2. Studies of Cancer in Humans

2.1 Case series

Table 7 summarizes the seroprevalence of antibodies to HCV in 30 series of patients with hepatocellular carcinoma (HCC). In most, the prevalence was determined by first-generation assays. In general, the prevalence is high among Japanese cases, relatively high in European populations and relatively low in Chinese and Africans. In those series in which data were included for cases grouped by HBV surface antigen (HBsAg) status, the prevalence of antibodies to HCV was generally substantially higher among the HBsAg-seronegative cases.

2.2 Cohort studies

Kiyosawa *et al.* (1990b) studied 58 patients (41 men and 17 women) with chronic hepatitis C (by first-generation ELISA) admitted to Shinshu University Hospital, Japan, between January 1970 and April 1990. All had a history of blood transfusion, and 20 had had clinical acute hepatitis in the past. Twenty-six patients were diagnosed at the time of entry into the study with chronic active hepatitis and 28 with chronic persistent hepatitis. Serum samples were collected serially for a mean of 13.2 years. Among 54 patients who remained seropositive for HCV antibodies throughout the follow-up period, 10 (18.5%) developed HCC. Among four patients who converted from seropositivity to seronegativity, there was no case of HCC. In a further study including some of these patients, Yousuf *et al.* (1992) reported on 16 of 62 HCV-seropositive patients who developed HCC after a mean interval of 9.5 years. [Insufficient detail was provided on the selection of the cases, and no information was given on length of follow-up between the two comparison groups, precluding calculation of expected numbers.]

Seeff *et al.* (1992) studied 545 patients with post-transfusion non-A, non-B hepatitis (diagnosed by exclusion) and 930 matched controls who had received transfusions but did not develop non-A, non-B hepatitis. The subjects were drawn from among participants in five prospective studies of post-transfusion non-A, non-B hepatitis in the USA conducted between 1967 and 1980. Of the 568 cases, 76% were male. The 984 controls were similar to cases with respect to age, sex, race, treatment centre, receipt of immune globulin, history of alcoholism, number of units of blood transfused and date transfused. Cause of death was

Table 7. Prevalence of antibodies to HCV in case series of patients with hepatocellular carcinoma (HCC)

Reference	Location	Period	Assay	Prevalence of antibodies to HCV						Comments
				All HCC patients		HBsAg-positive patients		HBsAg-negative patients		
				Total	%	Total	%	Total	%	
Africa										
Levrero *et al.* (1991)	Senegal	1980–88	ELISA	93	NR	NR	27	NR	68	
Robson *et al.* (1991)	South Africa	NR	ELISA and confirmation by Abbott neutralization EIA; C100-3	30	7	11	37	NR		
Bukh *et al.* (1993)	South Africa	1987–90	HCV RNA and 2nd-gen. ELISA	128	20	71	10	57	33	
Americas										
El-Ashmawy *et al.* (1992)	USA	1985–88	Confirmation by RIBA	38	26	11	45	27	19	Liver transplant patients
McHutchison *et al.* (1992)	USA	NR	1st- and 2nd-gen. EIA and RIBA	46	52	NR		NR		
Asia										
Kiyosawa *et al.* (1990a)	Japan	1958–89		83	73	29	35	54	94	21 patients with a history of transfusion all seropositive before HCC developed
Ohkoshi *et al.* (1990)	Japan	NR	ELISA	100	58	42	19	58	86	
Nishioka *et al.* (1991)	Japan	NR	ELISA	180	51	75	15	105	76	
Watanabe *et al.* (1991)	Japan	NR	ELISA (C100) P22	125 125	55 69	23 23	4 4	102 102	67 83	

Table 7 (contd)

Reference	Location	Period	Assay	Prevalence of antibodies to HCV						Comments
				All HCC patients		HBsAg-positive patients		HBsAg-negative patients		
				Total	%	Total	%	Total	%	
Asia (contd)										
Hagiwara *et al.* (1992)	Japan	NR	C100-3	NR		NR		39	74	All cases HBsAg-seronegative; 5/10 seronegatives were HCV RNA seropositive by PCR
Lee *et al.* (1992)	China	NR	ELISA	326	13	243	4	83	37	
Leung *et al.* (1992)	Hong Kong	1986–90	ELISA	424	7	341	4	83	19	Male to female ratio,7:1
Shimizu, S. *et al.* (1992)	Japan	1985–89	2nd-gen. ELISA (83%) and RIBA (58%)	NR		NR		24	58	All cases were in alcoholics
Yuki *et al.* (1992)	Japan	NR	C100-3	148	70	38	32	110	83	
Chien *et al.* (1992)	Japan	NR	ELISA (C100-3) C25 or C100-3	NR NR		NR NR		268 268	63 83	
Sheu *et al.* (1992b)	China	1988–90	1st- and 2nd-gen. assays	NR		NR		31	68	
Kiyosawa & Furuta (1992)	Japan	1971–80 1981–90	NR	112 267	34 59	65 86	8 5	47 181	81 88	
Takeda *et al.* (1992)	Japan	1980–89	ELISA	100	51	27	11	73	66	
Sun *et al.* (1993)	China	NR	2nd-gen. ELISA	112	5	NR		NR		

Table 7 (contd)

Reference	Location	Period	Assay	Prevalence of antibodies to HCV						Comments
				All HCC patients		HBsAg-positive patients		HBsAg-negative patients		
				Total	%	Total	%	Total	%	
Europe										
Colombo *et al.* (1989)	Italy	1975–88	ELISA (C100-3)	132	65	41	54	91	70	
Simonetti *et al.* (1989)	Italy	1982–88	ELISA	200	76	31	58	169	79	
Amitrano *et al.* (1990)	Italy	1989	ELISA	29	62	NR		NR		
Sbolli *et al.* (1990)	Italy	1981–89	ELISA	78	61	8	13	70	64	
Vargas *et al.* (1990)	Spain	NR	NR	81	54	NR		NR		
Benvegnù *et al.* (1991)	Italy	NR	2nd-gen. RIBA	40	65	NR		NR		14 seropositives negative in 1st-gen. assay
Levrero *et al.* (1991)	Italy	1980–88	ELISA	74	NR	NR	30	NR	76	
Nalpas *et al.* (1991)	France	1982–89	ELISA	55	58	12	75	35	57	
Farinati *et al.* (1992)	Italy	NR	2nd-gen. ELISA and RIBA	97	64	NR	43	NR		
Garson *et al.* (1992b)	Switzerland	NR	2nd-gen. EIA and RIBA	40	35	7	14	33	39	
Baur *et al.* (1992)	Austria	NR	ELISA	54	22	22	18	32	25	Any HBV marker

NR, not reported; HBsAg, HBV surface antigen; ELISA, enzyme-linked immunosorbent assay; EIA, enzyme immunoassay; RIBA, recombinant immunoblot assay; PCR, polymerase chain reaction

determined from death certificates for 545 (96.0%) of the cases and 930 (94.5%) controls as at February 1992, giving an average of 18 years of follow-up. No testing for antibodies to HCV was done. One case of HCC was found in the hepatitis group and two in the controls [estimated RR, 1.0]. [There were too few cases of HCC for the study to be informative.]

Verbaan *et al.* (1992) followed 566 patients (331 men and 235 women), with a mean age at entry into the study of 52.1 years, in a hospital in Malmö, Sweden, from whom liver biopsy samples were taken for assessment of chronic liver disease between 1978 and 1989. Causes of death were obtained from death certificates or autopsy records. Sera stored at the time of biopsy were tested for antibodies to HCV by first- and second-generation ELISA, and sera positive in these two tests were retested by second-generation RIBA. Of the 566 patients, 78 (13.8%) were seropositive by RIBA. Eleven cases of HCC developed over the follow-up period; two were diagnosed at the time of inclusion in the study. The proportion of deaths due to HCC in the seropositive group (5/23, 22%) was significantly different ($p = 0.01$) from that in the seronegative group (6/130, 5%).

In a cohort study from Taiwan, China, serum samples from 9691 male adults were collected and frozen during 1984–86 (Yu & Chen, 1993). A total of 35 cases of HCC were identified between 1984 and 1990 and matched individually by age, time of sample collection and residence to two HBsAg-seropositive and two HBsAg-seronegative controls from the original cohort. Samples were analysed for HBsAg status by a radioimmunoassay, for antibodies to HCV by an enzyme immunoassay and for serum testosterone level. Seven of the 35 cases and four of the 140 controls were seropositive for antibodies to HCV [crude relative risk (RR), 9] (multivariate-adjusted RR, 12; 95% confidence interval (CI), 2.4–58).

2.3 Case–control studies

For those studies in which estimated odds ratios (OR) are not provided, the Working Group calculated them using the crude data. The estimates are therefore not adjusted for other factors.

2.3.1 *First-generation assays*

Table 8 (p. 191) gives a summary of the case–control studies in which first-generation antibody tests were used (see section 1.2) and includes available information on the study period and source of control subjects.

(a) Africa

Coursaget *et al.* (1990) reported on 80 cases of HCC and 136 adult controls in Senegal. Sera were collected between 1982 and 1986. The seroprevalence of antibodies to HCV was 37.5% among the cases and 3% among the controls [OR = 20]. [No data were given on the age and sex distribution of subjects nor how they were selected.]

Kew *et al.* (1990) studied 380 southern African blacks (322 men, 58 women) with histologically confirmed HCC and compared them with 152 controls matched for race, sex, age and rural or urban status. The seroprevalence of antibodies to HCV among the cases (29%) was higher than that among the controls (1/152) [OR, 62]. Of the 196 HBsAg-seronegative cases, 32% were seropositive for HCV antibodies, compared with 26% of the 184 HBsAg-seropositive cases. [The periods of collection of data and sera were not given.]

(*b*) *Americas*

In a study conducted in the USA, Hasan *et al.* (1990) studied retrospectively a total of 87 HCC patients who had been diagnosed between January 1978 and March 1989 at the University of Miami Hospital and Clinic. Diagnosis was made either histologically, cytologically or by level of serum α-fetoprotein with at least one positive imaging study. Cases with alcoholic liver disease, haemochromatosis or α_1-antitrypsin deficiency were excluded. Controls were 200 consecutive blood donors. Forty percent of the cases and 0.5% of the controls were seropositive for antibodies to HCV (tested by the method of Kuo *et al.*, 1989, see p. 168) [OR, 134]. The seropositivity among cases varied by ethnicity, being found among the HBsAg-seronegative patients in 35% of 37 whites, 80% of 20 hispanics and the one black; the only Asian was seronegative. The seroprevalence of antibodies to HCV among the 41 cases with no evidence of past HBV infection was 49%, that among the 18 seropositive only for anti-HBc was 61%, and that among the 28 cases who were HBsAg seropositive was 14%. All cases who were HCV antibody seropositive and HBsAg seronegative had evidence of cirrhosis. [The sex and age distribution of the HBsAg-seropositive cases and of the controls were not given. It was not evident when the case blood samples were collected in relation to diagnosis or whether case and control samples were collected at different times.]

Yu *et al.* (1990) evaluated data on 51 cases (in 35 males and 16 females; mean age, 59.5 years) and 128 controls (81 males; mean age, 58.7 years) obtained in 1984–89 from Los Angeles County, California (USA) (for details, see p. 80 of the monograph on HBV). Of the cases, 29% were seropositive for antibodies to HCV, as were 4% of the controls. An OR of 11 was found for the association between HCV antibody seropositivity and HCC after adjustment for age and sex. The OR for antibodies to HCV in subjects with no HBV markers was 4.8; that for any HBV marker among the HCV seronegative subjects was 4.4. Ten cases and no control had evidence of any serological marker of HBV infection and were HCV seropositive ($p < 0.0005$). The authors noted that the serum specimens of the controls were drawn on average six years before those of the cases.

Di Bisceglie *et al.* (1991a) studied 99 cases of HCC seen at Johns Hopkins Oncology Center, Baltimore, USA, between January 1987 and May 1988 (for details, see p. 81 of the monograph on HBV). Controls consisted of 98 consecutive adult patients with other cancers seen between November 1987 and January 1988. The seroprevalence of antibodies to HCV was 13% among the cases and 2% among the controls; the OR for an association with HCC was 7.3.

(*c*) *Asia*

Saito *et al.* (1990) studied the seroprevalence of antibodies to HCV among 253 (207 men and 46 women; mean age, 61 years) patients with HCC diagnosed clinically and pathologically in several hospitals in Japan and from whom blood specimens were obtained at the time of diagnosis. For comparison, they evaluated 148 patients with other cancers (95 men, 53 women; mean age, 61.1 years). The seroprevalence of antibodies to HCV was 55% among cases and 10% among controls [OR, 11]. The prevalence among the cases varied by HBV status. The authors noted an association between history of transfusion and seropositivity for HCV antibodies in the controls [$p = 0.003$] but not in the cases, and that the control rate was

higher than that seen in Japanese blood donors (about 1%). [No data were given on the period of study.]

In a case–control study in Qidong County, Jiangsu, China, 50 cases of HCC diagnosed during 1988 were compared with 50 population controls individually matched to cases by age, sex and place of residence (Xu *et al.*, 1990). Four cases and no control were seropositive for antibodies to HCV ($p = 0.059$); one of these cases was seronegative for HBsAg, while the remaining three were seropositive.

Jeng and Tsai (1991) studied 48 HCC patients (35 men, 13 women; mean age, 62.0 years) in Taiwan, China, who were HBsAg seronegative and who had been recruited after admission to Kaohsiung Medical College Hospital between January 1988 and June 1990. Diagnosis was made histologically or cytologically. Controls were 54 HBsAg-seronegative individuals (46 men, eight women; mean age, 52.3 years) who were seen for normal physical check-ups during the same period. Seroprevalence for antibodies to HCV differed significantly between the two groups (60% *versus* 0 [$p = 0.0001$]). The authors also reported a significant difference between HCV antibody seroprevalence in these cases and in 81 HBsAg-seropositive HCC cases seen during the same period (23.5%; $p = 0.0001$).

Srivatanakul *et al.* (1991) conducted a matched case–control study on HCC in cases seen in Thailand in 1987–88 (Parkin *et al.*, 1991; described in detail on p. 85 of the monograph of HBV). There was no association between the presence of antibodies to HCV and HCC (OR, 1.3); the prevalence among cases was 6% and that among the controls was 5%.

Yu *et al.* (1991) conducted a matched case–control study in Taiwan, China, of HCC cases from two major teaching general hospitals newly diagnosed between August 1986 and July 1987 on the basis of either pathological examination or serum α-fetoprotein levels, confirmed by imaging. There were 127 cases (121 men, 6 women; mean age, 50.4 years). Controls were selected from household registration lists, were matched for age, sex, ethnicity and residence and were recruited during the same period. The seroprevalence of antibodies to HCV was 11% among the cases and 2% among the controls. In a matched analysis, the univariate estimate of the OR associated with seropositivity for HCV antibody was 7.0, and the ratio remained elevated after control for HBsAg status, HBeAg status, smoking habits, habitual alcohol use and peanut consumption. The seroprevalence of antibodies to HCV was higher in the 17 HBsAg-seronegative cases (29%) than in the 110 HBsAg-seropositive cases (8%). Among the HBsAg-seronegative subjects, the OR for HCV seropositivity and HCC was 16. The OR for co-infection as compared with seropositivity for neither marker could not be estimated, with nine cases and no control observed.

HCV antibody status was measured in 1989–90 using an enzyme immunoassay in 42 cases of HCC and in 4818 blood donors enrolled from the Military Hospital in Riyadh, Saudi Arabia (Al Karawi *et al.*, 1992). The seroprevalence of antibodies to HCV was 31% in cases and 1.5% among blood donors [crude OR, 30; 95% CI, 15–60]. The seroprevalence of antibodies to HCV was higher among HBsAg-seronegative HCC cases (42%) than among seropositive HCC cases (13%). [No information was available on the age or sex of the study subjects.]

In a study that overlapped partially with that of Yu *et al.* (1991), Chuang *et al.* (1992) studied 128 cases of HCC (in 112 men, 16 women; mean age, 54.3 years) and 384 age- and

sex-matched community controls in Taiwan, China (described on p. 85 of the monograph on HBV). Twenty percent of the cases and 3% of controls were seropositive for HCV antibodies. Among the cases, the seroprevalence was 45% for the 29 who were HBsAg seronegative and 12% for the 99 who were HBsAg seropositive. The OR for HCC was 27 among the subjects seropositive for HCV antibody and HBsAg seronegative. The OR for HCC for the joint presence of HBsAg and HCV antibody seropositivity as compared with the presence of neither was 40. [The period of data collection was not given.]

(*d*) *Europe*

The first study on HCV and HCC, reported by Bruix *et al.* (1989), was conducted in Barcelona, Spain. The cases were 96 (67 men, 29 women; mean age, 63.4 years) consecutive patients with HCC confirmed by ultrasonography and biopsy or with elevated serum α-fetoprotein level. The control group comprised 177 hospitalized surgical controls without liver disease (119 men, 58 women; mean age, 54.3 years). The seroprevalence of antibodies to HCV was 75% among cases and 7% among controls. Among cases, the seroprevalence varied somewhat by risk category: all of four cases with porphyria and cirrhosis, 81% of 43 with cirrhosis of unknown etiology, 77% of 30 with alcoholic cirrhosis, 56% of nine HBsAg-seropositive cases, none of three with a previously normal liver and 71% of seven with previous liver status unknown. The seroprevalence of antibodies to HCV in the case group was significantly higher than that in the control group without liver disease [OR, 38]. [Data on time period of serum collection were not provided.]

Caporaso *et al.* (1991) reported on 332 consecutive patients with cirrhosis seen in a medical centre in Naples, Italy, between January 1988 and May 1990. HCC was diagnosed in 88 of these by ultrasonographic examination and serum α-fetoprotein levels and/or by cytology. The patients with HCC were more likely than the 244 control patients with only cirrhosis to be male (88 *versus* 61%) and to be older (mean age, 64.5 years *versus* 57.0). The seroprevalence of antibodies to HCV among the cases was 72% and that among the controls was 55% [RR, 2]. After control for age and sex, seropositivity for antibodies to HCV was a significant predictor of HCC ($p = 0.009$).

Poynard *et al.* (1991) studied 2015 patients admitted in 1982–89 to the hepatogastroenterology service of the Antoine Béclère Hospital in Clamart, France, for alcoholism and alcoholic liver disease, using a uniform protocol. All patients were classified as alcoholic on the basis of a history of consumption of at least 50 g alcohol per day in the year before admission. Serum samples were available for testing for antibodies to HCV from 469 patients with documented liver cirrhosis (51 HCC cases and 418 controls). Diagnosis was made histologically or on the basis of α-fetoprotein levels, confirmed by ultrasonography and other imaging. The presence of cirrhosis was determined in all subjects either by biopsy or by use of an established algorithm. The seroprevalence of antibodies to HCV was 41% among the cases and 26% among the controls, giving an OR for an association with HCC of [2.0]; this estimate did not vary markedly with the presence of HBsAg. On multivariate analysis, age, male sex, HBsAg seropositivity and HCV antibody seropositivity were all significant risk factors for HCC; HCV antibody seropositivity was the weakest ($p = 0.04$). [Sex ratio and age range within the group were not available.]

Tzonou *et al.* (1991) re-evaluated subjects from an earlier case–control study (Trichopoulos *et al.*, 1987; described on p. 88 of the monograph on HBV). As reported previously (Kaklamani *et al.*, 1991), only strong seropositivity for antibodies to HCV (twice the recommended limit) was specifically related to HCC; weakly positive results were related to cases of metastatic liver cancer, suggesting that weakly positive results were false-positives. In the present report, subjects were categorized as strongly positive, weakly positive or negative. For 185 HCC cases and 432 hospital controls, the prevalence of strong reactivity to HCV antibodies was 39% among the cases and 7% among the controls: the OR was 6.2 after adjustment for age, sex, smoking and HBsAg status. The joint presence of seropositivity for HCV antibodies and HBsAg gave an OR of 20; that for HCV-seropositive and HBsAg-seronegative subjects was 4.8 ($p < 0.05$). The prevalence of antibodies to HCV was lower among the 99 HBsAg-seronegative cases (28%) than among the 85 HBsAg-seropositive cases (51%).

Simonetti *et al.* (1992) evaluated 212 consecutive HCC patients (161 men, 51 women; mean age, 62.4 years) admitted to a hospital in Palermo, Italy, between June 1982 and December 1988. Diagnosis was based on biopsy or serum α-fetoprotein level, confirmed by ultrasonography or tomography. Controls were matched for age and sex and were drawn from among patients hospitalized for chronic non-hepatic disease during the same period (mean age, 62.2). Of the cases, 71% were seropositive for antibodies to HCV compared with 5% of the controls; the OR for an association with HCC was 69 after control for markers of HBV. Of the cases, 197 had confirmed cirrhosis (based on clinical criteria in 60 cases and by biopsy in 137 cases), and a second comparison group was assembled of 197 patients with cirrhosis matched for age and sex (mean age, 61.5), who were hospitalized during the same period. Of the cirrhotic cases of HCC, 74% were seropositive for HCV antibody, as were 62% of their controls; the OR for an association with HCC was 2.0 after control for markers of HBV and alcohol abuse.

2.3.2 *Second-generation assays*

Table 9 summarizes the case–control studies in which antibody to HCV was screened using second-generation assays.

(*a*) *Africa*

A case–control study was carried out in Maputo, Mozambique, on 178 HCC patients admitted to the department of gastroenterology of the central hospital of that city and 194 blood donors from the same hospital (Dazza *et al.*, 1993). HCV antibody status was investigated using a second-generation enzyme immunoassay with confirmation by a line immunoassay. Eleven cases and four controls were seropositive for HCV antibody, yielding an age-adjusted OR of 1.1, which was substantially different from the crude OR of 3.1. The OR for seropositivity to HCV antibody was 1.4 (95% CI, 0.4–5.3) among HBsAg-seronegative individuals. The mean age of cases was 40.8 years and that of the controls, 31.3 years; the mean age of HCV antibody-seropositive cases was 53.9 and that of seronegative cases was 35.1. [The controls were substantially younger than the cases, but the age ranges were not given, and the statistical methods used to control for age were not described.]

Table 8. Summary of results of case–control studies of hepatocellular carcinoma and the prevalence of antibody to HCV as measured by first-generation assays

Reference and location	Subjects	Seroprevalence of antibodies to HCV				OR[a]	95% CI	Study period and comments
		Cases		Controls				
		No.	%	No.	%			
Africa								
Coursaget *et al.* (1990); Senegal	NR	80	37.5	136	3	[20]	[6.9–57]	1982–86; stringent cut-off used for assay
Kew *et al.* (1990); South Africa	Men and women	380	29	152	0.7	[62]	[11–353]	Unmatched hospital controls
Americas								
Hasan *et al.* (1990); USA	NR	87	40	200	0.5	[134]	[23–787]	Cases, 1978–89; unspecified for controls; blood donor controls
Yu, *et al.* (1990); USA	Men and women	51	29	128	4	11	3.5–31	Cases, 1984–89; community controls, 1978–82; all estimates adjusted for age and sex
	Subjects with no evidence of HBV infection					4.8	1.3–18	
	Subjects with any HBV marker					∞	15–∞	
Di Bisceglie *et al.* (1991a); USA	Men and women	99	13	98	2	7.3	1.8–48	Cases, 1/1987–5/1988; controls, 11/1987–1/1988. Controls, other cancer patients
Asia								
Saito *et al.* (1990); Japan	Men and women	253	55	148	10	[11]	[5.9–19]	Controls, other cancer patients. Among controls, significant association of HCV seroprevalence and history of blood transfusion ($p = 0.003$)
Xu *et al.* (1990); China	Men and women	50	4	50	0	∞		Population controls ($p = 0.06$)

Table 8 (contd)

Reference and location	Subjects	Seroprevalence of antibodies to HCV				OR[a]	95% CI	Study period and comments
		Cases		Controls				
		No.	%	No.	%			
Asia (contd)								
Jeng & Tsai (1991); Taiwan, China	Men and women	48	60	54	0	∞		[$p < 0.0001$]; 1988–90; non-hospital controls. 11 subjects were HBsAg seronegative
Srivatanakul *et al.* (1991); Thailand	Men and women	63	6	63	5	1.3	0.2–8.7	1987–88; hospital controls; matched analysis; subjects matched on sex, age, area of residence, hospital
Yu *et al.* (1991); Taiwan, China	Men and women	127	11	127	2	7.0	1.6–31	1986–87; community controls; matched analysis; subjects matched for age, sex, ethnicity and residence
						24	2.2–254	Conditional multivariate analysis with adjustment for matching factors plus HBsAg status, HBeAg status, smoking, habitual alcohol use and peanut consumption
	HCV seropositive/HBsAg seropositive *versus* neither					∞	14–∞	
Al Karawi *et al.* (1992); Saudi Arabia	NR	42	31	4818	1.5	[30]	[15–60]	1989–90; blood donor controls; no data on age or sex
Chuang *et al.* (1992); Taiwan, China	Men and women	128	20	384	3	[6.9]	3.5–14	Community controls
	HBsAg seronegative					27	9.8–75	
	HBsAg seropositive, HCV seropositive *versus* neither					40	13–128	
Europe								
Bruix *et al.* (1989); Spain	Men and women	96	75	177	7	[38]	[18–133]	Hospital controls
Caporaso *et al.* (1991); Italy	Men and women	88	72	244	55	[2.0]	[1.2–3.4]	1988–90; all subjects had cirrhosis

Table 8 (contd)

Reference and location	Subjects	Seroprevalence of antibodies to HCV				OR[a]	95% CI	Study period and comments
		Cases		Controls				
		No.	%	No.	%			
Europe (contd)								
Poynard *et al.* (1991); France	Men and women	51	41	418	26	[2.0]	[1.1–3.7]	1982–89; all subjects were alcoholics and had cirrhosis.
Tzonou *et al.* (1991); Greece	Men and women	185	39	432	7	6.2	3.6–11	1976–84; hospital controls; all estimates adjusted for age, sex, residence; seropositivity for HCV based on 'strongly positive' results
	HCV seropositive/HBsAg seropositive *versus* neither					20	2.5–158	
	HBeAg seropositive or anti-HBe seropositive *versus* neither					∞	5.8–∞	
	HCC with cirrhosis					11	5.3–25	
Simonetti *et al.* (1992); Italy	Men and women Hospital controls	212	71	212	5	69.1	15–308	1982–88; conditional multivariate analysis with control for sex and age plus HBsAg and anti-HBc status
	Controls with cirrhosis	197	74	197	62	2.0	1.3–3.2	1982–88; conditional multivariate analysis with control for age and sex plus HBsAg and anti-HBc status and alcohol abuse

NR, not reported

[a]Cornfield limits; all estimates are unadjusted unless otherwise specified.

A series of 49 cases of HCC and 134 adult controls from the general population were re-investigated in Senegal with regard to HCV antibody status using both a first-generation ELISA test for C100 and an anti-core/anti-C33c recombinant assay (Coursaget *et al.*, 1992). Seropositivity to HCV antibody was found in six (12%) cases and two (1.5%) controls [crude OR, 9.2; 95% CI, 1.8–47]; seropositivity was confirmed by neutralization assay in two cases and one control [crude OR, 5.7]. [No details were provided on the methods or time of recruitment of cases and controls or on potential confounding variables.]

(*b*) *Asia*

Tanaka *et al.* (1991) conducted a case–control study in Fukuoka, Japan, on 91 cases of HCC (in 73 men and 18 women; median ages, 59.0 and 56.5 years, respectively) in 1985–89. A total of 410 controls (291 men, 119 women; median ages, 57.0 and 56.0, respectively) were identified during examinations at public health centres in 1986–89. Most of the cases had evidence of pre-existing liver disease (76 cirrhosis, nine chronic hepatitis). The prevalence of antibodies to HCV was measured with a first-generation enzyme immunoassay, and positive sera were re-tested using a second-generation assay (RIBA). The seropositivity rates in the initial assay were not related to duration of storage of the sera. Seropositivity to HCV antibody was 51% among cases and 3% among controls. The OR for an association with HCC was 52 after adjustment for demographic factors. The seroprevalence of antibodies to HCV was low among patients who were HBsAg seropositive (5%).

In a case–control study carried out in Hanoi, Viet Nam, described in detail in the monograph on HBV (p. 85), HCV antibody status was investigated using a confirmatory line immunoassay (Cordier *et al.*, 1993). Seropositivity to HCV antibody was seen in three of 152 male cases and two of 241 male hospital controls (OR, 2.0). All were HBsAg seronegative (OR, 38; 95% CI, 2.8–1443).

(*c*) *Europe*

Stroffolini *et al.* (1992) studied 65 cases of HCC diagnosed in four teaching hospitals in Palermo, Italy, between January and December of 1990 (described in detail on p. 89 of the monograph on HBV). All cases had cirrhosis; controls were hospitalized patients with non-hepatic chronic diseases. The seroprevalence of antibodies to HCV was determined using a second-generation enzyme immunoassay; positive samples were confirmed by RIBA. Seropositivity to HCV antibody was seen in 66% of cases and 13% of controls (OR, 27 after control for age, gender and HBV markers). Five cases and no control were seropositive for both HCV antibody and HBsAg (OR, 77); the OR for HCV antibody seropositivity alone was 21 and that for HBsAg alone, 13.

Zavitsanos *et al.* (1992) re-tested serum samples obtained during the case–control study described above in Athens, Greece (Tzonou *et al.*, 1991; see also p. 88 of the monograph on HBV). Samples from 181 HCC cases, 35 patients with metastatic liver cancer and 416 hospital controls with no malignant neoplasm or liver disease were examined. The sera had been collected between April 1976 and October 1984 at nine major hospitals in Athens. In the present study, sera were tested by second-generation enzyme immunoassay with confirmation empirically determined using a variety of supplemental enzyme immunoassays with other recombinant peptides and an inhibition assay using C100; a random sample of 32 HCC

patients and 13 hospital controls was also tested by RIBA-2. The authors again assumed that the cases of metastatic liver cancer were true negatives. The final algorithm for positivity was repeated reactivity to the second-generation enzyme immunoassay at an absorbance to cut-off ratio of ≥ 3.0, with confirmation based on supplemental assays with seropositivity for antibody against another viral protein or by inhibition. On the basis of these tests, the OR for an association with HCC was 10.

2.4 Modifying effects of seropositivity for hepatitis B surface antigen

At least six case–control studies presented results according to HCV antibody status (assessed by first-generation tests) separately for HBsAg-seropositive and HBsAg-seronegative individuals, allowing analyses of the separate effects of each virus and their combined effects. A further five studies presented similar results based on second-generation tests (Table 10). With both the first- and second-generation tests, the ORs for HCC in subjects infected with HBV or HCV alone are increased. The OR associated with HCV alone is in general higher in studies based on second-generation tests [combined results: OR, 26; 95% CI, 16–43] than in other studies [combined results: OR, 14; 95% CI, 10–20]. The combined effect of the two viruses cannot be described accurately, given the small numbers of subjects.

The results summarized in Table 10 should be considered with caution, since the estimated ORs were not adjusted for confounding factors such as sex and age. Comparisons between the columns of the table may be valid if it can be assumed that the uncontrolled confounding effect of sex, age and other possible factors varies across the exposure categories.