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

F Donato¹, U Gelatti¹, RM Limina¹ and G Fattovich²

¹Institute of Hygiene, Epidemiology and Public Health, University of Brescia, Brescia, Italy and ²Department of Gastroenterology, University of Verona, Verona, Italy

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.

Keywords: hepatocellular carcinoma; risk factors; epidemiology; hepatitis B virus; hepatitis C virus; alcohol

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
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, hence 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

| Characteristics of the research | Inclusion criteria |
|---------------------------------|--------------------|
| 1. Outcome investigated         | HCC                |
| 2. Language                     | English            |
| 3. Area in which the research   | Southern Europe: European countries bordering on the Mediterranean sea |
| 4. Time period                  | For some minor risk factors, when few studies were available for this area, we extended the analysis to include those performed outside 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.

Oncogene
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 than 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 (Benvenù 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 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 (Benvenù et al., 1994; Chiaramonte et al., 1995) showed an additive synergism between the two infections, while the others (Zoli et al., 1996; Del Olmo et al., 1998; Benvenù 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 (Liu, 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 hypothesized; 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 immunosassays 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).
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). 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 then 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 Arico`, 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,

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., 1998). 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.

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 |
|----------------------|-----------|-----------------------------------|------------------|-----------|----------------------|
|                      |           |                                   |                  |           | HBV alone | HCV alone | HBV and HCV |
| Benvegnu` et al. (1994) | Italy     | 46.3 (8–96 months)                | 246              | 28        | 3.16 (5/41) | 2.55 (17/173) | 10.37 (6/15) |
| Benvegnu` et al. (2004) | 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) | 1.53 (23/186) | 1.81 (2/21)  |
| Sangiovanni et al. (2004) | Italy    | 148 (1–213 months)                | 209              | 76        | 0.88 (9/40) | 1.16 (64/152) | 1.32 (3/17)  |
| Zoli et al. (1996)    | Italy     | 54 (7–77 months)                  | 94               | 22        | 3.03 (3/22) | 5.87 (14/53)  | 5.85 (5/19)  |

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

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.
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 the risk of HCC. 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 (Bellantini 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 Arico`, 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<sup>a</sup> (559/1028) | OR<sup>b</sup> (95% CI)<sup>c</sup> |
|-------------------|----------------------|---------------------------------------|----------------------------------|
| 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)               |

<sup>a</sup>A total of 16 cases and two controls with both HBV and HCV infections excluded. <sup>b</sup>Adjusted for age, sex and education. <sup>c</sup>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 | Incidence rate per 100 person-years (HCC cases/patients at risk) |
|-------------------|----------------------|---------------------------------------------------------------|
|                   |                      | Incidence rate (HCC cases/patients at risk)                   |
|                   |                      | HBV positive                                                  |
|                   |                      | HCV positive                                                  |
|                   |                      | Both HBV/HCV positive                                         |
| None              | No                   | —                                                             |
| None              | Yes                  | 0.72 (7/199)                                                 |
| HBV               | No                   | 0.32 (1/54)                                                   |
| HBV               | Yes                  | 0.05 (1/65)                                                   |
| HCV               | No                   | 1.57 (23/264)                                                |
| HCV               | Yes                  | 0.07 (2/104)                                                  |
| Both              | No/yes               | 1.16 (64/52)                                                  |
|                  |                      | 0.10 (2/6)                                                    |
|                  |                      | 3.70 (14/53)                                                  |
|                  |                      | 3.70 (14/53)                                                  |
|                  |                      | 6.76 (7/12)                                                   |

Incidence rates computed per 100 person-years. *Follow-up obtained by the authors. †Updated by the authors. ‡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.

At present, there are few data on the interaction between HBV infection and alcohol intake that also take account of HCV 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 (Pessicone 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 0.5 (0.1–0.7) for 5 or more cups/day (Galietti 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 38.2 to 9.0. These findings suggest a substantial reduction of HCC risk associated with either HBV 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, hyper-insulinemia 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/m²) 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/m² (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).

### Table 6

| 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: Male 2.49 (1.5–4.1) | Female 1.23 (0.5–3.0) |
| Lagliu et al. (2000)| Greece | Case-control  | 333/360           | 1995–1998           | OR: 1.86 (0.99–3.51) |                  |
| La Vecchia et al. (1997)| Italy | Case-control  | 428/1502          | 1984–1996           | OR: Male 2.4 (1.5–3.8) | Female 2.0 (1.0–4.2) |
| Verlato et al. (2003)| Italy | Cohort        | 7148              | 1987–1996           | SMR: Male 1.80 (1.29–2.46) | Female 1.97 (1.26–2.91) |

*Adjusted for age, sex, area of residence, smoking status, total carrot, green vegetables and fresh fruit consumption. *Adjusted for age, sex, education, hepatitis B or C virus (HBV or HBC) infection, smoking status and alcohol consumption. *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. *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/m², and 2.9 for BMI ≥ 30 kg/m² 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/m² and no diabetes as the reference category and BMI ≥ 30 kg/m² 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 ≥ 30 kg/m² 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 ≥ 25 kg/m² and 1.4 in those with BMI < 25 kg/m² (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, 2.4 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 (Bellantani and Tiribelli, 2001), and a recent multicentre Italian study found that only 6.4% of 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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