (33 points; 1950–1982), which were calculated by the 5-year moving average method, and that in birth year j (3 points; 1983–1985) was assumed as the same as that in the birth year cohort of 1982. The number of HBV carriers with horizontal infection (CH^M_j and CH^F_j) was calculated by subtracting the estimated number of HBV carriers with vertical infection (CV^M_j and CV^F_j) from the estimated total number of HBV carriers.

Estimation of the number of HBV carriers taking into account the mortality rate at 2005

Multiplying the numbers of HBV carriers in 1-year notched birth groups by the corresponding sex- and age-specific survival rates at 2005, the numbers of HBV carriers presumed to be alive at 2005 were obtained. They were summed to estimate the total numbers of HBV carriers.

Statistical analysis

We constructed 95% CI for the rate of HBV carriers with vertical infection using 95% CI for production of two positive rates (see Appendix for details). The χ^2-test was used for comparison of HBV carrier rates with vertical and horizontal infections. A *P*-value less than 0.05 was considered to indicate statistical significance.

RESULTS

HBV carrier rates and proportions of vertical and horizontal transmissions during 1950–1985

FIGURE 2 ILLUSTRATES HBV carrier rates in men and women born between 1950 and 1985 in Japan. HBV carrier rates decreased gradually both in men and women during these years, from 1.75% to 0.30% and from 1.27% to 0.23%, respectively.

Contribution of vertical or horizontal infection to carrier rates was estimated by a mathematical model described in Methods. The rate of HBV carriers with vertical infection stayed constant at approximately 0.3% in birth cohorts between 1950 and 1980, and decreased to 0.20% in the birth cohort of 1985. By a remarkable contrast, the rate of HBV carriers with horizontal infection decreased gradually between 1950 and 1985 both in men and women, from 1.43% to 0.10% and from 0.95% to 0.03%, respectively. The extent of decrease, in the rate of HBV carriers with horizontal infection, was higher for men than women (1.33% vs 0.92%, $P < 0.001$). The difference in HBV carrier rate with horizontal infection between men and women was statistically significant in groups born before 1972 ($P < 0.05$).

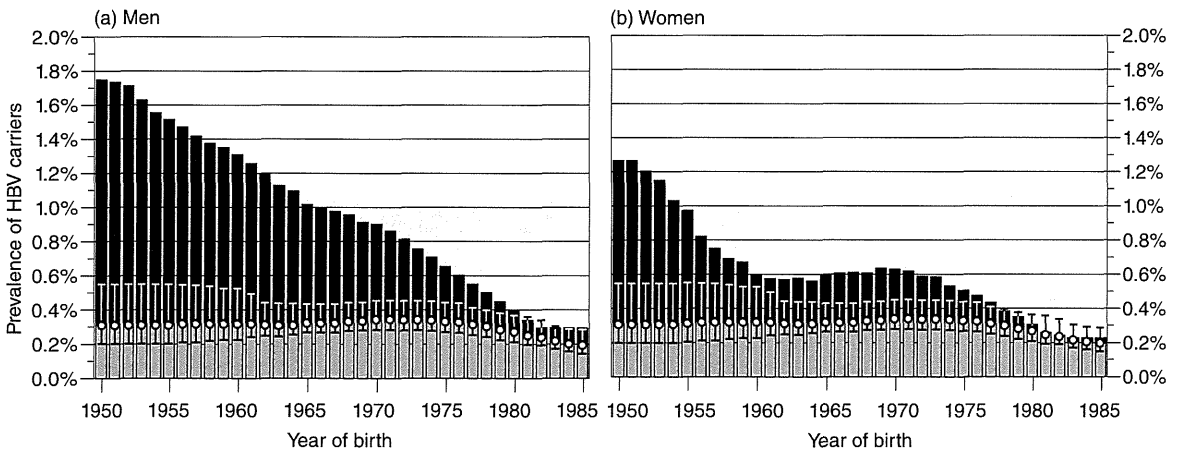

Figure 2 Hepatitis B virus (HBV) carrier rates and proportions of vertical and horizontal transmissions in yearly birth groups during 1950–1985. HBV carrier rates of vertical and horizontal transmissions are shown. Bars indicate the 95% confidence interval of vertical transmission rate. (a) Men, (b) women. ■, horizontal transmission; ▨, vertical transmission.

Estimation of the numbers of HBV carriers with vertical and horizontal infections in birth groups notched by 1 year between 1950 and 1985

Estimated numbers of HBV carriers with vertical and horizontal transmissions in 1-year notched birth cohorts during the 36 years between 1950 and 1985 are illustrated in Figure 3, both for men and women. The results are summarized in Table 1.

The estimated total number of HBV carriers born between 1950 and 1985 was 522 500. Of them, the estimated number of HBV carriers with vertical infection was 197 574, and those with horizontal infection was

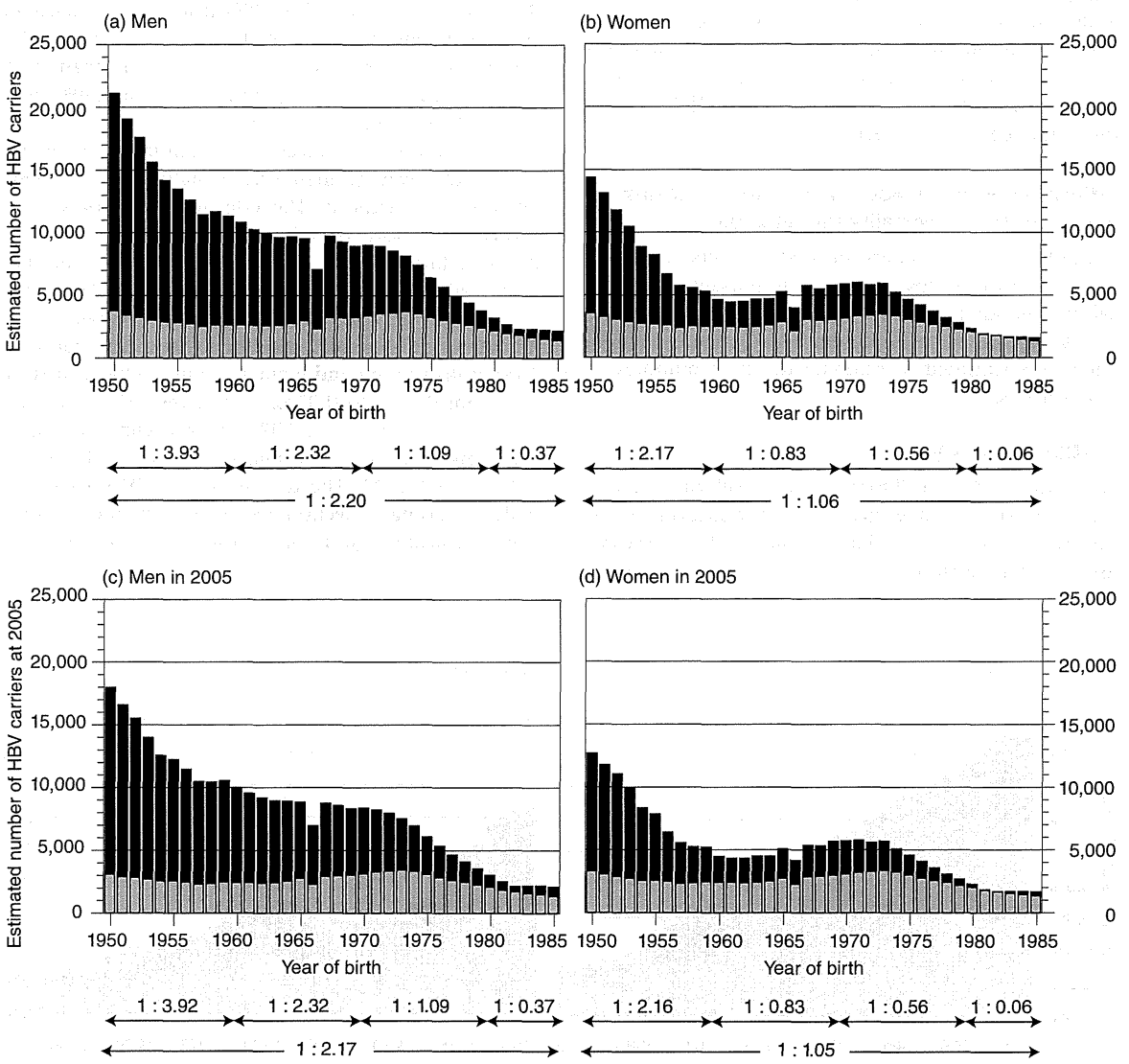

Figure 3 Estimated numbers of hepatitis B virus (HBV) carriers with vertical or horizontal transmission who were born during the 36 years between 1950 and 1985 and alive in 2005 in yearly birth groups. The ratio of the number of HBV carriers between vertical and horizontal transmissions are shown below for indicated time periods. (a) Men, (b) women, (c) men in 2005, (d) women in 2005. ■, horizontal transmission; ▨, vertical transmission.

Table 1 Estimated numbers of hepatitis B virus carriers with vertical or horizontal transmission who were born during the 36 years between 1950 and 1985 and alive in 2005

	Estimated number of HBV carriers (95% confidence interval)		
	Total	With vertical transmission	With horizontal transmission
Whole			
Total	522 500 (355 488–693 606)	197 574 (149 505–288 709)	324 926 (205 983–404 896)
Men	324 945 (235 765–414 592)	101 673 (76 948–148 542)	223 273 (158 817–266 047)
Women	197 555 (119 723–279 014)	95 901 (72 557–140 167)	101 654 (47 166–138 847)
In 2005			
Total	486 038 (329 981–646 011)	185 871 (140 826–271 096)	300 168 (189 155–374 914)
Men	297 031 (215 484–379 016)	93 773 (71 094–136 631)	203 258 (144 390–242 385)
Women	189 007 (114 497–266 995)	92 098 (69 732–134 465)	96 909 (44 765–132 529)

324 926; they accounted for 37.81% and 62.19% of total carriers, respectively, with a ratio of 1:1.64. Of 324 945 HBV carrier men, 101 673 had vertical infection and 223 273 had horizontal infection, accounting for 31.29% and 68.71%, respectively, with a ratio of 1:2.20.

Likewise, of 197 555 HBV carrier women, 95 901 had vertical infection and 101 654 had horizontal infection; they accounted for 48.54% and 51.46%, respectively, with a ratio of 1:1.06.

Moreover, the ratio between the number of HBV carriers with vertical infection and those with horizontal infection increased remarkably in men from 1:3.93 in the birth cohort during 1950–1959 to 1:2.32 during 1960–1969 and to 1:1.09 during 1970–1979, and reached the highest at 1:0.37 during 1980–1985. Similarly, in women, the ratio between the number of HBV carriers with vertical infection and those with horizontal infection increased steadily with 1:2.17, 1:0.83, 1:0.56 and 1:0.06 in birth cohorts during 1950–1959, 1960–1969, 1970–1979 and 1980–1985, respectively. However, during 1950–1985, while the number of HBV carriers with vertical infection decreased slightly, the number of HBV carriers with horizontal infection declined remarkably, both for men and women.

In women, the number of horizontal transmissions increased slightly during 1963–1973. This was, however, not the case for men in whom horizontal transmission decreased steadily through the study period (1950–1985).

Estimation of the number of HBV carriers taking into account the mortality rate at 2005

The number of live HBV carriers of a given birth year was calculated, taking into account the corresponding mortality rate at 2005 for the birth groups of 1950–1985 (Fig. 3). The estimated number of HBV carriers born between 1950 and 1985 and alive in 2005 is 486 038, corresponding to 93.02% of the 522 500 carriers who were born during the 36 years. Of them, the estimated number of HBV carriers with vertical infection is 185 871 (38.24%) and those with horizontal infection is 300 168 (61.76%), with a ratio of 1:1.61 (Table 1). Of 297 031 HBV carrier men, 93 773 (31.57%) had vertical transmission, and 203 258 (68.43%) had horizontal infection, with a ratio of 1:2.17. Likewise, of 189 007 HBV carrier women, 92 098 (48.73%) had vertical infection, and 96 909 (51.27%) had horizontal infection, with a ratio of 1:1.05.

In addition, the ratio between the number of HBV carriers alive in 2005 with vertical infection and those with horizontal infection in men also increased considerably from 1:3.92 in the birth cohort during 1950–1959 to 1:2.32 in 1960–1969, then to 1:1.09 in 1970–1979 and peaked at 1:0.37 in 1980–1985. Likewise for women, in birth cohorts during 1950–1959, 1960–1969, 1970–1979 and 1980–1985, the ratio between the number of HBV carriers alive in 2005 with vertical infection and those with horizontal infection was 1:2.16, 1:0.83, 1:0.56 and 1:0.06, respectively, showing an increasing trend between 1950 and 1985. As a whole, through the 36-year period, the number of HBV carriers alive in 2005 with horizontal infection decreased to an extent higher than those with vertical infection.

Thus, 93.02% of HBV carriers born during 1950–1985 would be alive in 2005. In the total of 486 038 carriers living in 2005, there were 297 031 (61.11%) men and 189 007 (38.89%) women with a ratio of 1.57:1 (Table 1). Overall, horizontal transmission was approximately sesquialteral as frequent as vertical transmission among HBV carriers. Proportion of horizontal

transmission was higher in men than women who carried HBV (68.43% vs 51.27% [1.33:1], $P < 0.001$).

DISCUSSION

PERSISTENT HBV INFECTION in a given community had been maintained by two principal routes. One of them is the vertical infection from mothers who carry HBV to their babies, and the other is the horizontal infection, typically during an early childhood.[13–15] The contribution of vertical or horizontal transmission to establish HBV carrier state is subject to host and viral factors, and varies among different countries. In Asian countries where HBV is endemic, with the prevalence of HBsAg of 8% or higher,[16] the vertical infection had been the main route of transmission for establishing HBV carrier state.

In 1980, plasma-derived hepatitis B (HB) vaccine was produced and introduced to babies born to mothers infected with HBV. As the HB vaccine was very efficient in preventing vertical infection,[17] the World Health Organization recommended universal vaccination of all babies, regardless of the mother's infection with HBV. By the end of 2011, the HB vaccine was introduced to 179 (93%) of the 193 member states; 93 (52%) recommended the first dose within 24 h of birth to prevent vertical transmission of HBV.[18] Hence, the contribution of vertical transmission to establish persistent HBV infection, in comparison with that of horizontal transmission, has to be examined in persons born before the implementation of HB vaccine to prophylaxis of vertical transmission.

Japan is a country of low endemicity for HBV, with the prevalence of HBsAg of less than 2% at the same level as those in Australia and New Zealand, although they belong to Asia.[16] Because the vertical infection was regarded as the principal route for establishing persistent HBV carrier state, a national program for passive and active immunoprophylaxis of babies born to carrier mothers was launched in 1986.[8] Initially, only the babies born to carrier mothers with HBeAg were indicated to immunoprophylaxis, in view of a high efficacy (~90%) of transmitting persistent HBV infection to their babies.[3–7,19] Since 1995, the indication was expanded to include babies born to carrier mothers without HBeAg.

In this study, we estimated the contribution of vertical and horizontal transmissions to the HBV carrier state in Japan. To avoid the effect of immunoprophylaxis, persons born during the 36 years between 1950 and 1985, before the start of immunoprophylaxis in 1986, were examined. A mathematical model was devised to estimate the number of HBV carriers with vertical infection. It involved HBsAg positive rates stratified by sex and birth year examined in the first-time blood donors during 1995–2000 in Japan,[11] and retrieved from the fact sheet on HBV by the National Institute of Infectious Diseases.[7] HBeAg positive rates among HBsAg positive women in 10-year age groups were reported by Sasaki *et al.*[12] The efficacy of vertical infection was assumed to be 90% for carrier mothers with HBeAg and 10% for those without HBeAg.[7] Then, the number of HBV carriers with horizontal infection was obtained by subtracting the number of HBV carriers with vertical infection from the total number of HBV carriers.

There were remarkable differences between the contribution of vertical and horizontal infections to the HBV carrier rate in birth cohorts during 1950–1985 (Fig. 2). The rate of HBV carriers with vertical infection stayed constant at 0.3% through the 36 years. By contrast, the rate of carriers with horizontal infection kept decreasing through these years, from 1.43% to 0.10% in men, and from 0.95% to 0.03% in women.

Numbers of HBV carriers with vertical and horizontal infections were obtained for persons born during 1950–1985 (Table 1). However, they would not represent numbers of HBV carriers in recent years, because some of them would have been deceased. Hence, numbers of HBV carriers with vertical and horizontal infections were obtained for persons who would be alive in 2005, the year when sex- and age-specific prevalence rates of HBsAg were determined in the first-time blood donors.[20]

The estimated number of HBV carriers who were born between 1950 and 1985 and alive in 2005 is 486 038, corresponding to 93.02% of the 522 500 carriers who were born during the 36 years. Of them, the estimated number of HBV carriers with vertical infection is 185 871 and those with horizontal infection is 300 168, accounting for 38.24% and 61.76%, respectively, with a ratio of 1:1.61. Proportion of horizontal infection was greater for men than women who carried HBV (68.43% vs 51.27%, $P < 0.001$). The higher proportion of horizontal infection in men than women would be due to increased chances of body contacts during physical activities in boys than girls, resulting in the break of skin and contamination with HBsAg positive blood. In addition, immune responses to protect from persistent HBV infection might have been lower in boys than girls.

It is of a particular note that the HBV carrier rate had already decreased during 1950–1985 in Japan, before the immunoprophylaxis program was started in 1986. The decrease was due to reduction of horizontal HBV infection (Fig. 2). The diminution of horizontal HBV

infection would be due to many factors, including improved socioeconomic environments, improved awareness of risks for infection, such as sharing a toothbrush and shaver, and advanced medical maneuvers and equipment, as well as careful vaccination procedures. Disposable needles and syringes, introduced in the early 1960s and universally distributed in the late 1970s in Japan, would have given the highest impact on decreasing horizontal transmission to establish the HBV carrier state.

It may be worth mentioning that, in women, the number of horizontal transmission increased slightly during 1963–1973 (Fig. 3). This was, however, not the case for men in whom horizontal transmission decreased steadily through the study period (1950–1985). It is not certain why horizontal infection of HBV increased preferentially in women during 1963–1973. However, vertical infection increased in women also during this period. Therefore, when the ratio of vertical to horizontal transmission was compared during four timespans of approximately 10 years in the entire study period (Fig. 3), the ratio kept increasing throughout 1950–1985 in women (from 1:2.16 to 1:0.06), as in men (1:3.92 to 1:0.37).

It has to be pointed out that this study has limitations. The HBV carrier rate of pregnant mothers may have been underestimated, because HBsAg positive rates in the first-time blood donors surveyed during 1995–2000 were used to estimate it.[11] Hence, the number of HBV carriers with vertical infection might have been underestimated. Moreover, the loss of HBsAg in the natural course, which is reported in recent years,[21] might have influenced the estimation in this study toward underestimation of HBV carriers.[22,23] However, such a large-scale survey in the 1990s with the standardized HBsAg screening level throughout the entirety of Japan had not existed except in the first-time blood donors, the database used for this mathematical model. In addition in the 1990s, the questionnaires to donors before their donation had not been strict enough to exclude the high-risk group of HBV and HCV infections than in the 2000s. Therefore, it may be worthwhile to estimate the proportion of the number of HBV carriers with vertical and horizontal infections in the first-time blood donors nowadays.

These constraints notwithstanding, contribution of horizontal infection to establish HBV carrier state, in comparison with that of vertical infection, decreased remarkably over years in Japan, even before the immunoprophylaxis of babies born to HBV carrier mothers was implemented in 1986. Furthermore, the prevalence of HBsAg in children decreased sharply from 0.75% (78/10 437) in the children born during 1978–1980 to 0.04% (12/32 049) in those during 1986–1990.[24] It is to be hoped that yearly trends of horizontal and vertical transmissions to establish HBV carrier states during the post-World War II era, described herein, may help in decision-making in Japan, whether to keep resorting solely to selective vaccination, or step toward mass vaccination of babies and/or teenagers. Increasing horizontal transmission for the persistent HBV carrier state transmitted by sexual contacts, typically with a foreign subgenotype A2,[25–29] must be taken into considerations in making such decisions. For this purpose, it is imperative to estimate the number of horizontal infection with genotype A, which is expected to have accumulated rapidly, by extensive epidemiological surveys in Japan.

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APPENDIX

WE CONSTRUCTED 95% confidence intervals (CI) for the rate of hepatitis B virus (HBV) carriers with vertical infection using 95% CI for production of two positive rates. In the Appendix, we show the conduction of 95% CI for production of two positive rates. Let n_1 and n_2 be numbers of two independent groups, x_1 and x_2 be numbers of positive in each group, and $p_1 = x_1 / n_1$, $p_2 = x_2 / n_2$ be positive rates in each group. Then, the 95% CI of log p_1 and log p_2 are asymptotically normal distributed with asymptotic variance $(1-p_1)/n_1p_1$ and $(1-p_2)/n_2p_2$. Thus, 95% CI for product of two positive rates is given by $p_1p_2 \exp\left(\pm 1.96\sqrt{(1-p_1)/n_1p_1 + (1-p_2)/n_2p_2}\right)$.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

High-sensitivity *Lens culinaris* agglutinin-reactive alpha-fetoprotein assay predicts early detection of hepatocellular carcinoma

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Abstract
Background Prognosis of patients with hepatocellular carcinoma (HCC) remains poor because HCC is frequently diagnosed late. Therefore, regular surveillance has been recommended to detect HCC at the early stage when curative treatments can be applied. HCC biomarkers, including *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3), are widely used for surveillance in Japan. A newly developed immunoassay system measures AFP-L3 % with high sensitivity. This retrospective study aimed to evaluate clinical utility of high-sensitivity AFP-L3 (hs-AFP-L3) as a predictor of early stage HCC in surveillance at a single site.
Methods Of consecutive 2830 patients in the surveillance between 2000 and 2009, 104 HCC-developed and 104 non-HCC patients were selected by eligibility criteria and propensity score matching. Samples were obtained from the HCC patients who had blood drawn annually for 3 years prior to HCC diagnosis.
Results In the present study, hs-AFP-L3 was elevated 1 year prior to diagnosis in 34.3 % of patients. The survival rate of patients with the hs-AFP-L3 $\geq$ 7 % at 1 year prior to diagnosis was significantly lower than that of patients with hs-AFP-L3 $<$ 7 %.
Conclusions Elevation of hs-AFP-L3 was early predictive of development of HCC even at low AFP levels and in absence of ultrasound findings of suspicious HCC. The hs-AFP-L3 should be added to surveillance programs with US because elevated hs-AFP-L3 may be a trigger to perform enhanced imaging modalities for confirmation of HCC.

Keywords Surveillance · A propensity score analysis · High-sensitivity AFP-L3 · DCP · HCC

Abbreviations

HCC	Hepatocellular carcinoma
AFP	Alpha-fetoprotein
AFP-L3	*Lens culinaris* agglutinin-reactive fraction of AFP
hs-AFP-L3	High-sensitivity AFP-L3
US	Ultrasound
DCP	Des-gamma-carboxy prothrombin
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
ALT	Alanine aminotransferase
MRI	Magnetic resonance imaging

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Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of death from cancer worldwide [1], and poor prognosis is reported because HCC is frequently diagnosed at late stages and is often untreatable. Therefore, surveillance for HCC has been advocated to detect HCC at

early stages when curative treatments can be applied [2, 3]. Global liver associations, including the American Association for the Study of Liver Disease (AASLD), the European Association for the Study of the Liver (EASL), and the Asian Pacific Association for the Study of the Liver (APASL), recommend regular surveillance on patients at high risk for HCC [4–6]. The most common tests used for surveillance are alpha-fetoprotein (AFP) tests and ultrasound (US). EASL and APASL adopt AFP and US in their guidelines, while AASLD recommends only US. Interpretation of US can be challenging when routine screening and comparison to previous imaging results are impossible or when US are performed by different institutes or instruments, whereas HCC biomarker values can be used independently with appropriate cutoff values. The Japan Society of Hepatology (JSH) has recommended not only US but also assays of three biomarkers: AFP, *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3), and des-gamma-carboxy prothrombin (DCP) [7].

However, AFP levels are often elevated even in patients with benign liver diseases. The low specificity of AFP has been a cause of concern for use as a HCC marker [8–10]. In contrast, a rate of AFP-L3 in total AFP (AFP-L3 %) has been reported to be highly specific for HCC in many studies [11–13]; however, accurate measurements of AFP-L3 % have been limited to patients having AFP >20 ng/mL by insufficient analytical sensitivity on a conventional assay system that is a liquid-phase binding assay (LiBASys) [14]. Recently, a micro-total analysis system (μTAS) based lectin-affinity electrophoresis using microfluidics technology has enabled accurate measurements of AFP-L3 % even at low AFP [15]. The high-sensitivity AFP-L3 (hs-AFP-L3) assay has demonstrated improvement in clinical sensitivity and predicting of prognosis in HCC patients with AFP < 20 ng/mL [16–18]. The Liver Cancer Study Group of Japan has reported that 37 % of HCC patients had low AFP (<15 ng/mL) at the HCC diagnosis [19]. They also show that 34 % of patients had tumors with maximum diameter of <2 cm. Early HCC is a distinct clinical entity with a high rate of surgical cure and detection of early HCC results in long-term survival [20]. However, elevated AFP is not always observed in patients with such small tumors. Therefore, the hs-AFP-L3 assay which can measure serum levels at low AFP is expected to improve detection of HCC at the early stage. Moreover, lower cutoff values for hs-AFP-L3 has been considered to improve clinical sensitivity [16–18].

In this study, clinical utility in early prediction of development of HCC in our study cohort under surveillance using hs-AFP-L3 and analyzed retrospectively is reported.

Patients and methods

Patients

The study protocol was approved by the Institutional Ethics Committee of Ogaki Municipal Hospital in January 2009 and was in compliance with the Declaration of Helsinki. Written informed consent for use of stored serum samples for the study was obtained from the enrolled patients.

Between 2000 and 2009, a total of consecutive 2830 patients positive for hepatitis B surface antigen (HBsAg) or anti-hepatitis C virus (HCV) antibody who visited the Department of Gastroenterology and Hepatology at Ogaki Municipal Hospital were prospectively enrolled in our HCC surveillance. Of the 2830, 1214 patients met eligibility criteria: HBsAg- or HCV RNA-positive for more than 6 months, follow-up period of >3 years before HCC diagnosis, availability of sera sampled at least twice at 12-month intervals, maximal tumor diameter <3 cm and 3 nodules or less at diagnosis, and no oral intake of warfarin which is a DCP-inducing agent.

Of these 1214 patients, 114 patients had HCC and 1100 patients had no evidence of HCC during follow-up period. To reduce the confounding effects of covariates between HCC and control patients, we selected patients using propensity score matching. Six covariates including age, gender, etiology (HBV or HCV), Child-Pugh classification, platelet number, and alanine aminotransferase (ALT) except tumor markers were used. We computed the propensity score by using logistic regression with the independent variable including age (<65 years or ≤65 years), sex (female or male), etiology (HBV or HCV), Child-Pugh classification (A, B, or C), platelet count (>150 × $10^3/m^3$ or ≤150 × $10^3/m^3$), and ALT activity (≤40 IU/mL or >40 IU/mL) as shown in previous reported cut-off values according to the previous reports [21, 22]. This model yielded a *c* statistic of 0.832 (95 % confidence interval [CI], 0.797–0.866), indicating a strong ability to differentiate between HCC and control patients. Calibration was assessed using the Hosmer–Lemeshow goodness-of-fit test [23]. The *P* value of the calculated propensity score was 0.647 based on the Hosmer–Lemeshow test and showed an absence of bias. We were able to match 104 HCC developed patients to 104 non-HCC developing patients. Table 1 shows demographics of HCC and non-HCC groups. The median of tumor size was 1.9 cm. The 69 % of HCC patients had single tumor and the 86 % of HCC patients were at TNM stage I and II.

Surveillance and diagnosis

According to Clinical Practice Guidelines for Hepatocellular Carcinoma in Japan [7], we performed US and three

Table 1 Demographics and propensity score matching

Characteristics		HCC (n = 104)	Non-HCC (n = 104)	P value
Age (years)	Median (range)	67 (37–81)	68 (14–84)	0.980
Gender	Male/female	58 (56 %)/46 (44 %)	58 (56 %)/46 (44 %)	0.889
Etiology	B/C/B + C	14 (13 %)/89 (86 %)/1 (1 %)	14 (13 %)/89 (86 %)/1 (1 %)	1.000
Child-Pugh classification	A/B/C	82 (79 %)/18 (17 %)/4 (4 %)	84 (81 %)/17 (16 %)/3 (3 %)	0.907
ALT (IU/L)	Median (range)	49 (7–361)	46 (12–321)	0.582
Platelet ($\times 10^4/mm^3$)	Median (range)	10.1 (3.2–34.0)	12.1 (2.1–41.4)	0.150
Tumor size (cm)	Median (25 %, 75 % quartile)	1.9 (1.5, 2.3)	NA	NA
Tumor number	Single/Multiple	72 (69 %)/32 (31 %)	NA	NA
TNM stage	I/II/III	49 (47 %)/41 (39 %)/14 (14 %)	NA	NA

biomarker studies (AFP, AFP-L3, and DCP) every 3–4 months and dynamic magnetic resonance imaging (MRI) every 12 months for cirrhosis patients under surveillance. For patients with chronic hepatitis, we performed US and three biomarker studies every 6 months. For diagnostic confirmation of HCC, patients had a dynamic MRI when US suggested progression in nodular lesion, change of echo pattern in nodules, or increased biomarkers: continuous elevation of AFP or increase to AFP 200 ng/mL or more, AFP-L3 15 % or more, or DCP 40 mAU/mL or more. The hs-AFP-L3 assay was not available for the surveillance of those days.

Forty-five patients were diagnosed as HCC histologically (surgical specimen, 39 patients; US-guided needle biopsy specimens, 6 patients). The remaining 59 patients were diagnosed as HCC as typical findings of dynamic MRI including hypervascular in the arterial phase with washout in the portal venous or delayed phase [4].

Treatments

Individual decisions for a primary treatment were generally made on the basis of the guidelines for HCC in Japan [7]. Patients were initially assessed for eligibility for resection. When patients declined or were deemed ineligible for resection, they underwent locoregional ablative therapy (LAT) as a second option or transcatheter arterial chemoembolization (TACE) as a third one. Of the enrolled 104 patients, 99 patients underwent resection (n = 39), LAT (n = 23), or TACE (n = 37: including patients with both LAT and TACE). Five patients did not receive any treatment for HCC. No patient underwent liver transplantation.

Imaging modalities

B-mode US was performed with an Aplio XV or XG ultrasound system (Toshiba Medical System, Tokyo, Japan) equipped with a convex probe (PUT-375BT). MR imaging was performed using a superconducting scanner operating at 1.5 T (Signa Twin Speed; General Electric Medical Systems, Milwaukee, WI). MR images were obtained in the axial plane with a phased-array multicoil for the body. To scan whole livers, the section thickness was 8–10 mm with 2- and 3-mm intersectional gaps, depending on liver size. Breath-hold T1-weighted in-phase and out-of-phase fast spoiled gradient-recalled echo (SPGR, 200/dual echo [4.3/2.1] [TR/TE], 80° flip angle, one signal averaged) MR images were obtained with a field of view of 36–42 cm and a 256 × 192 matrix during a 22-s acquisition time. T2-weighted fat suppression fast spin-echo (2000/85 [TR/TE], two signal averaged) MR images with respiratory synchronization were obtained with a field of view of 36–42 cm and a 352 × 256 matrix. Breath-hold double arterial dynamic fast SPGR images (115/1.2 [TR/TE], 70° flip angle, one signal averaged) were obtained with a field of view of 36–42 cm and 512 × 192 matrix during a 12-s acquisition time. Dynamic MR images were obtained before and after an antecubital intravenous bolus injection of 0.1 mmol/kg of gadopentetate dimeglumine (Magnevist; Bayer in Japan, Tokyo, Japan) followed by 15–20 ml of a sterile normal saline flush. The optimum timing of start of scanning was decided for each case after 1 ml test injection of gadopentetate dimeglumine. The scan times were about 25, 40, and 60 s, and 2–2.5 min after initiation of the contrast injection, representing the early hepatic artery, late hepatic artery, portal vein, and equilibrium phase, respectively. All MR images except T2-weight MR images were obtained using array spatial sensitivity encoding technique (ASSET).

Assays of hs-AFP-L3, AFP, and DCP

For this retrospective study, the measurements of hs-AFP-L3, AFP, and DCP were achieved by using a microchip capillary electrophoresis and liquid-phase binding assay on μTASWako i30 auto analyzer (Wako Pure Chemical Industries, Ltd.) [16]. Analytical sensitivity of the μTAS is 0.3 ng/mL AFP, and percentage of AFP-L3 can be

measured when AFP-L3 is over 0.3 ng/mL. Analytical sensitivity of LiBASys is 0.8 ng/mL AFP, but AFP-L3 % can not be calculated at AFP < 10 ng/mL.

Samples were obtained from 104 HCC patients who had blood drawn annually for 3 years prior to the HCC diagnosis and stored at −80 °C until the measurements. In the HCC patients, stored serum samples at −3 years (over 30 months before, $n = 94$), −2 years (from 18 to 30 months before, $n = 97$), −1 year (from 6 to 18 months before, $n = 103$), and 0 year ($n = 104$) at the time of the HCC diagnosis were measured. In the non-HCC patients, similarly, stored serum samples at −3 years ($n = 99$), −2 years ($n = 104$), and −1 year ($n = 102$), and 0 year ($n = 104$) from the end of follow-up were measured.

Statistical analysis

To evaluate the diagnostic accuracy and predictive values of AFP, hs-AFP-L3, and DCP, sensitivity and specificity were calculated with cutoff values in the guidelines [7]. Furthermore, cutoff values of 5, 7, and 10 % for hs-AFP-L3 were used for this retrospective study according to previous reports [13, 16]. Serial changes of three biomarkers before the diagnosis of HCC were analyzed by Wilcoxon matched pair signed rank test. For the evaluation of prognosis, the long-term survival of patients with HCC was determined by the Kaplan–Meier method and the log-rank test was used to compare the survival rates. The values were considered significant when P value was <0.05. The analyses were performed using JMP10 statistical software (SAS Institute Japan, Japan).

The propensity score matching was performed with SPSS, version 18.0 for Windows (International Business Machines Corporation, Tokyo, Japan).

Results

Dynamic changes of biomarkers

The dynamic changes of hs-AFP-L3, AFP, and DCP in HCC patients at −3, −2, −1, and 0 year before diagnosis are shown in Fig. 1a, b, and c. The levels of hs-AFP-L3 at −1 year were significantly elevated from the levels at −2 years ($P = 0.0001$). The levels of hs-AFP-L3 at −0 year were significantly elevated from the levels at −1 year ($P = 0.0003$, Table 2). AFP and DCP were significantly elevated between −1 and 0 year ($P = 0.0315$

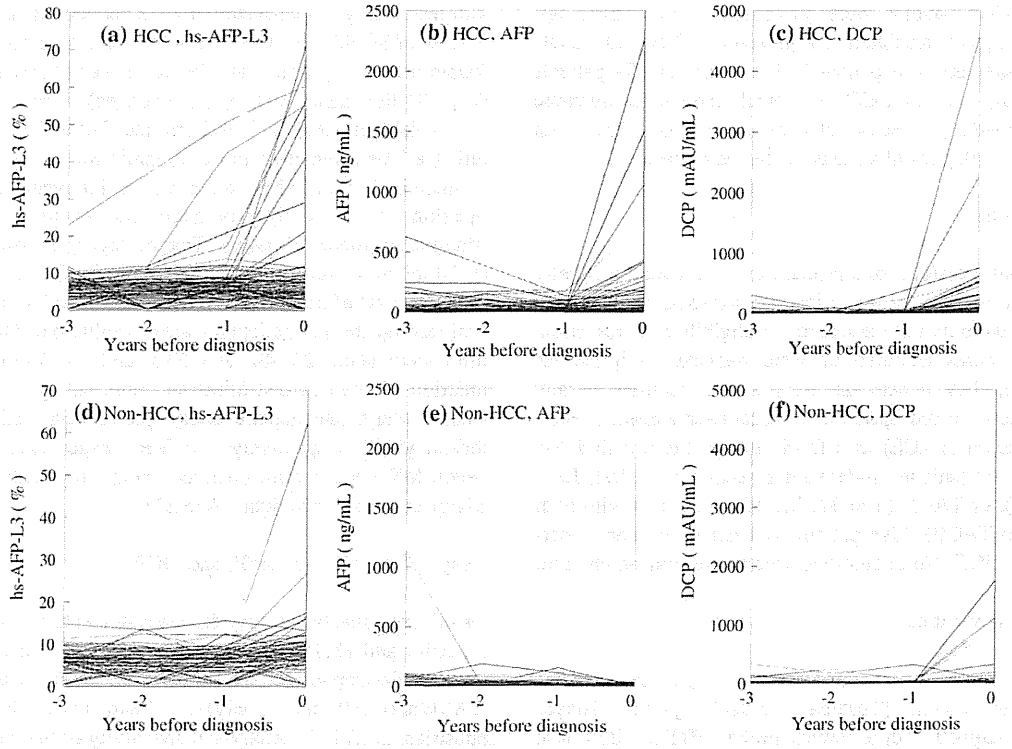

Fig. 1 Dynamic changes of biomarkers: **a** hs-AFP-L3, **b** AFP, and **c** DCP in each HCC patient ($n = 104$), and **d** hs-AFP-L3, **e** AFP, and **f** DCP in each non-HCC patient ($n = 104$)

Table 2 Serial changes of three biomarkers in HCC patients (Wilcoxon matched pair signed rank test)

Analyte	*P* value		
	At −3 year and −2 year	At −2 year and −1 year	At −1 year and 0 year
hs-AFP-L3	0.2935	0.0001	0.0003
AFP	0.4278	0.5359	0.0315
DCP	0.0926	0.6302	<0.0001

and $P < 0.0001$, respectively, Table 2). In non-HCC patients, no significant differences were observed for any markers (Fig. 1d–f). Only hs-AFP-L3 in HCC patients were significantly elevated 1 year prior to HCC diagnosis.

Sensitivity and specificity at diagnosis

Diagnostic sensitivity and specificity were evaluated for the hs-AFP-L3, AFP, DCP, and the combination of biomarkers (Table 3). The sensitivity was calculated by using HCC patient samples at diagnosis ($n = 104$) and the specificity was calculated by using non-HCC patient samples at −3 years ($n = 100$) to ensure that none had developed HCC for the following 3 years. Of the 104 HCC patients, 43 patients (41.3 %) had AFP < 10 ng/mL at which the conventional assay was not able to calculate AFP-L3 %. The sensitivity and specificity for hs-AFP-L3 were 11.5 and 100.0 %, respectively at a cutoff value of 15 %. A cutoff value of 7 % improved the sensitivity to 39.4 %. A combination assay with hs-AFP-L3, AFP, and DCP resulted in sensitivity of 60.6 % at diagnosis.

Sensitivity and specificity for 3 years before diagnosis

We calculated sensitivities using HCC samples at 3, 2, and 1 years prior to diagnosis. Similarly, specificities were calculated by using non-HCC samples (Table 4). The sensitivity and specificity for hs-AFP-L3 at −1 year were 34.3 and 74.7 %, respectively. The sensitivities at −1 year for AFP and DCP were 35.0 and 12.1 %, respectively. In HCC patients, hs-AFP-L3 turned positive at 34 patients (33.3 %) and stayed in positive at 27 patients (26.2 %) for two years till the diagnosis of HCC. In contrast, hs-AFP-L3 turned positive at 25 patients (24.3 %) and stayed in positive at 22 patients (21.4 %) for 2 years till the end of follow-up in non-HCC patients.

Table 3 Sensitivity and specificity at diagnosis

Analyte	Cutoff	Sensitivity (%)	Specificity (%)
hs-AFP-L3	5 %	50.9	51.0
	7 %	39.4	77.0
	10 %	16.3	96.0
	15 %	11.5	100.0
AFP	20 ng/mL	41.4	90.4
	200 ng/mL	12.5	99.0
DCP	40 mAU/mL	34.6	94.0
All biomarkers	7 % + 200 ng/mL + 40 mAU/mL	60.6	76.0

Comparison of tumor characteristics and survival rates

Comparing tumor characteristics at detection of HCC by a level of hs-AFP-L3 at −1 year, the tumor size, the number of tumors, and TNM stage between patients with hs-AFP-L3 ≥ 7 % and <7 % ($P = 0.064$, 0.821, and 0.504, respectively) were not statistically significant. The number of patients receiving curative treatments such as resection and LAT was significantly higher in patients with hs-AFP-L3 < 7 % ($P = 0.020$) (data not shown).

During the follow-up period after the diagnosis that was ranged from 4 to 110 months (median of 39 months), the survival rate of patients with hs-AFP-L3 ≥ 7 % was significantly lower than that of patients with hs-AFP-L3 < 7 % by using values at −1 year ($P = 0.039$) (Fig. 2). There was no statistical significance between patients with DCP ≥ 40 mAU/mL and patients with DCP < 40 mAU/mL ($P = 0.831$). No patients had AFP > 200 ng/mL at −1 year. The survival rate of patients with hs-AFP-L3 ≥ 7 % had a lower tendency than that of patients with hs-AFP-L3 < 7 % at HCC diagnosis ($P = 0.1501$).

Triggers to perform MRI for suspicious HCC and positivity rates for hs-AFP-L3

In this study population, US was performed median of 4 times between −1 year and diagnosis day. The 104 HCC

Table 4 Sensitivity and specificity for three years before diagnosis

Analyte	Year	Sensitivity (%)	Specificity (%)
hs-AFP-L3 ≥7 %	−1	34.3	74.7
	−2	25.3	80.6
	−3	24.5	77.0
AFP ≥20 ng/mL	−1	35.0	86.4
	−2	31.0	83.0
	−3	33.0	86.0
DCP ≥40 mAU/mL	−1	12.1	93.9
	−2	8.4	94.9
	−3	4.3	94.0

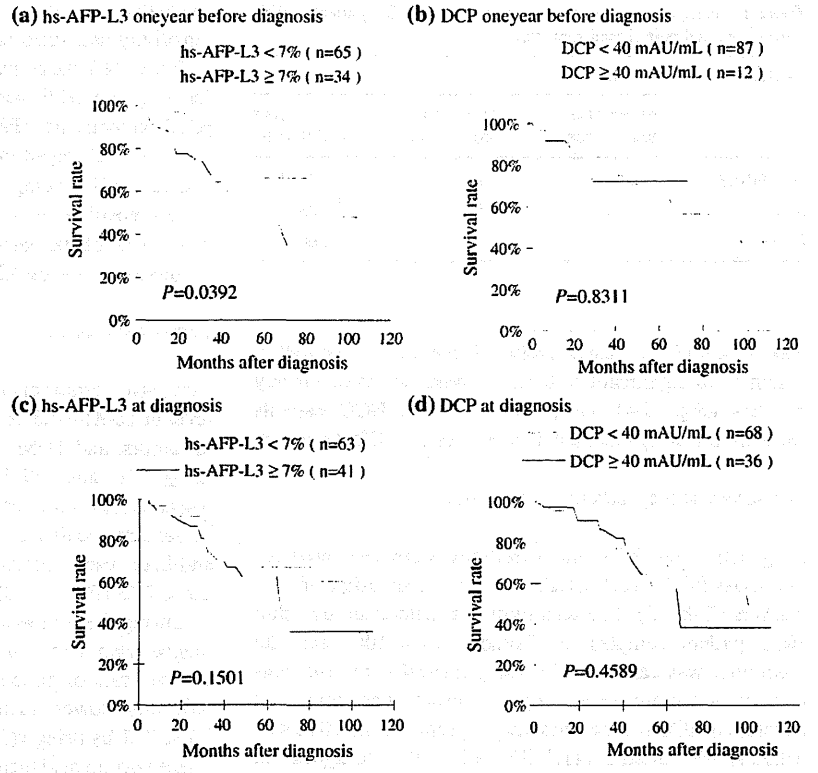

Fig. 2 Survival rates by levels of biomarkers: **a** hs-AFP-L3 and **b** DCP 1 year before, **c** hs-AFP-L3 and **d** DCP at diagnosis

patients were classified into three groups by a trigger to perform MRI for diagnostic confirmation (Table 5). US findings triggered MRI for 86 patients. The 86 patients were classified further by US findings: increase of the tumor number (51/86), increase of the tumor size (18/86), or change of the echo pattern in nodules (17/86). Five patients were monitored by MRI as results of elevated biomarkers. The remaining 13 patients were screened by MRI instead of US because interpretation of US was difficult in patients who were obese or had severe liver atrophy.

In the present retrospective study for hs-AFP-L3, 29.6 % of patients who were diagnosed with HCC by the trigger of US had hs-AFP-L3 ≥ 7 % 1 year prior to the diagnosis day. In the patients who had changes of the echo pattern in nodules, the positivity rate for hs-AFP-L3 at −1 year was 50.0 % and relatively higher compared to the other groups by US.

Table 5 Triggers to perform MRI for suspicious HCC and positivity rates for hs-AFP-L3

Triggers to perform MRI	*n*	hs-AFP-L3 >7 % At −1 year (%)	hs-AFP-L3 >7 % At diagnosis (%)
(a) Ultrasound	86	29.6	36.0
Increase of the tumor number	51	27.7	39.2
Increase of the tumor size	18	16.7	11.1
Change of the echo pattern in nodules	17	50.0	52.9
(b) Biomarkers	5	80.0	60.0
(c) Others	13	46.2	53.8

Discussion

Most studies on HCC biomarkers have focused on the accuracy at the time of diagnosis and the prediction of prognosis. So far there are a few studies which have evaluated early prediction of development of HCC in patients at high risk for HCC by biomarkers.

Taketa et al. [24] have reported that AFP-L3 values elevated above the cutoff value of 15 % with an average of 4.0 ± 4.9 months before the detection of HCC by imaging techniques. Sato et al. [25] also have demonstrated that lectin-reactive AFP elevated 3–18 months before the detection. However, only samples with AFP levels higher than 30 ng/mL were measured in their study. Recent data

indicated that the elevated AFP is not typical at HCC diagnosis for patients under in surveillance in Japan. Therefore, hs-AFP-L3 is expected to be more useful at low levels of AFP. Even though there were some differences in AFP concentration among the studies, they reported that elevation of AFP-L3 prior to diagnosis was associated with development of HCC.

Shiraki et al. [26] detected the small tumor <2 cm in maximum diameter in more than half of the patients. In the study population, they demonstrated clinical utility of lectin-reactive AFP as an early indicator while low AFP was reported limiting of the early recognition of HCC. Shimauchi et al. [27] demonstrated that AFP-L3 and DCP values showed elevated in about half of the patients at 6 months before the recognition of HCC by imaging techniques. These two markers were mutually complementary. In our study, DCP was not significantly elevated 1 year prior to diagnosis.

Lok et al. [28] have reported in a retrospective study of AFP and DCP values in patients in the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis Trial who had blood drawn every 3 months for 12 months prior to HCC diagnosis. They have concluded that the biomarkers are needed to complement ultrasound in the detection of early HCC but neither DCP nor AFP is optimal. For the study, early stage HCC was defined as a single tumor nodule <3 cm in diameter with no evidence of vascular invasion or metastasis, and only 61.5 % of patients presented with early stage HCC. In our study, median of tumor size was 1.9 cm and all patients with <3 cm. Tumor volume doubling time is reported to be 90–132 days [29] and it may take a half year or 1 year for a nodule to develop from <2 cm to >3 cm. Therefore, HCC patients in our study were diagnosed 1 year earlier than the patients in Lok's study. Clinically the tumor size between <2 cm and 3 cm is one of the factor for making decisions of treatments, and it has been reported that survival rate of patients with tumor size <2 cm is higher [20]. Therefore, HCC should be diagnosed at the earlier stage with tumor <2 cm in order to achieve better outcome.

It is well known that AFP-L3 concentration correlates well with AFP; however, AFP-L3 % is not correlated with AFP [24, 30]. AFP-L3 % is a marker that is independent of AFP. Therefore, we have used AFP-L3 % for analysis.

In the present study, hs-AFP-L3 was significantly elevated 1 year prior to HCC diagnosis in 34.3 % of patients at a cutoff value of 7 %. Tamura et al. [16] reported that a cutoff value of 7 % is most appropriate for discriminating HCC from benign liver disease using this assay. Therefore, patients with elevated hs-AFP-L3 value under surveillance should be followed up closely. The specificity of 80 % or less before diagnosis may actually mislead because the non-HCC patients selected by matching with the HCC patients were potentially higher risk group for HCC and would likely develop HCC later.

In previous studies, elevated AFP-L3 has been reported to be correlated to a shorter doubling time of tumor volume, increased hepatic arterial supply, and pathologic features such as infiltrative tumor growth pattern, capsule infiltration, vascular invasion, and intrahepatic metastasis [31, 32]. These findings are often difficult to diagnose by various imaging modalities in small HCCs. Such blood supply changes typically result in change of echo pattern in nodules. In this study, therefore, high positivity rates for hs-AFP-L3 at −1 year in the patients who had such changes of echo pattern may be associated with developing HCC. The survival rate of patients with hs-AFP-L3 > 7 % at −1 year was significantly poorer compared to patients with hs-AFP-L3 < 7 %. However, differences of the detected tumor size and number were not statistically significant between patients with hs-AFP-L3 ≥ 7 % and <7 %. AFP-L3-positive HCC nodules may be aggressive and have high malignancy potential even though the tumor size is small. Therefore, it may be useful in early detection of the aggressive tumor to perform enhanced imaging techniques such as MRI for patients with elevated hs-AFP-L3. Survival rate of patients with the hs-AFP-L3 elevation at HCC diagnosis showed a poorer tendency; however, there were no statistical differences. HCC treatments were done just after the HCC diagnosis. Therefore, HCC tumors in patients with the hs-AFP-L3 elevation 1 year before HCC diagnosis might have 1 year to grow. This 1 year may reflect the difference of survival of two groups. DCP is a good marker for poor prognosis of HCC. However, the difference of overall survival between patients with DCP ≥40 and <40 mAU/mL was not observed due to the early stage (small) HCC without obvious vascular invasion.

AFP is a good marker to distinguish high-risk group for HCC development in the future [22]; however, AFP was not elevated 1 year prior to HCC development. AFP-L3 was elevated 1 year prior to diagnosis of small HCC in 34.3 % of patients.

Interpretation of US can be challenging without comparison to previous imaging results and performance of US can be limited in patients who are obese or have severe background liver cirrhosis. In the present study, sensitivity of the combined three biomarkers was 60.6 % at diagnosis, and measurements of biomarkers are expected to complement to US in surveillance.

In conclusion, elevation of hs-AFP-L3 was early predictive of development of HCC even at low AFP levels and in absence of US findings of suspicious HCC. Prognosis of patients with elevated hs-AFP-L3 was significantly poorer. HCC may be diagnosed earlier to receive curative treatments by the elevated hs-AFP-L3 as a trigger of enhanced imaging techniques. Additional prospective studies are

expected to demonstrate whether routine measurements of hs-AFP-L3 in HCC surveillance can improve overall patient survival.

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Conflict of interest All authors declare that the authors report no conflicts of interest.

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