2. Studies of Cancer in Humans

Reports of epidemiological studies of liver cancer relevant to these monographs have employed a variety of terms to describe this disease, for example, liver cancer, primary liver cancer, primary hepatocellular carcinoma and hepatocellular carcinoma. We chose to use only the term hepatocellular carcinoma (HCC) in describing these studies. This choice was made because most of the studies have specified primary cancer of the liver or HCC, and the vast majority of primary liver cancers in most areas of the world are HCC (Colombo, 1992). A large number of case–control studies and fewer cohort studies have been conducted on the association between HBV and HCC. The many case reports, case series and descriptive studies are therefore not described in detail.

HBsAg is the marker of infection with HBV most often measured in epidemiological studies of HBV infection and HCC. While it is not often measured twice, six months apart (as required by the strict definition of carrier status), its presence in adults without acute hepatitis has generally been taken to indicate chronic infection with the virus.

The presence of other markers of HBV infection has also been documented in many studies, but most studies in which these markers were measured were not analysed with the intention of estimating their relationship with HCC. In addition, in some countries, the large majority of the population may have been exposed to HBV, and most have some marker of infection. Thus, an appropriate—uninfected—reference population of sufficient size may not be available for comparison with individuals with anti-HBc or anti-HBs alone or in combination. Lastly, in populations in which a suitable reference group may exist, it has usually not been reported separately.

2.1 Case series and case reports

2.1.1 *Hepatocellular carcinoma*

Payet *et al.* (1956) appear to have been the first to have suggested that HCC is a consequence of chronic viral hepatitis. Within five years of the identification of HBsAg (then called Australia antigen) by Blumberg *et al.* (1965), its significance as a marker of chronic viral hepatitis had been appreciated, and case reports and case series had given rise to the suspicion that it was linked to liver cancer. Okochi and Murakami (1968) appear to have been the first to have found HBsAg in a case of liver cancer (one of 19), but they made no specific comment as to its relevance. Wright *et al.* (1969) found no HBsAg-seropositive patients among 11 with liver neoplasms in the United Kingdom. In reporting the presence of HBsAg in sera from 2 of 42 cases of HCC in Hong Kong but not in 11 East African or 12 US cases, Smith and Blumberg (1969) stated the hypothesis that the antigen and its underlying viral infection were linked to HCC. Sherlock *et al.* (1970) reported HBsAg seropositivity in five male patients with HCC, three from Greece, one from the United Kingdom and one from Sierra Leone; Hadziyannis *et al.* (1970) found HBsAg in sera from 4 of 13 Greek patients with HCC superimposed on active cirrhosis and in six other cases of HCC.

With the development and application of more sensitive tests for HBsAg, case series of HCC have consistently shown apparently high proportions seropositive for the antigen

(Blumberg & London, 1982, 1985). The earlier reports were comprehensively reviewed by Szmuness (1978). Reports of HBsAg seropositivity in the rare cases of HCC in children also appeared (Shimoda *et al.*, 1980), often linking the infection in the child to chronic infection in the mother (Ohaki *et al.*, 1983). Instances of multiple HCC have been reported in families, often with an apparently high prevalence of HBsAg in the serum in both the cases and unaffected members of the family (Tong *et al.*, 1979; Tong & Govindarajan, 1988; Alberts *et al.*, 1991).

HbsAg, and sometimes HBV DNA, have also been reported in liver tissue from patients with HCC (see, for example Tanaka & Mori, 1985; Tanaka *et al.*, 1986), usually in non-neoplastic hepatocytes and rarely in the cancer cells (Tanaka & Mori, 1985).

2.1.2 *Other cancers*

In several case series of patients with cholangiocarcinoma, the prevalence of HBsAg (Okuda *et al.*, 1980) or of markers of HBV in liver tissue (Bunyaratvej *et al.*, 1979; Suwangool, 1979) appeared to be lower than that among cases of HCC and similar to that reported in subjects without HCC.

In two early studies of the presence of HBsAg in sera from patients with a range of cancers (e.g. leukaemias, Hodgkin's disease, breast cancer, lymphoma, multiple myeloma), prevalences of 1.1–2.8% were observed (Viola *et al.*, 1972; Al-Sarraf *et al.*, 1973). In a study of pancreatic tissue from patients who had undergone surgery, immunoperoxidase staining revealed HBsAg in five (7%) patients with pancreatic cancer (Hohenberger, 1985). Planes *et al.* (1976) noted that the prevalence of HBsAg in the sera of patients with malignant lymphoma was related to treatment and that infection with HBV may have occurred as a result of chemotherapeutic immunodepression, parenteral injection, transfusion or a prolonged stay in a hospital environment. In several reports of high prevalences of HBV markers in children with cancer, mainly lymphatic and haematopoietic cancers (Mikhailov *et al.*, 1986; Pontisso *et al.*, 1987; Jackowska *et al.*, 1990), it was considered possible that infection had occurred after the cancer developed.

2.2 Descriptive studies

A strong geographical correlation has been found between the incidence of HCC and the prevalence of HBsAg seropositivity (see, for instance, Szmuness, 1978; Maupas & Melnick, 1981; Lin *et al.*, 1986; Hsing *et al.*, 1991). A number of studies have also shown a high prevalence of HBsAg seropositivity in migrants from countries where the risk for HCC is high (see, for example, Szmuness *et al.*, 1978b).

2.3 Cohort studies

Cohort studies of HBV and HCC can be divided into three broad groups: studies of general population groups, studies of blood donors and studies of populations with pre-existing disease.

2.3.1 *Prospective studies of general population groups* (Table 5, p. 73)

Beasley and colleagues recruited 22 707 male Government employees in Taiwan, China, to evaluate their risk for HCC (Beasley & Lin, 1978; Beasley *et al.*, 1981; Beasley & Hwang,

1991). Subjects were recruited at the time of routine physical examinations offered by the Government between March 1976 and June 1978. Study subjects were Chinese men aged from their twenties to over 70; 82% were 40–59 years of age. Follow-up for cancer incidence and mortality from all causes was conducted through medical and life insurance records and also by annual physical examinations of both HBV carriers and a subset of non-carriers. At the time of enrolment into the study, 3454 of the 22 707 men were HBsAg seropositive, 15 570 were anti-HBs seropositive and anti-HBc seropositive, 2411 were only anti-HBc positive and 1272 had no HBV marker. As at 30 June 1989, none of the HBsAg-seronegative men who were re-tested had become HBsAg seropositive. All HBsAg-seropositive men were re-tested annually; about 1% per year became HBsAg seronegative. At 30 June 1989, 194 cases of HCC had occurred, 184 (95%) in the HBsAg seropositive men, conferring a relative risk (RR) of 103 (95% CI, 57–205) as compared with HBsAg-seronegative subjects. The remaining 10 cases of HCC all occurred among the 17 981 anti-HBc-seropositive men. Among HBsAg-seronegative men, the difference in risk between those who were anti-HBc seropositive and those with no marker of HBV infection was not significant. The occurrence of HCC in the entire population was related to age, evidence of cirrhosis, HBeAg seropositivity and IgM anti-HBe seropositivity. The only causes of marked excess mortality found in relation to HBV markers were HCC and cirrhosis.

Tu *et al.* (1985) studied all men over the age of 40 from four communes on Chongming Island, China, for development of HCC; 98% (12 222 men) of those eligible participated and were followed for three years. Of the 12 222 men, 1971 (16.1%) were classified as HBV carriers (defined as being either HBsAg seropositive or anti-HBs seronegative and anti-HBc seropositive at the time of the survey). Thirty-seven of the 70 deaths from HCC occurred among the HBV carriers and 33 occurred among the non-carriers, giving an RR of 6.7 [95% CI, 4.2–10.7]. The authors also studied the effects of water source, cigarette smoking, maize consumption (as a measure of exposure to aflatoxin B_1) and alcohol consumption on the risk for HCC. A significantly higher rate of HCC was seen among carriers who smoked 20 cigarettes or more per day. None of the other factors had a significant effect.

Yeh *et al.* (1989) conducted a prospective study of the effects of HBV and aflatoxins on risk for HCC in the southern Guangxi Autonomous Region, China. The entire cohort consisted of 7917 men who were between the ages of 25 and 64 at enrolment, lived in one of five communities and did not have HCC at the initiation of the study. The cohort was assembled between July 1982 and June 1983, at which time demographic information was collected and a blood sample was drawn; the sera were banked. In 1987, the sera of 2072 men were tested for HBsAg. The 2072 men included 149 men who had died as of 31 July 1986, of whom 76 had died of HCC, and a 25% random sample of the men (1923) still alive. Four live controls were matched to each death from HCC. Of the 76 cases, 69 had occurred in HBsAg-seropositive men (90.7%), whereas 68 of the 304 matched controls were HBsAg seropositive (22.4%) (RR, 39; 95% CI, 16–117). In a geographical analysis of these data, mortality from HCC was not correlated with the mean prevalence of HBsAg seropositivity by commune, but the range of HBsAg seropositivity was narrow (19.5–24.8%). A positive correlation was found between the estimated mean level of aflatoxin B_1 consumed in food and the rate of HCC by commune, and the range of aflatoxin B_1 levels was broad (0.3–51.8 mg/person per year).

Ding *et al.* (1988) carried out a prospective investigation of residents of the Guangxi Autonomous Region, China. A total of 22 830 people [sex distribution unspecified] over the age of 20 years were stratified on the basis of HBsAg status, hepatic enlargement, alanine aminotransferase levels and residence in an area with a high or low rate of HCC. The average period of follow-up was 6.8 years. The highest RR for HCC occurred in HBsAg-seropositive people with evidence of both liver enlargement and abnormal liver function, regardless of place of residence. In the group from the high-rate area, 30.6% who were HBsAg seropositive and 2.7% who were HBsAg seronegative developed HCC. In the group from the low-rate area, 10% of the HBsAg-seropositive and 2.4% of the HBsAg-seronegative people developed HCC. In the total population, the RR for HCC, adjusted for age, was 8.2 [95% CI, 4.5–15] in HBsAg-seropositive as compared with HBsAg-seronegative subjects.

In a study in Shanghai, China, an association between HBsAg seropositivity and HCC was reported among men participating in a prospective study of diet and cancer (Ross *et al.*, 1992). Between January 1986 and September 1989, 18 244 male subjects aged 45–64 were enrolled in the cohort; 22 cases of HCC had occurred by 1 March 1990. A nested case–control study was conducted with 140 controls matched by age (within one year), sample date (within one month) and residence, who had no history of liver cancer when the case was diagnosed. Five cases have been confirmed by biopsy. Conditional logistic regression, controlling for education, urinary aflatoxins, smoking and alcohol use, yielded an RR of 8.5 (95% CI, 2.8–26) for HBsAg seropositivity as compared with HBsAg seronegativity. The RR associated with detectable urinary aflatoxins was 3.8 (1.2–12), adjusted for educational level, HBsAg seropositivity, smoking and alcohol use; the RR associated with both HBsAg seropositivity and detectable urinary aflatoxins was 60 (95% CI, 6.4–562).

In the Japan–Hawaii Cancer Study of 7498 men of Japanese ancestry born between 1900 and 1919 and living in Hawaii, sera were collected in 1967–70 and stored. From 1967 through 1980, 18 histologically confirmed incident cases of HCC were identified; serum was available for 16. In a case–control analysis (Nomura *et al.*, 1982), each of the 16 cases was matched to three controls from the study cohort by age and serum collection date. Ten of the cases were seropositive for HBsAg as compared with none of the controls ($p < 0.0001$). Another indicator of persistent infection, anti-HBc without anti-HBs, was detected in 7 of the 16 patients and in two of the 48 controls.

Iijima *et al.* (1984) and Sakuma *et al.* (1988) studied prospectively two overlapping cohorts of male Japanese employees of Japan National Railways. The first cohort was identified in 1973 and 1978. A total of 6918 men, 126 of whom were found to be HBsAg seropositive at the time of the study (1.8%) were followed to March 1985. Average follow-up was 8.5 years (range, 6.5–11.5 years) and was conducted using annual health examinations, self-reported disease (mandatory for any illness lasting more than six days), follow-up of all men who failed to report for an annual examination, and death certificates. Four HCCs developed in the HBsAg-seropositive group and six in the HBsAg-seronegative group, giving an RR of 30 [95% CI, 8.1–77]. The second cohort consisted of 25 547 men who were identified in 1977–79; the purpose of this study was to determine whether HBeAg status among HBsAg-seropositive men ($n = 513$) was associated with the risk for developing HCC. Average follow-up to 1985 was 7.3 years. RRs were calculated in relation to the risks for HBsAg-seronegative men. HCC was observed in 21 HBsAg-seronegative and 9 HBsAg-

seropositive individuals. The RR was 50 [95% CI, 0.66–280] for the 30 HBsAg-seropositive, HBeAg-seropositive men (one HCC), 9.5 [1.1–34] for the 238 HBsAg-seropositive, anti-HBe-seropositive carriers (two HCCs) and 29 [11–63] for the 245 HBsAg-seropositive, HBeAg- and anti-HBe-seronegative carriers (six HCCs).

A population-based study of risk for HCC was conducted among Alaskan natives (Alward *et al.*, 1985; Heyward *et al.*, 1985; McMahon *et al.*, 1990a,b). About two-thirds of the Alaskan native population was classified according to HBV infection status in a statewide screening programme begun in 1983 with the establishment of a HBV carrier registry. The authors identified 1400 HBV carriers (824 men, 576 women) and followed them up until 1 July 1987 (when 1292 were left) for a total of 7815 person-years. The study cohort comprised people of all three Alaskan native groups (85% Inuit, 7.5% Indian, 7.5% Aleut). Review of HBV sequelae from January 1975 to July 1987 showed that 20 cases of HCC had occurred, 19 of which were histologically confirmed. The annual incidence among men was 387/100 000, and that among women was 63/100 000. The incidence of HCC in HBsAg-seronegative people was estimated from the prevalence of HBsAg seronegativity in the Alaskan native population (97%) and the seven HCCs diagnosed in HBsAg-seronegative Alaskan natives between 1975 and 1987, to give an RR of 148 [95% CI, 59–305] for carriers *versus* non-carriers (McMahon *et al.*, 1990a).

2.3.2 *Prospective studies of blood donors* (Table 5)

Oshima *et al.* (1984) followed a cohort of 8646 HBsAg-seropositive male Japanese blood donors in the Osaka Red Cross Blood Center, who were found to be HBsAg seropositive in 1972–75. Follow-up was conducted by examining data in the Osaka Cancer Registry and the death certificate files of Osaka Prefecture through the end of 1980. The mean length of follow-up was 6.2 years (range, 5–8.5 years). The expected number of cases was based on the age-specific incidence rates among the general population of Osaka. The study cohort developed 20 HCCs during the follow-up period, with 3.0 expected, giving a significant RR of 6.6 [95% CI, 4.0–10]. In a nested case–control analysis, alcohol drinking was significantly related to the risk of developing HCC, with RRs of 5.5 (95% CI, 1.2–26) for moderate drinkers and 8.0 (95% CI, 1.3–50) for heavy drinkers in comparison with non-drinkers.

Fukao (1985) identified 1000 HBsAg-seropositive and 10 000 HBsAg-seronegative blood donors from blood donation records in Miyagi Prefecture, Japan. All cohort members were men over the age of 30 years who had donated blood between 1971 and 1977. The HBsAg-seronegative group was matched 10:1 to the HBsAg-seropositive group by age, district of residence and month of blood donation. Cancer incidence was determined from the records of the Migayi Prefectural Cancer Registry for the years 1971–80. Three HCCs were detected in the HBsAg-seropositive group and one in the HBsAg-seronegative group, giving an RR of 30 [95% CI, 6.0–88] in carriers as compared with non-carriers.

Tokudome *et al.* (1987, 1988) estimated the risk for HCC among HBV carriers in Japan. A cohort of 3769 HBsAg-seropositive women was identified from among blood donors at the Fukuoka Red Cross Blood Center by examining Red Cross records of donations between the years 1977 and 1982. Mortality follow-up was completed until 1985; vital status was determined by checking against each donor's home residence card. Death certificates were

obtained for each donor known to be dead. The 220 women (5.8%) who were lost to follow-up were assumed in the analysis to be alive at the end of the study. The average period of follow-up was five years. Seventeen deaths occurred during the study period, four of which were due to HCC. In comparison with the age-adjusted mortality rates among all women in Fukuoka Prefecture in 1980, the RR for HCC was 5.6 [95% CI, 1.5–14] (Tokudome *et al.*, 1987). In a cohort of 2595 HBsAg-seropositive male blood donors identified during 1977–79 and followed up to 1983 (9.2% lost to follow-up; average length of follow-up, six years), 15 HCCs developed as compared with 2.1 expected on the basis of age-specific mortality rates for Fukuoka Prefecture in 1980. The RR in HBsAg-seropositive men in relation to the general male population was 7.3 [95% CI, 4.1–12] (Tokudome *et al.*, 1988).

Prince and Alcabes (1982) identified a cohort of HBsAg-seropositive men from four sources in the USA: the health departments of New Jersey, New York State and New York City and blood donors in the Greater New York Blood Program. The three health departments maintain lists of HBsAg-seropositive people, most of whom are blood donors who were found to be HBsAg seropositive during routine testing. Men identified as being HBsAg seropositive between 1971 and 1979 and who were residents of either New York City (n = 5353) or New York State (n = 1497) were included. Causes of death were determined by record matching with the New York State and New York City health departments. Four deaths from HCC occurred (three in New York City), as compared with 0.40 expected on the basis of mortality rates for the general populations of New York City and New York State [RR, 10; 95% CI, 2.7–25].

Dodd and Nath (1987) conducted a mortality study of people who had given blood during 1971–80 in the national Red Cross Donor Deferral Registry and identified 15 166 HBsAg-seropositive and 18 144 HBsAg-seronegative people. Vital status was determined by matching records with those of the Social Security Administration, and cause of death was determined from death certificates. The mean length of follow-up for the HBsAg-seropositive group was 3.7 years and that for the HBsAg-seronegative group, 3.3 years. Men comprised 70% of the HBsAg-seropositive group and 63% of the HBsAg-seronegative group. Six HCCs occurred in the HBsAg-seropositive group and none in the HBsAg-seronegative group. The authors give a standardized mortality ratio (SMR) for HCC of 27 [95% CI, 10–39] on the basis of mortality rates in the general population.

A prospective mortality study of HBsAg-seropositive people who donated blood in England and Wales between 1971 and 1981 (Hall *et al.*, 1985) comprised 2880 men and 1054 women, of whom more than 92% could be followed for death to the end of 1983. Seventy-seven cohort members died during the period. Five deaths from HCC were reported among men in contrast to the 0.1 expected, giving a RR of 42 (95% CI, 13–98). No death from HCC occurred among the female cohort members (0.02 expected).

2.3.3 *Prospective studies of populations with pre-existing disease*

The incidence of HCC in HBsAg-seropositive and HBsAg-seronegative subjects has been studied among patients with pre-existing liver disease, as indicated variously by abnormal liver function, 'chronic hepatitis' and cirrhosis. The RRs for HCC associated with HBsAg seropositivity in these studies were generally small, e.g. [RR, 2.1 (95% CI, 0.3–17)] (Liaw *et al.*, 1986). Their interpretation is complicated by the fact that causes of liver disease

other than HBV may also be associated with an increased risk for HCC (Liaw *et al.*, 1986; Dodd & Nath, 1987; Colombo *et al.*, 1991; Johnson, 1991; Kato *et al.*, 1992).

2.4 Case–control studies

2.4.1 *Hepatocellular carcinoma*

Many case–control studies have been published on the relationship between HCC and HBV infection. The prevalences of seropositivity are summarized in Table 6 (p. 90). In all studies, tests for HBV markers were performed on one occasion only, and 'carriers' were taken to be subjects in whom HBsAg was detected at that time. Unless otherwise noted in the Table, testing for HBV markers was done by radioimmunoassay. RRs, as measured by the odds ratio (OR) and 95% Cornfield confidence intervals (CIs), were calculated by the Working Group wherever the data reported in the original papers allowed it. In general, only ORs and *p* values are reported in the text, while ORs and CIs are reported in Table 6. Studies of clinical series (typically, patients with liver disease) in which cases of HCC were a subgroup but in which there was no specifically defined control group were not included.

(*a*) *Africa*

Prince *et al.* (1970) reported a comparison of patients with HCC and other chronic liver diseases with various control groups in relation to the prevalence of HBsAg seropositivity. They tested sera from subjects from Senegal, Uganda and the USA using two serological assays—agar-gel diffusion and high-voltage immunoelectroosmophoresis; the latter was 10 times more sensitive than the former. The prevalences of HBsAg seropositivity among the subjects examined were: in Senegal, 42% of 210 HCC patients, 9% of 201 adult males and 12.7% of 959 army personnel; in Uganda, 12% of 34 HCC patients, 23% of 26 with cirrhosis and 2% of 311 healthy subjects [OR for HCC, 6.8; 1.8–25]; in the USA, 4% of 55 HCC patients, 29% of 42 with chronic active hepatitis, 8% of 124 with cirrhosis and 0.1% of 55 956 blood donors. The authors remarked that, although the controls were not matched to the cases by age, sex or place of residence, hepatitis virus appeared to play an important role in at least a proportion of the cases of chronic liver disease.

Vogel *et al.* (1972) reported the results of a study conducted among in-patients at Mulago Hospital, Kampala, Uganda, or the Solid Tumour Centre in Uganda between October 1969 and May 1970 (Vogel *et al.*, 1970) and January 1970 to May 1971. The 90 HCC cases (in 73 men and 17 women) had a significantly higher frequency of HBsAg in their sera (40%) than the 224 (149 non-neoplastic and 75 neoplastic) controls combined (151 men, 73 women) (3%; $p < 0.001$). Control patients with cirrhosis or hepatitis were excluded; histological confirmation was available for 71 of the HCC cases. There was no difference between cases and controls with respect to anti-HBs seropositivity (28–37%). A nonsignificant association was noted between HBsAg seropositivity in HCC patients and the presence of cirrhosis (44% in comparison with 27% without cirrhosis). Serum was tested by complement fixation, counter immunoelectrophoresis and passive haemagglutination.

Kew *et al.* (1974) investigated 75 male Bantu miners in South Africa with HCC confirmed at necropsy. The control group of 18 377 healthy miners was comparable with respect to age and tribal distribution. Testing of sera for HBsAg by complement fixation and

Table 5. Prospective studies of HBV surface antigen (HBsAg)-seropositive people for the development of hepatocellular carcinoma (HCC)

Region, reference, location	Subjects (age)	HBsAg seroprevalence	Mean duration of follow-up (years)	No. of cases of HCC	Annual incidence (cases/ 100 000)	RR (95% CI)
America						
Nomura *et al.* (1982) Hawaii, USA	Men of Japanese ancestry (born 1900–19) Controls	7498 HBsAg+ 16 HCCs (10 HBsAg+) 48 (0 HBsAg+)	11.8	16	[18]	[∞], $p < 0.0001$ Nested case-control analysis
Prince & Alcabes (1982) New York, USA	Blood donors; men (20–> 50 years)	6850 HBsAg+	4.4	4	[13, crude]	[10; 2.7–26] SMR using HCC mortality in New York
Dodd & Nath (1987) USA	Red Cross blood donors (age at death from HCC, 30–64 years)	15 166 HBsAg+ 18 144 HBsAg-	3.68 3.26	6 0	11 0	27 [10–39] SMR using HCC mortality in US population in 1975
McMahon *et al.* (1990a) Alaska, USA	Alaskan native population; men, women; average age, ~ 22 years	1400 HBsAg+; 59% male	5.58	20	Men, 387 Women, 63	148 [59–305] Compared with estimated incidence in general population
Asia						
Oshima *et al.* (1984) Osaka, Japan	Blood donors; men (more than half < 30 years)	8646 HBsAg+	6.2	20	[37]	6.6 (4–10) Compared with incidence in Osaka general population
Fukao (1985) Miyagi, Japan	Blood donors; men (> 30 years)	1000 HBsAg+ 10 000 HBsAg- Matched by age, residence, month of donation	NR	3 1	45	30 [6.0–88]

Table 5 (contd)

Region, reference location	Subjects (age)	HBsAg seroprevalence	Mean duration of follow-up (years)	No. of cases of HCC	Annual incidence (cases/ 100 000)	RR (95% CI)
Asia (contd)						
Tu *et al.* (1985) Chongming Island, China	Men (> 40 years) from 4 communes	1971 HBsAg+ or anti-HBc+ and anti-HBs – 10 251 HBsAg –	3	37 33	651 (carriers) 98.6 (non-carriers)	6.7 [4.2–11]
Tokudome *et al.* (1987) Fukuoka, Japan	Blood donors; women (age, NR)	3769 HBsAg+	5.05	4	[21]	5.6 [1.5–14] Ccompared with adjusted mortality in Fukuoka population
Tokudome *et al.* (1988) Fukuoka, Japan	Blood donors; men (age, NR)	2595 HBsAg+	5.86	15	[98.6]	7.3 [4.1–12] Compared with adjusted mortality in Fukuoka population
Ding *et al.* (1988) Guangxi, China	Men and women (> 20 years)	1839 HBsAg+ 9233 HBsAg –	6.8	[41] [39]	NR	[5.3; 3.8–7.2]
Sakuma *et al.* (1988) Japan	Railway workers; 6918 men	126 HBsAg+ 6792 HBsAg –	8.5 8.5	4 6	[374] [10]	30 [1–77]
Sakuma *et al.* (1988) Japan	Railway workers; men (40.55 years)	513 HBsAg+ 25 034 HBsAg –	7.3 7.3	9 21	[240] [12]	[21; 9.6–40]
Yeh *et al.* (1989) Guangxi, China	Men (25–64 years) from 5 communes	2072 tested for HBsAg 76 HCC (69 HBsAg+) 304 controls (68 HBsAg+)	3.8	 69 HBsAg+ 7 HBsAg –	NR	 39 (16–117) Nested case–control analysis
Beasley & Hwang (1991) Taiwan, China	Government employees; men (82% 40–59 years)	3454 HBsAg+ 19 253 HBsAg –	[11.25] [10.28]	184 10	474 [52]	103 (57–205)

Table 5 (contd)

Region, reference, location	Subjects (age)	HBsAg seroprevalence	Mean duration of follow-up (years)	No. of cases of HCC	Annual incidence (cases/ 100 000)	RR (95% CI)
Asia (contd)						
Ross *et al.* (1992) Shanghai, China	Men (45–64 years)	18 224 men 22 HCCs (12 HBsAg+) 140 controls (15 HBsAg+)	1.9 years	12 HBsAg+ 10 HBsAg–	NR	8.5 (2.8–26) Nested case-control analysis
Europe						
Hall *et al.* (1985) United Kingdom	Blood donors (age, NR)	2880 HBsAg+ men 1054 HBsAg+ women	NR	5 0	NR	42 (13–98)

NR, not reported

counter immunoelectrophoresis revealed a difference in the prevalence of persistent HBV infection: 40% *versus* 7% [$p < 0.05$]. No significant relationship was noted between HBsAg seropositivity and the presence of cirrhosis (46% in comparison with 31% without cirrhosis) among cases. [No information was provided as to how and when cases and controls were identified.]

A study conducted at Le Dantec Hospital, Dakar, Senegal, between October 1972 and July 1974 involved 165 cases of HCC (in 127 men and 38 women), 154 controls with other cancers (102 men, 52 women) and 328 non-cancer controls (226 men, 102 women) (Michon *et al.*, 1975; Prince *et al.*, 1975). A diagnosis of HCC was histologically confirmed for 80 cases. Controls were matched to cases on sex, age (within 10 years) and admission date (within 30 days) and were of similar ethnicity and religion; non-cancer controls were free of liver disease. A higher frequency of HBsAg seropositivity was reported among the cases than in each control group: 61.2% *versus* 11.8 and 11.3% in the two control groups, respectively [$p < 0.05$]. The prevalence of anti-HBs seropositivity (tested by passive haemagglutination) alone was lower among cases (18.2%) than controls (45.4 and 42.1%, respectively) (Michon *et al.*, 1975). In 94 documented cases of HCC, the presence of cirrhosis was not related to HBsAg seropositivity but was related to anti-HBs seropositivity (6% *versus* 20%; $p < 0.05$) (Prince *et al.*, 1975).

Larouzé *et al.* (1976) matched 28 cases of HCC (in 22 men and 6 women) from Le Dantec Hospital, Dakar, Senegal, to healthy individuals from the same urban neighbourhood or rural village, of the same sex, age and ethnic group. Blood was also collected from parents and siblings. Histological confirmation was obtained in 24 cases. Although no significant association was observed between the presence of HBsAg (by radioimmunoassay) and HCC (79% in cases, 57% in controls; $p = 0.15$ [OR, 2.8]), the prevalence of HBsAg among the controls was very high. Cases were markedly less likely to be seropositive for anti-HBs (by passive haemagglutination) (25% *versus* 64%; $p = 0.006$) and more likely to be seropositive for anti-HBc (by counter immunoelectrophoresis and immunodiffusion) (89% *versus* 64%; $p = 0.05$). [Neither the time frame nor the case selection method was described.]

In a study in Addis Ababa, Ethiopia, HBsAg seroprevalence was compared in 46 HCC cases (in 31 men and 15 women) and 90 healthy hospital employees without a history of liver disease, blood transfusion, leprosy, leukaemia or Down's syndrome (Tsega *et al.*, 1976; Tsega, 1977). The cases from whom serum was collected were a subset of 100 consecutive HCC patients admitted to St Paul's or Haile Selassie I hospitals between June 1972 and November 1974. The diagnosis of HCC was confirmed by biopsy in 25 cases. HBsAg seropositivity was significantly associated with the occurrence of HCC: 50% among cases, 7% among controls [OR, 14, $p < 0.001$].

Tabor *et al.* (1977) analysed serum samples from 47 cases of HCC confirmed by biopsy in Uganda (previously studied by Vogel *et al.*, 1972), 19 in Zambia and 27 in the USA; the controls were 50 in-patients with melanoma or Kaposi's sarcoma in Uganda, 40 healthy Zambian villagers (from the same geographic region as the cases) and three US blood donor groups (6726 total), respectively. Evidence of active HBV infection (HBsAg seropositivity with or without anti-HBs seropositivity or anti-HBc seropositivity with anti-HBs seronegativity) was significantly associated with HCC in each comparison: 72% of cases in Uganda, 68% in Zambia and 41% in the USA. Anti-HBs was tested by radioimmunoassay

and passive haemagglutination and anti-HBc by complement fixation and counter immuno-electrophoresis.

In a study involving blood donor controls, 32 histologically confirmed cases of HCC in 19 men and 13 women in Mozambique were studied (Reys *et al.*, 1977). Their 60% HBsAg seropositivity was significantly higher than the 3–15% [9% overall] observed in 231 male African blood donors from the Hôpital Central de Maputo by subgroup ($p < 0.01$). Anti-HBs was measured by radioimmunoprecipitation (12% compared with 33–49% had anti-HBs). [Neither the methods of subject selection nor the time period were further specified in either study.]

Van Den Heever *et al.* (1978) conducted a study at H.F. Verwoerd Hospital in Pretoria, South Africa, during 1973–76 of 92 histologically confirmed cases of HCC from the Pretoria area (in 75 men and 17 women) matched to 92 orthopaedic out-patients from the same area, of the same age and sex with no history of liver disease; all subjects were black. The association between seropositivity for HBsAg and HCC status was significant (34% of cases, 9% of controls; $p < 0.01$) [RR, 5.3].

In another case–control study in South Africa (Kew *et al.*, 1979), a 62% seroprevalence of HBsAg was found among 289 blacks (280 men, nine women) with histologically confirmed HCC over a four-year period; the prevalence in the 213 healthy controls (gold miners matched by age, sex and ethnic group) was significantly lower, 11% ($p < 0.001$). The cases had been referred consecutively to the South African Primary Liver Cancer Unit from mine hospitals or admitted to two teaching hospitals; most were miners, and the majority were not from South Africa. A significant inverse association was observed for anti-HBs reactivity and HCC (17% in cases, 42% in controls; $p < 0.001$) [OR, 0.28]. In a subset of 74 cases and 104 controls tested for anti-HBc, a significant difference was found (89% *versus* 38%; $p < 0.001$). A detailed serological analysis of 131 HBsAg-seropositive (98 cases, 33 controls) and 222 HBsAg-seronegative (50 cases, 172 controls) male miners was performed (Kew *et al.*, 1981), using the more sensitive radioimmunoassay to detect HBeAg, anti-HBe and anti-HBc. Significant positive correlations with HCC were observed for anti-HBc ($p < 0.01$) and anti-HBe ($p < 0.05$) among the HBsAg-seronegative subjects, and significant inverse relationships for anti-HBe ($p < 0.02$) among HBsAg-seropositive individuals and for anti-HBs ($p < 0.05$) among HBsAg-seronegative subjects. No relationship was observed between HBsAg seropositivity and the presence of cirrhosis among HCC cases (Kew *et al.*, 1979). [The case identification period was not further specified. The later study (Kew *et al.*, 1981) may have included additional subjects not in the earlier one.]

Seventy-six cases of HCC (in 60 men and 16 women) and 33 controls matched for age, sex and tribe were studied by Bowry and Shah (1980) in Nairobi, Kenya. The cases attended Kenyatta National Hospital between January 1976 and April 1979; histological or cytological confirmation was obtained for 56. Of the controls, 28 were relatives of hospital patients and five were hospital patients. HBsAg was detected (by passive haemagglutination) in 51% of cases and 6% of controls [$p < 0.05$]. A report published a year later by the same group (Bowry *et al.*, 1981) probably involved a subset of 60 of these HCC cases [subject selection was not described]. Seropositivity only for anti-HBc was found in eight [13%] of the 60 HCC cases, 15% of 20 matched hospital controls and 4% of 104 volunteer blood donors;

the prevalence was higher among the 13 HCC patients with known cirrhosis (31%). [The blood donors were younger than the cases.]

Coursaget *et al.* (1981) conducted a study in Senegal of 134 cases of HCC (in 114 men and 20 women) diagnosed at Le Dantec Hospital and 100 blood donor controls from the National Blood Center of Dakar, in which anti-HBc was measured by counter immunoelectrophoresis. A significant association was found between HCC and active HBV infection [OR, 14] (67% *versus* 13%; $p < 10^{-6}$). A significantly lower frequency of past infection (HBsAg seronegativity and anti-HBs seropositivity or anti-HBc seropositivity by radioimmunoassay) was noted for cases (33%) in comparison with controls (74%; $p < 10^{-6}$). HBsAg was detected in 63% of the HCC patients and in 12% of the 100 blood donors [OR, 12], 14% of 833 rural country dwellers (72 men, 761 women) [OR, 11] and 25% of 560 leprosy patients from the Pavillon de Malte (410 men, 150 women) [OR, 50]. Two earlier reports of this study (Maupas *et al.*, 1977; Coursaget *et al.*, 1980) showed consistent findings. [Little detail could be found as to subject selection.]

Gombe (1984) found a significantly higher prevalence of HBsAg seropositivity among 65 cases of HCC (in 47 men and 18 women) (74%) than among 120 blood donors (115 men, five women) (9% [$p < 0.05$]) or 71 other cancer controls (13 men, 58 women) (3%; $p < 0.05$) in the Congo. The blood donors were tested in two centres by radioimmunoassay and passive haemagglutination, and the HCC cases and cancer controls from the Brazzaville General Hospital by passive haemagglutination. [The process for selecting subjects was not clear.]

Sebti (1984) in Rabat, Morocco, reported a significant but weaker association between HCC and HBsAg seropositivity (17% in 46 cases and 5% in 379 controls) [OR, 4.2]. The HCC patients were a subset of 63 cases hospitalized at Avicenne Hospital between 1976 and 1983; the controls were healthy subjects and people with non-hepatic disease. [The process for selecting subjects was not clear.]

Another study by Kew *et al.* (1986a) focused on southern African blacks living in an urban environment. Markers of HBV infection were assayed in 62 urban-born patients with histologically confirmed HCC (41 men, 21 women) and in pair-matched urban-born hospital controls, matched according to race, sex, age, tribe (when possible), hospital and ward. Subjects were identified from two large general hospitals, Baragwanath in Soweto and Hillbrow in Johannesburg. HBsAg was detected significantly more frequently among the cases (40%) than the controls (3%) ($p < 0.001$). No difference was observed with regard to past infection (HBsAg seronegativity and anti-HBs or anti-HBc seropositivity). [Information about the period of subject selection was not provided.]

Otu (1987) studied 200 consecutive, histologically confirmed cases of HCC (in 180 men and 20 women) at the University of Calabar Teaching Hospital, Nigeria, between January 1978 and December 1982. Two symptomless controls matched for sex and age (within five years) were selected per case from the general out-patient department. HBsAg was detected in 49% of the cases and 8% of the controls ($p < 0.01$). [Little detail was provided about selection of controls.]

Gashau and Mohammed (1991) compared the prevalence of HBV markers in 65 HCC patients (57 men, eight women) and 69 sex- and age-matched healthy controls in Nigeria. The cases of HCC were examined consecutively at the University of Maiduguri Teaching Hospital

between 1986 and 1987; needle biopsy was used for diagnosis in 21. The controls were examined at the hospital during the same period; most were blood donors. The cases had a significantly higher rate of HBsAg seropositivity (65%), measured by ELISA and reverse passive haemagglutination, than the controls (35%) [OR, 3.2]. Seropositivity for anti-HBc, measured by ELISA, was about the same in those tested in the two groups; HBeAg seropositivity was higher and that of anti-HBe lower in the cases. [Few details were provided about the controls.]

Mohamed *et al.* (1992) examined the prevalence of current (HBsAg seropositivity) and past (seronegative for HBsAg and seropositive for anti-HBs or anti-HBc) infection among 101 black South Africans with HCC, matched for ethnic origin, sex, age (within two years) with patients from the wards of Baragwanath Hospital, Johannesburg (77 men, 24 women). Controls were excluded if they had a disease caused by alcohol abuse or were unable to answer questions. Histological confirmation was obtained for 85 cases of HCC. Among men, 35% of cases and 5% of controls were currently HBsAg seropositive; the OR, adjusted for alcohol and smoking, was 7.5 (95% CI, 2.2–25). Among women, HBsAg seropositivity was 25% for cases and 4% for controls, with an adjusted OR of 12 (95% CI, 1.0–154). [The time frame for subject selection was not given.]

At the Parirenyatwa Teaching Hospital in Zimbabwe, Tswana and Moyo (1992) studied 182 HCC cases (in 128 men and 54 women) and 100 non-liver disease patient controls (50 men, 50 women). Pregnant women, cigarette smokers and alcohol consumers were excluded from the study. The diagnosis of HCC was made clinically and confirmed by α-fetoprotein level. Controls were selected randomly and were comparable to the cases with respect to age and sex; subjects were 20–65 years old. A significant correlation ($p < 0.0001$) was reported between HBsAg seroprevalence and HCC [OR, 10] (56% in cases *versus* 11% in controls); anti-HBc was detected in 54% of cases and 4% of controls [OR, 29; $p < 0.05$]. Among HBsAg-seropositive subjects, the seroprevalence of HBeAg was 20% among cases and 9% among controls. HBV markers were determined by ELISA. [The time frame for the study was not given.]

Ryder *et al.* (1992) studied HCC and HBV infection in the Gambia between 1 December 1981 and 30 November 1982. In a community-based surveillance system, they identified 70 cases (in 61 men and nine women); 44 were confirmed histologically. Patients were interviewed within one month of diagnosis in their village, at which time the person living closest to the case, of the same sex and age (within five years) was identified as a control. All potential subjects agreed to participate, and the two groups were not significantly different with respect to length of residence, alcohol consumption, smoking habits or family size. After adjustment for age, the following associations were reported: HBsAg (64 cases and 67 controls tested), OR, 6.9 ($p < 0.01$); anti-HBs (63 cases and 68 controls tested), OR, 0.31 (not significant); HBeAg (63 cases and 68 controls tested), OR, undefined ($p < 0.001$); and anti-HBe (62 cases and 66 controls tested), OR, 2.3 (not significant). The prevalence of HBsAg seropositivity was 63% among cases and 21% among controls; that of HBeAg was 17% among cases and 0 among controls. In all instances, the relationships were strongest among people under 50 years of age.

Serological HBV markers were investigated by the immunoperoxidase procedure in 40 cases of HCC selected in 1985 among 223 cases collected at the Department of Pathology

of the University Hospital and Medical School of Kinshasa, Zaire, and in 68 age- and sex-matched controls selected from among blood donors (Kashala *et al.*, 1992). The proportion of seropositive individuals among cases of HCC was significantly higher for HBsAg (57.6% *vs* 7.35%) and for anti-HBeAg (27.5% *vs* 16.2%) but was significantly lower for anti-HBs (25% *vs* 63%), and no significant difference was observed for anti-HBc, HBe or HBV DNA.

(*b*) *Americas*

Yarrish *et al.* (1980) analysed sera collected from patients attending hospitals in Philadelphia, USA, between 1968 and 1977. The sera from 34 HCC cases (in 28 men and six women) were then matched to those of 38 patients (30 men, eight women) with colon cancer, 45 (36 men, nine women) with lung cancer and 56 blood donors (48 men, eight women) matched for age (within five years) and sex. All but one HCC case was histologically confirmed. Blood donor samples were collected prior to routine screening for HBsAg and were stored on average 39 months longer than the sera from HCC cases; the sera of the colon cancer controls were stored for four months less and those of the lung cancer controls for 17 months less than those of the HCC cases. Five HCC cases (15%) were seropositive for HBsAg (assayed by radioimmunoassay), which was significantly higher than in any control group ($p < 0.05$). Seropositivity for anti-HBs, as assayed by passive haemagglutination, was not significantly different between cases and controls. [The details of subject selection were not provided.]

In the Japan–Hawaii Cancer Study, described in detail on p. 69, Nomura *et al.* (1982) found a significant excess of HBsAg in HCC patients in Hawaii (63%, with none in controls).

Austin *et al.* (1986) performed a multicentre study of 67 HCC patients (45 men, 22 women), aged 18–84, from 12 US hospitals, for whom HBsAg status was known (49 assayed by radioimmunoassay, 18 from medical records). The 18% (all in men) HBsAg prevalence among these cases was significantly higher ($p = 0.0002$) than the 0 prevalence for the 63 controls, who had no liver disease or a condition related to tobacco use and were matched by sex, year of birth (within five years), race and current residence (59 assayed by radioimmunoassay, four from records). Of the people seronegative for HBsAg who were tested, 7/40 (18%) of cases and 5/58 (9%) of controls were seropositive for anti-HBs [not significant]. [Details were not given about the time and method of case selection.]

Yu *et al.* (1990) described a study of black and white residents of Los Angeles County, USA, 18–74 years of age. Histologically confirmed incident cases of HCC were identified between January 1984 and August 1989; 392 cases were eligible, but only 51 (12 blacks, 39 white; 35 men, 16 women) were analysed, either because of death (290), refusal (29), inability to locate (9), incorrect diagnosis (7) or serum sample depletion (6). Controls were selected from among 404 community control subjects used in a case–control study of lymphoma from 1978 to 1982; 128 of 404 controls were randomly selected to be frequency matched by sex and age (10-year intervals) to the cases (1 black, 127 white; 81 men, 47 women). All interviews were performed in the subjects' homes. The age- and sex-adjusted ORs for the various HBV markers, tested by radioimmunoassay with no markers as the reference level, were: HBsAg, infinity ($p = 0.002$); anti-HBc, 7.3 ($p < 0.0005$); anti-HBs, 5.2 ($p < 0.0005$). Neither

adjustment for level of education nor a separate analysis of US-born or white subjects affected the results.

In a study conducted in Baltimore, USA, 99 consecutive histologically confirmed HCC patients at the Johns Hopkins Oncology Center were compared between January 1987 and May 1988 with 98 consecutive patients with other malignancies seen at the same centre between November 1987 and January 1988 (Di Bisceglie *et al.*, 1991). The cases were from the eastern half of the USA and were referred for inclusion in therapeutic radiation trials. No significant difference was reported between the two groups with regard to age, sex or race. HBsAg and IgM anti-HBc were detected only in the cases (7% and 8%, respectively), at significantly higher prevalences than in the controls ($p = 0.009$ and 0.004). Cases and controls were similar with regard to the presence of the other HBV markers measured. Anti-HBc was determined by enzyme immunoassay. [The fact that the patients had advanced disease might have affected HBsAg levels.]

(*c*) *Asia*

In Taiwan, China, Tong *et al.* (1971) examined the prevalence of detectable antigen in 55 cases of HCC (in 52 men and three women) and 943 male personnel at the Tsoying Naval Base. The cases were from the Chinese Veterans' Hospital, and 25 diagnoses were confirmed by needle biopsy or autopsy. No subject had Down's syndrome, leprosy, leukaemia or a recent transfusion (within 12 months); the controls had no past or present liver disease. A significant difference in HBsAg seropositivity was found by a modified immunodiffusion technique: 80% among cases and 15% among controls ($p < 0.001$) [OR, 23]. The cases were older than the controls (mean, 48 *versus* 30 years). [No details were provided concerning the timing or selection of subjects.]

Simons *et al.* (1972) studied HBsAg seroprevalence among 156 Chinese HCC patients in Singapore; 114 (87 men, 27 women) had been reported previously (Simons *et al.*, 1971). The control groups consisted of 1516 male blood donors, 260 women attending antenatal clinics and 207 patients investigated for suspected nasopharyngeal carcinoma at one of the hospitals; all controls were Chinese. HBsAg seropositivity was markedly higher among the HCC cases (35%) than in any of the control groups (8%, 2% and 6%, respectively). [The associations calculated by the Working Group were all statistically significant; the blood donors and women attending antenatal clinics are combined in Table 6 as 'normal controls'. No details were found concerning selection of controls.]

Lee (1975) compared the prevalence of HBsAg seropositivity in 100 cases of HCC (in 85 men and 15 women) and 120 patient controls (98 men, 22 women) in Hong Kong with no history of liver disease or blood transfusion; the diagnosis of HCC was confirmed by biopsy in 81 cases. Testing by immunoelectroosmophoresis and complement fixation showed a significant difference (49% *versus* 9%; $p < 0.001$). [No data were provided on the timing or process of subject selection or on the gender distribution of the cases.]

Chainuvati *et al.* (1975) reported a higher frequency of HBsAg seroprevalence, measured by crossover immunoelectrophoresis, among 49 HCC patients with cirrhosis (16%) than among 87 hospitalized controls (74 men, 13 women) without liver disease (2%) in Thailand ($p < 0.005$). No HBsAg was found in eight HCC patients without cirrhosis. The

cases in this study had been identified between August 1972 and April 1973 and were histologically confirmed.

Kubo *et al.* (1977) studied 124 cases of HCC (in 107 men and 17 women) seen at Kurume University and Chiba University Schools of Medicine, Japan; the diagnosis was histologically confirmed in 108 cases. Healthy employees of the Japan National Railways (290 men, nine women) seen at regular physical check-up were used as controls. The difference in seroprevalence of HBsAg between cases and controls was significant (46% *versus* 4%; $p < 0.01$) [OR, 20], as were smaller differences in anti-HBc seropositivity (73% *versus* 30%; $p < 0.01$) [OR, 6.2] and the presence of any marker (81% *versus* 34%; $p < 0.01$) [OR, 8.1]. No difference between cases and controls was observed for anti-HBs, as tested by passive haemagglutination. Immune adherence haemagglutination was used to detect anti-HBc. [No details were given as to when subjects were diagnosed.]

In Taiwan, China, 127 HCC cases admitted to the National Taiwan University Hospital between May 1974 and December 1976 were compared with 729 healthy controls (Chen & Sung, 1978). The controls comprised 241 40–67-year-old adults from a cancer education programme and 488 18–22-year-old university students receiving a regular check-up in 1975. HCC was histologically confirmed in 63 cases. HBsAg (assayed by reverse passive haemagglutination) was found in 83% of cases and 15% of controls [OR, 28; $p < 0.05$], and anti-HBs (detected by passive haemagglutination) in 14% and 45%, respectively [OR, 0.21; $p < 0.05$]. An analysis of 68 HCC cases with and without cirrhosis revealed no association with HBsAg seropositivity (81% in each group). [The gender breakdown for the subjects was not given.]

Chien *et al.* (1981) conducted a study among Chinese HCC patients seen at the Taiwan Veterans General Hospital (Tong *et al.*, 1971). The 102 cases (in 97 men and five women) were matched by age and sex to 100 healthy controls from the out-patient clinic; histological confirmation was obtained for 36 cases. A larger proportion of cases than of controls were seropositive for HBsAg (71% *versus* 12%) [OR, 18] or anti-HBc (98% *versus* 84%) [OR, 4.7], and a lower proportion for anti-HBs (27% *versus* 54%). HBsAg-seropositive patients had higher levels of HBeAg and lower levels of anti-HBe than controls. All differences except those for HBeAg and anti-HBe were significant [$p < 0.05$].

Lam *et al.* (1982) performed a study in Hong Kong which included 107 Chinese cases (in 95 men and 12 women) of HCC at the Queen Mary Hospital, who were matched by sex and age (within five years) to 107 control (94 men, 13 women) trauma patients in the orthopaedic ward of the same hospital; 106 cases were histologically confirmed. Between March 1977 and September 1980, 149 Chinese HCC patients were admitted to the hospital, 72% of whom were interviewed; controls were interviewed within one month of the index case. After adjustment for age and sex, the OR for HCC associated with the presence of HBsAg was 21 (95% CI, 10–46).

In the Republic of Korea, Sjøgren *et al.* (1984) reported an HBsAg seroprevalence of 82% among 110 histologically confirmed HCC cases (in 90 men and 20 women) and 14% among 63 controls with other cancers matched for sex and age ($p < 0.001$). Subjects were identified between 1973 and 1981 at St Mary's Hospital in Seoul; the control patients had no evidence of liver disease, although five had metastatic liver cancer (Chung *et al.*, 1983). IgM anti-HBc was present in 74 cases (67%) and one control (2%) ($p < 0.001$) [OR, 127]; the

association between HCC and presence of IgM anti-HBc was also seen among the HBsAg-seropositive subjects (81% *versus* 11%; $p < 0.005$). [No details of subject selection methods were given.]

A report from Yamanashi Prefecture, Japan (Inaba *et al.*, 1984), described a matched analysis of 62 cases (in 49 men and 13 women) of HCC from seven hospitals between April 1977 and August 1979. Patient controls with no hepatic disease were selected by sex, age (within five years) and hospital. Confirmation of the diagnosis in liver biopsies was obtained for 36 of the HCC cases. There was a significant, 10-fold increase in risk for liver cancer associated with HBsAg seropositivity, assayed by reverse passive haemagglutination (36% in cases *versus* 3% in controls) ($p < 0.01$), and a weaker association with anti-HBs (27% *versus* 18%) ($p < 0.05$) [crude OR, 1.7].

A matched analysis of cases of HCC in Guangxi Autonomous Region, China, revealed a high OR (17; 95% CI, 4.3–99) for the relationship between HBsAg seropositivity and HCC (Yeh *et al.*, 1985a,b), based on 50 cases (in 47 men and three women) and 49 controls without liver disease matched by sex, age (within five years) and ward/clinic; 86% of the cases were seropositive for HBsAg *versus* 22.45% of the controls. Case identification began on 1 July 1982 and continued until 50 cases were obtained at the College Hospital; only four diagnoses were based on histological examination. In the HBsAg-seropositive subjects assayed, HBeAg reactivity was not significantly greater in HCC patients (31%) than in controls (18%); the seroprevalence of anti-HBe was lower among cases than controls (50% *versus* 64%) (Luo *et al.*, 1988).

In Riyadh, Saudi Arabia, a significant difference ($p < 0.001$) was found between cases of HCC and local population controls for HBsAg seropositivity [60% *versus* 12%] (Arya *et al.*, 1988). The 30 histologically confirmed cases (in 25 men and five women) were a subset of cases in 75 local HCC patients hospitalized at King Fahad Central Hospital from September 1984 to October 1985, from whom serum was available. The control group comprised 326 patients aged 20–> 40 treated for minor ailments in the area. Among the HBsAg-seropositive subjects, a significant inverse association was reported between HCC and anti-HBe seroprevalence [OR, 0.15] (24% in cases *versus* 67% of controls; $p < 0.01$), but no significant association was seen for HBeAg [OR, 2.7]. ELISA was used to assay all HBV markers. [Little detail was available about control selection.]

A matched analysis by Lu, C.Q. *et al.* (1988) of 30 HCC patients and 60 matched controls with other tumours or anorectal diseases in the same hospital in Tianjin, China, showed significant associations with reactivity to HBsAg ($p < 0.001$) (OR, 5) and anti-HBc ($p < 0.05$) (OR, 39). The prevalences among the cases were 57% and 83%, respectively, and those among controls were [17%] and [20%]. HBV markers were assayed by passive haemagglutination and ELISA.

Lingao (1989) conducted a study in the Philippines of 340 HCC cases (in 288 men and 52 women) individually matched by age and sex to asymptomatic population-based controls from five rural areas. About 90% of the diagnoses of HCC were confirmed histologically. The presence of HBsAg was evaluated by reverse passive haemagglutinin, radioimmunoassay and ELISA only in patients with HBV infection and was significantly higher for HCC patients than for the controls [75% *versus* 14%; $p < 0.0001$; OR, 19 (12–29)]. Among the HBsAg-reactive subjects, a significant association was found between HCC and anti-HBe

seropositivity (73% *versus* 52%; $p < 0.01$) [RR, 2.6] but not with HBeAg; these markers were determined by gel diffusion followed by radioimmunoassay and ELISA. Among 99 cirrhosis patients studied, the HBsAg seroprevalence was 58%. In a preliminary study of 104 histopathologically confirmed HCC cases (in 88 men and 16 women), which probably represented a subset of the larger case group (Lingao *et al.*, 1981), the control group consisted of 84 asymptomatic controls (42 men, 42 women). [No details of subject selection were provided.]

In the study of Yeh *et al.* (1990) in southern Guangxi Autonomous Region, China (see p. 68), 91% of 76 HCC cases were HBsAg seropositive compared with 22% of 304 controls (OR, 39; 95% CI, 16–117).

In a study by Tsukuma *et al.* (1990) in Japan, of 229 (192 men, 37 women) newly diagnosed HCC patients admitted to the Center for Adult Diseases in Osaka between November 1983 and June 1987, 221 (96.5%) were interviewed; 87 cases were histologically confirmed. One control per male case and two per female case (266 in all) were selected from among patients admitted for gastroenterology, people admitted for health check-ups and those admitted for gastroenterological endoscopy. People with liver disease, malignancy, smoking- and alcohol-related disease, and lack of HBsAg testing were excluded. All subjects were interviewed at admission or at the time of endoscopic examination. In the analysis, confounding by sex, age, history of blood transfusion, heavy drinking, cigarette index and family history of liver cancer was controlled by unconditional logistic regression; the OR for HBsAg seropositivity was 14 (95% CI, 5.7–36; $p < 0.0001$). HBsAg was determined by reverse passive haemagglutination; the results were abstracted from medical records.

Lin *et al.* (1991) studied cases of HCC and hospital controls at the Chang-Gung Memorial and Kaohsiung Medical College Hospitals in Taiwan, China, and interviewed them between 20 February 1985 and 20 December 1986. Preliminary results were reported previously (Lu, S.N. *et al.*, 1988). The subjects were 243 hospitalized or out-patient cases of HCC (in 218 men and 25 women) and 302 orthopaedic and ophthalmic in-patient controls (260 men, 42 women). Two controls were matched to each case by age (within three years) and sex, but some subsequently refused to have blood drawn; the authors stated that no significant difference was found between cases and controls with regard to age or sex. Adjustment for age, sex and hepatitis markers yielded significantly increased risks in association with seropositivity for HBsAg (OR, 10; 77% in cases *versus* 19% in controls) and HBeAg (OR, 3.2; 18% *versus* 2%) and significantly decreased risks in association with seropositivity for anti-HBs (OR, 0.1; 28% *versus* 78%) and anti-HBc (OR, 0.1; 97% *versus* 100%). [No information was given about how many subjects refused to have blood drawn or how many cases were histologically confirmed.]

Chen *et al.* (1991) examined 200 male HCC patients from the same two hospitals in Taiwan who were recruited consecutively between September 1985 and July 1987. Healthy community controls were matched individually to cases by age (within three years), ethnic group and residence, using a roster from household registration offices. Seventeen female pairs had also been identified but were excluded owing to their small number. Only two cases and three controls were seronegative for markers of HBV infection; thus, the authors focused on detection of HBsAg and HBeAg. In comparison with those seronegative for both antigens, those only seropositive for HBsAg had a 17-fold higher risk for HCC (95% CI, 7.4–38) and those seropositive for both had an OR of 58 (95% CI, 27–124). [Refusal rates

were not provided. The cases of HCC overlapped with those analysed by Lin *et al.* (1991); the number of histologically confirmed diagnoses was not stated.]

Srivatanakul *et al.* (1991) studied subjects from three hospitals in several areas of northeast Thailand as part of a larger study of liver cancer (Parkin *et al.*, 1991). Sixty-five cases (in 47 men and 18 women) were compared with 65 controls matched by sex, age (within five years), residence and hospital. Controls were either in-patients or clinic patients with nonmalignant, nonhepatic diseases and diseases unrelated to tobacco or alcohol. All subjects were under 75 years, were recruited during 1987–88 and were interviewed in hospital; histological confirmation was obtained for 20 HCC cases. Conditional multivariate analysis, controlling for consumption of alcohol, shrimp paste, powdered peanuts and fresh vegetables and for betel-nut chewing gave a significant OR of 15 (95% CI, 2.3–103) for the relationship between HCC and seropositivity for HBsAg ($p < 0.001$); 42% of the cases were HBV carriers *versus* 8% of the controls. HBV markers were determined by ELISA.

A study in Japan involved 204 patients with HCC (31 were not studied 'for logistic reasons') admitted to Kyushu University Hospital between December 1985 and June 1989 (Tanaka, K. *et al.*, 1988, 1992). The cases were diagnosed within one year of identification, were aged 40–69 and were residents of Fukuoka or Saga Prefecture (168 men, 36 women). The diagnosis of HCC was confirmed by histology in 82 cases. The 410 controls selected were residents of Fukuoka City, had undergone a health examination between January 1986 and July 1989 at a nearby public health centre, did not have chronic liver disease and had had a blood specimen taken; they were frequency-matched by sex and age to the cases (291 men, 119 women). Cases and controls were similar with respect to education and occupation and were interviewed in the hospital wards or at the health centre. The OR for HBsAg seropositivity as a risk factor for HCC was 14 (95% CI, 5.9–33) after adjustment for sex, age, history of blood transfusion, family history of liver disease, alcohol consumption and smoking (19% prevalence in cases *versus* 2% in controls). Case sera were tested by radioimmunoassay or reverse passive haemagglutination and control sera by the latter method.

In Kaohsiung Medical College Hospital, Taiwan, China, Chuang *et al.* (1992) studied 128 histologically or cytologically confirmed cases of HCC (in 112 men and 16 women) and 384 community controls (336 men, 48 women) matched for age (within five years) and sex; no significant difference was found in age and sex distributions. HBsAg was detected in 77% of the cases, which was significantly higher than in controls (28%) ($p < 0.001$). Using these data, Leandro and Duca (1993) calculated an OR of 14 for HBsAg seropositivity (95% CI, 7.8–25).

A case–control study of HCC was carried out in Hanoi, Viet Nam, between 1989 and 1992 (Cordier *et al.*, 1993). A total of 152 male cases were recruited from two hospitals and frequency-matched on sex, age, hospital and residence to 241 controls admitted to the abdominal surgical departments of the same hospitals. HBsAg status was investigated using a second-generation ELISA test. One hundred and thirty-eight (93%) cases and 44 (18%) controls were seropositive for HBsAg (OR, 62; 95% CI, 30–128). The effect of alcohol consumption was significant only among HBsAg-seronegative individuals.

(*d*) *Europe*

Trichopoulos *et al.* (1978) reported the findings of a study of 80 HCC patients (69 men, 11 women; 47 histologically confirmed cases) admitted to one of eight large hospitals in

Athens, Greece, between April 1976 and June 1977. Two control patients were matched by sex and age (within five years) to each case, who had diagnoses exclusive of neoplasm and liver disease. After Mantel–Haenszel adjustment for age and sex, the OR for an association between HCC and active HBV infection (HBsAg seropositivity or anti-HBc seropositivity and anti-HBs seronegativity) was 10 ($p < 0.001$) by comparison with people with no evidence of active infection. No relationship with any antigen was observed in 40 metastatic liver cancer patients (OR, 1.2). In addition, the presence only of anti-HBs did not confer a greater risk for HCC than the absence of HBV markers (OR, 0.8; 95% CI, 0.3–2.1). Among the HCC cases, the prevalence of active HBV infection was significantly higher in the 45 with cirrhosis (67%) than in the 35 without cirrhosis (26%) ($p < 0.001$).

The prevalence of active HBV infection (HBsAg seropositivity or anti-HBc seropositivity and anti-HBs seronegativity) was determined in 34 cases of HCC (22 with cirrhosis) and 100 healthy general population controls of similar age and sex in Barcelona, Spain (Pedreira *et al.*, 1980). Eighteen cases were verified by biopsy. A strong association was observed, with 52% of cases and 5% of controls having this HBV status [OR, 21; $p < 0.05$]. The prevalence was somewhat higher for HCC cases without cirrhotic liver (58%) than for those with (50%); 38% of 139 cirrhotic patients (47 alcoholics, 92 not) had active HBV infection. HBsAg was determined by reverse passive haemagglutination. [No details of subject selection methods were given.]

Goudeau *et al.* (1981) reported the prevalence of HBV markers in 46 histologically confirmed cases of HCC (in 39 men and seven women) and in 10 000 blood donors in Tours, France. All subjects were Caucasian. Significant differences were found for HBsAg seroprevalence (4% *versus* 0.5%; $p < 0.01$) and the seroprevalence of anti-HBc alone (15% *versus* 2%; $p < 10^{-6}$). [No information was given on the timing and method of subject selection.]

De Franchis *et al.* (1982) studied 42 subjects with HCC (33 men, nine women) and two groups of controls matched for age (within five years) and sex, comprising 42 patients with chronic liver disease and 84 patients with diagnoses other than neoplasm or liver disease. Subjects were identified from 1974 at the University Hospital in Milan, Italy, and histological examination was used to diagnose HCC. The cases were significantly different from the other hospital controls with respect to all HBV markers analysed, the greatest differences ($p < 0.0005$) being for the presence of HBsAg (36% *versus* 2%) [OR, 23], of anti-HBc (95% *versus* 51%) [OR, 19], of HBeAg (19% *versus* 0%) and of active infection (seropositivity for HBsAg or a high titre of anti-HBc alone; 44% *versus* 2%) [OR, 32]. The only significant associations ($p < 0.05$) for HCC cases in the comparison with chronic liver disease controls were for HBsAg seropositivity (12% of controls) [OR, 4.1], anti-HBs seropositivity (43%) [OR, 0.14] and active infection (17%) [OR, 3.9]. The direction of the associations between HCC and markers of HBV infection was the same in comparison with both control groups. [No information was given about the timing of subject selection.]

In a multicentre case–control study in Italy (Pagliaro *et al.*, 1982), a significant OR of 14 (1.4–∞) was reported for HBsAg seropositivity in relation to HCC (80% confirmed histologically) in a matched analysis of 50 case–control pairs (37 men, 13 women). Consecutive prevalent cases were collected from 23 hospitals and university medical departments from December 1974 through December 1976; controls were diagnosed with non-surgical

diseases other than liver disease. Matching was by sex, age (within five years), hospital and admission date (within six months). HBsAg seropositivity was assayed by the same method for each case–control pair, by radioimmunoassay, counter electroimmunophoresis or reverse passive haemagglutination.

A study from 17 centres in Italy of patients newly diagnosed with HCC during 1979–80 (Pagliaro *et al.*, 1983) comprised 286 HCC cases with cirrhosis (in 250 men and 36 women), who were compared with 3629 patients with cirrhosis (2340 men, 1289 women), and 64 HCC cases without cirrhosis (in 52 men and 12 women), who were compared with 1545 patients with chronic, non-hepatic disease (1038 men, 507 women). The latter control group represented a random sample of non-surgical disease. Among the cirrhotic subjects, cases were significantly different from controls for seropositivity for all HBV markers except HBeAg and anti-HBe: HBsAg, 30% *versus* 17% ($p < 0.0005$); anti-HBs, 26% *versus* 37% ($p < 0.005$); anti-HBc alone, 26% *versus* 16% ($p < 0.005$). Among those without liver cirrhosis, a significant difference was reported for seropositivity for HBsAg (33% *versus* 2%; $p < 0.0005$). HBsAg was detected by radioimmunoassay or ELISA; anti-HBs and anti-HBc were assayed by radioimmunoassay in a subset of the sample that was similar to the whole group with respect to sex, age and HBsAg reactivity. [The data were incompletely reported.]

In London, United Kingdom, 27 consecutive HCC patients (20 men, seven women) who had undergone liver biopsy between 1979 and 1981 were compared with 112 hospital in-patient and staff controls (60 men, 52 women) (Bassendine *et al.*, 1983); the subjects were Caucasian. The cases had significantly higher frequencies than the controls of HBsAg (15%; controls, 0.9%; $p < 0.005$), anti-HBc (48%; controls, 11%; $p < 0.005$) and anti-HBe (26%; controls, 7%; $p < 0.001$). [Details about the control selection process were not available.]

An association with HBsAg seropositivity was reported by Pirovino *et al.* (1983) in Switzerland: 31% in 65 HCC cases and 17% in 115 liver cirrhosis controls ($p < 0.05$) [RR, 2.3]. The cases were a subset of 75 histologically confirmed HCC cases diagnosed between 1975 and 1982 at the City Hospital Waid in Zurich on whom HBV testing was done. [Although all of the controls had cirrhosis, it is not known what proportion of cases did.]

Filippazzo *et al.* (1985), in Palermo, Italy, enrolled 120 consecutive in-patients with HCC (99 men, 21 women) between December 1980 and December 1983 and three controls from the same hospital matched for sex and age (within five years), who had either cirrhosis, solid tumour or chronic non-neoplastic disease. Biopsy or laparoscopy was used to verify the diagnosis in 62 cases of HCC. The difference in HBsAg prevalence between cases (17%) and controls was greater for the two non-cirrhotic control groups (2–3%) than for the cirrhotic controls (15%). [The prevalences reported in the paper did not correspond exactly to those calculable from the data given. No information was provided on how HBsAg status was determined.]

Colloredo Mels *et al.* (1986) conducted a study in Bergamo, Italy, which included 72 histologically confirmed HCC cases (in 60 men and 12 women), 57 of whom also had cirrhosis. Cases were identified between January 1980 and December 1984, as were two control groups from the same hospital: 199 without liver disease (159 men, 40 women) and 156 with liver cirrhosis (114 men, 42 women). The OR for the relationship between seropositivity for HBsAg and HCC was 11.5 among the non-cirrhotic patients ($p < 0.001$) and 1.0 among the cirrhotic patients: 47% of the HCC cases without cirrhosis, 7% of their

controls and 28% of those with cirrhosis and 26% of their controls were seropositive for HBsAg. The authors calculated an overall OR of 4.6 on the basis of an HBsAg seroprevalence of 9.1% in the general population. Among the subset of HBsAg-seronegative subjects assayed, no significant difference was found for the prevalence of past HBV infection in either comparison. [The sample sizes were small, and the means of subject selection could not be determined.]

A case–control study in Greece (Trichopoulos *et al.*, 1987) involved 194 cases of HCC (in 81 cirrhotic subjects out of 173 men and 21 women) and 456 in-patient controls (400 men, 56 women). Cases admitted to eight hospitals in Athens between April 1976 and October 1984 were interviewed in hospital; 113 were confirmed by histology. Controls with diagnoses other than neoplasm or liver disease were selected from the same hospitals (as well as the hospital for accidents and orthopaedic disorders) and were also interviewed in hospital. All subjects were Caucasians of Greek nationality and were comparable with respect to education and birthplace. Data on about one-third of these subjects were analysed previously (see above: Trichopoulos *et al.*, 1978; 1980a), but all assays were repeated for this study. Multiple logistic regression was used to control for age, sex and anti-HCV status (Kaklamani *et al.*, 1991). A significant, 11-fold association was observed between HBsAg seropositivity (46% in cases; 7% in controls) and HCC on the basis of 185 cases (in 166 men and 19 women; 108 confirmed by histology) and 432 controls (381 men, 51 women). This estimate was slightly lower than the earlier one, in which HCV infection was not controlled for (OR, 14; 95% CI, 8.0–24); the association with HBsAg seropositivity was stronger in comparison with subjects with no HBV marker (OR, 19; 95% CI, 10–38) (Trichopoulos *et al.*, 1987). An additional analysis of these cases according to the presence of cirrhosis (Tzonou *et al.*, 1991) revealed a stronger association with HBsAg seropositivity among 78 cases with cirrhosis (65% positive; OR, 33) than those 107 without (32% positive; OR, 6.7), after control for age, sex, anti-HCV seropositivity and smoking.

Vall Mayans *et al.* (1990) investigated 96 cases of HCC (in 67 men and 29 women; 83 cases with cirrhosis) admitted to the University Hospital in Barcelona, Spain, between October 1986 and March 1988; 74 cases were confirmed histologically or cytologically. Two controls were chosen for each case from the same hospital and matched for sex and age (within five years), within one month after identification of the case; controls with diagnoses related to the risk factors of interest (HBV infection, alcohol consumption, smoking and oral contraceptive use) were not eligible, leaving 199 controls for analysis. Cases and controls were Caucasian and had comparable histories of occupation and blood transfusion. All interviewing took place in hospital. A significant, nearly five-fold association was observed for HBsAg seropositivity after adjustment for age and sex (OR, 4.9, exact 95% CI, 1.3–22); the seroprevalence of HBsAg was low (9% in cases, 2% in controls). The OR for anti-HBc seropositivity (2.3; 95% CI, 1.3–3.9) was also significant, 50% of cases and 31% of controls being seropositive. The authors reported that adjustment for alcohol drinking did not change the association with HBV infection.

Leandro *et al.* (1990) studied 457 patients with liver cirrhosis in Italy: 140 (117 men, 23 women) had confirmed cases of HCC, and 317 without HCC (209 men, 108 women) were used as controls. HCC patients were diagnosed in 1980–88 at a hospital in either Bari or Bergamo; the controls were admitted to the same centres between 1 January 1984 and

3 December 1985. After control for age and sex by logistic regression, the association with HBsAg seropositivity was significant ($p < 0.05$), with an OR of 2.3 [similar to the crude estimated OR of 1.8 (95% CI, 1.1–3.1)]. [The ORs are related to the probability of developing HCC given the presence of pre-existing cirrhosis.]

A study was carried out in four cities in Italy (Stroffolini *et al.*, 1992) to investigate HBsAg seropositivity in 65 incident cases of HCC with underlying cirrhosis (in 47 men and 18 women) admitted to four teaching hospitals during 1990. Patients with chronic nonhepatic disease, matched for age (within five years) and sex and admitted consecutively to the same hospitals in the same year were selected as controls (75 men, 23 women). Multiple logistic regression methods were used to control for age, sex, anti-HCV status and HBV markers. A significant OR of 12 (95% CI, 3.1–41) was found for the association between HBsAg reactivity and HCC; 25% of cases and 6% of controls were seropositive for the antigen. HBV markers were determined by ELISA. [Not all cases were confirmed histologically.]

(*e*) *International collaborative studies*

Not included in Table 6 are the results of two large collaborative studies. In one, patients with liver disease (including HCC) were compared with healthy subjects in Burma, China, Hong Kong, India, Indonesia, Japan, Kenya, Papua New Guinea, the Philippines and Thailand (Nishioka *et al.*, 1975). HBsAg was determined by immune adherence haemagglutination and anti-HBs by passive haemagglutination. The seroprevalence of HBsAg ranged from 33 to 80% among HCC patients and from 3 to 18% among healthy controls; that for anti-HBs was 0–26% among HCC patients and 12–43% among controls. In each country, HBsAg seropositivity was always higher and anti-HBs lower among HCC cases than among controls.

In the other collaborative study, data on blacks in Senegal, Burundi and Mali were combined (Coursaget *et al.*, 1984, 1985) to give a total of 453 HCC patients, 221 cirrhotic patients and 7051 adult controls. HBsAg seropositivity was 58–65% among HCC cases, 4–17% among controls and 63% among cirrhotic patients. HBeAg was detected in 25% of cases and 13–19% of controls, and anti-HBe was detected in 60% of cases and 75% of controls. An analysis of HBsAg-seropositive Senegalese subjects (Coursaget *et al.*, 1986a) revealed an OR of 6.2 (95% CI, 4.1–9.6) for HCC; HBsAg/IgM complexes were detected in 14% of controls, 40% of cirrhotics and 50% of HCC cases. [No details were provided about subject selection.]

Some studies have addressed immunohistochemical identification of HBV antigens in liver tissues and detection of HBV DNA in serum or liver tissue. Their results are important in elucidating pathogenetic mechanisms but cannot provide directly interpretable estimates of effect parameters like the OR, since it is inherently difficult to assess the suitability of the comparison groups. These studies provide strong support for the hypothesis that HBV is an important factor in the etiology of HCC (Nayak *et al.*, 1977; Tan *et al.*, 1977; Turbitt *et al.*, 1977; Omata *et al.*, 1979; Bréchot *et al.*, 1981a, 1985; Röckelein & Hecken-Emmel, 1988; Sjøgren *et al.*, 1988; Guan *et al.*, 1989).

Table 6. Summary of results of case–control studies of hepatocellular carcinoma and presence *versus* absence of hepatitis B surface antigen (HBsAg)

Reference and location	Subjects	Seroprevalence of HBsAg				OR	95% CI	Comments[a]
		Cases		Controls				
		No.	%	No.	%			
Africa								
Prince *et al.* (1970); Uganda	Sex unspecified	4	12	6	2	[6.8]	[1.8–25]	Blood donor controls
Vogel *et al.* (1972); Uganda	Women and men	90	40	224	3	[19]	[7.6–45]	Adjusted for age and sex; testing by CF, CEP and PHA
Kew *et al.* (1974); South Africa	Men	75	40	18 377	7	[8.7]	[5.3–14]	Mineworkers; testing by CEP and CF
Michon *et al.* (1975); Prince *et al.* (1975); Senegal	Women and men	165	61	154	12	[11]	[5.8–19]	Adjusted for age
	Controls with other cancer							
	Controls without cancer	165	61	328	11	[14]	[8.7–24]	
Larouzé *et al.* (1976); Senegal	Women and men	28	79	28	57	[2.8]	[0.74–10]	
Tsega *et al.* (1976); Tsega (1977); Ethiopia	Women and men	46	50	90	7	[14]	[4.6–44]	
Tabor *et al.* (1977)	Women and men							
Uganda		47	47	50	6	[14]	[3.8–51]	
Zambia		19	63	40	8	[21]	[4.7–96]	
USA		27	30	6726	0.02	[134]	[53–337]	
Reys *et al.* (1977); Mozambique	Women and men	32	60	231	9	[15]	[5.9–37]	Male controls; solid-phase RIA + CEP
Van Den Heever *et al.* (1978); South Africa	Women and men	92	34	92	9	[5.3]	[2.2–14]	Blacks
Kew *et al.* (1979) South Africa	Women and men	289	62	213	11	[13]	[7.6–21]	Blacks; solid-phase RIA
Bowry and Shah (1980); Kenya	Women and men	76	51	33	6	[16]	[3.4–106]	Testing by PHA

Table 6 (contd)

Reference and location	Subjects	Seroprevalence of HBsAg				OR	95% CI	Comments[a]
		Cases		Controls				
		No.	%	No.	%			
Africa (contd)								
Coursaget *et al.* (1981); Senegal	Women and men	134	63	100	12	[12]	[5.9–26]	
	Blood donor controls							
	Rural controls	134	63	833	14	[11]	[6.9–16]	
	Leprosy patient controls	134	63	560	25	[5.0]	[3.3–7.5]	
Gombe (1984); Congo	Women and men	65	74	120	9	[32]	[11–87]	Adjusted for sex; testing by RIA or PHA
	Blood donor controls							
	Other cancer controls	65	74	71	3	[55]	[12–256]	Adjusted for sex
Sebti (1984); Morocco		46	17	379	5	[4.2]	[1.6–11]	
Kew *et al.* (1986a); South Africa	Women and men	62	40	62	3	[20]	[4.3–132]	Blacks
Otu (1987); Nigeria	Women and men	200	49	400	7.5	[12]	[7.3–19]	
Gashau & Mohammed (1991); Nigeria	Women and men	65	65	69	36	[3.2]	[1.5–7.0]	Testing by ELISA and reverse PHA
Mohamed *et al.* (1992); South Africa	Men	77	35	77	5	7.5	2.2–25	Adjusted for alcohol intake and smoking; blacks
	Women	24	25	24	4	12	1.0–154	
Tswana & Moyo (1992); Zimbabwe	Women and men	182	56	100	11	[10]	[5.0–22]	Testing by ELISA
Ryder *et al.* (1992); Gambia	Women and men	70	63	70	21	6.9		Adjusted for age; $p < 0.01$; 64 cases, 67 controls tested
Kashala *et al.* (1992); Zaire	Women and men	40	57.6	68	7.4	[17]	[5.7–51]	Testing by immunoperoxidase

Table 6 (contd)

Reference and location	Subjects	Seroprevalence of HBsAg				OR	95% CI	Comments[a]
		Cases		Controls				
		No.	%	No.	%			
Americas								
Yarrish *et al.* (1980); USA	Women and men	34	15	38	0			$p < 0.05$; control sera stored 4 months less than case sera
	Controls with colon cancer							
	Controls with lung cancer	34	15	45	0			$p < 0.05$; control sera stored 17 months less than case sera
	Blood donor controls	34	15	56	0			$p < 0.02$; control sera stored 39 months longer than case sera
Nomura *et al.* (1982); Hawaii, USA	Men	16	63	48	0			$p < 0.0001$; subjects of Japanese ancestry
Austin *et al.* (1986); USA	Women and men	67	18	63	0	-	3.8–∞	$p = 0.0002$
Yu *et al.* (1990); USA	Women and men	51	10	128	0	-	3.8–∞	Adjusted for age and sex
Di Bisceglie *et al.* (1991); USA	Women and men	99	7	98	0			$p = 0.009$
Asia								
Tong *et al.* (1971); China	Women and men	55	80	943	15	[23]	[11–49]	Male controls; testing by modified ID
Simons *et al.* (1972); Singapore	Women and men	156	35	1776	7	[7.6]	[5.1–11]	Controls were male blood donors and female antenatal clinic attendees; testing by immune adherence HA
	Normal controls							
	Suspected cancer controls	156	35	207	6	[8.9]	[4.4–18]	
Lee (1975); Hong Kong	Women and men	100	49	120	9	[9.5]	[4.4–21]	Testing by immunoelectroosmophoresis and CF
Chainuvati *et al.* (1975); Thailand	Women and men	49	16	87	2	[8.3]	[1.5–59]	Testing by cross-over immunoelectrophoresis
	Cases with cirrhosis							
	Cases without cirrhosis	8	0	87	2	Not significant		

Table 6 (contd)

Reference and location	Subjects	Seroprevalence of HBsAg				OR	95% CI	Comments[a]
		Cases		Controls				
		No.	%	No.	%			
Asia (contd)								
Kubo *et al.* (1977); Japan	Women and men	124	46	299	4	[20]	[9.9–43]	RIA + reverse PHA + CEP
Chen & Sung (1978); China	Women and men	127	83	729	15	[28]	[17–46]	Testing by reverse PHA [$p < 0.05$]
Chien *et al.* (1981); China	Women and men	102	71	100	12	[18]	[8.0–40]	
Lam *et al.* (1982); Hong Kong	Women and men	107	82	107	18	21	10–46	Adjusted for age and sex
Chung *et al.* (1983); Sjøgren *et al.* (1984); Republic of Korea	Women and men	110	82	63	14	[27]	[11–70]	Solid-phase RIA
Inaba *et al.* (1984); Japan	Women and men	62	36	62	3	10		Matched-pairs analysis; $p < 0.01$; 59 controls tested; testing by reverse PHA
Yeh *et al.* (1985a,b); Luo *et al.* (1988); China	Women and men	50	86	49	22	17	4.3–99	Matched analysis
Arya *et al.* (1988); Saudi Arabia	Women and men	30	60	326	12	[11]	[4.6–27]	ELISA
Lu, C.Q. *et al.* (1988); China	NR	30	57	60	17	5		Matched analysis; $p < 0.001$
Lingao (1989); Philippines	Women and men HBV-infected subjects	329	75	238	14	[19]	[12–29]	Adjusted for sex
Yeh *et al.* (1989); China	Men	76	91	304	22	39	16–117	Matched analysis

Table 6 (contd)

Reference and location	Subjects	Seroprevalence of HBsAg				OR	95% CI	Comments[a]
		Cases		Controls				
		No.	%	No.	%			
Asia (contd)								
Tsukuma *et al.* (1990); Japan	Women and men	229	19	266	2	14	5.7–36	Adjusted for sex, age, history of blood transfusion, heavy drinking, cigarette index and family history of liver cancer; testing by reverse PHA
Lin *et al.* (1991); China	Women and men	243	77	302	19	10		Adjusted for age, sex and other hepatitis markers; $p < 0.05$
Chen *et al.* (1991); China	Men	200	20	200	2	58	27–124	Matched-pair analysis; HBsAg(+) and HBeAg(+)
		200	64	200	19	17	7.4–38	Matched-pair analysis; HBsAg(+) and HBeAg(–)
Srivatanakul *et al.* (1991); Thailand	Women and men	65	42	65	8	15	2.3–103	Adjusted for alcohol, shrimp paste, powdered peanut and fresh vegetable consumption, betel-nut chewing, by conditional multivariate regression; ELISA
Tanaka *et al.* (1992); Japan	Women and men	204	19	410	2	14	5.9–33	Adjusted for sex, age, history of blood transfusion, family history of liver disease, alcohol consumption and smoking amount; testing by RIA or reverse PHA
Chuang *et al.* (1992); China	Women and men	128	77	384	28	[9.9]	[5.9–17]	Adjusted for anti-HCV status
Cordier *et al.* (1993); Viet Nam	Men	152	93	241	18	62	30–128	Testing by second-generation ELISA

Table 6 (contd)

Reference and location	Subjects	Seroprevalence of HBsAg				OR	95% CI	Comments[a]
		Cases		Controls				
		No.	%	No.	%			
Europe								
Trichopoulos *et al.* (1978); Greece	Women and men	80	49	160	8	10	5.2–21	Adjusted for age and sex; $p < 0.001$; HBsAg seropositivity or anti-HBc seropositivity and anti-HBs seronegativity
Pedreira *et al.* (1980); Spain	Women and men	34	52	100	5	[21]	[7–66]	Testing by reverse PHA; $p < 0.005$; HBsAg seropositivity or anti-HBc seropositivity and anti-HBs seronegativity
Goudeau *et al.* (1981); France	Women and men	46	4	10 000	0.5	[10]		$p < 0.01$
De Franchis *et al.* (1982); Italy	Women and men	42	36	42	12	[4.1]	[1.2–15]	
	Controls with chronic liver disease							
	Other hospital controls	42	36	84	2	[23]	[4.5–155]	
Pagliaro *et al.* (1982); Italy	Women and men	50	NR	50	NR	14	1.4–∞	Matched analysis; testing by RIA, CEP or reverse PHA
Pagliaro *et al.* (1983); Italy	Women and men	286	30	3629	17	[2.1]		$p < 0.0005$; testing by RIA or ELISA
	Subjects with cirrhosis							
	Subjects without cirrhosis	64	33	1545	2	[24]		$p < 0.0005$
Bassendine *et al.* (1983); UK	Women and men	27	15	112	0.9	[19]	[1.9–476]	
Pirovino *et al.* (1983); Switzerland	Women and men	65	31	115	17	[2.3]	[1.0–4.9]	
Filipazzo *et al.* (1985); Italy	Women and men	120	18	120	14	[1.4]	[0.68–2.9]	Adjusted for age
	Controls with cirrhosis							
	Controls with solid tumour	120	18	120	3	[6.8]	[2.2–20]	Adjusted for age
	Controls with chronic disease	120	18	120	4	[5.3]	[1.9–15]	Adjusted for age

Table 6 (contd)

Reference and location	Subjects	Seroprevalence of HBsAg				OR	95% CI	Comments[a]
		Cases		Controls				
		No.	%	No.	%			
Europe (contd)								
Colloredo Mels *et al.* (1986); Italy	Women and men	15	47	199	7	12		$p < 0.001$
	Subjects without cirrhosis							
	Subjects with cirrhosis	57	28	156	26	1		NS
Kaklamani *et al.* (1991); Greece	Women and men	185	46	432	7	11	6.7–19	Adjusted for age, gender and anti-HCV status
Trichopoulos *et al.* (1987); Tzonou *et al.* (1991); Greece	Cases with cirrhosis	78	65 of 81	432	7	33	15–70	Adjusted for age, gender, anti-HCV status and smoking (percentages calculated from the data of Trichopoulos *et al.*; no. with cirrhosis from Tzonou *et al.*)
	Cases without cirrhosis	107	32 of 113	432	7	6.7	3.6–12	Adjusted for age, gender, anti-HCV status and smoking
Vall Mayans *et al.* (1990); Spain	Women and men	96	9	190	2	4.9	1.3–22	Adjusted for age and sex
Leandro *et al.* (1990); Italy	Women and men	140	23	317	14	2.3		Adjusted for age and sex; $p < 0.05$
Stroffolini *et al.* (1992); Italy	Women and men	65	25	98	6	11	3.1–41	Adjusted for age, gender, anti-HCV and HBV markers; testing by ELISA

OR, odds ratio; CI, confidence interval; NR, not reported. The estimates in square brackets were calculated by the Working Group and are unadjusted unless otherwise indicated in the comments.

[a]Serological testing for HBV markers was by radioimmunoassay (RIA), unless otherwise specified. ID, immunodiffusion; HA, haemagglutination; CF, complement fixation; CEP, counter immunoelectrophoresis; PHA, passive HA, ELISA, enzyme-linked immunosorbent assay; NS, not significant; HCV, hepatitis C virus; HBeAg, hepatitis B envelope antigen

(f) Factors that modify the risk for HCC associated with HBV

Male HBsAg carriers are more likely to develop HCC than female carriers (Anthony, 1984; Coursaget *et al.*, 1987), and there is some evidence that establishment of the carrier state prenatally or early in life is associated with a higher OR for HCC than establishment of a similar state in adulthood (Larouzé *et al.*, 1976; London, 1981; Hsieh *et al.*, 1992).

Several factors other than HBV have been evaluated as causally associated with HCC. In particular, aflatoxins, drinking of alcoholic beverages and oral contraceptives have been determined to be human carcinogens (IARC, 1987, 1988, 1993). Whether these factors modify the effect of HBV in the causation of HCC is inconclusive. The modifying effect of concurrent infection with HCV on the action of HBV is discussed in the monograph on HCV (p. 165).

2.4.2 *Cholangiocarcinoma*

Two case–control studies found no association between HBsAg seropositivity and the occurrence of cholangiocarcinoma. Parkin *et al.* (1991) conducted a case–control study in north-east Thailand involving 103 cases and 103 hospital controls matched for sex, age and hospital; patients with tobacco- and alcohol-related disease and other liver disease were excluded. No association was found with HBsAg seropositivity (OR, 1.0; 95% CI, 0.4–2.7). In Taiwan, China, Chen and Sung (1978) reported that of seven cases one (14%) was HBsAg seropositive, giving a similar rate to that seen among 729 controls (15%).

2.4.3 *Other cancers*

The relationship between HBsAg seroprevalence (as measured by reverse passive haemagglutination) and the occurrence of oral and uterine cervical cancer was examined in one study (Vijayakumar *et al.*, 1984). The subjects analysed were 350 oral cancer patients (232 men, 118 women), 150 cervical carcinoma patients and 100 healthy controls (50 men, 50 women); all were 40–60 years old and had no history of jaundice. Significant differences ($p < 0.001$) were found for all sex-specific comparisons between cases and controls: the seroprevalence of HBsAg was 11% in male and 12% in female oral cancer cases, 13% in cervical cancer cases and 4% in both male and female controls. [No data were provided on social class, nor was it clear whether the carrier state preceded treatment of the disease.]