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REVIEW

Southern Europe as an example of interaction between various environmental factors: a systematic review of the epidemiologic evidence

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Hepatitis B virus (HBV), hepatitis C virus (HCV) and alcohol consumption are major causes of hepatocellular carcinoma (HCC) worldwide. We performed a systematic review of epidemiologic studies carried out on HCC aetiology in Southern Europe, an area with an intermediate–high prevalence of these agents as well as of putative risk factors such as tobacco smoking, diabetes and obesity. To retrieve the articles, we performed a Medline search for titles and abstracts of articles. After the Medline search, we reviewed the papers and reference lists to identify additional articles. A synergism between HCV infection and HBV infection, overt (hepatitis B virus antigen (HbsAg) positivity) or occult (HBsAg negativity with presence of HBV DNA in liver or serum), is suggested by the results of some studies. The pattern of the risk for HCC due to alcohol intake shows a continuous dose–effect curve without a definite threshold, although most studies found that HCC risk increased only for alcohol consumption above 40–60 g of ethanol per day. Some evidence supports a positive interaction of alcohol intake probably with HCV infection and possibly with HBV infection. A few studies found that coffee has a protective effect on HCC risk due to various risk factors. Some data also support a role of tobacco smoking, diabetes and obesity as single agents or preferably co-factors in causing HCC. In countries with a relatively high alcohol consumption and intermediate levels of HCV and HBV infections (1–3% of population infected by each virus), such as Mediterranean countries, the three main risk factors together account for about 85% of the total HCC cases, leaving little space to other known risk factors, such as haemochromatosis, and to new, still unrecognised, factors as independent causes of HCC.
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Introduction

Recently, various reviews have focused on the aetiology of hepatocellular carcinoma (HCC) but none of them has attempted a systematic evaluation of the available literature in terms of quantitative rather than qualitative risk estimate of the role of the main risk factors for the disease in the world, namely hepatitis B virus (HBV), hepatitis C virus (HCV) and alcohol consumption (Bosch *et al*., 2004; McGlynn and London, 2005).

The HCC incidence rates in subjects with a definite major cause show substantial differences between populations, as shown in a recent review (Fattovich *et al*., 2004): the risk of HCC development is about twice as high in Japan as it is in Europe and the US among subjects with HCV-related cirrhosis, and 50% higher in Taiwan and Singapore compared to Europe among subjects with HBV-related cirrhosis. Therefore, an evaluation of the role of HCC risk factors should be done in a homogeneous area. Southern Europe has favourable conditions for investigating HCC aetiology: (1) intermediate incidence of HCC and cirrhosis in a global picture; (2) intermediate–high prevalence of the three main causes and of other putative risk factors, such as tobacco smoking and metabolic factors (diabetes, obesity, metabolic syndrome) and (3) both cohort and case–control studies carried out in the last few decades.

Aim of the study

The aim of this review was to evaluate the role of each major risk factor for HCC – namely HBV and HCV infection and alcohol intake – alone and in combination, in an area with an intermediate–high prevalence of these factors, using a systematic approach. We also assessed the role played by tobacco smoking, coffee drinking, diabetes and obesity. The global impact of major risk factors on the burden of HCC in Southern Europe was also assessed.

Methods

Literature search and study selection

A scheme of the criteria followed for the study selection is given in Table 1. The outcome measure was HCC and all the

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articles had to be written in English. As regards the geographical area, we first retrieved all the articles published on the issue, regardless of where the research had been performed. We excluded articles published before 1989 because of the lack of evaluation of HCV infection, as antibody to hepatitis C virus (anti-HCV) antibodies were not detectable before that date. Then, we selected the studies on populations living in Southern Europe to estimate the absolute and relative effect of each factor investigated. However, when discussing the results of the evaluation, we also considered studies performed outside this area. Furthermore, we took into account the results of studies investigating aetiology of cirrhosis since 90% of HCC cases have underlying cirrhosis in South Europe and cirrhosis from any cause predisposes to HCC (Fattovich *et al*., 2004).

In agreement with other authors (Bosch *et al*., 2004), we considered the following factors as 'major' causes of HCC in Southern Europe: HBV and HCV infection and alcohol intake. Since the main aim of this review was to assess the interaction between HCC risk factors, we included epidemiologic studies that investigated the role of at least two of these factors. We included studies investigating HBV and HCV infection even if they did not evaluate alcohol intake, but excluded studies investigating alcohol intake that did not take account of both HBV and HCV infection. This is because heavy alcohol intake is associated with HCV infection and possibly HBV infection in Southern Europe (Bhattacharya and Shuhart, 2003), and the HCC risks due to HBV and HCV infections are greater than that due to alcohol intake alone, thence the former may confound the effect of alcohol. In fact, two case–control studies in Mediterranean countries found that HCV and HBV infection confounded negatively the risk of HCC due to alcohol drinking (Kuper *et al*., 2000a; Donato *et al*., 2002).

We also evaluated the role of the following 'minor' risk factors for HCC, which are common in the area: tobacco smoking and coffee consumption, diabetes and obesity. We only considered studies that investigated these risk factors when controlling for the main ones, to avoid confounding. For some of these factors, however, few data were available from Southern Europe studies, thus we extended the analysis to studies performed outside this area. We did not evaluate other environmental factors, such as sex hormones, dietary items and occupational exposure, for which literature data are inconsistent (Yu and Yuan, 2004; McGlynn and London, 2005).

We excluded studies investigating HCV infection if they had used first-generation anti-HCV tests, because of their different sensitivity for subjects with and without liver disease, resulting in overestimation of the relative risk for HCV infection (Zavitsanos *et al*., 1992). A meta-analysis of HBV and HCV infection and HCC showed substantial differences in summary odds ratios between studies using first-generation anti-HCV ELISA and those using second- or third-generation tests (Donato *et al*., 1998). For HBV infection, only hepatitis B virus antigen (HbsAg) was considered as a marker of current infection. For the other factors, any evaluation of the patient was considered suitable.

We included both cohort and case–control published studies for which the following data were available: number of subjects at risk and of HCC cases according to aetiology for cohort studies and the mean or median duration of follow-up; number of HCC cases and controls according to aetiology for case–control studies. Cohort (longitudinal) studies were included if they had enrolled patients with cirrhosis or chronic liver disease untreated for HBV or HCV infection. Case–control studies were included if they had recruited HCC patients as cases as well as subjects without chronic liver diseases as controls.

To retrieve the articles, we performed a Medline search (update: 31st December 2005) for titles and abstracts of articles using the following terms: hepatocellular carcinoma, risk factors, hepatitis C virus, hepatitis B virus, alcohol, tobacco smoking, coffee, diabetes, body mass index (BMI), obesity and metabolic syndrome. After the Medline search, we reviewed the papers and reference lists to identify additional articles. We contacted the authors of studies that contained relevant information but did not report the results in a way that suited our analysis.

Data extraction and epidemiological measures of occurrence and association

We analysed data only from papers that reported, or allowed us to compute, estimates of the incidence rates for cohort studies and of the odds ratios (ORs) for case–control studies, for each risk factor. For this purpose, for cohort studies an approximate of the person-years was computed by multiplying

Table 1 Study inclusion criteria used to estimate the epidemiologic measures of absolute and relative effect of risk factors for HCC

Characteristics of the research	*Inclusion criteria*
1. Outcome investigated	HCC
2. Language	English
3. Area in which the research was conducted	Southern Europe: European countries bordering on the Mediterranean sea For some minor risk factors, when few studies were available for this area, we extended the analysis to include those performed outside
4. Time period	Studies published after 1989
5. Risk factors investigated	• Major risk factors for HCC (HBV, HCV, alcohol intake): at least two of the three • Minor risk factors for HCC (tobacco smoking, coffee drinking, diabetes, obesity and metabolic syndrome): when controlling for the major risk factors
6. Exposure measurement	• HBV: HBsAg detection • HCV: second or third generation ELISA test or HCV RNA qualitative test • HBV- and HCV-positive subjects not undergoing anti-viral therapy • For all the others factors, any evaluation was considered suitable
7. Study design	Cohort (longitudinal) and case–control studies

HBV, hepatitis B virus; HBsAg, hepatitis B virus antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ELISA, enzyme-linked immunosorbent assay.

subjects at risk by mean duration of follow-up; the incidence rates are expressed for 100 person-years. Other measures of associations (relative risks, rate ratios, hazard ratios, standardized mortality/incidence ratios) were also considered. When more than one publication from the same study population was available, only one was used, usually the most complete.

Since we retrieved few data to evaluate interaction, with a high degree of heterogeneity among the studies, we did not compute summary measures of associations.

The epidemiologic interaction between any two of major risk factors for HCC was assessed according to Rothman (1986), since this definition has practical biological and clinical implications. In brief, there is 'independence' (absence of interaction) between two factors if the excess relative risk for both exposures is approximately equal to the sum of the excess relative risks for each factor alone. Conversely, there is 'synergy' (positive interaction) if the excess relative risk for both exposures is greater than the sum of the excess relative risks for each factor alone, and 'antagonism' (negative interaction) if the excess relative risk for both exposures is less than the sum of those for each factor alone. For instance, when computing the incidence rates (IRs) for HCC due to two factors, A and B, in a cohort study, and given that IR (A and B), IR (A), IR (B) and IR (neither A nor B) are the IRs among people with both A and B, A only, B only and neither A nor B risk factor, respectively, the above-mentioned definitions of synergism and antagonism mean that the IR for factors A and B is given by

(a) IR (A and B) $\approx$ IR (A) + IR (B) − IR (neither A nor B) if the two factors are independent;
(b) IR(A and B) > IR (A) + IR (B) − IR (neither A nor B) if there is synergy;
(c) IR(A and B) < IR (A) + IR (B) − IR (neither A nor B) if there is antagonism.

The population attributable risks (aetiologic fraction) for the three main risk factors, separate and together, were computed as suggested (Rockhill *et al*., 1998) to estimate the global impact of these factors on the incidence of HCC, and thence the disease burden that could theoretically be eliminated if the effects of these factors are contrasted successfully.

Viral hepatitis: hepatitis B and C virus infections and their interaction

Overt hepatitis B virus infection

Hepatitis B virus and HCV infections both increase the risk of developing HCC in humans (IARC, 1994). A synergism between the two infections has been hypothesized, since some researchers found higher liver damage with dual infection that the infection with one virus only (Fong *et al*., 1991; Crespo *et al*., 1994), and others reported a higher HCC incidence among cirrhotics with dual compared to single infections (Benvegnù *et al*., 1994). A meta-analysis published in 1998, which included 32 case–control studies, showed a synergism between HBV and HCV infections: the OR for co-infection was greater than the sum, and lower than the product, of those for each infection alone (Donato *et al*., 1998). Substantial differences, however, were found according to geographical area: the summary OR for HBsAg positivity was lower in Mediterranean countries compared to East Asia countries where HBV infection is highly endemic (China, Taiwan, South Korea), and the OR for anti-HCV/HCV RNA was higher in the former than the latter area.

Table 2 presents an update of the results of the meta-analysis restricted to studies performed in South Europe countries. All the studies had sparse data, especially as regards the number of controls with concurrent infections. In the Brescia HCC study (Donato *et al*., 1997, 2002), which is the largest one, the OR for dual infection was moderately higher than the sum of those for each infection alone, suggesting a more than additive but less than multiplicative interaction, in agreement with results from the meta-analysis.

Six cohort studies performed in South Europe among patients with clinical diagnosis of cirrhosis with HBV and/or HCV infections were retrieved and are set out in Table 3. Only two of them (Benvegnù *et al*., 1994; Chiaramonte *et al*., 1998) showed an additive synergism between the two infections, while the others (Zoli *et al*., 1996; Del Olmo *et al*., 1998; Benvegnù *et al*., 2004; Sangiovanni *et al*., 2004) showed independence between the two infections in causing HCC.

Among studies performed outside South Europe, a recent meta-analysis of 32 Chinese case–control studies showed a moderate synergism between the two infections for HCC risk, with ORs of 15.6 for HBsAg positivity alone, 8.1 for anti-HCV positivity alone and 35.7 for positivity for both markers (Shi *et al*., 2005).

Reciprocal, negative confounding has been observed between HBV and HCV infections in the above-mentioned meta-analysis, since the crude ORs for HBsAg and anti-HCV/HCV RNA were higher than those adjusted for each other in almost all studies where both estimates could be compared (Donato *et al*., 1998). The reciprocal negative confounding is probably due to interference between the two viruses. Hepatitis C virus superinfection on the HBsAg carrier status can in fact suppress HBV replication or terminate the HBsAg carrier status (Liaw, 1995). On the other hand, HCV replication has also been found to be suppressed by active HBV replication in patients with chronic hepatitis B (Pontisso *et al*., 1993). Some findings indicate that the viruses show alternative dominance in replication in patients with dual infection (Koike *et al*., 1995). A recent longitudinal virological evaluation of HBV and HCV co-infected Italian patients showed that the behaviour of each virus is independent of the other, determining a synergistic effect in terms of liver damage (Raimondo *et al*., 2006). This indicates, in line with available data regarding the biological mechanisms of carcinogenesis by these viruses, that they may act through common as well as different pathways in the carcinogenic process. The use of a common pathway could explain the interference phenomenon, and thence the reciprocal negative confounding, while activity at different points could explain the synergism between the two infections. The common pathway might be liver cirrhosis, which is found in 80–90% of patients with either HBV- or HCV-related HCC in Western countries, whereas different mechanisms of a direct carcinogenic effect for HBV and HCV infection in causing HCC have been hypothesised; these are dealt with in detail in other papers in this review.

Occult hepatitis B virus infection

Hepatitis B virus infection determined by the detection of the virus by HBV DNA PCR but not by the current and otherwise sensitive immunoassays for HBsAg is defined as occult HBV infection (Hu, 2002; Torbenson and Thomas, 2002). Occult HBV infection has been found among people affected by liver disease in various parts of the world (Brechot *et al*., 2001; Hu, 2002; Torbenson and Thomas, 2002), and in a high proportion of subjects with cryptogenic chronic liver disease in one study (Chan *et al*., 2002). Possible explanations for the seronegativity include mutations in the immunodominant loop of HBV

surface antigen altering HBsAg antigenicity or strong suppression of viral replication and gene expression that can also involve wild-type strains.

Two recent studies investigated the association between occult HBV infection and HCC in Italy. In the Brescia HCC study, HBV DNA was investigated in the sera of 203 HCC cases and 38 controls who were HCV RNA-positive, and in the sera of 196 cases and 479 controls who were anti-HCV-negative (Donato *et al*., 2005). All subjects were HBsAg-negative. An increased risk for HCC owing to occult HBV infection was found among both HCV RNA-negative (OR = 8.9; 95% CI: 5.5–14.5) and HCV RNA-positive (OR = 3.6; 1.4–9.2) subjects. Among anti-HCV negatives, an interaction was found between occult HBV infection and intake of >60 g/day of ethanol, with OR = 11.8 (5.9–23.5) in subjects without and OR = 99.2 (43.4–227) in those with occult HBV infection. Of 27 HCC cases without risk factors for the disease ('cryptogenic'), 14 (51.9%) had occult HBV infection, with OR = 8.2 (3.4–19.6). Since the study investigated HBV DNA in sera only, whereas occult HBV infection can be detected most effectively by examining liver tissue, the proportion of HCC cases and controls with occult HBV infection may have been underestimated by about 30% (Pollicino *et al*., 2004). The overall impact of occult HBV infection as a cause of HCC by itself, however, seems modest in the area, since only 14 of 574 total HCC cases (2.3%) did not have evident risk factors ('cryptogenic') and may have been caused by 'pure' occult HBV infection. The role of occult HBV was also evaluated in an Italian cohort study performed in Messina, Sicily (Squadrito *et al*., 2006), with HBV DNA search performed in liver samples. The study found that, among 134 HBsAg-negative patients with chronic liver disease, mostly caused by HCV, nine developed HCC, eight of which had occult HBV infection as well (seven HCV positive, one with cryptogenic liver disease), during the follow-up (median: 83 months). Therefore, surprisingly, this study suggests that among HBsAg-negative patients with chronic liver hepatitis, mostly due to HCV infection, very few cases of HCC occur in the absence of occult HBV infection in this area.

In the Brescia HCC study, an interaction was found between occult HBV infection and heavy alcohol intake. Although the interaction between occult HBV and HCV infection could not be assessed properly, the study showed that the OR for HCC was about threefold higher for dual occult HBV and HCV infection compared to HCV infection alone, which is in line with the 2.9 OR for occult HBV infection for having HCC (cases) compared to having chronic hepatitis or cirrhosis (controls) among HCV RNA-positive subjects in a multicentre Italian study (Pollicino *et al*., 2004). In the Messina study (Squadrito *et al*., 2006), a strong interaction was evident between occult HBV infection and HCV infection: among 124 patients with HCV chronic hepatitis, seven of the 50 with concurrent occult HBV and HCV infection developed HCC (incidence rate: 2.18/100 person-years) compared to one of the 74 without occult HCV infection (incidence rate: 0.19/100 person-years).

In line with some recent comments (Marrero and Lok, 2004), these findings suggest that (a) there is little room for an independent role of occult HBV infection as a cause of HCC in the absence of other factors; (b) on the other hand, occult HBV infection may contribute substantially to HCC development as a co-factor, especially in the presence of HCV infection or alcohol intake. These findings support the hypothesis that the role of HCV infection alone as a cause of HCC may be lower than that believed and that various factors can influence HCC development in persons with chronic HCV infection (Heathcote, 2004).

Table 2 Number of cases and controls and estimates of the odds ratios (ORs) and 95% confidence interval (95% CI) for each HBsAg and anti-HCV combination in case–control studies conducted in South Europe using second- or third-generation anti-HCV or HCV RNA tests

Reference	*Country*	*Total no. ca/co*	*HBsAg-negative, anti-HCV-negative*	*HBsAg-positive, anti-HCV-negative*		*HBsAg-negative, anti-HCV-positive*		*HBsAg-positive, anti-HCV- positive*	
			ca/co	*ca/co*	*OR (95% CI)*	*ca/co*	*OR (95% CI)*	*ca/co*	*OR (95% CI)*
Donato *et al*. (1997)[a]	Italy	598/1031	231/937	115/52	13.3 (8.9–20.0)	221/40	38.4 (25.4–58.2)	16/2	67.7 (14.6–314.4)
Hadziyannis *et al*. (1995)	Greece	65/130	20/116	37/11	19.5 (8.6–44.5)	5/2	14.5 (2.6–79.9)	3/1	17.4 (10.7–176)
Kuper *et al*. (2000b)[b]	Greece	333/360	83/339	198/12	67.4 (35.2–137.9)	41/1	167.5 (27.6–6791.1)	11/0	—
Stroffolini *et al*. (1992)	Italy	65/99	11/80	11/6	13.3 (4.1–43.3)	38/13	21.3 (8.7–51.8)	5/0	—

ca/co: cases/controls. [a]Updated by the authors. [b]Square brackets: data computed on the basis of reported data (crude ORs). HBsAg, hepatitis B virus antigen; HCV, hepatitis C virus.

Table 3 Incidence rates of HCC in cohort studies of patients with cirrhosis

Reference	*Country*	*Mean duration of follow-up (range)*	*Patients at risk*	*HCC cases*	*HBV and HCV infection Incidence rate[a] (HCC cases/patients at risk)*		
					HBV alone	*HCV alone*	*HBV and HCV*
Benvegnù *et al*. (1994)	Italy	46.3 (8–96 months)	246	28	3.16 (5/41)	2.55 (17/173)	10.37 (6/15)
Benvegnù *et al*. (2004)[b]	Italy	93 (14–194 months)	187	39	2.08 (5/31)	2.37 (27/147)	4.69 (4/11)
Chiaramonte *et al*. (1998)	Italy	64.5 (12–175 months)	259	51	1.69 (6/66)	3.81 (34/166)	7.58 (11/27)
Del Olmo *et al*. (1998)	Spain	63.1 months	361	26	0.35 (1/54)[c]	1.53 (23/286)	1.81 (2/21)[c]
Sangiovanni *et al*. (2004)[b]	Italy	148 (1–213 months)	209	76	0.88 (9/40)[c]	1.16 (64/152)	1.32 (3/17)[c]
Zoli *et al*. (1996)	Italy	54[d] (7–77 months)	94	22	3.03 (3/22)	5.87 (14/53)	5.85 (5/19)

[a]Incidence rates per 100 person-years, calculated from published grouped data. [b]Updated by the authors. [c]Delta infection excluded. [d]Calculated by the authors. HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

In fact, some studies have shown that many HCV-positive subjects in the Mediterranean area with HCC also had a history of heavy alcohol intake or occult HBV infection. An Italian multicentre cross-sectional study found that 61.6% of anti-HCV-positive HCC cases also had occult HBV infection (Pollicino *et al*., 2004). In addition, other studies found a high proportion of occult HBV infections among HCV-positive HCC cases (Brechot *et al*., 2001) and that, in subjects with chronic HCV hepatitis, cirrhosis was more common among those with than those without occult HBV infection (Cacciola *et al*., 1999). In the Messina study, eight of nine HCC cases which developed in subjects with HCV-related chronic hepatitis were co-infected with occult HBV (Squadrito *et al*., 2006). In the Brescia HCC study, 37.4% of the HCV RNA-positive and HBsAg-negative cases with HCC had HBV DNA in their sera, and 42.4% had a history of heavy alcohol intake, leaving a minority of HCC cases with HCV infection alone. Overlapping of HCV infection with other risk factors for HCC may also explain the substantial differences in the risk of HCC occurrence in cohort studies among HCV-infected subjects in various areas of the world and further research is mandatory.

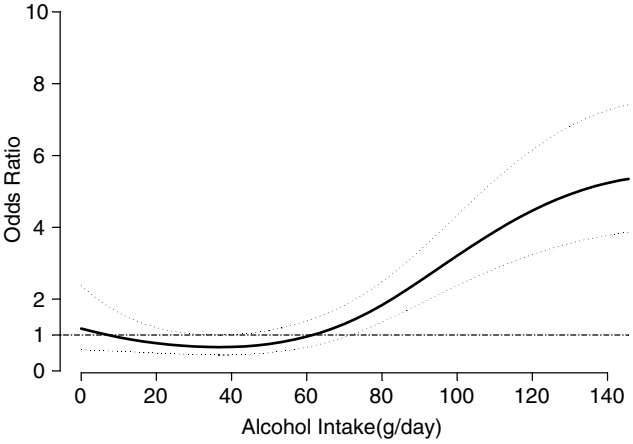

Figure 1 Odds ratios and their 95% confidence intervals (dotted lines) for HCC in men according to alcohol intake, obtained by fitting spline regression models that included age, residence, HBV antigen and HCV as covariates in the Brescia HCC study.

Alcohol drinking: the role of alcohol as a risk factor for HCC alone and combined with hepatitis C virus or hepatitis B virus infection

Alcohol drinking by itself is a cause of chronic liver disease (IARC, 1988; Mandayam *et al*., 2004; Morgan *et al*., 2004). Cohort studies among patients with alcohol-related diseases enrolled in referral centres as well as population-based cohort studies all have shown an increased incidence of HCC among people with heavy alcohol intake compared to the general population (Fattovich *et al*., 2004). A fundamental issue, however, is the dose–effect relationship between alcohol intake and the risk of HCC, which can change substantially when other risk factors are present. First of all, the association between alcohol intake and HCC risk will be considered when controlling for HCV and HBV infection, and thence the interaction with them will be considered.

Dose–effect relationship and threshold of safe intake

The hypothesis of a dose-effect relationship between alcohol intake and the risk of developing a clinically evident liver disease is biologically plausible but not easy to demonstrate, because most epidemiological studies conducted on alcohol and HCC did not have enough power to investigate more than two or three categories of intake.

Figure 1 shows the dose–effect association between alcohol intake and HCC risk in men from the Brescia HCC study. Other case–control studies carried out in South Europe have demonstrated a dose–effect relationship of increased risk for both cirrhosis and HCC with increasing alcohol intake, when also adjusting for HBV and HCV infection (Corrao *et al*., 1993, 1997; Bellentani *et al*., 1994; Corrao and Aricò, 1998; Kuper *et al*., 2000a). On the same line, two meta-analyses conducted by Corrao *et al*. (1998a, 2004) on the risk of cirrhosis and of various neoplasms, including liver cancer, show a continuous curve of increasing risk of disease with increasing alcohol intake.

Cohort studies performed in the USA and Northern Europe all showed an increased risk of developing cirrhosis or HCC due to alcohol drinking, although some of them found a dose–effect relationship (Klatsky and Armstrong, 1992; Becker *et al*., 2002) whereas others found a threshold effect of 50–75 g/day, after which the risk does not increase further (Sorensen *et al*., 1998; Kamper-Jorgensen *et al*., 2004). Various factors may have caused these contrasting results, mainly confounding by other factors, inaccuracy in estimating the level of intake during follow-up, and the low power of the studies.

Assuming that a dose–effect relationship exists, one important point is whether there is a 'low' alcohol intake that may be regarded as not harmful to the liver. In the Brescia HCC study, no statistically significant effect was defined below 60 g/day, although a 60% higher risk was observed for 40–60 g/day in men and a 40% higher risk for 21–40 g/day of intake in women (Donato *et al*., 2002). An update of the data,

to include additional HCC cases and controls recruited in 1999–2002 (Covolo *et al*., 2005), giving a total of 598 cases and 1031 controls, shows the following results as regards the ORs for HCC for 1–20, 21–40 and 41–60 g/day of alcohol consumption (reference categories: no consumption, men and women together): 1.04 (95% CI: 0.54–1.99; 61 cases and 146 controls), 1.11 (0.56–2.18; 58 cases and 149 controls) and 1.52 (0.78–2.99; 66 cases and 177 controls) (data provided by the authors). Although none of these estimates was statistically significant at the 0.05 *P*-value, the increasing trend supports the hypothesis of a continuous relationship, and suggests that even small doses of alcohol intake may lead to an increase in HCC risk. Interestingly, a recent USA case–control study found an OR of 1.5 for 20–40 g/day and an OR of 2.1 for >40 g/day of alcohol intake among HBV- and HCV-negative subjects (Yuan *et al*., 2004).

Studies on cirrhosis aetiology are in line with the above-mentioned data. The meta-analyses by Corrao *et al*. (1998a) showed a continuous curve of increasing risk for liver cirrhosis due to alcohol intake without any evident threshold. They found a significant increase of the risk of liver cirrhosis even for 25 g/day, the lowest level of intake considered, using the results of six studies performed in Mediterranean areas between 1978 and 1997, although a high degree of heterogeneity was found among them, most of which did not take account of possible confounders. A recent cohort study among patients with alcoholic fatty liver in the UK found that those who drank >40 g/day of alcohol were at risk of developing cirrhosis (Teli *et al*., 1995). A cross-sectional population-based study in Italy found no increased risk of alcoholic liver disease below 30 g/day (Bellentani *et al*., 1997).

Taken together, these findings suggest that a value of 40 g of ethanol per day is a reasonable proposal for a safe level of intake. A lower level (20 g/day) could be proposed for female subjects, based on some findings of a higher susceptibility to alcohol damage (Mandayam *et al*., 2004).

The pathogenetic mechanisms whereby alcohol intake can lead to development of cirrhosis and HCC are discussed in detail in some recent reviews (Poschl and Seitz, 2004; McKillop and Schrum, 2005; Voigt, 2005).

One common view is that the increased risk of HCC among people with alcohol-related disease is due to development of cirrhosis. In fact, population-based cohort studies in North Europe showed that the risk of HCC was about 10-fold higher among subjects with hospital discharge diagnosis of cirrhosis, with or without alcoholism, compared to those with diagnosis of alcoholism without cirrhosis, suggesting that cirrhosis is a necessary intermediate for the development of HCC among subjects with alcoholism (Adami *et al*., 1992; Sorensen *et al*., 1998; Kuper *et al*., 2001). A role of alcohol in the absence of cirrhosis as a 'pure' carcinogen seems of minor importance.

Interaction with hepatitis C virus

The interaction between heavy alcohol intake alone and HCV infection has been evaluated in only a few studies. Table 4 sets out the results of the Brescia HCC study on the interaction between a given alcohol intake, 60 g/day or more, and HCV or HBV infection. A more than additive but less than multiplicative synergism is evident between an alcohol intake of 60 g/day or more and each virus hepatitis infection.

When considering the interaction between alcohol intake and hepatitis virus infection in terms of dose–effect instead of all-or-none relationship, the curves of HCC risk for alcohol intake in subjects with and without HCV and HBV infection are shown in Figure 2: for each level of alcohol intake, the highest risks are found among subjects with HCV infection, followed by those with HBV infection, and finally by those without hepatitis virus infection, with parallelism between the curves. In particular, the dose–effect curve for subjects with HCV infection shows a further increase in risk due to virus infection for 40 g/day of alcohol intake, suggesting that even a low alcohol intake cannot be regarded as safe in subjects with HCV infection.

The results of some cohort studies carried out in South Europe countries with separate data on people with alcohol intake alone and combined with HBV and HCV infections are detailed in Table 5. All of them had low power for investigating the separate effects of each risk factor alone and combined with the others. The largest study in the series, the Spanish study carried out by del Olmo *et al*. (1998) among cirrhotics, showed no differences between those with HCV infection alone and combined with heavy alcohol intake.

Some Italian studies on the aetiology of cirrhosis confirm the hypothesis of a synergism between alcohol intake and HCV or HBV infection (Corrao and Aricò, 1998). In agreement with these findings, in a French study among subjects with HCV infection, age at infection and duration of infection were associated linearly with fibrosis stage, both associations being modified by alcohol intake: patients who consumed 50 g/day or more of alcohol had a higher fibrosis

Table 4 Interaction between HBV or HCV infection and heavy alcohol intake (>60 g/day of ethanol for at least 10 years) in the Brescia HCC case–control study (Donato *et al*., 2002, data updated by the authors)

HBV/HCV infection	*Heavy alcohol intake*	*Cases/controls*[a] *(559/1028)*	*OR*[b] *(95% CI)*[c]
None	No	34/530	Reference
None	Yes	192/406	7.4 (4.7–11.5)
HBV	No	47/30	25.3 (13.7–46.5)
HBV	Yes	66/22	46.8 (24.4–89.6)
HCV	No	126/26	65.5 (36.8–116.5)
HCV	Yes	94/14	122.3 (57.6–259.5)

[a]A total of 16 cases and two controls with both HCV and HBV infections excluded. [b]Adjusted for age, sex and education. [c]95%CI: 95% confidence interval. HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

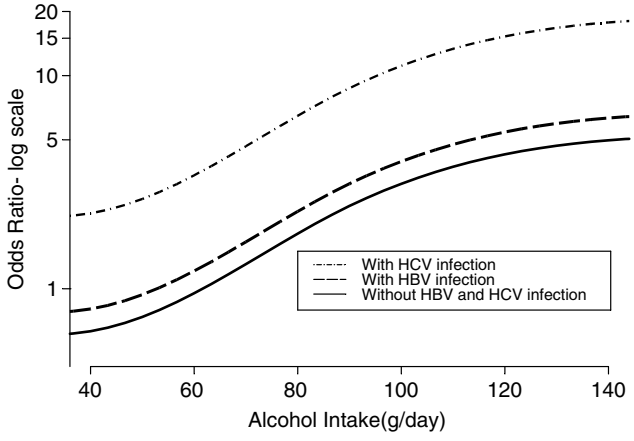

Figure 2 Odds ratio for HCC according to alcohol intake and the presence of HBV or HCV, obtained by fitting spline regression models that included age and residence as covariates, hepatitis B surface antigen and HCV RNA in the Brescia HCC study.

Table 5 Incidence rates of HCC according to HBV or HCV infection and heavy alcohol intake (more than 60 g/day of ethanol for at least 10 years) in cohort studies carried out in South Europe

HBV/HCV infection	*Heavy alcohol intake*	Benvegnù *et al.* (2004)[a,b] *(cirrhotics) (Italy)*	Del Olmo *et al.* (1998)[c] *(cirrhotics) (Spain)*	Manno *et al.* (2004) *(non-cirrhotics) (Italy)*	Sangiovanni *et al.* (2004)[c] *(cirrhotics) (Italy)*	Zoli *et al.* (1998)[a] *(cirrhotics) (Italy)*
		Incidence rate (HCC cases/patients at risk)	*Incidence rate (HCC cases/patients at risk)*	*Incidence rate (HCC cases/patients at risk)*	*Incidence rate (HCC cases/patients at risk)*	*Incidence rate (HCC cases/patients at risk)*
None	No	—	—	— (0/92)	1.32 (8/34)	—
None	Yes	—	0.72 (7/199)	0.05 (1/65)	1.87 (2/8)	4.44 (5/25)
HBV	No	2.34 (4/22)	0.32 (1/54)	— (0/193)	0.88 (9/40)	3.03 (3/22)
HBV	Yes	1.84 (1/7)	— (0/23)	0.07 (2/104)	0.10 (2/6)	3.70 (1/6)
HCV	No	3.08 (28/117)	1.57 (23/286)	—	1.16 (64/152)	5.87 (14/53)
HCV	Yes	2.80 (6/22)	1.31 (6/99)	—	2.04 (13/28)	3.70 (2/12)
Both	No/yes	4.69 (4/11)	1.47 (2/27)	—	1.32 (4/18)	6.76 (7/23)

Incidence rates computed per 100 person-years. [a]Follow-up obtained by the authors. [b]Updated by the authors. [c]Daily intake of more than 60 g of ethanol in women and more than 80 g in men for more than 10 years. HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

stage for each level of the variables concerning HCV infection, as shown in Figure 3 (Poynard *et al*., 1997).

On the same line, a USA cross-sectional study on 800 patients with chronic HCV infection undergoing liver biopsy found that both mean fibrosis score and the risk for fibrosis increased linearly with increasing alcohol intake, suggesting that also light and moderate alcohol intake may play a role in fibrosis (Monto *et al*., 2004), and another Italian study found that alcohol consumption increased the rate of fibrosis progression and decreased the response to interferon therapy in patients with chronic hepatitis C (Loguercio *et al*., 2000). The Italian population-based Dionysos study showed a higher rate of cirrhosis among HCV-positive subjects who drank >30 g/day of alcohol compared to those who drank less, and that all the five patients with HCC among 162 HCV RNA-positive subjects examined were alcohol abusers, with a mean alcohol intake of 122 g/day (Bellentani *et al*., 1999). Other cross-sectional and cohort studies carried out among patients with chronic HCV infection found that alcohol intake favoured the development of cirrhosis and HCC (Roudot-Thoraval *et al*., 1997; Ikeda *et al*., 1998; Aizawa *et al*., 2000; Harris *et al*., 2001), and caused the development of HCC at a lower age among alcohol drinkers than non-drinkers (Yotsuyanagi *et al*., 2004). Reciprocally, studies among alcoholics showed a higher severity of liver disease and a higher risk of HCC in the presence of HCV infection (Mendenhall *et al*., 1991), and a higher HCC incidence among patients with alcoholic liver cirrhosis with HBsAg or Anti-HCV than those without hepatitis virus infection (Yamanaka *et al*., 2001).

In actual fact, although the adverse effects of light to moderate alcohol intake on severity of hepatitis C have not been clearly shown, there are few doubts that alcohol has a deleterious effect on HCV-related liver disease (Regev and Jeffers, 1999; Peters and Terrault, 2002; Bhattacharya and Shuhart, 2003). Therefore, reducing alcohol intake as a public health policy probably leads to a substantial reduction in the burden of HCV-related cirrhosis and HCC in Western countries.

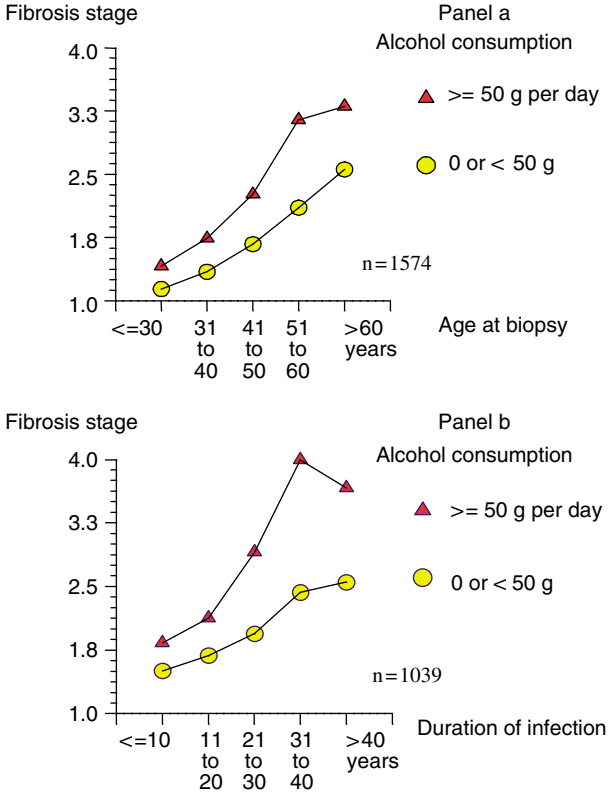

Figure 3 Relationship between stage of fibrosis, age at biopsy and duration of infection according to the alcohol consumption ($\geqslant 50$ g/day) (panels a and b) (Reprinted from The Lancet, 349, 9055, Poynard T *et al*.: Natural history of liver fibrosis progression in patients with chronic hepatitis C, 825–832, Copyright (1997), with permission from the Elsevier.)

Interaction with hepatitis B virus

At present, there are few data on the interaction between HBV infection and alcohol intake that also take account of HCV infection. The Brescia HCC study suggests a synergism between HBV infection and heavy alcohol intake (Table 4). However, the positive interaction seems weaker than that between HCV infection and alcohol, since the two curves of the dose–effect relationship of HCC risk with alcohol intake

with and without HBV infection are near, and the difference between them is not statistically significant (Figure 2). An Italian case–control study on aetiology of cirrhosis found a more than additive interaction between alcohol intake and HBV infection at each level of intake (Corrao *et al*., 1998b). A Greek case–control study showed a higher HCC risk for an alcohol intake of 60 g/day and over among subjects with HBsAg and/or anti-HCV positivity compared to those without hepatitis virus infections (Kuper *et al*., 2000a). Although the study did not evaluate the interaction of alcohol intake with HBV and HCV separately, HBV was much more prevalent than HCV infection in both cases and controls, and thence it was the interaction between alcohol and HBV infection to be assessed mainly. A lower age at HCC diagnosis among alcoholic patients with HBsAg positivity compared to HBsAg-positive but not alcoholic patients also suggests that the presence of both conditions accelerates the progression of chronic hepatitis B to HCC (Yotsuyanagi *et al*., 2004).

Studies performed outside the Mediterranean area were limited by the small number of subjects enrolled with both HBV infection and heavy alcohol intake, with a low prevalence of HBV infection in the USA and North Europe and low prevalence of heavy alcohol intake in East Asia (Morgan *et al*., 2004). Large population-based cohort studies performed in East Asia showed no interaction between alcohol consumption and HBV infection on HCC risk (Yu *et al*., 1997; Sun *et al*., 1999; Evans *et al*., 2002; Wang *et al*., 2003; Jee *et al*., 2004). Low levels of alcohol consumption were, however, defined in these studies for 'exposed' subjects: 25 g/day (Jee *et al*., 2004), 'habitual' or 'weekly' consumption (Yu *et al*., 1997; Sun *et al*., 1999; Evans *et al*., 2002; Wang *et al*., 2003). Therefore, no conclusion can be drawn from these studies on the interaction between medium–high alcohol intake and HBV infection.

Interactions with other factors

Very little information is available on the interaction between alcohol drinking and other risk factors in Western countries. A recent Italian study found a synergism between vinyl chloride monomer and alcohol intake in increasing the risk of both cirrhosis and HCC (Wong *et al*., 2003; Mastrangelo *et al*., 2004). No data are available on the interaction of alcohol intake with aflatoxins and chemicals; interactions with tobacco smoking, diabetes and obesity will be discussed later.

Tobacco smoking

An etiological role of tobacco smoking in HCC is biologically plausible, as cigarette smoke contains several chemicals which can be metabolized and then activated as carcinogens in the liver (Staretz *et al*., 1997; Wang *et al*., 1998). Furthermore, a strong correlation has been observed between HCC risk and DNA-adducts of 4-aminobiphenyl and polycyclic aromatic hydrocarbons, which are animal carcinogens and components of tobacco smoking (Wang *et al*., 1998; Chen *et al*., 2002).

Tobacco smoking was indicated as a risk factor for liver cancer in a recent review (Vineis *et al*., 2004). However, discrepancies among studies have been noted. Should tobacco smoking act as a liver carcinogen, the risk associated with the habit is probably weak, difficult to detect and easily confounded by other risk factors. An association between alcohol drinking and tobacco smoking has in fact been observed in Western countries (Doll *et al*., 1994), and thence the former may confound the risk of HCC due to tobacco smoking. Furthermore, although no association has been noted between tobacco smoking and HBV or HCV infection, these factors too may confound the effect of smoking.

To be sure of avoiding confounding, the HCC risk for tobacco smoking should be evaluated among subjects negative for all the main risk factors for HCC. Indeed, a significant positive association between cigarette smoking and HCC was found among subjects without HBV and/or HCV infection in two recent case–control studies, from Greece (Kuper *et al*., 2000a) and the USA (Yuan *et al*., 2004). Other case–control studies performed in East Asia also showed a significant association between tobacco smoking and liver cancer risk after stratification and/or adjustment for the main risk factors for HCC (Chen *et al*., 1991; Yu *et al*., 1991). A Chinese study found an increased risk for HCC for cigarette smoking enrolling individuals who died from cirrhosis as controls, which meant controlling indirectly for major risk factors for chronic liver disease (Chen *et al*., 2003). A case–control study in Taiwan showed a dose–response relation between hepatic levels of 4-aminobiphenyl DNA-adducts and the OR for HCC in HBsAg-negative subjects (Wang *et al*., 1998). A cohort study performed in Italy among cirrhotics of various aetiology (Sangiovanni *et al*., 2004) found a hazard ratio of 1.7 for HCC development among smokers compared to non-smokers when controlling for aetiology of cirrhosis, the risk for smokers of >20 being higher than that for smokers of 1–20 cigarettes/day (Sangiovanni A, personal communication).

However, two community-based cohort studies provided contrasting results on the risk of HCC in subjects uninfected by HBV or HCV, only one of them showing an association between tobacco smoking and HCC (Mori *et al*., 2000; Sun *et al*., 2003). No association between cigarette smoking and HCC was evident in the Brescia HCC study, even when restricting the analysis to subjects negative for HBV and HCV infection and alcohol consumption (Gelatti *et al*., 2005b), although a limit of the study was the small number of HCC subjects negative for all the main risk factors for the disease. No role of tobacco smoking was found also in two small case–control studies carried out in Greece and Spain (Vall Mayans *et al*., 1990; Hadziyannis *et al*., 1995). Among some large cohort studies performed in East Asia, a few showed an increased risk of death from liver cancer among smokers compared to non-smokers (Goodman *et al*., 1995; Liaw and Chen, 1998; Liu *et al*., 1998; Mizoue *et al*., 2000), but those that took account of HBV and HCV infection and alcohol intake did not find the association (Mori *et al*., 2000; Evans *et al*., 2002; Sun *et al*., 2003).

Tobacco smoking alone may be unable to cause HCC but it may sustain the activity of other risk factors, therefore the interactions between tobacco smoking and other risk factors should be explored thoroughly. A Greek case–control study found some evidence of an interaction between tobacco smoking and HCV infection in HCC risk (Tzonou *et al*., 1991). Some of the cohort studies performed in East Asia did indeed find a synergistic interaction on HCC risk between tobacco smoking and HCV (Mori *et al*., 2000; Sun *et al*., 2003) or HBV infection (Mori *et al*., 2000; Wang *et al*., 2003), whereas another one did not find an interaction between smoking and HBV infection (Evans *et al*., 2002). Two French studies conducted on patients with chronic hepatitis C found an association between cigarette smoking and severity of hepatic lesions, irrespective of alcohol consumption, suggesting that smoking could aggravate the progression of HCV-related liver disease (Pessione *et al*., 2001; Hezode *et al*., 2003). The cited Taiwan study on the liver levels of 4-aminobiphenyl DNA-adducts in HCC cases and in controls unaffected by liver diseases found that HBsAg-positive subjects with the highest levels of these adducts had an OR for HCC greater

than the sum of the ORs for each factor alone (Wang *et al*., 1998).

An interaction between alcohol drinking and tobacco smoking for HCC risk controlling for HBV and HCV infections was found in the above-mentioned Greek study (Kuper *et al*., 2000a). Furthermore, a USA cohort study found an increased risk of alcoholic cirrhosis among cigarette smokers as compared to non-smokers (Klatsky and Armstrong, 1992).

Coffee drinking

Coffee drinking has been investigated widely as a possible risk factor for various neoplasms, although no meaningful associations, either positive or negative, have been definitively established (IARC, 1991). Some data, however, suggest that coffee may have beneficial effects on the liver.

In the Brescia HCC study, an inverse association was found between coffee intake and HCC, with a dose–effect relationship, the ORs for HCC for coffee drinking, taking non-drinking subjects as a reference, being: 0.8 (95% CI 0.4–1.3) for 1–2 cups/day, 0.4 (0.2–0.8) for 3–4 cups/day and 0.3 (0.1–0.7) for 5 or more cups/day (Gelatti *et al*., 2005a). The OR for HCC for each of the main risk factors decreased for drinking >2 compared to 0–2 cups/day of coffee: the OR for drinking >80 g/day of ethanol declined from 5.7 to 3.3, the OR for HBsAg positivity from 16.4 to 7.3 and the OR for HCV RNA positivity from 38.2 to 9.0. These findings suggest a substantial reduction of HCC risk associated with either HCV or HBV infection or heavy alcohol intake. Two other case–control studies performed in Italy and Greece found a protective effect of coffee drinking on HCC risk (Gallus *et al*., 2002a), showing an odds ratio of 0.7 for drinkers of three or more cups of coffee per day. Confirmation of these results comes from two population-based Japanese cohort studies, which observed a reduced risk of developing HCC for coffee drinking after controlling for alcohol drinking, tobacco smoking and other confounders (Inoue *et al*., 2005; Kurozawa *et al*., 2005).

Other data support the hypothesis that coffee drinking helps to protect the liver. Firstly, several studies carried out on different populations found an inverse relation between coffee drinking and the serum levels of gamma-glutamyltransferase and aminotransferase (Casiglia *et al*., 1993; Pintus and Mascia, 1996; Poikolainen and Vartiainen, 1997; Tanaka *et al*., 1998; Honjo *et al*., 2001). Some Japanese studies found that coffee inhibits the induction of GGT in the liver by alcohol consumption (Tanaka *et al*., 1998) and that the inverse relation between coffee drinking and serum gamma-glutamyltransferase was progressively steeper with increasing alcohol consumption (Tanaka *et al*., 1998; Honjo *et al*., 1999). Secondly, some cohort and case–control studies performed in various countries, including Italy, found an inverse relation between coffee consumption and risk of cirrhosis (Klatsky and Armstrong, 1992; Klatsky *et al*., 1993; Corrao *et al*., 2001, Gallus *et al*., 2002b; Tverdal and Skurtveit, 2003). It is worth noting that both the cohort and the case–control studies found an inverse dose–effect relationship between coffee intake and cirrhosis, and one of them found that the association was not attributable to caffeine (Corrao *et al*., 2001). Recently, a large USA population-based cohort study found that subjects who drank >2 cups of coffee per day had less than half the rate of hospital or death diagnosis of chronic liver disease or cirrhosis and that protection by coffee was limited to subjects at higher risk of liver diseases, in a median follow-up of 19 years (Ruhl and Everhart, 2005). Finally, some experimental studies suggest that coffee drinking can reduce the incidence of chemical-induced liver cancer (Tanaka *et al*., 1998). The mechanisms whereby coffee may protect the liver from harmful agents are totally unknown, and are discussed elsewhere (Sharp *et al*., 1999; Gelatti *et al*., 2005a).

Taken together, these findings suggest that coffee by itself may be a protective agent for the liver, irrespective of the cause of the chronic liver disease.

Metabolic factors

The carcinogenic potential of non-alcoholic steatohepatitis (NASH) and of metabolic disorders has recently gained intense scientific attention (Marchesini *et al*., 2005). Non-alcoholic steatohepatitis represents a stage within the spectrum of non-alcoholic fatty liver disease (NAFLD) which ranges from fatty liver to NASH and cirrhosis in patients who have not consumed alcohol in amounts known to be injurious to the liver (Neuschwander-Tetri and Caldwell, 2003). An alcohol consumption of 20 g per day is the upper limit now generally accepted for the diagnosis of NAFLD (Falck-Ytter *et al*., 2001). Pure steatosis is considered very benign and non-progressive and NASH to be slowly evolving, although it can lead to cirrhosis and eventually to HCC (Choudhury and Sanyal, 2004).

In a representative sample of the general population in Northern Italy as part of the Dionysos Project, the prevalence of NAFLD, diagnosed by ultrasonography, was similar in subjects with and without suspected liver disease (25 vs 20%, respectively) (Bedogni *et al*., 2005) and within the range (20–30%) estimated in Western countries on the basis of clinical series, autopsy studies and convenience samples from the general population (Neuschwander-Tetri and Caldwell, 2003). However, the prevalence of NASH in the general population is largely unknown since laboratory and ultrasonography screening methods are unable to diagnose steatohepatitis. Non-alcoholic fatty liver disease has been associated with metabolic syndrome-related conditions, such as obesity, diabetes, hyperinsulinemia or insulin resistance, and hyperdyslipidemia, suggesting that NAFLD might be the liver component of the metabolic syndrome (Falck-Ytter *et al*., 2001; Marchesini *et al*., 2003; Bedogni *et al*., 2005). Indeed, it is estimated that approximately 90% of patients with obesity (body mass index (BMI) >30 kg/m^2) have some form of fatty liver disease, including NASH in about 20% and NASH-related cirrhosis in 2–3% of cases, and that 50–75% of patients with type II diabetes have some form of NAFLD (Neuschwander-Tetri and Caldwell, 2003).

Non-alcoholic steatohepatitis and hepatocellular carcinoma

There is some evidence that HCC may develop as the last step in the natural history of progressive NASH, based on case reports of HCC in patients with NASH-related cirrhosis from various parts of the world, including Southern Europe (Cotrim *et al*., 2000; Zen *et al*., 2001; Shimada *et al*., 2002; Cuadrado *et al*., 2005). Almost all the HCC cases had obesity and/or type II diabetes, and they were all negative for HBsAg and anti-HCV, with biopsy-based diagnosis of NASH-related cirrhosis. Diagnosis of HCC was simultaneous in some cases, whereas in others it occurred up to 10 years after diagnosis of NASH-related cirrhosis. A small study of 42 patients with NASH followed up for 21 years found one patient who developed cirrhosis and then HCC (Powell *et al*., 1990). Two cohort studies of patients with NASH-related cirrhosis have been published recently, from France (Ratziu *et al*., 2002) and

Australia (Hui *et al*., 2003). In the French study, which was retrospective, none of 10 patients with cryptogenic cirrhosis and without comorbidities (e.g. NAFLD, NASH, obesity and/or diabetes) developed HCC during a mean follow-up of 3.5 years, whereas three of 22 subjects with obesity-related cryptogenic cirrhosis developed HCC during a mean follow-up of 1.8 years (incidence of 0.8 per 100 person-years, recalculated from the original paper) (Ratziu *et al*., 2002). In the Australian study, which was prospective, none of 23 cases with NASH-associated cirrhosis, defined by strict clinicopathologic criteria, developed HCC during a mean follow-up of 5 years. However, the short length of the follow-up in this study does not allow us to rule out the occurrence of HCC as a late complication of the condition under study. Overall, these results suggest that the incidence of HCC may be low in NASH-associated cirrhosis. A Danish population-based small cohort study found a fourfold increased risk of liver cancer incidence among patients hospitalized for non-alcoholic/unspecified fatty liver, after excluding those with previous diagnosis of cirrhosis (Sorensen *et al*., 2003).

Recent studies suggest that cryptogenic cirrhosis may represent a late stage of NASH, which has lost its features of necroinflammation and steatosis in up to 80% of patients. Cryptogenic cirrhosis accounts for 3.5% of all cases of cirrhosis in Italy (Stroffolini *et al*., 2004). In an Italian study, 23 patients with cryptogenic cirrhosis and HCC were compared with 115 age-matched patients with viral- and alcohol-associated cirrhosis and HCC: the former were more likely to have clinical features suggestive of NASH, including precirrhosis BMI $>30\,kg/m^2$ (41 vs 16%), type II diabetes (50 vs 20%), dyslipidemia and insulin resistance (Bugianesi *et al*., 2002). Similarly, patients with cryptogenic chronic liver disease and HCC who underwent surgical resection were compared with matched patients with alcohol- and chronic viral hepatitis-related HCC in a French study: patients with cryptogenic chronic liver disease, compared to patients with alcohol abuse and those with chronic viral hepatitis, had a significantly higher prevalence of obesity (50 vs 17 and 14%), diabetes (56 vs 17 and 11%) and $>20\%$ steatosis (61 vs 17 and 19%) (Regimbeau *et al*., 2004). These findings support the hypothesis that NASH is a risk factor for HCC and that it may explain a considerable proportion of cryptogenic HCC cases. In these studies, however, the prevalence of cryptogenic HCC was 7% (Bugianesi *et al*., 2002) and 9% (Regimbeau *et al*., 2004), much lower than the 29% reported in a USA study with a high prevalence of obesity and obesity-related metabolic disorders (Marrero *et al*., 2002).

Diabetes and hepatocellular carcinoma

Some case–control and population-based cohort studies conducted in Italy and Greece provided evidence that diabetes is associated with a 2–3-fold increased risk of HCC (Table 6) (Braga *et al*., 1997; La Vecchia *et al*., 1997; Lagiou *et al*., 2000; Verlato *et al*., 2003). The case–control study conducted in Greece found an approximately twofold increase in the OR for HCC among subjects with a history of diabetes, with or without adjustment for HBV and HCV infection and alcohol consumption (Lagiou *et al*., 2000). Three USA case–control studies that investigated the association between diabetes and HCC found a 2–4-fold increase in the risk for HCC when taking account of the main risk factors for the disease (Hassan *et al*. 2002; Yuan *et al*., 2004; Davila *et al*., 2005). One of them found that the OR for diabetes did not change when restricting the analysis to HBV- and HCV-negative subjects (Yuan *et al*., 2004).

Two population-based cohort studies carried out in Northern Europe on subjects with type II diabetes found a higher number of HCC cases than expected according to incidence rates in the general population, the ratio between observed and expected cases (relative risk) being 2.1–4.7, which was higher in men than women (Adami *et al*., 1996; Wideroff *et al*., 1997). The observed-to-expected ratio of liver cancer cases declined somewhat, but was still about 2 in men, after excluding patients with a hospital record diagnosis of hepatitis, alcohol-related disease, cirrhosis, haemochromatosis and jaundice in both studies. Interestingly, a French prospective cohort study found that diabetes was significantly and independently associated with HCC occurrence among patients with cirrhosis due to alcohol, with a relative risk of 1.6 in a mean follow-up of 4.2 years (N'Kontchou *et al*., 2006).

Further evidence comes from some recently published large cohort studies from the USA and Korea, which used self-reported questionnaire-based data, hospital discharge diagnosis databases or medical evaluations (Nair *et al*., 2002; Coughlin *et al*., 2004; El-Serag *et al*., 2004; Jee *et al*., 2005). They all found a twofold increased incidence in mortality rate for liver cancer among diabetics compared to non-diabetics, after controlling for BMI, heavy alcohol intake and other confounders. Furthermore, the Korean study found that elevated fasting serum glucose was an independent risk factor for liver cancer incidence, especially in men, with a dose–response relation (Jee *et al*., 2005).

Overall, these studies support the hypothesis that diabetes can increase the risk of HCC about twofold in men, and less in women. However, since the HCC risk for diabetes may be

Table 6 Odds ratios (ORs) or standardized mortality ratio (SMR) for hepatocellular carcinoma for diabetes mellitus in studies carried out in South Europe

Reference	*Country*	*Type of study*	*No. cases/controls*	*No patients at risk*	*Time period*	*Results (95% CI)*
Braga *et al*. (1997)	Italy	Case-control	320/1408		1984–1993	OR[a]: Male 2.49 (1.5–4.1) Female 1.23 (0.5–3.0)
Lagiou *et al*. (2000)	Greece	Case-control	333/360		1995–1998	OR[b]: 1.86 (0.99–3.51)
La Vecchia *et al*. (1997)	Italy	Case-control	428/1502		1984–1996	OR[c]: Male 2.4 (1.5–3.8) Female 2.0 (1.0–4.2)
Verlato *et al*. (2003)	Italy	Cohort		7148[d]	1987–1996	SMR: Male 1.80 (1.29–2.46) Female 1.97 (1.26–2.91)

[a]Adjusted for age, sex, area of residence, smoking status, total carrot, green vegetables and fresh fruit consumption. [b]Adjusted for age, sex, education, hepatitis B or C virus (HBV or HBC) infection, smoking status and alcohol consumption. [c]Adjusted for age, sex, education, area of residence, alcohol and tobacco consumption, history of hepatitis and liver cirrhosis, body mass index and family history of liver cancer. [d]Type II diabetes.

confounded by major risk factors for liver cancer, and only a few studies controlled for confounding adequately, the association between diabetes and HCC cannot yet be considered as definitely proved. Moreover, the temporal pattern is a matter of concern since diabetes may be secondary to cirrhosis of an unrelated cause, which in turn predisposes the subject to HCC, and no studies published so far have reported the date of diagnosis of both diabetes and cirrhosis.

Obesity and hepatocellular carcinoma

Some population-based cohort studies from Central and Northern Europe and from the USA found that obesity is associated with a 2–4-fold increased risk of liver cancer, higher among men than women (Moller *et al*., 1994; Wolk *et al*., 2001; Calle *et al*., 2003; Samanic *et al*., 2004; Rapp *et al*., 2005). These results should be considered with caution, however, as some of these studies found no statistically significant increase in liver cancer risk and no trend of increasing risk with increasing BMI (Rapp *et al*., 2005), did not control accurately for all major risk factors for HCC (Moller *et al*., 1994) or controlled only for hospital discharge of alcoholism and diabetes (Calle *et al*., 2003; Samanic *et al*., 2004), or found no increased risk for liver cancer due to obesity when excluding patients with diabetes (Wolk *et al*., 2001). By contrast, in a large USA cohort study of male veterans hospitalized with a diagnosis of obesity, excess risks for liver cancer were also observed when the analysis was restricted to white men without a history of diabetes or alcoholism (Samanic *et al*., 2004). In the same study, however, a reduced – rather than increased – risk for obesity was observed among black men (Samanic *et al*., 2004).

A cohort study carried out in France on 771 patients with alcoholic or HCV-related cirrhosis found an association between BMI and HCC, with a dose–effect relation: the hazard ratio (HR) for HCC was 2.0 for a BMI of 25–30 kg/m^2, and 2.9 for BMI $\geqslant 30\,kg/m^2$ in patients with alcoholic cirrhosis, when controlling for confounders; the corresponding figures for patients with HCV-related cirrhosis were 1.7 and 2.9 (N'Kontchou *et al*., 2006). A USA cohort study of transplant candidates found that obesity was an independent risk factor for HCC in patients with alcoholic cirrhosis (OR 3.2) and cryptogenic cirrhosis (OR 11), but not in patients with hepatitis C, hepatitis B, primary biliary cirrhosis or autoimmune hepatitis, when taking account of diabetes and other confounders (Nair *et al*., 2002). On the other hand, no increased risk of liver cancer due to obesity was evident when adjusting for alcohol intake and other confounders in a population-based case–control study from Canada (Pan *et al*., 2004) and in a prospective population-based cohort study from Japan (Kuriyama *et al*., 2005).

Overall, these results provide some evidence in favour of the hypothesis that obesity can contribute to HCC global burden, even though a number of inconsistencies among studies suggest that factors linked to obesity, such as alcohol consumption and diabetes, and possibly HCV and HBV infection, may confound the association between obesity and HCC risk, and no definite conclusion can be drawn as to the role of obesity as a risk factor for HCC *per se*.

Interaction between metabolic disorders, alcohol consumption and hepatitis virus infections

Information concerning the interaction between metabolic disorders and major risk factors for HCC is still limited. In the above-mentioned USA study by Nair *et al*. (2002), the presence of obesity was associated with an increased HCC risk in patients with alcoholic and cryptogenic but not HCV or HBV-related cirrhosis, whereas in the French study by N'Kontchou *et al*. (2006) obesity increased HCC risk in both alcoholic and HCV-related cirrhosis. However, neither of these studies investigated the association of metabolic factors with HCC risk in the absence of the three major risk factors for HCC, thus preventing us from evaluating epidemiologic interactions between these factors.

It is well known that obesity and type II diabetes are closely related (Haslam and James, 2005), therefore it is difficult to disentangle the role of each of them as a single cause of HCC. A French cohort study in patients with alcoholic or HCV-related cirrhosis evaluated the role of both overweight and diabetes when also controlling for aetiology of cirrhosis (N'Kontchou *et al*., 2006). When considering BMI $< 25\,kg/m^2$ and no diabetes as the reference category and BMI $\geqslant 30\,kg/m^2$ as the risk condition for overweight (obesity), patients with diabetes only had a relative risk (RR, computed as HR using Cox proportional hazard models) of 1.4, those with BMI $\geqslant 30\,kg/m^2$ and no diabetes had an RR of 2.1, and those with both factors had an RR of 6.0, which is greater than the sum and greater than the product of the RRs for each factor alone, thus indicating that patients with alcoholic or HCV cirrhosis who are both obese and diabetic are at the highest risk of HCC occurrence. An Italian case–control study found a positive interaction between diabetes and overweight: the OR for diabetes was 3.3 among subjects with BMI $\geqslant 25\,kg/m^2$ and 1.4 in those with BMI $< 25\,kg/m^2$ (La Vecchia *et al*., 1997). On the other hand, a Korean cohort study showed a linear increase in HCC risk with increasing fasting serum glucose for each category of BMI, thus showing no interaction between diabetes and BMI on HCC risk (Jee *et al*., 2005).

Few data are available on the interaction between metabolic factors and alcohol intake. The above-mentioned Italian case–control study found an interaction between diabetes and alcohol consumption: the OR for diabetes was 4.0 in subjects who had >4 drinks/day and 2.4 in non-drinkers (La Vecchia *et al*., 1997). Two USA case–control studies also showed a synergism between diabetes and alcohol intake on the risk of HCC, although based on a small number of control subjects with both exposures (Hassan *et al*., 2002; Yuan *et al*., 2004).

Recently, much attention has been drawn to the metabolic aspects of HCV infection. A Spanish cross-sectional study found an OR of about 4 for having type II diabetes or impaired fasting glucose among patients with HCV-related chronic hepatitis compared to those with non-HCV chronic hepatitis (Lecube *et al*., 2004) and a USA cohort study showed that among people at high risk for diabetes, those with HCV infection were more than 11 times as likely as those without HCV infection to develop diabetes (Mehta *et al*., 2003). Experiments on transgenic mice have provided evidence for the contribution of HCV in the development of insulin resistance in HCV infection, which eventually leads to type II diabetes (Shintani *et al*., 2004). Overall, epidemiologic and biological data both suggest that the association between HCV infection and diabetes is real and appears to be causally linked, at least in predisposed individuals (older and overweight), as recently reviewed (Mehta *et al*., 2001; Ratziu *et al*., 2005). However, few data are available on the interaction between diabetes and HCV infection in HCC risk. A large USA population-based case–control study showed a significant interaction between diabetes and HCV infection on HCC risk, the adjusted OR being 2.9 for diabetes only, excluding subjects with major risk factors for the disease, 24.4 for HCV infection alone and 36.9 for both conditions together (Davila *et al*., 2005).

In conclusion, European studies and reports from the USA and other countries provide some evidence that diabetes and obesity are associated with the development of HCC. Whether

the development of HCC is related to the metabolic effects of obesity and diabetes or to underlying NASH-related cirrhosis remains unclear. Insulin resistance and compensatory hyperinsulinemia are cardinal features of obesity and diabetes. Indeed, hyperinsulinemia, disturbance of the insulin-like growth factor axis that stimulates hepatic cell proliferation and inhibits apoptosis, alteration in hepatocyte proliferation and apoptosis encountered in a fatty liver, increased risk of genomic mutations through lipid peroxidation and excess free-radical activity, and other mechanisms could explain the development of HCC prior to the occurrence of inflammation and cirrhosis in obese and/or diabetic subjects (Bugianesi, 2005; Ratziu and Poynard, 2005). Alternatively, the metabolic effects of obesity and diabetes may increase the risk of HCC through the development of NASH-associated cirrhosis.

The global impact of major risk factors for hepatocellular carcinoma in the Mediterranean area

To estimate the global impact of major risk factors for HCC, we computed the attributable risk for all the factors, using the data from the Brescia HCC study, which provided estimates of the ORs adjusted for each other and other confounders by multiple logistic regression and of the prevalence of these risk factors among an unselected series of HCC cases (Donato *et al*., 2002). To this end, we dichotomized alcohol intake at 60 g/day of ethanol ('heavy' alcohol intake) and computed the adjusted ORs for each combination of the three factors examined.

The population attributable risks (ARs) are shown in Figure 4. HCV and HBV infection and heavy alcohol intake together account for 88.5% of the total HCC cases. Three other studies conducted in Italy on the aetiology of HCC and cirrhosis confirm these results. A multicentre case–control study on the aetiology of symptomatic cirrhosis showed that alcohol intake was responsible for the highest proportion of cases, followed by HCV and HBV infection, and the three risk factors together accounted for 85.5% of the total cases (Corrao *et al*., 1998c). In agreement with these results, the population-based Dionysos study showed that the same factors were responsible for 92.4% of all the cases of cirrhosis and HCC in the area, considering >30 g/day of ethanol as a risk factor for liver diseases (Bellentani and Tiribelli, 2001), and a recent multicentre Italian study found that only 6.4% of

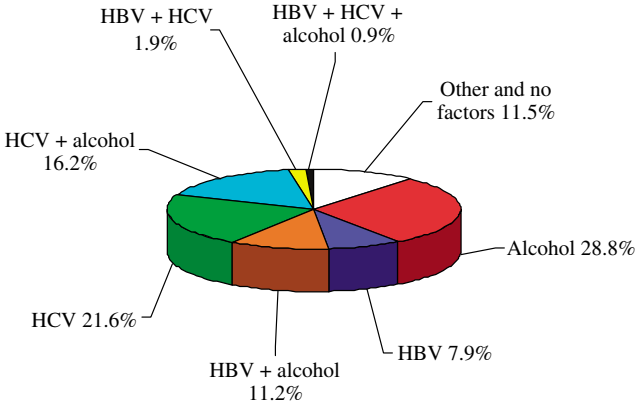

Figure 4 Population attributable risk for HCC due to HBV infection, HCV infection and heavy alcohol intake (daily intake of more than 60 g of ethanol) in the Brescia HCC study.

341 HCC cases had neither HBV or HCV infection nor alcoholic liver disease (Sagnelli *et al*., 2005).

In the Brescia area, North-East Italy, alcohol has the highest impact on HCC risk, since it is responsible for 28.9% of the HCC cases as a single agent and for 28.3% of the cases when combined with HCV and HBV infection, followed by HCV infection and HBV infection. The role of alcohol intake may be lower in other Mediterranean areas, however, as shown by the different proportions of HCC cases with alcoholic liver disease in various parts of Italy in the mentioned multicentre Italian study (Sagnelli *et al*., 2005), and by the lower proportion of HCC cases with alcohol intake >60 g/day in the Greek study (Kuper *et al*., 2000b) compared to the Brescia HCC study.

The proportion of HCC cases that cannot be attributed to the three main risk factors for HCC is 10–15% according to the results of the Brescia HCC study and the other Italian studies on aetiology of cirrhosis and HCC (Donato *et al*., 1997, 2002; Corrao *et al*., 1998c; Bellentani and Tiribelli, 2001). Some of these HCC cases may be due to low–moderate alcohol intake (20–60 g/day) and to some host causes of cirrhosis and HCC, such as hemochromatosis, genetic susceptibility and inherited metabolic diseases (McGlynn and London, 2005). This leaves little room for 'new' unknown risk factors as single causes of chronic liver disease, and suggests that the role of tobacco smoking, diabetes, obesity and other environmental factors as single causes of liver disease is limited in Southern Europe. On the other hand, they might contribute to the global burden of HCC as co-factors, increasing the activity of major risk factors.

Conclusions

Our systematic review of epidemiologic studies carried out on HCC aetiology in Southern Europe confirmed that HBV and HCV infection and alcohol consumption are the main causes of HCC in an area with an intermediate–high prevalence of these agents.

A positive interaction (synergism) between these factors in causing HCC is difficult to demonstrate due to the limited power of studies investigating interactions between factors. A synergism between HCV infection and overt or occult HBV infection has been found in some case–control studies performed in Italy on the aetiology of HCC and cirrhosis, which is major determinant of HCC by itself. The pattern of the risk for HCC because of alcohol intake shows a continuous dose–effect curve without a definite threshold, although most studies found that HCC risk increased only for alcohol consumption above 40–60 g of ethanol per day. Most studies with accurate control of confounding show a significant increase in HCC risk at a level of 40 g of ethanol per day (possibly 20 g/day in women). There is some evidence that alcohol intake interacts probably with HCV infection and possibly with HBV infection, increasing the risk due to each infection alone. Some data suggest that even relatively low levels of alcohol consumption may facilitate the evolution of hepatitis virus-related disease. Some data also support a role of tobacco smoking, diabetes and obesity as single agents or preferably co-factors in causing HCC. A protective effect of coffee on HCC risk due to various risk factors has been found in a few studies, although no definite proof has yet been provided. Hepatitis C virus and HBV infection and alcohol intake together account for about 85% of the total cases of HCC in Mediterranean countries, leaving little room to other, already known risk factors, such as haemochromatosis and other genetic diseases, and to new, still unrecognised factors as

independent causes of HCC. Instead, there are increasing findings supporting an indirect role of 'minor' factors, such as tobacco smoking, diabetes and obesity, in favouring the development of HCC in people with one of the main risk factors for liver disease, especially HCV infection, but well-designed prospective studies controlling for confounding by major risk factors for HCC are needed before firm conclusions can be drawn.

Abbreviations

anti-HCV, antibody to hepatitis C virus; BMI, body mass index; HBsAg, hepatitis B virus antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IR, incidence rate; NAFLD, nonalcoholic fatty liver disease; NASH, non alcoholic steatohepatitis; OR, odds ratio; RR, relative risk.

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References

Adami HO, Chow WH, Nyren O, Berne C, Linet MS, Ekbom A *et al*. (1996). *J Natl Cancer Inst* **88**: 1472–1477.
Adami HO, Hsing AW, McLaughlin JK, Trichopoulos D, Hacker D, Ekbom A *et al*. (1992). *Int J Cancer* **51**: 898–902.
Aizawa Y, Shibamoto Y, Takagi I, Zeniya M, Toda G. (2000). *Cancer* **89**: 53–59.
Becker U, Gronbaek M, Johansen D, Sorensen TI. (2002). *Hepatology* **35**: 868–875.
Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. (2005). *Hepatology* **42**: 44–52.
Bellentani S, Pozzato G, Saccoccio G, Crovatto M, Croce LS, Mazzoran L *et al*. (1999). *Gut* **44**: 874–880.
Bellentani S, Saccoccio G, Costa G, Tiribelli C, Manenti F, Sodde M *et al*. (1997). *Gut* **41**: 845–850.
Bellentani S, Tiribelli C. (2001). *J Hepatol* **35**: 531–537.
Bellentani S, Tiribelli C, Saccoccio G, Sodde M, Fratti N, De Martin C *et al*. (1994). *Hepatology* **20**: 1442–1449.
Benvegnù L, Fattovich G, Noventa F, Tremolada F, Chemello L, Cecchetto A *et al*. (1994). *Cancer* **74**: 2442–2448.
Benvegnù L, Gios M, Boccato S, Alberti A. (2004). *Gut* **53**: 744–749.
Bhattacharya R, Shuhart MC. (2003). *J Clin Gastroenterol* **36**: 242–252.
Bosch FX, Ribes J, Diaz M, Cleries R. (2004). *Gastroenterology* **127**: S5–S16.
Braga C, La Vecchia C, Negri E, Franceschi S. (1997). *Eur J Cancer* **33**: 629–634.
Brechot C, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Brechot P. (2001). *Hepatology* **34**: 194–203.
Bugianesi E. (2005). *Aliment Pharmacol Ther* **22**(Suppl 2): 40–43.
Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P *et al*. (2002). *Gastroenterology* **123**: 134–140.
Cacciola I, Pollicino T, Squadrito G, Cerenzia G, Orlando ME, Raimondo G. (1999). *N Engl J Med* **341**: 22–26.
Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. (2003). *N Engl J Med* **348**: 1625–1638.
Casiglia E, Spolaore P, Ginocchio G, Ambrosio GB. (1993). *Eur J Epidemiol* **9**: 293–297.
Chan HL, Tsang SW, Leung NW, Tse CH, Hui Y, Tam JS *et al*. (2002). *Am J Gastroenterol* **97**: 1211–1215.
Chen CJ, Liang KY, Chang AS, Chang YC, Lu SN, Liaw YF *et al*. (1991). *Hepatology* **13**: 398–406.
Chen SY, Wang LY, Lunn RM, Tsai WY, Lee PH, Lee CS *et al*. (2002). *Int J Cancer* **99**: 14–21.
Chen ZM, Liu BQ, Boreham J, Wu YP, Chen JS, Peto R. (2003). *Int J Cancer* **107**: 106–112.
Chiaramonte M, Stroffolini T, Vian A, Stazi MA, Floreani A, Lorenzoni U *et al*. (1998). *Cancer* **85**: 2132–2137.
Choudhury J, Sanyal AJ. (2004). *Semin Liver Dis* **24**: 349–362.
Corrao G, Aricò S. (1998). *Hepatology* **27**: 914–919.
Corrao G, Aricò S, Lepore R, Valenti M, Torchio P, Galatola G *et al*. (1993). *J Clin Epidemiol* **46**: 601–607.
Corrao G, Aricò S, Zambon A, Torchio P, Lepore AR, Busellu G *et al*. (1997). *J Hepatol* **27**: 470–476.
Corrao G, Bagnardi V, Zambon A, La Vecchia C. (2004). *Prev Med* **38**: 613–619.
Corrao G, Bagnardi V, Zambon A, Torchio P. (1998a). *Alcohol Alcohol* **33**: 381–392.
Corrao G, Torchio P, Zambon A, Ferrari P, Arico S, di Orio F. (1998b). *Eur J Epidemiol* **14**: 447–456.
Corrao G, Zambon A, Bagnardi V, D'Amicis A, Klatsky A, Collaborative SIDECIR Group. (2001). *Ann Epidemiol* **11**: 458–465.
Corrao G, Zambon A, Torchio P, Arico S, La Vecchia C, di Orio F. (1998c). *J Hepatol* **28**: 608–614.
Cotrim HP, Parana R, Braga E, Lyra L. (2000). *Am J Gastroenterol* **95**: 3018–3019.
Coughlin SS, Calle EE, Teras LR, Petrelli J, Thun MJ. (2004). *Am J Epidemiol* **159**: 1160–1167.
Covolo L, Gelatti U, Talamini R, Garte S, Trevisi P, Franceschi S *et al*. (2005). *Cancer Causes Control* **16**: 831–838.
Crespo J, Lozano JL, de la Cruz F, Rodrigo L, Rodriguez M, San Miguel G *et al*. (1994). *Am J Gastroenterol* **89**: 1147–1151.
Cuadrado A, Orive A, Garcia-Suarez C, Dominguez A, Fernandez-Escalante JC, Crespo J *et al*. (2005). *Obes Surg* **15**: 442–446.
Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. (2005). *Gut* **54**: 533–539.
del Olmo JA, Serra MA, Rodriguez F, Escudero A, Gilabert S, Rodrigo JM. (1998). *J Cancer Res Clin Oncol* **124**: 560–564.
Doll R, Peto R, Hall E, Wheatley K, Gray R. (1994). *BMJ* **309**: 901–911.
Donato F, Boffetta P, Puoti M. (1998). *Int J Cancer* **75**: 347–354.
Donato F, Ribero ML, Menatti E, Gelatti U, Covolo L, Nardi G *et al*. (2005). *Dig Liver Dis* **37**: A47 (abstract).
Donato F, Tagger A, Chiesa R, Ribero ML, Tomasoni V, Fasola M *et al*. (1997). *Hepatology* **26**: 579–584.
Donato F, Tagger A, Gelatti U, Parrinello G, Boffetta P, Albertini A *et al*. (2002). *Am J Epidemiol* **155**: 323–331.
El-Serag HB, Tran T, Everhart JE. (2004). *Gastroenterology* **126**: 460–468.
Evans AA, Chen G, Ross EA, Shen FM, Lin WY, London WT. (2002). *Cancer Epidemiol Biomarkers Prev* **11**: 369–376.

Falck-Ytter Y, Younossi ZM, Marchesini G, McCullough AJ. (2001). *Semin Liver Dis* **21**: 17–26.
Fattovich G, Stroffolini T, Zagni I, Donato F. (2004). *Gastroenterology* **127**: S35–S50.
Fong TL, Di Bisceglie AM, Waggoner JG, Banks SM, Hoofnagle JH. (1991). *Hepatology* **14**: 64–67.
Gallus S, Bertuzzi M, Tavani A, Bosetti C, Negri E, La Vecchia C *et al*. (2002a). *Br J Cancer* **87**: 956–959.
Gallus S, Tavani A, Negri E, La Vecchia C. (2002b). *Ann Epidemiol* **12**: 202–205.
Gelatti U, Covolo L, Franceschini M, Pirali F, Tagger A, Ribero ML, *et al*. Brescia HCC Study Group. (2005a). *J Hepatol* **42**: 528–534.
Gelatti U, Covolo L, Talamini R, Tagger A, Barbone F, Martelli C *et al*. (2005b). *Int J Cancer* **115**: 301–306.
Goodman MT, Moriwaki H, Vaeth M, Akiba S, Hayabuchi H, Mabuchi K. (1995). *Epidemiology* **6**: 36–41.
Hadziyannis S, Tabor E, Kaklamani E, Tzonou A, Stuver S, Tassopoulos N *et al*. (1995). *Int J Cancer* **60**: 627–631.
Harris DR, Gonin R, Alter HJ, Wright EC, Buskell ZJ, Hollinger FB *et al*. (2001). *Ann Intern Med* **134**: 120–124.
Haslam DW, James WP. (2005). *Lancet* **366**: 1197–1209.
Hassan MM, Hwang LY, Hatten CJ, Swaim M, Li D, Abbruzzese JL *et al*. (2002). *Hepatology* **36**: 1206–1213.
Heathcote EJ. (2004). *Gastroenterology* **127**(5 Suppl 1): S294–S302.
Hezode C, Lonjon I, Roudot-Thoraval F, Mavier JP, Pawlotsky JM, Zafrani ES *et al*. (2003). *Gut* **52**: 126–129.
Honjo S, Kono S, Coleman MP, Shinchi K, Sakurai Y, Todoroki I *et al*. (1999). *Ann Epidemiol* **9**: 325–331.
Honjo S, Kono S, Coleman MP, Shinchi K, Sakurai Y, Todoroki I *et al*. (2001). *J Clin Epidemiol* **54**: 823–829.
Hu KQ. (2002). *J Viral Hepatitis* **9**: 243–257.
Hui JM, Kench JG, Chitturi S, Sud A, Farrell GC, Byth K *et al*. (2003). *Hepatology* **38**: 420–427.
Ikeda K, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Koida I *et al*. (1998). *J Hepatol* **28**: 930–938.
Inoue M, Yoshimi I, Sobue T, Tsugane S, JPHC Study Group. (2005). *J Natl Cancer Inst* **97**: 293–300.
International Agency for Research on Cancer (IARC) (1988). *Alcohol Drinking IARC Monographs*, vol. 44. International Agency for Research on Cancer: Lyon, France.
International Agency for Research on Cancer (IARC) (1991). *Coffee, Tea, Mate, Methylxanthines and Methylglyoxal IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, vol. 51. International Agency for Research on Cancer: Lyon, France.
International Agency for Research on Cancer (IARC) (1994). *Hepatitis Viruses IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, vol. 59. International Agency for Research on Cancer: Lyon, France.
Jee SH, Ohrr H, Sull JW, Samet JM. (2004). *J Natl Cancer Inst* **96**: 1851–1856.
Jee SH, Ohrr H, Sull JW, Yun JE, Ji M, Samet JM. (2005). *JAMA* **293**: 194–202.
Kamper-Jorgensen M, Gronbaek M, Tolstrup J, Becker U. (2004). *J Hepatol* **41**: 25–30.
Klatsky AL, Armstrong MA. (1992). *Am J Epidemiol* **136**: 1248–1257.
Klatsky AL, Armstrong MA, Friedman GD. (1993). *Ann Epidemiol* **3**: 375–381.
Koike K, Yasuda K, Yotsuyanagi H, Moriya K, Hino K, Kurokawa K *et al*. (1995). *J Med Virol* **45**: 236–239.
Kuper H, Tzonou A, Kaklamani E, Hsieh CC, Lagiou P, Adami HO *et al*. (2000a). *Int J Cancer* **85**: 498–502.
Kuper H, Ye W, Broomè U, Romelsjo A, Mucci LA, Ekbom A *et al*. (2001). *Hepatology* **34**: 714–718.
Kuper HE, Tzonou A, Kaklamani E, Hadziyannis S, Tasopoulos N, Lagiou P *et al*. (2000b). *Cancer Causes Control* **11**: 171–175.
Kuriyama S, Tsubono Y, Hozawa A, Shimazu T, Suzuki Y, Koizumi Y *et al*. (2005). *Int J Cancer* **113**: 148–157.
Kurozawa Y, Ogimoto I, Shibata A, Nose T, Yoshimura T, Suzuki H *et al*. JACC Study Group. (2005). *Br J Cancer* **93**: 607–610.
La Vecchia C, Negri E, Decarli A, Franceschi S. (1997). *Int J Cancer* **73**: 204–207.
Lagiou P, Kuper H, Stuver SO, Tzonou A, Trichopoulos D, Adami HO. (2000). *J Natl Cancer Inst* **92**: 1096–1099.
Lecube A, Hernandez C, Genesca J, Esteban JI, Jardi R, Simo R. (2004). *Diabetes Care* **27**: 1171–1175.
Liaw KM, Chen CJ. (1998). *Tob Control* **7**: 141–148.
Liaw YF. (1995). *Hepatology* **22**: 1101–1108.
Liu BQ, Peto R, Chen ZM, Boreham J, Wu YP, Li JY *et al*. (1998). *BMJ* **317**: 1411–1422.
Loguercio C, Di Pierro M, Di Marino MP, Federico A, Disalvo D, Crafa E *et al*. (2000). *Alcohol Alcohol* **35**: 296–301.
Mandayam S, Jamal MM, Morgan TR. (2004). *Semin Liver Dis* **24**: 217–232.
Manno M, Camma C, Schepis F, Bassi F, Gelmini R, Giannini F *et al*. (2004). *Gastroenterology* **127**: 756–763.
Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R *et al*. (2003). *Hepatology* **37**: 917–923.
Marchesini G, Forlani G, Bugianesi E. (2005). *Ann Med* **37**: 333–346.
Marrero JA, Fontana RJ, Su GL, Conjeevaram HS, Emick DM, Lok AS. (2002). *Hepatology* **36**: 1349–1354.
Marrero JA, Lok AS. (2004). *Gastroenterology* **126**: 347–350.
Mastrangelo G, Fedeli U, Fadda E, Valentini F, Agnesi R, Magarotto G *et al*. (2004). *Environ Health Perspect* **112**: 1188–1192.
McGlynn KA, London WT. (2005). *Best Pract Res Clin Gastroenterol* **19**: 3–23.
McKillop IH, Schrum LW. (2005). *Alcohol* **35**: 195–203.
Mehta SH, Brancati FL, Strathdee SA, Pankow JS, Netski D, Coresh J *et al*. (2003). *Hepatology* **38**: 50–56.
Mehta SH, Strathdee SA, Thomas DL. (2001). *Epidemiol Rev* **23**: 302–312.
Mendenhall CL, Seeff L, Diehl AM, Ghosn SJ, French SW, Gartside PS *et al*. the VA Cooperative Study Group. (1991). *Hepatology* **14**: 581–589.
Mizoue T, Tokui N, Nishisaka K, Nishisaka S, Ogimoto I, Ikeda M *et al*. (2000). *Int J Epidemiol* **29**: 232–237.
Moller H, Mellemgaard A, Lindvig K, Olsen JH. (1994). *Eur J Cancer* **30A**: 344–350.
Monto A, Patel K, Bostrom A, Pianko S, Pockros P, McHutchison JG *et al*. (2004). *Hepatology* **39**: 826–834.
Morgan TR, Mandayam S, Jamal MM. (2004). *Gastroenterology* **127**: S87–S96.
Mori M, Hara M, Wada I, Hara T, Yamamoto K, Honda M *et al*. (2000). *Am J Epidemiol* **151**: 131–139.
Nair S, Mason A, Eason J, Loss G, Perrillo RP. (2002). *Hepatology* **36**: 150–155.
Neuschwander-Tetri BA, Caldwell SH. (2003). *Hepatology* **37**: 1202–1219.
N'Kontchou G, Paries J, Tin Tin Htar M, Ganne-Carrie N, Costentin L, Grando-Lemaire V *et al*. (2006). *Clin Gastroenterol Hepatol* (in press).
Pan SY, Johnson KC, Ugnat AM, Wen SW, Mao Y, Canadian Cancer Registries Epidemiology Research Group. (2004). *Am J Epidemiol* **159**: 259–268.

Pessione F, Ramond MJ, Njapoum C, Duchatelle V, Degott C, Erlinger S *et al*. (2001). *Hepatology* **34**: 121–125.
Peters MG, Terrault NA. (2002). *Hepatology* **36**: S220–S225.
Pintus F, Mascia P. (1996). *Eur J Epidemiol* **12**: 71–76.
Poikolainen K, Vartiainen E. (1997). *Am J Epidemiol* **146**: 1019–1024.
Pollicino T, Squadrito G, Cerenzia G, Cacciola I, Raffa G, Crax A *et al*. (2004). *Gastroenterology* **126**: 102–110.
Pontisso P, Ruvoletto MG, Fattovich G, Chemello L, Gallorini A, Ruol A *et al*. (1993). *Gastroenterology* **105**: 1529–1533.
Poschl G, Seitz HK. (2004). *Alcohol Alcohol* **39**: 155–165.
Powell EE, Cooksley WG, Hanson R, Searle J, Halliday JW, Powell LW. (1990). *Hepatology* **11**: 74–80.
Poynard T, Bedossa P, Opolon P. (1997). *Lancet* **349**: 825–832.
Raimondo G, Brunetto MR, Pontisso P, Smedile A, Maina AM, Saitta C *et al*. (2006). *Hepatology* **43**: 100–107.
Rapp K, Schroeder J, Klenk J, Stoehr S, Ulmer H, Concin H *et al*. (2005). *Br J Cancer* **93**: 1062–1067.
Ratziu V, Bonyhay L, Di Martino V, Charlotte F, Cavallaro L, Sayegh-Tainturier MH *et al*. (2002). *Hepatology* **35**: 1485–1493.
Ratziu V, Heurtier A, Bonyhay L, Poynard T, Giral P. (2005). *Aliment Pharmacol Ther* **22**(Suppl 2): 56–60.
Ratziu V, Poynard T. (2005). Hepatocellular carcinoma in NAFLD. In: Farrell G, De la Hall P, George J, McCullough AJ (eds). *Fatty Liver Disease NASH and Related Disorders*. Blackwell: Oxford (UK). pp 263–275.
Regev A, Jeffers LJ. (1999). *Alcohol Clin Exp Res* **23**: 1543–1551.
Regimbeau JM, Colombat M, Mognol P, Durand F, Abdalla E, Degott C *et al*. (2004). *Liver Transpl* **10**: S69–S73.
Rockhill B, Newman B, Weinbarg C. (1998). *Am J Public Health* **88**: 15–19.
Rothman KJ. (1986). *Modern epidemiology*. Little, Brown and Co.: Boston.
Roudot-Thoraval F, Bastie A, Pawlotsky JM, Dhumeaux D, The Study Group for the Prevalence and the Epidemiology of Hepatitis C Virus. (1997). *Hepatology* **26**: 485–490.
Ruhl CE, Everhart JE. (2005). *Gastroenterology* **129**: 1928–1936.
Sagnelli E, Stroffolini T, Mele A, Almasio P, Coppola N, Ferrigno L *et al*. (2005). *J Med Virol* **75**: 522–527.
Samanic C, Gridley G, Chow WH, Lubin J, Hoover RN, Fraumeni Jr JF. (2004). *Cancer Causes Control* **15**: 35–43.
Sangiovanni A, Del Ninno E, Fasani P, De Fazio C, Ronchi G, Romeo R *et al*. (2004). *Gastroenterology* **126**: 1005–1014.
Sharp DS, Everhart JE, Benowitz NL. (1999). *Ann Epidemiol* **9**: 391–393.
Shi J, Zhu L, Liu S, Xie WF. (2005). *Br J Cancer* **92**: 607–612.
Shimada M, Hashimoto E, Taniai M, Hasegawa K, Okuda H, Hayashi N *et al*. (2002). *J Hepatol* **37**: 154–160.
Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S *et al*. (2004). *Gastroenterology* **126**: 840–848.
Sorensen HT, Friis S, Olsen JH, Thurlstrup AM, Mellemkjaer L, Linet M *et al*. (1998). *Hepatology* **28**: 921–925.
Sorensen HT, Mellemkjaer L, Jepsen P, Thulstrup AM, Baron J, Olsen JH *et al*. (2003). *J Clin Gastroenterol* **36**: 356–359.
Squadrito G, Pollicino T, Cacciola I, Caccamo G, Villari D, La Masa T *et al*. (2006). *Cancer* **106**: 1326–1330.
Staretz ME, Murphy SE, Patten CJ, Nunes MG, Koehl W, Amin S *et al*. (1997). *Drug Metab Dispos* **25**: 154–162.
Stroffolini T, Chiaramonte M, Tiribelli C, Villa E, Simonetti RG, Rapicetta M *et al*. (1992). *J Hepatol* **16**: 360–363.
Stroffolini T, Sagnelli E, Almasio P, Ferrigno L, Craxi A, Mele A, Italian Hospitals Collaborating Groups. (2004). *Dig Liver Dis* **36**: 56–60.
Sun CA, Wu DM, Lin CC, Lu SN, You SL, Wang LY *et al*. (2003). *Am J Epidemiol* **157**: 674–682.
Sun Z, Lu P, Gail MH, Pee D, Zhang Q, Ming L *et al*. (1999). *Hepatology* **30**: 379–383.
Tanaka K, Tokunaga S, Kono S, Tokudome S, Akamatsu T, Moriyama T *et al*. (1998). *Int J Epidemiol* **27**: 438–443.
Teli MR, Day CP, Burt AD, Bennett MK, James OF. (1995). *Lancet* **346**: 987–990.
Torbenson M, Thomas DL. (2002). *Lancet Infect Dis* **2**: 479–486.
Tverdal A, Skurtveit S. (2003). *Ann Epidemiol* **13**: 419–423.
Tzonou A, Trichopoulos D, Kaklamani E, Zavitsanos X, Koumantaki Y, Hsieh CC. (1991). *Int J Cancer* **49**: 377–380.
Vall Mayans M, Calvet X, Bruix J, Bruguera M, Costa J, Esteve J *et al*. (1990). *Int J Cancer* **46**: 378–381.
Verlato G, Zoppini G, Bonora E, Muggeo M. (2003). *Diabetes Care* **26**: 1047–1051.
Vineis P, Alavanja M, Buffler P, Fontham E, Franceschi S, Gao YT *et al*. (2004). *J Natl Cancer Inst* **96**: 99–106.
Voigt MD. (2005). *Clin Liver Dis* **9**: 151–169.
Wang LY, Chen CJ, Zhang YJ, Tsai WY, Lee PH, Feitelson MA *et al*. (1998). *Am J Epidemiol* **147**: 315–323.
Wang LY, You SL, Lu SN, Ho HC, Wu MH, Sun CA *et al*. (2003). *Cancer Causes Control* **14**: 241–250.
Wideroff L, Gridley G, Mellemkjaer L, Chow WH, Linet M, Keehn S *et al*. (1997). *J Natl Cancer Inst* **89**: 1360–1365.
Wolk A, Gridley G, Svensson M, Nyren O, McLaughlin JK, Fraumeni JF *et al*. (2001). *Cancer Causes Control* **12**: 13–21.
Wong RH, Chen PC, Wang JD, Du CL, Cheng TJ. (2003). *J Occup Environ Med* **45**: 379–383.
Yamanaka T, Shiraki K, Nakazawa S, Okano H, Ito T, Deguchi M *et al*. (2001). *Anticancer Res* **21**: 2937–2940.
Yotsuyanagi H, Hashidume K, Suzuki M, Maeyama S, Takayama T, Uchikoshi T. (2004). *Alcohol Clin Exp Res* **28**: 181S–185S.
Yu MC, Tong MJ, Govindarajan S, Henderson BE. (1991). *J Natl Cancer Inst* **83**: 1820–1826.
Yu MC, Yuan JM. (2004). *Gastroenterology* **127**: S72–S78.
Yu MW, Hsu FC, Sheen IS, Chu CM, Lin DY, Chen CJ *et al*. (1997). *Am J Epidemiol* **145**: 1039–1047.
Yuan JM, Govindarajan S, Arakawa K, Yu MC. (2004). *Cancer* **101**: 1009–1017.
Zavitsanos X, Hatzakis A, Kaklamani E, Tzonou A, Toupadaki N, Broeksma C *et al*. (1992). *Cancer Res* **52**: 5364–5367.
Zen Y, Katayanagi K, Tsuneyama K, Harada K, Araki I, Nakanuma Y. (2001). *Pathol Int* **51**: 127–131.
Zoli M, Magalotti D, Bianchi G, Gueli C, Marchesini G, Pisi E. (1996). *Cancer* **78**: 977–985.