Hepatitis Viruses, Alcohol, and Tobacco in the Etiology of Hepatocellular Carcinoma in Italy

Silvia Franceschi,[1] Maurizio Montella,[3] Jerry Polesel,[5] Carlo La Vecchia,[6,7] Anna Crispo,[3] Luigino Dal Maso,[5] Pietro Casarin,[8] Francesco Izzo,[4] Luigi G. Tommasi,[5] Isabelle Chemin,[2] Christian Trépo,[2] Marina Crovatto,[9] and Renato Talamini[5]

[1]International Agency for Research on Cancer; [2]Institut National de la Sante et de la Recherche Medicale Unité 271, Lyons, France; [3]Servizio di Epidemiologia, Istituto Tumori ''Fondazione Pascale,'' Cappella dei Cangiani; [4]Divisione di Chirurgia D, Istituto Tumori ''Fondazione Pascale,'' Cappella dei Cangiani, Naples, Italy; [5]Unità di Epidemiologia e Biostatistica, Centro di Riferimento Oncologico, Istituto Nazionale Tumori, Aviano, Italy; [6]Istituto di Ricerche Farmacologiche ''Mario Negri''; [7]Istituto di Statistica Medica e Biometria, Università degli Studi di Milano, Milan, Italy; [8]Divisione di Medicina 3, Ospedale ''S. Maria degli Angeli''; and [9]Divisione di Microbiologia e Immunologia, Ospedale ''S. Maria degli Angeli,'' Pordenone, Italy

Abstract

Mortality rates of hepatocellular carcinoma (HCC) are high in Italy compared with other Western countries. To elucidate further the role of hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol drinking, and tobacco smoking in the etiology of HCC, we carried out a hospital-based case-control study in two areas of Italy: the province of Pordenone in the Northeast and the town of Naples in the South. A total of 229 HCC cases (median age, 66 years) and 431 controls (median age, 65 years) answered a questionnaire and provided blood samples between 1999 and 2002. Odds ratios (OR), percent attributable risks, and corresponding 95% confidence intervals were computed using unconditional multiple logistic regression. ORs for hepatitis B surface antigen (HBsAg) positive versus HBsAg negative and for anti-HCV antibody positive versus anti-HCV antibody negative were 20.2 and 15.6, respectively. Positivity for both markers was associated with an OR of 51.6. Sensitive molecular techniques applied to sera in a subset of HCC cases disclosed a very small number of occult hepatites. Maximal lifetime alcohol intake of ≥35 versus <7 drinks/wk was associated with an HBV/HCV adjusted OR of 5.9. Tobacco smoking was unrelated to HCC risk overall but seemed to enhance HCC risk among virus carriers. Overall, 61% of HCC were attributable to HCV, 13% to HBV, and 18% to heavy alcohol drinking. In conclusion, our study confirms the importance of HCV in HCC etiology in Italy where the widespread dissemination of the virus dates back four or five decades. (Cancer Epidemiol Biomarkers Prev 2006;15(4):683–9)

Introduction

Upward trends in incidence or mortality of hepatocellular carcinoma (HCC) have been reported in the United States (1), Japan (2), and in several European countries, including Italy (3), over the last two or three decades. These increases have been chiefly attributed to the spread of hepatitis C virus (HCV; ref. 1), which occurred earlier in Japan and Southern Europe than in the United States (3, 4) and has been mainly sustained by i.v. drug use and the use of contaminated needles in medical procedures (5). Although the role of heavy alcohol drinking has long been recognized (6-9), the contribution of cigarette smoking to HCC has been established only recently (10, 11).

To elucidate further the interplay of viruses and lifestyle, we have conducted a case-control study of HCC in two areas of Italy, characterized by different levels of alcohol consumption (12) and HCV prevalence (13, 14). Sensitive molecular techniques for viral genomes were added to serologic assays to obtain a more accurate figure of the proportion of HCC attributable to hepatitis B virus (HBV) or HCV.

Materials and Methods

Between January 1999 and July 2002, we conducted a case-control study on the association of HBV and HCV with HCC in the province of Pordenone in the Northeast of Italy and the town of Naples in the South. The original study included another case group (lymphohematopoietic neoplasms) whose relationship with HBV and HCV has already been reported (15, 16).

Cases. Cases in the present report included patients <85 years of age with incident HCC, who had not yet received any cancer treatment at study entry. They were admitted to the National Cancer Institute in Aviano, the ''Santa Maria degli Angeli'' General Hospital in Pordenone, and the ''Pascale'' National Cancer Institute, plus four General Hospitals in Naples (i.e., the hospitals to which the majority of HCC patients from the two study areas were referred). A total of 261 HCC cases were identified. Three cases refused to participate in the study and 29 HCC cases were interviewed but could not give a blood sample, thus leaving 229 cases (median age, 66 years; range, 43-84 years) for whom both questionnaire and blood sample were available. Histologic or cytologic confirmation was available from the majority (78.2%) of HCC cases whereas diagnosis was based on ultrasound, tomography, and elevated α-fetoprotein levels in the remaining HCC cases. According to the Child-Pugh classification, 59.0% of HCC cases were classified as class A, 31.7% as class B, and 18.4% as class C. All medical and histocytologic records were reviewed by two authors (F.I. and L.G.T.).

Controls. The comparison group included patients <85 years of age admitted to the same hospitals where HCC cases had been interviewed for a wide spectrum of acute conditions. Patients whose hospital admission was due to diseases related to alcohol and tobacco use (e.g., respiratory diseases, peptic ulcer, lung cancer, head and neck cancer, etc.) or hepatitis viruses (e.g., hepatitis, cirrhosis, esophageal varices, etc.) were specifically excluded from the control group, as were those

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Requests for reprints: Silvia Franceschi, IARC, 150 cours Albert Thomas, 69372 Lyon cedex 08, France. Phone: 33-4-72-73-84-02; Fax: 33-4-72-73-83-45. E-mail: franceschi@iarc.fr

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with any chronic diseases that might have resulted in substantial lifestyle modifications (e.g., diabetes, cardio- and cerebro-vascular diseases, etc.). However, comorbidity for such diseases was not an exclusion criterion. Of 467 controls contacted, 462 accepted to participate. Blood samples were available for 431 controls; of these, 26% were admitted to the hospital for trauma, 23% for nontraumatic orthopaedic diseases, 25% for acute surgical conditions, 13% for eye diseases, and 13% for other illnesses. Controls were more often female and were younger than HCC cases as matching was conducted according to the distribution by age and gender of cancer cases in the entire study, which also included already published studies of lymphohematopoietic neoplasms (15, 16). In the present article on the substudy on HCC, however, the control group was restricted to persons ≥40 years of age (n = 431; median age, 65 years; range, 40-83 years) as no HCC case <40 years of age had been identified.

All study participants signed an informed consent form, according to the recommendations of the Ethical Committee of the Aviano National Cancer Institute, which approved the study.

Questionnaire. The questionnaire included information on sociodemographic characteristics, body size measures at various ages, medical history of selected diseases, and a food-frequency section.

The section on alcohol drinking included questions on drinking status (never, former, or current drinker), weekly number of drinks of the five most common alcoholic beverages or groups of beverages (i.e., wine, beer, liquors, after-dinner drinks, and grappa), age at starting and duration of the habit. Taking into account the different ethanol concentrations, one drink corresponded to ~125 mL of wine, 330 mL of beer, and 30 mL of hard liquor (i.e., ~12 g of ethanol). Never drinkers were individuals who had abstained from alcohol drinking throughout life. Former drinkers were individuals who had abstained from alcohol drinking for at least 12 months. Drinkers were asked to report any change in alcohol beverage intake to be able to compute average and maximal lifetime alcohol intake level. Maximal, rather than average, alcohol intake was used and current and former drinkers were combined in the present report to take into account the strong tendency of HCC cases to diminish or stop alcohol drinking as a consequence of liver disease before cancer onset.

The section on smoking habits included questions on smoking status (never, former or current smoker), daily number of cigarettes/cigars and grams of tobacco pipe smoking, age at starting and duration of the habit. Former or current smokers were subjects who had smoked at least 1 cigarette/d for at least 1 year. No cases and three controls smoked pipes or cigars only, and one case and three controls reported occasional use of pipes or cigars in addition to cigarettes. In our computations, 1 g of pipe-smoked tobacco corresponded to one cigarette, and one cigar to three cigarettes. Former smokers were defined as those who had abstained from any type of smoking for at least 12 months. Satisfactory reproducibility of questions on self-reported alcohol intake (17) and smoking habits (18) in our study populations has been previously reported.

HBV and HCV testing. Each case and control provided a 15-mL sample of blood (7.5 mL with vacutainer tubes with EDTA and 7.5 mL with vacutainer tubes without anticoagulant) on the day of interview (generally on the first day of hospital stay). Blood samples were centrifuged at 1,500 g for 10 minutes, extracted, distributed into different cryotubes of serum, buffy coat, and red blood cells, and then stored at −80°C until shipment to the laboratory of Microbiology and Immunology, General Hospital of Pordenone, where they were tested in a blind fashion.

Testing for anti-HBV surface antigen (anti-HBs) and anti-HBV core antigen (anti-HBc), markers of HBV infection, was done using microparticle enzyme immunoassay (AxSYM AUSAB and AxSYM Core TM, Abbott Diagnostic Division, Wiesbaden, Germany). Samples that gave borderline results were retested. HBV surface antigen (HBsAg) and HBeAg, markers of chronic carriage of HBV, were tested for using microparticle enzyme immunoassay (AxSYM HBsAg version 2.0 and AxSYM HBeAg version 2.0, Abbott Diagnostic Division) and confirmed using a neutralization test (AxSYM, Abbott Diagnostic Division). In this article, samples were deemed $HBsAg^+$ or $HBeAg^+$ if they were positive at the neutralization test. Seven controls reported HBV vaccination and were confirmed to be anti-HBs^+ and anti-HBc^-.

Sera were screened for antibodies against HCV (anti-HCV) using a third-generation microparticle enzyme immunoassay (AxSYM HCV version 3.0, Abbott Diagnostic Division). The assay detects antibodies to both structural and nonstructural proteins of the HCV genome. Its sensitivity is estimated to be 99% in patients with chronic liver disease, with a specificity of 97% in panels of sera (19). Positive samples were tested for anti-HCV using a third-generation line immunoassay (Innogenetics, Gent, Belgium) and for serum HCV RNA using the Roche Amplicor version 2.0 (Roche Molecular System, Pleasenton, CA). The limit of detection for the Roche Amplicor version 2.0 test for HCV RNA was 50 IU/mL. HCV genotyping of HCV RNA^+ samples was done using a second-generation line probe assay (Innogenetics).

HBV DNA and/or HCV RNA were tested for in serum samples of subsets of HCC cases among whom false negatives (occult infections) for either or both viruses were considered likeliest to have occurred. Both HBV DNA and HCV RNA were searched for in HCC cases who were negative for all viral markers (i.e., 32 cases for whom serum was available) or were anti-HBc^+ only (eight cases). HBV DNA alone was tested for in serum samples of anti-HCV^+ cases who were negative for all HBV markers or were anti-HBc^+ only (53 and 28 cases, respectively, for whom serum was available). HBV DNA detection was carried out in serum samples using a nested PCR on the S gene that can detect ~350 viral copies/mL (20, 21). HCV RNA was also tested for in serum samples using a semi-nested PCR on the 5′ noncoding region of the HCV genome that can detect ~430 viral copies/mL (22, 23). On account of the low proportion of HBV DNA^+ or HCV RNA^+ findings, PCR assays were not extended to all HCC cases and controls.

Statistical Methods. Adjusted odds ratios (OR) and corresponding 95% confidence intervals (95% CI) were calculated by means of unconditional multiple logistic regression including age (in 5-year groups plus a term for age as a continuous variable), gender, study center, years of education and, when mentioned, HBsAg and anti-HCV positivity (24). Percent attributable risks were computed using the distribution of the risk factors in HCC cases and corresponding 95% CIs were obtained (25). Attributable risk for a combination of different risk factors can be different than the sum of the attributable risks for the corresponding factors on account of departure from multiplicativity of ORs.

Results

Table 1 shows the distribution of HCC cases and controls by gender, age group, study center, and selected covariate. Twenty percent of cases and 32.3% of controls were female. Place of birth was not associated with HCC risk whereas an inverse relationship was found with educational level (OR for ≥12 versus <7 educational years, 0.3; 95% CI, 0.2-0.6). Type of occupation was not a significant determinant of HCC risk (Table 1).

Table 2 shows the relationship between HCC and alcohol drinking and tobacco smoking after adjustment for $HBsAg^+$ and anti-HCV^+. Drinking cessation was reported by 51.5% of HCC cases but only 11.1% of controls, and was associated with

Table 1. OR for HCC and corresponding 95% CI by selected sociodemographic variables (229 cases and 431 controls); Italy, 1999-2002

	Cases	Controls	OR (95% CI)
	n (%)	*n* (%)	
Gender			
Men	183 (79.9)	292 (67.7)	—
Women	46 (20.1)	139 (32.3)	—
Age group (y)			
40-54	19 (8.3)	89 (20.7)	—
55-64	67 (29.3)	119 (27.6)	—
65-74	106 (46.3)	155 (36.0)	—
≥75	37 (16.2)	68 (15.8)	—
Study area			
Pordenone	105 (45.9)	249 (57.8)	—
Naples	124 (54.2)	182 (42.2)	—
Place of birth			
North and Center	94 (41.1)	225 (52.2)	1*
South and Islands	135 (58.9)	206 (47.8)	1.42 (0.71-2.87)
Education (y)			
<7	153 (66.8)	245 (56.8)	1*
7-11	60 (26.2)	96 (22.3)	1.07 (0.71-1.61)
≥12	16 (7.0)	90 (20.9)	0.31 (0.17-0.57)
χ^2_1 for trend			10.28, $P < 0.01$
Occupation†			
White collar	54 (23.7)	144 (33.5)	1*
Blue collar	126 (55.3)	198 (46.1)	1.17 (0.74-1.84)
Farmer	24 (10.5)	27 (6.3)	1.71 (0.84-3.47)
Housewife	24 (10.5)	61 (14.2)	1.13 (0.51-2.49)

NOTE: OR and 95% CI were adjusted for gender, age, center, and education, when appropriate.
*Reference category.
†Sums may not add up to the total because of some missing values.

increased HCC risk (OR for former versus never drinkers, 4.0; 95% CI, 1.7-9.1). Indeed, when the relationship between maximal lifetime alcohol intake was evaluated separately among current and former drinkers, the highest ORs for each intake level were found among former drinkers (e.g., OR for ≥35 drinks/wk versus never drinkers, 11.9; 95% CI, 3.7-38.2; data not shown). Current and former drinkers were thus combined (Table 2). No significant risk increase was seen among light and moderate drinkers compared with never drinkers but the OR for an alcohol intake of ≥35 drinks/wk was 5.9 (95% CI, 2.3-15.7). In respect to cigarette smoking, the ORs were 0.8 among former smokers and 1.1 among current smokers, with no clear dose-response relation among current smokers (Table 2).

Self-reported history of HBV infection (9.3% of cases and 3.0% of controls; OR, 3.8; 95% CI, 1.7-8.4), HCV infection (53.2% of cases and 2.8% of controls; OR, 50.8; 95% CI, 24.9-104), and cirrhosis (19.6% of cases and 0.5% of controls; OR, 55.1; 95% CI, 12.9-235) showed strong associations with HCC risk whereas history of hepatitis A (1.3% of cases and 1.9% of controls) was unrelated to HCC risk (OR, 0.7; 95% CI, 0.2-2.8; data not shown).

The association between different HBV and HCV serologic markers and HCC risk is shown in Table 3. Sixteen percent of HCC cases, compared with 55.0% of controls, were negative for all serologic markers. Among anti-HCV$^-$ individuals, being HBsAg$^+$ was associated with an OR of 96.7 (95% CI, 18.9-495) whereas being anti-HBc$^+$ and/or anti-HBs$^+$ was unrelated to HCC risk. Among individuals negative for all HBV markers, being anti-HCV$^+$ showed an OR of 20.5 (95% CI, 10.5-39.9). Copresence of anti-HBc and/or anti-HBs did not materially modify the OR for being anti-HCV$^+$. The OR for individuals who were both HBsAg$^+$ and anti-HCV$^+$ was 51.6 (95% CI, 9.4-285); i.e., significantly lower than the OR expected if the effects of the two viruses were multiplicative on the OR scale (χ^2_1 for departure from multiplicativity = 9.90; $P < 0.001$). Overall, the OR for HBsAg$^+$ versus HBsAg$^-$ individuals was 20.2 (95% CI, 6.4-63.6) and the OR for anti-HCV$^+$ versus anti-HCV$^-$ individuals was 15.6 (95% CI, 10.0-24.2; Table 3). HBeAg was found in one HBsAg$^+$ case only and in no HBsAg$^+$ controls.

HCV RNA was detected in 144 of 149 (96.6%) anti-HCV$^+$ cases but in 30 of 46 (65.2%) corresponding controls (OR for HCV RNA$^+$ versus HCV RNA$^-$, 26.5; 95% CI, 15.8-44.7; Table 4). HCV genotype 1 was more frequently detected than genotype 2 but corresponding ORs were similar (27.1; 95% CI, 14.9-49.0 and 27.4; 95% CI, 11.6-64.5, respectively).

PCR-based assays revealed the presence of HCV RNA in two HCC cases (6.3% of 32 tested) who were negative for anti-HCV and all HBV markers, as well as in one case (12.5% of 8 tested) who was anti-HCV$^-$, HBsAg$^-$, and anti-HBc$^+$. HBV DNA was detected in one case (1.9% of 53 tested) who was anti-HCV$^+$ but negative for all HBV markers, as well as in another (3.6% of 28 tested cases) who was anti-HCV$^+$ and anti-HBc$^+$. On account of the small number of occult hepatites detected in serum samples, the findings in Table 3 were not materially changed by the addition of PCR-based information (data not shown).

Table 5 shows the joint effects of alcohol drinking, tobacco smoking and being HBsAg$^+$ or anti-HCV$^+$. Alcohol drinking was associated with HCC risk among both nonchronic carriers and chronic carriers of HBV or HCV. Heavy drinkers who were either HBsAg$^+$ or anti-HCV$^+$ showed an OR of 74.4 (95% CI, 22.9-242) that was lower, but compatible with multiplicativity of the effects of hepatitis viruses and alcohol (χ^2_1 for departure from multiplicativity = 2.11; $P = 0.15$). Current smoking was unrelated to HCC risk among HBsAg$^-$ and anti-HCV$^-$ individuals (OR, 1.0; 95% CI, 0.5-2.0) but seemed to enhance the adverse effect of hepatitis viruses (OR among HBsAg$^+$ or anti-HCV$^+$ individuals: 23.4 among never or former smokers and 44.3 among current smokers; Table 5). When the effect of smoking was examined among anti-HCV$^+$ individuals only, the findings of Table 5 were confirmed (OR for anti-HCV$^+$ current smokers, versus anti-HCV$^-$ never/former

Table 2. OR for HCC and corresponding 95% CI by alcohol drinking and tobacco smoking (229 cases and 431 controls); Italy, 1999-2002

	Cases	Controls	OR (95% CI)
	n (%)	*n* (%)	
Alcohol drinking			
Never	20 (8.7)	66 (15.3)	1*
Former	118 (51.5)	48 (11.1)	3.98 (1.74-9.09)
Current	91 (39.7)	317 (73.6)	0.84 (0.39-1.83)
Years since cessation†			
<5	46 (20.1)	11 (2.6)	6.34 (1.92-21.04)
≥5	72 (31.4)	37 (8.5)	2.56 (0.96-6.82)
Maximal lifetime alcohol intake (drinks/wk)‡			
<7	16 (7.0)	42 (9.7)	1.67 (0.55-5.13)
7-13	26 (11.4)	80 (18.6)	0.91 (0.35-2.38)
14-20	38 (16.6)	79 (18.3)	1.04 (0.41-2.65)
21-34	53 (23.1)	81 (18.8)	1.61 (0.61-4.29)
≥35	76 (33.2)	83 (19.32)	5.94 (2.25-15.67)
χ^2_1 for trend			14.44; $P < 0.01$
Tobacco smoking			
Never	66 (28.8)	146 (33.9)	1†
Former	89 (38.9)	172 (39.9)	0.82 (0.45-1.50)
Current	74 (32.3)	113 (26.2)	1.14 (0.61-2.15)
Cigarettes/d§			
1-14	38 (16.6)	59 (13.7)	1.15 (0.55-2.42)
≥15	36 (15.7)	54 (12.5)	1.13 (0.52-2.45)
χ^2_1 for trend			0.03; $P = 0.87$

NOTE: OR and 95% CI were adjusted for gender, age, center, education, and HBsAg or anti-HCV positivity.
*Reference category.
†Former drinkers only.
‡Current and former drinkers combined.
§Current smokers only.

Table 3. OR for HCC and corresponding 95% CI by serologic markers of HBV and HCV (229 cases and 431 controls); Italy, 1999-2002

HBsAg	Anti-HBs	Anti-HBc	Anti-HCV$^-$			Anti-HCV$^+$			All cases
			Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)	OR (95% CI)
			n (%)	*n* (%)		*n* (%)	*n* (%)		
−	−	−	36 (15.7)	237 (55.0)	1*	61 (26.6)	20 (4.6)	20.49 (10.53-39.89)	
−	+	+/−	13 (5.7)	107† (24.8)	0.84 (0.42-1.70)	43 (18.8)	11 (2.6)	28.50 (12.61-64.42)	→ 1*
−	−	+	8 (3.5)	39 (9.0)	1.24 (0.53-2.95)	36 (15.7)	13 (3.0)	19.66 (8.93-43.31)	
+	−	+	23 (10.9)	2 (0.5)	96.67 (18.88-495)	9 (3.9)	2 (0.5)	51.64 (9.36-285)	20.20 (6.42-63.60)
All cases			80 (34.9)	385 (89.3)	1*	149 (65.1)	46 (10.7)	15.58 (10.02-24.22)	

NOTE: OR and 95% CI were adjusted for gender, age, center and education.
*Reference category.
†Including seven controls who reported vaccination against HBV and were positive for anti-HBs only.

smokers 41.7; 95% CI, 18.1-96.1; data not shown). The rarity of HBsAg prevented a separate evaluation of the interaction between HBV and smoking.

Percent risk of HCC attributable to HBV, HCV, alcohol drinking, and tobacco smoking is shown in Table 6 overall and by gender and study center. The contribution of HCV (61%) in the two genders and centers combined far exceeded the contribution of HBV (13%) whereas 18% and 8% of HCC were attributable to alcohol drinking and tobacco smoking, respectively. Altogether, hepatitis viruses, alcohol, and tobacco accounted for 87% of HCC in our study population, but differences did emerge between genders and centers. The proportion of HCC explained by HCV infection was greater in women than in men whereas the contribution of alcohol and tobacco was only appreciable in men (Table 6). The attributable risk for alcohol drinking among men was larger among HBsAg$^+$ or anti-HCV$^+$ individuals (60%; 95% CI, 42-78%) than among HBsAg$^-$ and anti-HCV$^-$ ones (17%; 95% CI, 1-33%) whereas a significant contribution of tobacco smoking was found only among HBsAg$^+$ or anti-HCV$^+$ individuals (23%; 95% CI, 3-43%; data not shown). In addition, mainly on account of higher prevalence of hepatitis viruses in Naples than in Pordenone, attributable risk for the two infections was greater (89% versus 53%, respectively) and the contribution of tobacco smoking was only appreciable (21%) in Naples (Table 6). Conversely, the contribution of heavy drinking was greater in Pordenone (32%) than in Naples (6%, nonsignificant). Differences in attributable risks between men and women and between Pordenone and Naples reflected differences in the prevalence of viral infection and consumption of alcohol and tobacco, whereas the corresponding relative risks were similar in the two genders and study areas.

Discussion

Nearly two thirds of HCC cases in our study were attributable to HCV and 13% to HBV infection. The high OR and attributable risks we found for HCV in our study population are consistent with observations that suggest that the virus had already widely spread in Italy in the 1950s (26). The burden of HCC in countries like Italy or Japan (4) can, therefore, help to predict the future effect of HCV on liver cancer in countries like the United States (1), France (27), or Australia (28), where the spread of the virus is more recent and, on account of the three- to four-decade latency of HCV carcinogenesis, the cancer sequelae have not yet fully manifested. Indeed, based on mathematical modeling, it is estimated that HCV-related HCC will increase over the next 10 to 20 years in these countries (27-29).

The relative contribution of hepatitis viruses to the causation of HCC varies greatly worldwide (5), but the importance of HCV is increasing in many parts of the world (4, 30-33). Several studies showed that HCC related to HCV infection increased by ~2-fold in the United States from the early to the late 1990s (34, 35). Of 691 HCC patients referred to United States liver transplant centers between 1997 and 1999, 46.5% were anti-HCV$^+$, 15.4% HBsAg$^+$, and 4.7% had both viral markers present (36). Fifteen case-control studies of HCC carried out in China after third-generation HCV tests became available were included in a recent meta-analysis (30). Overall, 23.8% of HCC cases were anti-HCV$^+$/HCV RNA$^+$, but in two thirds of them HBsAg was found concurrently. HCV markers were found to be more prevalent than HBsAg among HCC cases older than 54 years, although not in younger ones, in the Gambia (33). Finally, a study of 503 cases of HCC in six European liver centers showed serologic HBV markers in 19% and HCV markers in 40.5% of the cases (37). Anti-HCV positivity was much more frequent than HBsAg positivity in Spain, Italy, and Germany, whereas the reverse was observed in Greece (37). In France and England (where the majority of HCC were serologically negative for both hepatitis viruses), the percentages of positivity for HBV and HCV were similar.

Our study shows that a substantial heterogeneity is present also within Italy as a consequence of the nearly double prevalence of HBV and HCV in Naples compared with the province of Pordenone. The 53% risk attributable to HBV and HCV in the Northeastern part of Italy resembles findings in other European countries (37) whereas the corresponding attributable risk in Naples (89%) is among the highest ever reported worldwide (5). HCV genotype 1 was confirmed to be the predominant genotype in Southern Europe (38), but the corresponding OR for HCC was similar to the OR for genotype 2.

The addition of sensitive PCR assays applied to serum samples did not materially decrease the low percentage (16%)

Table 4. OR for HCC and corresponding 95% CI by presence of anti-HCV and HCV RNA, and HCV genotype (229 cases and 431 controls); Italy, 1999-2002

	Cases	Controls	OR (95% CI)
	n (%)	*n* (%)	
Anti-HCV$^-$ and HCV RNA$^-$	80 (34.9)	385 (89.3)	1*
Anti-HCV$^+$ and HCV RNA$^-$	5 (2.2)	16 (3.7)	1.46 (0.50-4.31)
Anti-HCV$^+$ and HCV RNA$^+$	144 (62.9)	30 (7.0)	26.54 (15.75-44.74)
HCV genotype			
1	101 (44.1)	21 (4.9)	27.05 (14.94-48.98)
2	41 (17.9)	8 (1.9)	27.40 (11.64-64.50)
5	1 (0.4)	0 (—)	—
Unknown	1 (0.4)	1 (0.2)	—

NOTE: OR and 95% CI were adjusted for gender, age, center, and education.
*Reference category.

Table 5. OR for HCC and corresponding 95% CI by alcohol drinking, tobacco smoking and serologic markers of HBV and HCV (229 cases and 431 controls); Italy, 1999-2002

	$HBsAg^-$ and anti-HCV^-	$HBsAg^+$ and/or anti-HCV^+
Maximal lifetime alcohol intake (drinks/wk)		
<14		
Ca/Co	11:164	51:24
OR (95% CI)	1*	28.82 (12.84-64.69)
14-34		
Ca/Co	9:141	82:19
OR (95% CI)	0.68 (0.26-1.76)	47.60 (20.76-109)
≥35		
Ca/Co	37:78	39:5
OR (95% CI)	4.96 (2.19-11.24)	74.36 (22.89-242)
Tobacco smoking		
Never/former		
Ca/Co	42:281	113:37
OR (95% CI)	1*	23.42 (13.50-40.63)
Current		
Ca/Co	15:102	59:11
OR (95% CI)	1.02 (0.53-1.97)	44.27 (19.73-99.34)

NOTE: OR and 95% CI were adjusted for gender, age, center, and education.
Abbreviations: Ca, cases; Co, controls.
*Reference category.

of HCC cases that had no detectable sign of chronic hepatitis virus infection in our study. Only 2 of the 32 anti-HCV^- and $HBsAg^-$ cases who were tested using PCR assays were HCV RNA^+ and none were HBV DNA^+. The detection of occult hepatitis was even rarer in HCC cases who were $HBsAg^-$ but anti-HBc^+ regardless of the presence of anti-HCV. Thus, a small proportion of HCC seems to be unrelated to HBV and HCV even in populations like the Italian one where a large number of people had been infected by HBV or HCV in their lifetime (e.g., 40.4% and 10.7%, respectively, among controls in our study; Table 3).

A PCR assay–based analysis of sera from 503 European HCC patients detected HBV DNA (27 of 104; 26.0%) in a larger proportion of serologically negative patients than in our study, but found HCV RNA (13 of 182; 7.1%) in a similar proportion (37). Others have reported varying percentages of HBV or HCV occult hepatitis, depending on the types of samples (sera or liver biopsies) and individuals (e.g., blood donors versus individuals with liver disease) studied, the underlying prevalence of HBV and HCV in the source population, and the type of PCR conditions used (20, 39). The true prevalence of occult hepatitis is likely to be underestimated as viral levels are very low or undetectable even if sensitive molecular techniques are used. At least for HBV, however, a strong association between plasma viral DNA level and the risk of developing HCC has been shown (40) and there is no clear evidence that minimal viral replication is able in itself to provoke clinically relevant liver injury or cancer (39).

The evaluation of the joint effect of HBV and HCV on HCC risk is hampered in our study, as well as in previous work (31), by the small number of coinfection with the two viruses. We found, as in most previous studies (4, 9, 33, 41), a negative interaction in the joint effects of HBV and HCV according to a multiplicative scale, suggesting a lack of synergy between the two viruses in the induction of HCC. Furthermore, we rarely detected occult HBV hepatitis in the serum samples of anti-HCV^+ cases where HBV viral replication was suspected to be suppressed by HCV coinfection (39).

Anti-HBs^+ and anti-HBc^+, although not $HBsAg^+$, were positively correlated with the presence of anti-HCV, which was expected given the fact that the two viruses share some transmission routes (42). If we had computed ORs for individuals who were anti-HBC^+ and $HBsAg^-$ without stratification or adjustment for anti-HCV positivity, we would have found significant risk increases. The confounding effect of HCV infection may thus explain the increased HCC risk observed in some studies among individuals who were positive for markers of HBV infection, but not for markers of chronic carriage (9).

Our 55-fold increased risk of HCC among individuals who reported a history of cirrhosis is similar to the findings of previous case-control studies (43) and prospective studies (44). Alcohol drinking accounted for the majority of HCC unrelated to hepatites in our study. As the association was found only for high-intake levels (7, 45), the contribution of alcohol in our study was negligible among women and in Naples, where heavy drinking was much less frequent than in Pordenone (12), and reached an attributable risk of 40% among men who were nonchronic carriers of HBV or HCV. The persistence of the adverse effect of alcohol drinking many years after cessation of the habit suggests that alcohol has a role in both the initial and the final stages of liver carcinogenesis. The joint effects of heavy alcohol drinking and $HBsAg^+$ or anti-HCV^+ were compatible with multiplicativity of the ORs.

The association between tobacco smoking and HCC has been recently recognized (11) and seems to be especially strong in Asian populations where HBV infection is hyperendemic (10). In our present study, current and former smokers did not show significantly increased HCC risk but smoking contributed significantly to the burden of cancer among men who were chronic carriers of HBV or HCV.

Years of education were inversely related to HCC risk, but this association did not account for our study findings as education was adjusted for in all the analyses of viral markers, alcohol drinking, and tobacco smoking.

Table 6. Percent risk of HCC and corresponding 95% CI attributable to HBV, HCV, alcohol drinking, and tobacco smoking, overall and by gender and center (229 cases and 431 controls); Italy, 1999-2002

Risk factor	Percent attributable risks (95% CI)				
	Overall	Gender		Center	
		Men	Women	Pordenone	Naples
HCV (anti-HCV^+)	61 (54-68)	58 (50-66)	75 (62-89)	46 (35-57)	75 (66-83)
HBV ($HBsAg^+$)	13 (9-18)	14 (9-20)	8 (0-17)	7 (2-11)	19 (11-26)
Hepatitis viruses	72 (66-79)	70 (63-77)	83 (71-94)	53 (43-64)	89 (83-95)
Heavy alcohol drinking*	18 (9-27)	23 (13-34)	—	32 (18-47)	6 (−5-16)
Tobacco smoking†	8 (−2 to 19)	12 (0-24)	—	—	21 (6-36)
Hepatitis viruses, heavy alcohol drinking, and tobacco smoking	87 (81-94)	89 (82-96)	83 (70-97)	81 (70-93)	93 (87-99)

NOTE: Percent attributable risks and 95% CI were adjusted for age, education, and, when appropriate, gender and center.
*≥35 versus <35 drinks/wk.
†Current versus never/former.

The limitations of our present study include the possibility of selection bias in the inclusion of hospital controls. It is therefore reassuring that the prevalence of HBV and HCV positivity in our control group was consistent with the findings of previous surveys of hepatitis virus prevalence in Italy (13, 46, 47). Furthermore, individuals admitted to hospital for diseases related to tobacco smoking or heavy alcohol drinking were excluded and refusals to participate were very few, both among HCC cases and controls. Reporting of alcohol drinking and tobacco smoking may be more accurate in the Italian population than in many others as wine drinking is very common and smoking has been culturally acceptable until very recently (11).

Accurate testing for serologic markers of HBV and HCV (third-generation assays) in a centralized laboratory and performance of sensitive PCR-based assays on the subgroups of HCC cases where false negatives for HBV or HCV were a priori most probable are important strengths of our present study. Unfortunately, liver biopsies were rarely available and therefore could not be used for detection of viral genomes. Extension of PCR-based assays to serum samples in the totality of HCC cases and controls was not felt to be necessary on account of the low number of occult HBV and HCV infections detected. Such low numbers should not be due to a lack of sensitivity of the primers used. The region of HBV genome we targeted has been shown to be highly conserved (20, 21) and the HCV sequence we tested was the 5′ noncoding region, which allows for the recognition of all HCV genotypes (22, 23).

In conclusion, our study showed that 87% of HCC cases were attributable to four main causes that are in principle avoidable. Hepatitis-negative HCC cases in men were chiefly attributable to heavy alcohol drinking. Tobacco played a modest role overall, but it might potentiate viral carcinogenesis in $HBsAg^+$ or anti-HCV^+ individuals. Most importantly, our study confirmed the heavy burden of HCC due to HCV in countries such as Italy, where the widespread dissemination of the virus dates back four or five decades. If a lesson is to be learned from the current situation in Italy, a wide spectrum of measures should be implemented urgently in different parts of the world—from avoidance of use of contaminated blood and needles to early treatment of HCV-induced fibrosis (48)—depending on the phase of the HCV epidemic and health resources available.

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References

1. El Serag HB. Hepatocellular carcinoma: recent trends in the United States. Gastroenterology 2004;127 Suppl 1:S27–34.
2. Shibuya K, Yano E. Regression analysis of trends in mortality from hepatocellular carcinoma in Japan, 1972–2001. Int J Epidemiol 2005;34:397–402.
3. La Vecchia C, Lucchini F, Franceschi S, Negri E, Levi F. Trends in mortality from primary liver cancer in Europe. Eur J Cancer 2000;36:909–15.
4. Tanaka Y, Hanada K, Mizokami M, et al. Inaugural Article: A comparison of the molecular clock of hepatitis C virus in the United States and Japan predicts that hepatocellular carcinoma incidence in the United States will increase over the next two decades. Proc Natl Acad Sci U S A 2002;99:15584–9.
5. Di Bisceglie AM. Hepatitis C and hepatocellular carcinoma. Hepatology 1997;26 Suppl 1:S34–8.
6. La Vecchia C, Negri E, Decarli A, D'Avanzo B, Franceschi S. Risk factors for hepatocellular carcinoma in northern Italy. Int J Cancer 1988;4:872–6.
7. Kuper H, Tzonou A, Kaklamani E, et al. Tobacco smoking, alcohol consumption and their interaction in the causation of hepatocellular carcinoma. Int J Cancer 2000;85:498–502.
8. Wang LY, You SL, Lu SN, et al. Risk of hepatocellular carcinoma and habits of alcohol drinking, betel quid chewing and cigarette smoking: a cohort of 2416 HBsAg-seropositive and 9421 HBsAg-seronegative male residents in Taiwan. Cancer Causes Control 2003;14:241–50.
9. Yuan JM, Govindarajan S, Arakawa K, Yu MC. Synergism of alcohol, diabetes, and viral hepatitis on the risk of hepatocellular carcinoma in blacks and whites in the U.S. Cancer 2004;101:1009–17.
10. Chen ZM, Liu BQ, Boreham J, Wu YP, Chen JS, Peto R. Smoking and liver cancer in China: case-control comparison of 36,000 liver cancer deaths vs 17,000 cirrhosis deaths. Int J Cancer 2003;107:106–12.
11. IARC. Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 83: Tobacco Smoke and Involuntary Smoking. Lyon: IARC Press; 2004.
12. La Vecchia C, Pagano R, Negri E, Decarli A. Determinants of alcohol consumption in Italy. Int J Epidemiol 1987;16:295–6.
13. Bellentani S, Tiribelli C, Saccoccio G, et al. Prevalence of chronic liver disease in the general population of northern Italy: the Dionysos Study. Hepatology 1994;20:1442–9.
14. Montella M, Crispo A, Grimaldi M, et al. Prevalence of hepatitis C virus infection in different population groups in southern Italy. Infection 2005;33:9–12.
15. Talamini R, Montella M, Crovatto M, et al. Non-Hodgkin's lymphoma and hepatitis C virus: a case-control study from northern and southern Italy. Int J Cancer 2004;110:380–5.
16. Dal Maso L, Talamini R, Montella M, Crovatto M, Franceschi S. Hepatitis B and C viruses and Hodgkin lymphoma: a case-control study from Northern and Southern Italy. Haematologica 2004;89:ELT17.
17. Ferraroni M, Decarli A, Franceschi S, et al. Validity and reproducibility of alcohol consumption in Italy. Int J Epidemiol 1996;25:775–82.
18. D'Avanzo B, La Vecchia C, Talamini R, Franceschi S. Anthropometric measures and risk of cancers of the upper digestive and respiratory tract. Nutr Cancer 1996;26:219–27.
19. Colin C, Lanoir D, Touzet S, Meyaud-Kraemer L, Bailly F, Trépo C. Sensitivity and specificity of third-generation hepatitis C virus antibody detection assays: an analysis of the literature. J Viral Hepat 2001;8:87–95.
20. Chemin I, Zoulim F, Merle P, et al. High incidence of hepatitis B infections among chronic hepatitis cases of unknown aetiology. J Hepatol 2001;34:447–54.
21. Pichoud C, Seigneres B, Wang Z, Trépo C, Zoulim F. Transient selection of a hepatitis B virus polymerase gene mutant associated with a decreased replication capacity and famciclovir resistance. Hepatology 1999;29:230–7.
22. Li JS, Vitvitski L, Tong SP, Trépo C. Identification of the third major genotype of hepatitis C virus in France. Biochem Biophys Res Commun 1994;199:1474–81.
23. Li JS, Tong SP, Vitvitski L, Trépo C. Single-step nested polymerase chain reaction for detection of different genotypes of hepatitis C virus. J Med Virol 1995;45:151–5.
24. Breslow NE, Day NE. Statistical Methods in Cancer Research Vol. I: The analysis of case-controls studies. IARC scientific publication no 32. Lyon: International Agency for Research on Cancer; 1980.
25. Mezzetti M, Ferraroni M, Decarli A, La Vecchia C, Benichou J. Software for attributable risk and confidence interval estimation in case-control studies. Comput Biomed Res 1996;29:63–75.
26. Stanta G, Croce LS, Bonin S, Tisminetzky SG, Baralle FE, Tiribelli C. Cohort effect of HCV infection in liver cirrhosis assessed by a 25 year study. J Clin Virol 2000;17:51–6.
27. Deuffic S, Buffat L, Poynard T, Valleron AJ. Modeling the hepatitis C virus epidemic in France. Hepatology 1999;29:1596–601.
28. Law MG, Dore GJ, Bath N, et al. Modelling hepatitis C virus incidence, prevalence and long-term sequelae in Australia, 2001. Int J Epidemiol 2003;32:717–24.
29. Davis GL, Albright JE, Cook SF, Rosenberg DM. Projecting future complications of chronic hepatitis C in the United States. Liver Transpl 2003;9:331–8.
30. Shi J, Zhu L, Liu S, Xie WF. A meta-analysis of case-control studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma in China. Br J Cancer 2005;92:607–12.
31. Donato F, Boffetta P, Puoti M. A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma. Int J Cancer 1998;75:347–54.
32. Montella M, Crispo A, Izzo F, et al. HCV and hepatocellular carcinoma: A case-control study in an area of hyperendemicity. Int J Mol Med 2000;6:571–4.
33. Kirk GD, Lesi OA, Mendy M, et al. The Gambia Liver Cancer Study: Infection with hepatitis B and C and the risk of hepatocellular carcinoma in West Africa. Hepatology 2004;39:211–9.
34. Hassan MM, Frome A, Patt YZ, El Serag HB. Rising prevalence of hepatitis C virus infection among patients recently diagnosed with hepatocellular carcinoma in the United States. J Clin Gastroenterol 2002;35:266–9.
35. Davila JA, Morgan RO, Shaib Y, McGlynn KA, El Serag HB. Hepatitis C infection and the increasing incidence of hepatocellular carcinoma: a population-based study. Gastroenterology 2004;127:1372–80.

36. Di Bisceglie AM, Lyra AC, Schwartz M, et al. Hepatitis C-related hepatocellular carcinoma in the United States: influence of ethnic status. Am J Gastroenterol 2003;98:2060–3.
37. Bréchot C, Jaffredo F, Lagorce D, et al. Impact of HBV, HCV and GBV-C/HGV on hepatocellular carcinomas in Europe: results of a European concerted action. J Hepatol 1998;29:173–83.
38. Hatzakis A, Katsoulidou A, Kaklamani E, et al. Hepatitis C virus 1b is the dominant genotype in HCV-related carcinogenesis: a case-control study. Int J Cancer 1996;68:51–3.
39. Raimondo G, Pollicino T, Squadrito G. What is the clinical impact of occult hepatitis B virus infection? Lancet 2005;365:638–40.
40. Yu MW, Yeh SH, Chen PJ, et al. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. J Natl Cancer Inst 2005;97:265–72.
41. Kuper HE, Tzonou A, Kaklamani E, et al. Hepatitis B and C viruses in the etiology of hepatocellular carcinoma; a study in Greece using third-generation assays. Cancer Causes Control 2000;11:171–5.
42. IARC. Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 59: Hepatitis Viruses. Lyon: IARC; 1994.
43. La Vecchia C, Negri E, Cavalieri d'Oro L, Franceschi S. Liver cirrhosis and the risk of primary liver cancer. Eur J Cancer Prev 1998;7:315–20.
44. Adami HO, Hsing AW, McLaughlin JK, et al. Alcoholism and liver cirrhosis in the etiology of primary liver cancer. Int J Cancer 1992;51:898–902.
45. Donato F, Tagger A, Gelatti U, et al. Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. Am J Epidemiol 2002;155:323–31.
46. Guadagnino V, Stroffolini T, Rapicetta M, et al. Prevalence, risk factors, and genotype distribution of hepatitis C virus infection in the general population: a community-based survey in southern Italy. Hepatology 1997;26:1006–11.
47. Montella M, Crispo A, Serraino D, Rezza G, Franceschi S. Is the spread of HCV in Southern Italy attributable to iatrogenic transmission through unsterile injections? Eur J Cancer Prev 2003;12:85–6.
48. Omata M. Regression of liver fibrosis in patients treated by interferon. Intern Med 2004;43:887–8.