Review

Viral hepatitis and hepatocellular carcinoma
Peter P Michielsen*, Sven M Francque and Jurgen L van Dongen

Address: Division of Gastroenterology and Hepatology University Hospital Antwerp, Belgium
Email: Peter P Michielsen* - peter.michielsen@uza.be; Sven M Francque - sven.francque@uza.be; Jurgen L van
Dongen - jvandongen@pandora.be
* Corresponding author

Abstract

Background: Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in the world. The incidence of HCC varies considerably with the geographic area because of differences in the major causative factors. Chronic hepatitis B and C, mostly in the cirrhotic stage, are responsible for the great majority of cases of HCC worldwide. The geographic areas at the highest risk are South-East Asia and sub-Saharan Africa, here hepatitis B is highly endemic and is the main cause of HCC. In areas with an intermediate rate of HCC such as Southern Europe and Japan, hepatitis C is the predominant cause, whereas in low rate areas such as Northern Europe and the USA, HCC is often related to other factors as alcoholic liver disease. There is a rising incidence in HCC in developed countries during the last two decades, due to the increasing rate of hepatitis C infection and improvement of the clinical management of cirrhosis.

Methods: This article reviews the literature on hepatitis and hepatocellular carcinoma. The Medline search was carried out using these key words and articles were selected on epidemiology, risk factors, screening, and prevention of hepatocellular carcinoma.

Results: Screening of patients with advanced chronic hepatitis B and C with hepatic ultrasound and determination of serum alfa-fetoprotein may improve the detection of HCC, but further studies are needed whether screening improves clinical outcome.

Hepatitis B and C viruses (HBV/HCV) can be implicated in the development of HCC in an indirect way, through induction of chronic inflammation, or directly by means of viral proteins or, in the case of HBV, by creation of mutations by integration into the genome of the hepatocyte.

Conclusion: The most effective tool to prevent HCC is avoidance of the risk factors such as viral infection. For HBV, a very effective vaccine is available. Preliminary data from Taiwan indicate a protective effect of universal vaccination on the development of HCC. Vaccination against HBV should therefore be a health priority. In patients with chronic hepatitis B or C, interferon-alfa treatment in a noncirrhotic stage is protective for HCC development in responders, probably by prevention of cirrhosis development. When cirrhosis is already present, the protective effect is less clear. For cirrhosis due to hepatitis B, a protective effect was demonstrated in Oriental, but not in European patients. For cirrhosis due to hepatitis C, interferon-alfa treatment showed to be protective in some studies, especially in Japan with a high incidence of HCC in untreated patients. Virological, but also merely biochemical response, seems to be associated with a lower risk of development of HCC. As most studies are not randomized controlled trials, no definitive conclusions on the long-term effects of interferon-alfa
in HBV or HCV cirrhosis can be established. Especially in hepatitis C, prospective studies should be performed using the more potent reference treatments for cirrhotics, namely the combination of peginterferon and ribavirin.

**Epidemiology of hepatocellular carcinoma**

**Background**

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors, representing more than 5% of all cancers. The estimated annual number of cases exceeds 500,000 [1], with a mean annual incidence of around 3–4% [2]. In terms of relative frequencies, HCC ranks as the fifth most common cancer in the world, it is also the fifth among men and eighth among women; it is the second among cancers of the digestive tract after stomach cancer [3].

The incidence of HCC varies considerably with the geographic area because of differences in the major causative factors. The geographic areas at the highest risk are located in Eastern Asia, with age-adjusted incidence rates (AAIR) ranging from 27.6 to 36.6 per 100,000 in men; Middle Africa (AAIR 20.8–31.1/100,000) and some Western African countries (30–48/100,000). The geographic areas at lowest risk are Northern Europe, Australia, New Zealand and the Caucasian populations of North and Latin America (AAIR 1.5–3.0). In Southern Europe, AAIR is around 10 per 100,000 in men [3].

The most powerful risk factor for development of HCC is the existence of liver cirrhosis, regardless of its etiology [4]. Among cirrhotics, viral infection and high alcohol intake are associated with the highest risk [5-8].

Of the primary hepatitis viruses, only hepatitis B and C viruses cause HCC [9]. Hepatitis A and E viruses do not produce long-term pathological sequelae. Although hepatitis D virus (HDV) always occurs as co-infection with hepatitis B virus and leads to severe acute or chronic hepatic disease, there is controversy whether it increases the carcinogenic potential [10,11].

**Risk factors for development of HCC**

**Hepatitis B**

Hepatitis B virus (HBV) infection is a major public health problem. It is estimated that two billion people have been infected worldwide and 360 million suffer from chronic HBV infection [12]. Over 520,000 die each year, 50,000 from acute hepatitis B, 470,000 from cirrhosis and liver cancer. In South-East Asia hepatitis B is mostly acquired perinatally from an infected mother. In sub-Saharan Africa, it is mostly acquired in early childhood by horizontal infection, whereas in Northwestern Europe, North America and Australia infection is mainly through sexual contact or needle sharing among injecting drug users, with a peak incidence in the 15–25 age group [12]. Infection acquired perinatally and in early childhood is usually asymptomatic, becoming chronic in 90 and 30% of cases, respectively. In adults, infection resolves in >95% with loss of serum HBsAg and the appearance of anti-HBs. Chronic infection is characterized by the persistence of HBsAg for more than 6 months. Acute hepatitis B usually results in complete recovery with little if any risk of HCC. In cases with persistent HBV infection, HBV is one of the most important risk factors for HCC.

Chronic HBV infection presents as one of three potentially successive phases: *immune tolerant, immune active and low- or non-replicative*. In the *immune tolerant phase*, serum HBsAg and HBeAg are detectable, serum HBV DNA levels are high, serum aminotransferases are normal or minimally elevated. In the *immune active phase*, serum HBV DNA levels decrease and serum aminotransferase levels increase. Flares of aminotransferases may be observed, in some patients these flares are followed by HBeAg-anti-HBc seroconversion. Following this conversion, in the *low- or non-replicative phase* the HBV replication persists but at a very low level suppressed by the host immune response. HBV DNA in serum is undetectable by conventional, non-PCR based techniques. This phase is also called the ‘inactive carrier state’. It may lead to resolution of HBV infection where HBsAg becomes undetectable and anti-HBs is detected, anti-HBc staying positive as sign of contact with the virus. Recently it has been reported that HBV DNA can persist in the serum and liver tissue even after negativation of HBsAg [13]. Recent advances in molecular technology have allowed the isolation of HBV variants that either cannot produce HBeAg or produce it less efficiently, based on precore stop codon mutation and mutations in the core promoter region respectively. In patients with HBV variants, progressive liver damage occurs in parallel with relatively high levels of viremia. In perinatally infected people, the immunotolerant phase lasts till the age of 15–35 years, after which hepatitis flares may occur, leading eventually to viral remission. In patients infected during later childhood or adulthood, there is no immunotolerant phase.

Most studies on the risk of developing HCC in chronic HBV infection have been performed in the Far East. Here, most patients acquired the HBV infection as newborn infants [14]. It has been noted that the probability of acquiring HCC increases with severity of liver disease. The annual risk of HCC is 0.5% for asymptomatic HBsAg carriers and 0.8% for patients with chronic hepatitis B [15].
Patients with HBV-cirrhosis have a 1000 times higher risk of developing HCC compared to a HBsAg negative control group [16]. The incidence of HCC in compensated cirrhosis due to HBV from Asia was 2.7%. In Japan, the mean interval between the time of initial infection with HBV and the occurrence of HCC is 50 years. As most people here are infected at birth, HBV related liver cirrhosis usually develops in patients in their 40's and HCC in their 50's [17].

Few adequate studies have been performed in the West to address the issue of the incidence of HCC in persons who are positive for HBsAg. Most of the studies in Western countries have included small numbers of HBsAg positive patients and/or have not specifically analyzed the group of HBsAg carriers. There is also lack of uniformity in the timing of initiation of follow-up monitoring. In a cohort of 350 Western European patients with compensated cirrhosis followed for a mean period of 6 years, the 5-year cumulative incidence of HCC was 6% [18,19]. The incidence was 2.2% in a series of 179 untreated Caucasian patients [19,20]. In a retrospective analysis of cirrhotic European patients with HBV infection, the 5-year incidence of HCC was 9% irrespective of HBsAg or HBV DNA status at the time of diagnosis of cirrhosis [21].

The hepatitis B replication status seems to play an important role in determining the risk of development of HCC [22-24]. A recent study found that whereas the relative risk of HCC among men with HBsAg alone was 9.6 compared to those without HBsAg, the risk increased to 60.2 when they were positive for both HBsAg and HBeAg [23]. Another analysis showed that the level of HBV DNA is a prognostic marker for HBV-related HCC and that HCC patients with a less favorable course appear to either clear the virus poorly or to have a greater level of virus production [24]. It was recently demonstrated that positivity for anti-HBc alone in absence of HBsAg and anti-HCV is not rare in Japanese patients with HCC, which may indicate that HBV virus might be involved in so-called non-B HCC [25].

The entire nucleotide sequences of HBV genomes have been classified into 8 genotypes (A-H), with predominance of genotypes A and D in Western countries, and B and C in Southeast Asia and the Far East [26-29]. Several studies from the Far East evaluated the association between distinct genotypes and severity of liver disease. Genotype C was shown to be associated with the development of liver cirrhosis and HCC in Taiwan [30], China [31] and Japan [32], whereas genotype B was shown rarely to be associated with the development of HCC in China and Japan. In contrast, in Taiwan genotype B is the predominant type in patients with HCC who are younger than 35 years [30]. Another study from Taiwan showed that patients with genotype C had a greater tumor recurrence rate after curative resection of HCC compared with those with genotype B [33]. It was also shown that the likelihood of presence of T1762/A1764 mutations in the basal core promoter parallels the progression of liver disease, and that this mutation is found more frequent in HBV genotype C than B patients [34]. PreS deletions were shown to be more frequent in patients with HBV genotype C, and associated with more advanced disease such as liver cirrhosis and hepatocellular carcinoma [35].

**Hepatitis C**

Hepatitis C is also a major public health problem. There are more than 170 million people infected worldwide [36]. Approximately 80% of HCV infected patients develop chronic hepatitis C. About 20% of these patients will develop severe chronic hepatitis C and cirrhosis, which becomes detectable in the second and third decade after infection. The natural history of chronic hepatitis C infection is characterized by a predominantly asymptomatic course and a variable clinical outcome. For these reasons it is difficult to define the rate of progression to cirrhosis and HCC. The risk of cirrhosis in chronic hepatitis C is less than 10% in women infected at a young age and >30% in men infected after the age of 40 over a 20 year period [37,38]. Five prospective studies from Europe and the US have shown that during the first 10–15 years after initial infection, liver cancer is a rare occurrence [39-43]. In patients with hepatitis C, there is an increased risk of HCC coinciding with the establishment of cirrhosis with yearly incidence between 3–8% [6,7,44-47]. In Japan, the mean interval between infection and development of HCC is 30 years [48]. A study from the US shows a long time lag (mean 28 years, range 8–42) between transfusion-associated hepatitis and development of HCC [49].

There is conflicting information on the relationship between HCV genotype and progression to HCC in longitudinal studies. It is suggested by some authors that genotype 1b (most prevalent in Europe and Japan) is associated with a higher incidence of HCC than infection with other genotypes [50,51]. In other studies, however, this was not observed [52,53].

**Coinfection of HBV and HCV**

Both HBV and HCV are transmitted parenterally and coinfection is not uncommon in intravenous drug users and in countries with a high prevalence of HBV [54]. Coinfection of HBV and HCV seems to result in more severe liver disease than either infection alone [55]. The risk of developing HCC in subjects with both infections has been investigated in a meta-analysis of 32 epidemiological studies between 1993 and 1997 [56]. The odds ratio for development of HCC in HBsAg positive, anti-HCV/HCV
RNA negative subjects was 20.4; in HBsAg negative, anti-
HCV/HCV RNA positive subjects 23.6; and subjects posi-
tive for both markers 135. These data suggest a more than
additive but less than multiplicative effect of HBV and
HCV coinfection on the relative risk for HCC. The viruses
may act through common as well as different pathways in
the carcinogenic process.

It has been reported that HBV DNA is still present after
seroconversion of HBsAg in patients with hepatitis B. Se-
veral reports indicate that prior HBV infection, charac-
terized by presence of anti-HBc, affects the development of
HCC in patients infected with HCV [57-59]. Given these
data, in patients with chronic HCV infection, serologic
markers of past HBV infection should be checked, not just
HBsAg. Other authors, however, were not able to docu-
ment any adverse event of occult HBV infection on the
clinicopathologic course of chronic HCV infection [60].

In case of coinfection with HBV (whether active or past),
a more aggressive surveillance to detect early HCC could
be suggested [61]. However, to date screening and surveil-
lance programs have not demonstrated a significant sur-

In view of the role of HBV as cofactor in the development
of HCV related cirrhosis and HCC, vaccination of patients
with chronic hepatitis C against HBV has been advocated
with the presumption of avoiding additional liver injury
[62,63].

Coinfection of HBV and HDV
Verme et al [11] suggested that HBsAg positive patients
with HDV superinfection develop cirrhosis and HCC at an
erlier stage (mean age 48 year) than HBsAg carriers with-
out HDV infection (mean age 62 years).

Coinfection of HBV and HCV with HIV
Coinfection of HBV and HCV with HIV is common
because these diseases share the same routes of transmis-
sion. Recently a series of HCC in HIV-HCV coinfected
patients was published, indicating an unusually rapid
development of HCC in these patients [64]. This is not
surprising, as chronic hepatitis C is more aggressive in HIV
positive subjects, leading to cirrhosis and end-stage liver
disease in a shorter period of time [65].

Coinfection of HCV and S. mansoni
An Egyptian study showed that Schistosoma infection
increased the risk of HCC, only in the presence of HCV,
whereas isolated S. mansoni infection does not [66].

Role of alcohol consumption in HBV or HCV infection
Reports suggest that HBV and ethanol act synergistically to
promote HCC [67,68]. Habitual heavy drinking was
reported to be a significant risk factor for HCC in patients
with HCV-related liver cirrhosis by multiple logistic
regression analysis [57]. A recent study showed synergism
between alcohol drinking and HBV or HCV infection,
with approximately a twofold increase in the odds ratio
for each hepatitis virus infection for drinkers’ > 60 g/d,
with a more than additive but less than multiplicative risk
[69]. Although two case-control studies did not show a
relationship of alcohol consumption with the occurrence
of HCC [70,71], another case-control study found a posi-
tive interaction between HBsAg positivity and HCV RNA
positivity and heavy alcohol intake in the development of
HCC [72]. Furthermore, Hassan et al. [73] showed syner-
gistic interaction (more than additive) between heavy
alcohol consumption ≥ 80 ml/d and chronic HBV or HCV
infection (odds ratio 53.9) and insulin or non-insulin
dependent diabetes mellitus (odds ratio 9.9).

Incidence of HBV- and HCV-related HCC worldwide
Chronic hepatitis B and C infection are responsible for the
great majority of cases of HCC worldwide [9]. They also
account for the peculiar geographical distribution of the
tumor. The relative frequencies of HBV and/or HCV
related HCC in the world is illustrated in Table
[17,72,74-93]. The worldwide incidence of HCC varies
and is predominantly related to the regional prevalence of
chronic viral hepatitis and its associated chronic liver dis-
ease and cirrhosis. Aflatoxin intake has a role in the gene-
sis of HCC only in patients who have pre-existing chronic
hepatitis B [84].

In the Far East and sub-Saharan Africa, where HBV is
highly endemic, HBV is the main cause of HCC.

In areas with an intermediate rate of liver tumors such as
Southern Europe, Egypt and Japan, HCV is the predomi-
nant cause of HCC. Here HCC is mostly discovered at an
older age in patients with longstanding cirrhosis due to
HCV.

In regions with a low incidence of HCC such as Northern
Europe and the United States, HCC related to HCV or
HBV infection are found in a minority of cases and the
tumor is often related to other factors such as alcoholic
liver disease. In these low endemic areas, HCC is usually
discovered at an older age in patients with longstanding
cirrhosis due to alcohol abuse [72]. In France, ethanol is
still the leading cause of cirrhosis and was responsible for
60% of all HCC causes during the last decade [8].

Time trends in the incidence of HCC
An important epidemiological fact is the rising incidence
of HCC in developed countries during the last two dec-
dades [79,89,95,99].

(page number not for citation purposes)
In Japan, the HCC-related mortality rate has sharply increased since 1975 from 10/100,000 to almost 40/100,000 in 2000 [99]. An analysis of the Shinshu University Hospital (Japan) showed a change in etiology of the HCC [100]. Whereas in the 1971–1980 decade, hepatitis B was the predominant cause of HCC, in the 1991–1995 period hepatitis C was largely predominant (Table 3). However, the total numbers of yearly deaths because of HCC in HBsAg carriers’ stays constant, approximately 10% in the survey conducted in 1995. The rapid increase

### Table 1: Relative frequencies of HBV and HCV related HCC in the world

| Author [reference] | Country | Era | Sample size | HBsAg (%) | Anti-HCV (%) | HBsAg/anti HCV (%) | Other (%) |
|-------------------|---------|-----|-------------|-----------|--------------|-------------------|-----------|
| Chen, 1990 [74]   | Taiwan  | NR  | 66          | 35 (53.0) | 15 (22.7)    | 7 (10.6)          | 9 (13.6)  |
| Chuang, 1991 [75] | Taiwan  | NR  | 128         | 87 (68.0) | 13 (10.1)    | 12 (9.4)          | 16 (12.5) |
| Lee, 1992 [76]    | Taiwan  | NR  | 326         | 233 (71.5)| 31 (9.5)     | 10 (3.1)          | 52 (15.9) |
| Jeng, 1991 [77]   | Taiwan  | NR  | 129         | 62 (48.1) | 29 (22.5)    | 19 (14.7)         | 19 (14.7) |
| Leung, 1992 [78]  | Hong Kong| 1986–90| 424 | 341 (80.3) | 16 (3.8)    | 15 (4.0)          | 52 (12.3) |
| Nishioka, 1990 [79]| Japan   | NR  | 180         | 64 (35.6) | 80 (44.4)    | 11 (6.1)          | 25 (13.9) |
| Saito, 1990 [80]  | Japan   | NR  | 253         | 49 (19.4) | 136 (53.8)   | 2 (0.8)           | 66 (26.1) |
| Kiyosawa, 1990 [17]| Japan   | 1958–89| 83 | 19 (22.9) | 51 (61.4)    | 10 (12.0)         | 3 (3.6)   |
| Hassan, 2001 [81] | Egypt   | NR  | 33          | 5 (15.2)  | 12 (36.4)    | NR                | NR        |
| Kew, 1990 [82]    | South Africa | NR | 380 | 137 (36.1) | 63 (16.6)    | 47 (12.4)         | 127 (33.4) |
| Yu, 1990 [83]     | USA     | 1984–89 | 58 | 22 (37.9) | 36 (62.1)    | NR                | NR        |
| Di Bisceglie, 1991 [84]| USA | 1987–88| 99 | 7 (7) | 12 (12) | 1 (1) | 79 (79) |
| Hadziyannis, 1995 [85] | Greece | 1991–92 | 65 | 33 (50.8) | 5 (7.6) | 3 (4.5) | 23 (38.3) |
| Colombo, 1989 [86]| Italy   | 1975–88| 132 | 19 (14.4) | 64 (48.5)    | 22 (16.7)         | 27 (20.5) |
| Levrero, 1991 [87]| Italy   | 1980–88| 167 | 38 (22.8) | 82 (49.1)    | 15 (9.0)          | 32 (19.2) |
| Simonetti, 1992 [88]| Italy | 1982–88| 212 | 15 (7.1) | 133 (62.7)  | 18 (8.5)         | 46 (21.7) |
| Donato, 1997 [72]| Italy   | 1995–96| 172 | 37 (21.5) | 65 (37.8)    | 4 (2.3)           | 66 (38.4) |
| Stroffolini, 1998 [89]| Italy | 1996–97| 1083 | 125 (11.5)| 771 (71.2) | 55 (5.1)       | 132 (12.2) |
| Bruix, 1989 [90]  | Spain   | NR  | 96          | 4 (4.2)   | 67 (69.8)    | 5 (5.2)           | 20 (20.8) |
| Nalpas, 1991 [91] | France  | 1982–89| 55 | 3 (5.5)  | 28 (50.9)    | 9 (16.3)          | 15 (27.3) |
| Van Roey, 2000 [92]| Belgium| 90s | 154 | 37 (24.0) | 62 (40.0)    | NR                | 55 (36.0) |
| Haydon, 1997 [93] | Italy   | 1985–94| 80 | 13 (16.3) | 22 (27.5)    | 2 (2.5)           | 43 (53.8) |

NR: not reported; Bold: predominant cause

### Table 2: Time trends on the incidence of HCC in the world

| Author [reference] | Country | Number/100,000 era 1 | Number/100,000 era 2 |
|-------------------|---------|----------------------|----------------------|
| El Serag, 1999 [95]| USA     | 1976–80: 1.4         | 1991–95: 2.4         |
| El Serag, 2000 [96]| USA     | 1993–95: 2.3         | 1996–98: 7.0         |
| Benhamiche, 1998 [97] (men) | France | 1976–79: 7.5         | 1992–95: 10.2        |
| Stroffolini, 1998 [89]| Italy | 1969: 4.8            | 1994: 10.9           |
| Law, 2000 [98] (men) | Australia | 1983–85: 2.1         | 1995–96: 4.0         |
| Nishioka, 1991 [79]| Japan   | 1968–77: 9.5         | 1984–85: 16.0        |
| Yoshizawa, 2002 [99]| Japan | 1980: ca 10          | 2000: ca 40          |

### Table 3: Changing causes of HCC in Japan, 1971–95

| Author [reference] | Era     | Sample size | HBsAg (%) | Anti-HCV (%) | HBsAg/anti HCV (%) | Other (%) |
|-------------------|---------|-------------|-----------|--------------|-------------------|-----------|
| Kiyosawa, 1992 [100]| 1971–80| 112         | 60 (54%)  | 38 (34%)     | 5 (4%)            | 9 (8%)    |
|                   | 1981–90| 267         | 82 (31%)  | 159 (59%)    | 4 (2%)            | 22 (8%)   |
|                   | 1991–95| 162         | 21 (13%)  | 126 (78%)    | 5 (3%)            | 10 (6%)   |

Bold: predominant cause
of mortality due to HCC in Japan is mainly attributable (ca 80%) to persistent infection with HCV [99]. The hepatitis C epidemic in Japan originated due to intravenous drug use by the young generation after World War II during the late 40s and early 50s. It spread in the general population due to remunerated blood donors. Abrogation of paid blood donation in 1968, exclusion of blood units contaminated with HBV in 1973 and HCV in 1989 decreased the risk of posttransfusion hepatitis from > 50% in the 60s to almost zero at present. The incidence of HCV in Japan is decreasing. As the interval between the time of the initial infection with the hepatitis C virus and the development of HCC is 30 years [79], the growing incidence of HCC in Japan is expected to reach a plateau around the year 2015, and then to decrease [99].

Also in Italy the mortality rate of HCC is rising [89] from 4.8/100,000 in 1969 to 10.9/100,000 in 1994, reflecting the large cohort of subjects infected with HCV through iatrogenic route during the 50s and 60s when glass syringes were commonly used for medical treatment. Likewise in Australia, France and the United States of America (US) the HCC mortality is increasing, most probably because people infected with HCV have grown old and reach the cancer-bearing age [95-98]. In the US, an increase of about 80% in the incidence of HCC over the past 20–30 years is described, it is estimated that approximately 15,000 new cases occur each year. Also in France the incidence of HCC is steadily and markedly increased, the estimated number being about 4,000 per year [101].

Although the prevalence of HCV is declining in developed countries because of the decline in incidence in the 90s, the number of persons infected for ≥20 years is expected to increase substantially before peaking in 2015 [102].

Analysis of long-term serial HCV samples from the US and Japan suggest that HCV was introduced into the US population around 100 years ago and widely disseminated in the 1960s. In contrast, HCV was introduced in Japan >100 years ago and widely disseminated in the 1930s and 40s. The HCV genotype 1b population in Japan started to decrease around 1995 whereas HCV genotype 1a in the US is still growing exponentially. It is predicted that an increased HCC prevalence will occur in the US over the next two to three decades [103].

The reasons advocated for explaining the increased incidence of HCC are the increased rate of HCV infection and an improvement of the clinical management of cirrhotic patients. Enhancing the survival of patients with advanced cirrhosis leads to an increased incidence of HCC. In fact, a decade ago, most of the deaths in cirrhotic patients were due to digestive hemorrhage or bacterial infections, two conditions that are now efficiently prevented and cured [104]. Therefore, HCC has become the leading cause of death in patients with cirrhosis.

**Screening tests for HCC in patients with chronic viral hepatitis**

Despite knowledge of the risk factors for HCC, screening of HCC is controversial, as there have been no randomized controlled studies demonstrating the efficacy of screening for HCC. As HCC mostly occurs in patients with cirrhosis, or at least advanced fibrosis, most studies have been performed in these patients at risk. The most frequently used tests have been serum alfa-fetoprotein (AFP) and hepatic ultrasound (US).

There is one non randomized prospective cohort study suggesting that HCC was detected earlier and was more often resectable in patients who had twice yearly screening with serum AFP and hepatic US than in patients who had usual care [105].

Twenty-four studies, which included patients with chronic hepatitis B or C or both, addressed the sensitivities and specificities of screening tests [106].

Serum AFP for detection of HCC was evaluated in 19 studies. They were relatively consistent in showing that the sensitivity of serum AFP for detecting HCC increases from very low levels to moderately high levels of 60 to 80% as the threshold value decreased from 400 to 10 ng/mL, with corresponding specificity decreasing from 100 to 70–90%. A threshold between 10 and 19 ng/mL seems most appropriate as sensitivity usually is moderately high (45 to 100%), with a specificity of 70 to 90%. It has been shown that AFP is not always specific for HCC and titers can increase with flares of active hepatitis [107].

Seven studies evaluated screening with US, reporting high specificity of 95–100%, but variable sensitivity, varying from 11–99% [94].

A surveillance study combining US and AFP in 1,125 patients with HCV, HBV or both, reported a sensitivity of 100% when using a serum AFP > 10 ng/mL together with US, compared with a sensitivity of 75% using only AFP > 10 ng/mL and a sensitivity of 87% when using US alone [108].

Computed tomography and magnetic resonance imaging have a high sensitivity and specificity in detecting HCC, but are too expensive to be used in surveillance [1].

The surveillance intervals studied varied from 3 to 12 months. In a study of patients with hepatitis B, the most rapidly growing tumor increased from 1 to 3 cm in 5 months [109]. The ideal time for re-screening has not
been identified. Some investigators suggest a 4–5 month interval, others have suggested that a 6-month interval may be most appropriate [109,110]. It is suggested that in case of concomitant HBV and HCV infection serum AFP levels should be obtained every 3 months, and that persistent AFP levels should prompt an aggressive imaging search for HCC [61].

It can be concluded that screening patients with advanced chronic hepatitis B or C with AFP and US may improve detection of HCC, but further studies are needed whether screening improves clinical outcomes.

Pathogenesis of hepatitis B and C-induced hepatocellular carcinoma

Introduction

Epidemiologic data indicate that chronic hepatitis B and C are independent risk factors for development of HCC [7,16]. Furthermore, animal models confirm the oncogenic potential of HBV and HCV in the liver: transgenic mice for hepatitis B and C [110,111], and natural models such as the woodchuck infected with the woodchuck hepatitis virus, a hepadnavirus closely related to the HBV [112].

Carcinogenesis is believed to be a multistage process, occurring through a sequence of steps termed initiation, promotion and progression. This process evolves over several or many years. Tumor initiation begins in cells through mutations induced by exposure to carcinogens. DNA changes, maintained during successive cell divisions, activation of oncogenes and inactivation of suppressor genes lead to dysregulation of the cell division and to immortalization [113]. Tumor-initiated cells have a decreased responsiveness to both intercellular and intracellular signals that maintain normal cellular architecture and regulate homeostatic growth. Tumor promotion results in a further selective clonal expansion of initiated cells. During tumor progression, pre-malignant cells continue to develop progressive phenotypic changes and genomic instability (dysplasia), culminating as overt carcinoma [115].

More than 80% of HCC originate in cirrhotic livers. Macronodules (macrogenerative nodules and adenomatous hyperplasia), irregular hepatocyte regeneration, and some hyperplastic foci are considered as precancerous [116-119]. Large cell dysplasia and small cell dysplasia are considered to be risk factors for development of HCC [120-122].

HBV and HCV can be implicated in the development of HCC in an indirect way, through induction of inflammation, necrosis and chronic hepatocellular regeneration, or directly by means of viral proteins or, in the case of HBV, by creating insertional mutations by integration in the genome of the hepatocyte.

Indirect carcinogenicity of HBV and HCV

In most patients with chronic hepatitis B and/or C the occurrence of HCC is preceded by a process of longstanding inflammation. It is probable that malignant transformation is related to continuous or recurring cycles of hepatocyte necrosis and regeneration [123]. The resulting accelerated cell turnover rate may act as a tumor promoter by increasing the probability of spontaneous mutations or damage to DNA by exogenous factors. The accelerated rate of cell division leaves less time for altered DNA to be repaired before the cell divides again, resulting in transmission of altered DNA to the daughter cells. In this way a series of mutations may accumulate in individual cells over time. This process can lead to focal uncontrolled liver cell growth and eventual malignant cell transformation [115,124]. Another mechanism of induction of malignant transformation is the generation of mutagenic reactive oxygen species as a result of the inflammatory process, such as nitric oxide (NO), superoxide anion (O2−), hydroxyl radical (OH•) and hydrogen peroxide (H2O2) [124].

Evidence for a causal role for chronic necro-inflammation is provided by transgenic mice into which HBV preS/S genes have been introduced. These mice overproduce pre S1 protein that accumulates in the endoplasmatic reticulum of hepatocytes, producing severe and prolonged injury to these cells, initiating a response characterized by inflammation, regenerative hyperplasia and transcriptional deregulation that progresses ultimately to neoplasia [125].

Patterns of gene expression in cirrhosis and hepatocellular carcinoma have recently been shown to be of value in predicting prognosis. Kim et al could identify, using the complementary DNA microarray, a 273-gene signature that distinguished high risk types of cirrhosis (hepatitis B, hepatitis C, hereditary hemochromatosis) from low risk types (autoimmune hepatitis, PBC, alcoholic liver diseases) [126]. The same 273-gene signature was present in samples from patients with proven HCC. A subset of 30 genes was most significantly altered in both the high risk types of cirrhosis and the HCC patients. The TACSTD1, a gene associated with HCC development in other studies, is a lead gene in this gene signature. Lee et al could identify a limited number of genes that accurately predicted survival in a series of 91 HCC patients [127]. The genes involved are implicated in cell proliferation and apoptosis, but also in ubiquitination and histone modification. Delpuech et al identified distinct patterns of gene expression according to the viral aetiology [128]. Finally, Hann et al could demonstrate the presence of antibodies to differentially
expressed genes in hepatitis B and C, and this appeared to be linked with decreased survival [129]. These discoveries not only increase our insight in hepatocarcinogenesis, but may ultimately lead to the development of clinically valuable preneoplastic and prognostic blood markers.

**Direct carcinogenicity of HBV and HCV**

**Hepatitis B**

A significant proportion of HBV-related HCCs arise in an otherwise normal liver, implicating that the virus can also be directly oncogenic [124].

It has been demonstrated that HBV integrates into the DNA of the host cells. This integration may dysregulate the control mechanisms on the cell cycle by chromosomal abnormalities, production of viral proteins or alteration of human genes and proto-oncogenes. It is, however, controversial whether viral integration plays an important role in the process leading to development of HCC. The hepadnaviral integration process appears to involve recombination mechanisms that do not preserve the viral genome sequence. Thus it is impossible for the viral integrant to function as a template for subsequent virus replication. Several studies suggest that DNA integration sites are at random and that integration occurs at random times during the course of a chronic viral infection [130,131]. HBV integration can be present in chronically infected liver tissue without evidence of HCC [132]. Non-neoplastic hepatocytes may have a similar pattern of rearrangement of viral sequences following integration into human DNA.

**Chromosomal DNA instability**

Several studies have shown that HBV DNA integration enhances chromosomal instability. In many hepatic tumors large inverted duplication insertions, translocations and micro- and macrochromosomal deletions have been associated with HBV insertion [133-136]. These changes can result in loss of important cellular genes, sometimes involving tumor-suppressor genes and other genes involved in the regulation of regeneration and growth processes.

**Trans-activation of cellular genes**

HBV DNA may induce malignant transformation in another way.

Mammalian hepadnaviruses contain a gene (the HBX gene), of which the protein (HBX protein) can trans-activate several cellular promoters and upregulate their expression of different cellular and viral genes [137,138]. Integrated HBX, even when truncated, frequently encodes functionally active trans-activator proteins [139]. This protein has been shown to transform mouse fetal hepatocytes into a full malignant phenotype [140]. There are studies in transgenic mice with the HBX gene that developed multifocal areas of altered hepatocytes, adenomas and HCCs [110].

In contrast to mammalian hepadnaviruses associated with HCC, avian hepadnaviruses such as the duck hepatitis virus or heron hepatitis virus, lack the HBX gene and are not associated with HCC [123].

A gene that may be affected by the HBX gene is the p53 tumor suppression gene. This gene has been shown to play an important role in hepatocarcinogenesis. It is considered to negatively regulate the cell cycle. The HBX protein has been shown to complex p53 protein and to inhibit its function [141,142]. In a transgenic mouse model it was shown that HCC development correlates with p53 binding to HBX [143].

**Oncogenes**

It has been proposed that HBV acts as an insertion mutagen by integrating into the host genome and activating the cellular proto-oncogenes c-myc, ras and c-fos [144].

The preS2/S gene is integrated in most HCCs associated with HBV. When 3’-truncated it generates a truncated protein that is oncogenic by trans-activating proto-oncogenes c-myc and c-fos [145].

**Growth factors**

Growth factors and their receptors function as positive or negative modulators of cell proliferation and differentiation. Insulin-like growth factor-II and transforming growth factor-β expression correlate with HBX protein expression in animal models [146,147], suggesting trans-activation of these growth factors facilitating tumor formation.

**Role of PreS mutations**

PreS deletion mutants accelerate the storage of large envelope proteins in hepatocyte cytoplasm, which could induce cytotoxic effects toward the development of end-stage liver disease [148]. The accumulation of large envelope protein can activate cellular promoters by inducing endoplasmic reticulum stress [149]. Furthermore, Pre-S1 sequences can stimulate the transcription of transforming growth factor α (TGFα). Coexpression of TGFα and HBsAg could accelerate hepatocellular carcinoma by stimulation of hepatocyte proliferation [150].

**Allelic loss of chromosome 4q**

Allelic loss of chromosome 4q is one of the most frequent genetic aberrations found in HCC. It was found to be associated with HBV-related hepatocarcinogenesis, probably by inactivation of a putative tumor suppressor gene included in it [151].
**Hepatitis C**

In contrast to HBV, HCV is an RNA virus that lacks a reverse-transcriptase enzyme and cannot integrate into the host genome. Thus, insertional mutagenesis can be excluded as a pathogenic mechanism for the development of HCC associated with chronic HCV infection. The molecular pathogenetic mechanisms by which HCV contributes to cell transformation remain unclear.

One possibility is that the development of HCC is simply related to chronic necro-inflammatory liver disease. Overall, 97% of patients with HCV markers and HCC have cirrhosis [152,153], and most of the remainder develop HCC in the presence of chronic hepatitis.

An alternative mechanism of HCV-induced hepatocarcinogenesis may be that HCV has a direct oncogenic action. Viral replication might cause inappropriate expression of two growth factors that may be implicated in hepatic carcinogenesis: transforming growth factor-α and insulin-like growth factor II [154,155].

The non-structural HCV protein NS3 has both protease and helicase activity. HCV may therefore induce genomic instability and favor mutations through its helicase activity [156]. The protein also has an activity similar to protein kinase A, and could disturb cellular homeostasis [157].

The *HCV envelope protein* E2 and the non-structural protein NS5A inhibit RNA-dependent protein kinase, key mediator of the antiviral, antiproliferative and anti-oncogenic effect of interferon [158-160].

The *HCV core protein* has characteristics that imply that this protein could function as a gene-regulator [161,162]. The presence of the protein in transgenic mice can induce HCC [111]. After mutation, the HCV core protein can also inhibit tumor suppressor genes such as p53, as has been demonstrated in hepatic oncogenesis [163-165]. It has recently been shown that the HCV core protein induces nuclear factor κB (NF-κB), thereby suppressing TNF-α-induced apoptosis [166]. This anti-apoptosis may be a mechanism by which HCV leads to viral persistence and possibly to hepatocarcinogenesis.

**Prevention of hepatocellular carcinoma caused by viral hepatitis**

**Primary prevention**

The most effective tool to prevent HCC is avoidance of the risk factors such as viral infection by HBV or HCV. Any action diminishing the potential transmission of contaminated blood products (uncontrolled blood transfusion, needle sharing, invasive procedures without proper health standards) will decrease the likelihood of viral spread.

The major advance has come from the availability of an effective vaccine that protects against HBV.

In 1969, Taiwan was an hyperendemic area of HBV infection with a high rate of HBsAg positivity, 19% of the population being infected before the fourth decade of life. In 1976, HBsAg prevalence was > 80% in HCC in Taiwan [167]. In 1984 a program to control cirrhosis and HCC began. All neonates born to HBsAg positive mothers were given hepatitis B vaccine in order to counter perinatal infection. In 1986 all neonates were included in the program. As a consequence, there was a decrease in HBsAg positivity in six-year-olds from 10.6% in 1983–1984 to 0.8% in 1993–1994. There was a parallel decline in incidence of childhood HCC (6–14 years old), in the cohort born between 1980 and 1984. The incidence of liver cancer in children between 6 and 14 years old decreased to zero for children born in 1986 and 1987 [168]. The decline of HCC in children after universal vaccination can be considered as an early indicator of the effectiveness of vaccination in reducing the rate of HCC. Since the incidence of HCC in Taiwan peaks in the sixth decade of life, it may take 40 years or longer to see an overall decrease in the rate of HCC as a result of the vaccination program. Vaccination against HBV should become a health priority together with the promotion of adequate health standards.

Unfortunately, there is no vaccine against HCV. Up to now, the only effective method to prevent its transmission is the avoidance of contamination with infective blood products.

**Prevention of HCC in patients with previously acquired risk**

**Introduction**

Chronic viral carriage is one of the main risk factors for the development of HCC. Effective antiviral treatments have been developed in recent years and this has changed the management of viral infection.

Interferon-alfa is still considered the reference therapy for HBeAg positive chronic hepatitis B. However, its efficacy is limited, with seroconversion from anti-HBe negative to anti-HBe positive in up to 40%. Only <10% of patients become HBsAg negative [169]. Other possible treatments are antiviral drugs such as lamivudine and adefovir dipivoxil [12].

For the treatment of chronic hepatitis C, interferon-alfa monotherapy yielded only limited response. Combination with ribavirin led to a significant increase in sustained viral response to about 40% in treatment-naïve patients [170,171]. Recently, the combination of peginterferon-alfa and ribavirin improved the sustained
viral response rate to nearly 60% in treatment-naïve patients [172,173], and is now considered the reference treatment.

It is under debate whether interferon-alfa-based treatments are effective in declining the incidence of HCC in chronic hepatitis B and C.

**Anti-oncogenic effects of interferon-alfa**

HCC prevention by interferon-alfa might be the result of several direct or indirect mechanisms. Interferon has an antiproliferative and pro-apoptotic effect [174]. Interferon inhibits the expression of the \(c\)-\(myc\) oncogene and induces the expression of anti-proliferative factors and tumor suppressor genes [175-177]. In experimental animal models, the anti-neoplastic potential of interferon was demonstrated in already established tumors. In a transgenic mouse model it was demonstrated that early and prolonged administration of interferon diminished the severity of preneoplastic lesions and slowed down the development of HCC [178]. Interferon-alfa also could indirectly reduce the oncogenic risk by inhibition of synthesis of viral proteins which potentially dysregulate the cell cycle, and by enhancing the immune system eliminating not only infected hepatocytes but also initiated or fully malignant cells. Furthermore, interferon-alfa has an antifibrotic and anti-angiogenetic effect, which could also have an influence on tumor development [179].

**Interferon and antiviral treatment**

**Noncirrhotics**

In patients with chronic hepatitis B, clearance of the HBeAg after treatment with interferon-alfa is associated with improved clinical outcome in terms of survival and development of complications of cirrhosis [180]. Another study confirmed these results and showed a reduction of incidence of HCC in the responders [181]. As most of these patients were non-cirrhotics at entry of the study, the prophylactic effect of interferon on development of HCC can be explained by prevention of cirrhosis development. In Chinese patients with chronic hepatitis B infection, however, interferon-alfa was of no long-term benefit in inducing HBeAg conversion, or in the prevention of HCC and other cirrhosis-related complications [182].

**Cirrhotics**

Seven studies investigated the possible effect of interferon treatment on development of HCC in patients with already established cirrhosis [183-189] (Table 4). A meta-analysis was performed on these studies [190]. Interferon seemingly decreased the rate of HCC in all trials, while a significant difference was observed in 2 studies [183,186]. Virologic response was strongly associated with reduced risk for HCC in the studies of Oon [183] and Mazzella [184], suggesting that arrest of viral replication is a critical factor. Subgroup analysis in relation to ethnic origin of patients (European, Oriental) showed no preventive effect of interferon on the development of HCC in the European patients [190].

### Table 4: Studies of treatment with interferon-α for prevention of HCC in patients with hepatitis B-related cirrhosis

| Author [reference] | Country | Type of study | Interferon regimen (duration in weeks) | Follow-up (range) in months | Sample size | Rate of HCC (n/n) | Significance |
|--------------------|---------|---------------|----------------------------------------|-----------------------------|-------------|-----------------|-------------|
| Oon, 1992 [183]    | Singapore | NRCT, P       | 10 MU daily, 10 days/month (12)         | 12 (12–60)                  | T: 600      | C: 180          | T: 0/600 (0%) | Significant  |
| Mazzella, 1996 [184] | Italy   | NRCT, P       | 10 MU tiw (26)                         | 49 (12–119)                 | T: 34       | C: 28           | T: 2/34 (5.9%) | Not significant |
| Fattovich, 1997 [185] | Europe   | NRCT, P       | ≥ 300 MU (12–52)                       | 84 (80–92)                  | T: 40       | C: 50           | T: 3/40 (7.5%) | Not significant |
| Ikeda, 1998 [186] | Japan    | NRCT, P       | 12 MU/wk (26)                          | 84 (6–168)                  | T: 94       | C: 219          | T: 10/94 (10.6%) | Significant |
| IHCSG, 1998 [187] | Argentina, Germany, Italy, Saudi Arabia | NRCT, P | 9–30 MU/wk for 3–30 months | (36–250) | T: 49 | C: 97 | T: 8/49 (16.3%) | Not significant |
| Benvegnù, 1998 [188] | Italy | NRCT, P       | 6–10 MU (20–26)                        | 72                          | T: 10       | C: 18           | T: 0/10 (0%) | Not significant |
| Di Marco, 1999 [189] | Italy | NRCT, P       | 655 MU                                 | 93 (6–180)                  | T: 26       | C: 60           | T: 2/26 (7.7%) | NR |

NRCT: non-randomized controlled trial
P: prospective
T: treated
C: controls
MU: million units
NR: not reported

*Note: Table 4 data adapted from [180, 181]*
It should be noted that the studies are very heterogeneous and that none of them were randomized controlled trials, so that the results should be interpreted with caution.

A recent study showed a significant reduction of the risk of HCC in patients with chronic hepatitis B and advanced fibrosis or cirrhosis, treated with lamivudine for a maximum of five years, compared to placebo [191].

**Interferon treatment in HCV patients and HCC prevention**

**Noncirrhotics**

Three studies assessed whether interferon treatment prevents the development of HCC in noncirrhotic patients with chronic hepatitis C [192-194] comprising 3,798 noncirrhotic patients treated with interferon-alfa monotherapy. Pooled together, the incidence of HCC was 60/2,532 (2.37%) in sustained virological responders and 76/1,266 (5.29%) in nonresponders. In a study of 291 noncirrhotic patients with chronic hepatitis C who were nonresponders to interferon therapy and followed for 6–117 months after therapy, the incidence of HCC was significantly lower in patients who received > 500 MU of interferon. Patients with a transient response (i.e. relapse after end of treatment) had a significant lower rate of HCC development (4/166 = 2.4%) than nonresponders (12/125 = 9.6%) [195].

This anti-oncogenic benefit can presumably be explained by an arrest or slowing down of the cirrhotogenic process.

**Cirrhotics**

The findings of 13 studies of interferon treatment and development of HCC in HCV-infected patients with compensated cirrhosis are summarized in Table 5[45,184-186,194,196-204]. Only 3 studies were randomized [199,201,202,204], the remainders were observational cohort studies. Statistical combination of data is not possible because of different definitions of response (biochemical, virological), different dose schedules for interferon and different duration of follow-up. All studies showed a lower risk for development of HCC in the interferon-treated patients, suggesting that interferon may prevent HCC in compensated cirrhosis caused by hepatitis C. The overall result was largely influenced by three Japanese studies [194,198,201,202], which had the highest incidence of HCC in untreated patients (5–6% per year). This may be explained by intensiveness of the screening programs, but also by genetic, environmental or viral factors. Four European studies failed to document a significant reduction in risk of developing HCC [45,196,199]. In the studies of Fattovich et al [196] and Bruno et al [45], interferon-alfa treatment showed a decrease in incidence of HCC in univariate analysis. However, this was not present in multivariate analysis. In the study of Fattovich [196], a very low natural incidence of HCC was observed, rendering difficult to show a significant decrease. The prospective randomized controlled trial of Valla et al [199] also failed to show a significant effect of interferon treatment on the development of HCC. However, the number of patients in this study was limited and the follow-up relatively short. Also a recently published randomized controlled study from Italy comprising 51 interferon-treated and 71 untreated patients with compensated hepatitis C-cirrhosis, failed to demonstrate any reduced risk in development of HCC after a mean follow-up of 96.5 months [204].

In most studies, virological and/or biochemical response are associated with a lower risk of development of HCC, which is less clear in nonresponders. In the study of Imai et al. [198], patients with sustained biochemical response after interferon therapy were at low risk for development of HCC (risk ratio versus controls 0.06; 0.95 in nonresponders). Also in the study of Mazzella [184], a statistically significant effect of interferon treatment was demonstrated when biochemical responders were compared with controls but not when compared with nonresponders. In the study of Benvegnu et al. [188], the beneficial effect of interferon treatment on development of HCC was independent of the type of response. In the study of Yoshida et al [194] the risk for HCC was reduced especially among patients with sustained virological but also merely biochemical response that tested positive for HCV RNA. Okanoue et al [200] studied 1,148 patients with chronic hepatitis C treated with interferon-alfa, 40 of them having cirrhosis (fibrosis stage F4). They were followed for 1–7 years after therapy. The cumulative incidence of HCC was significantly decreased in sustained biochemical responders, compared to nonresponders and transient responders, in patients with stage F2 fibrosis, but not in the more advanced stages F3 and F4. In the study of Testino et al [204] HCC did also develop in sustained biochemical responders. Tanaka et al [205], however, demonstrated in 55 patients with HCV-cirrhosis that long-term administration of interferon prevented HCC in those with biochemical and virological response, whereas HCC only appeared in nonresponders.

The mechanisms by which an interferon treatment might reduce the risk of HCC development in cirrhosis caused by HCV independent of virological response remains speculative. Maintenance of serum transaminases at low levels may protect against the development of HCC as hepatocyte necrosis, cell damage and increase in hepatocyte replication result in increased DNA damage, influencing hepatocarcinogenesis. Other possible mechanisms for prevention of HCC are the direct and indirect effects of interferon. It is, however, perplexing that only 6 or 12 months of therapy can produce this benefit without virological response. Because of potential biases in the pub-
lished trials it is premature to advocate the use of interferon as established therapy in HCV infected patients with cirrhosis to prevent HCC. Prospective randomized controlled trials should reproduce the findings in large numbers of patients before a definitive conclusion on the long term effects of interferon in HCV cirrhosis can be established.

It must also be realized that a sustained virological response to interferon-alfa monotherapy can be obtained only in 0–8% of patients with cirrhosis [206-208]. New treatments are now available for chronic hepatitis C, which are more performant in difficulty to treat cases as patients with cirrhosis. The combination of interferon-alfa and ribavirin results in a sustained virologic response in up to 25% of cirrhotics due to hepatitis C [207]. A sustained virologic response of 32% was reported after peginterferon-alfa-2a monotherapy [207] and of 43% after combination of peginterferon-alfa2a and ribavirin [173]. It should be investigated in prospective trials, taking into account the sustained virological and biochemical responses if these more performant treatment regimens will also influence favorably the incidence of HCC, as no data on the long-term effects of these treatments are available up to now.

Secondary prevention
A few suiedes focus on the possible role of interferon in the secondary prevention of HCC recurrence in patients with chronic hepatitis B and C after curative resection or ablation.

Ikeda et al [209] showed that interferon prevented HCC recurrence after complete resection or ablation of the pri-
mary tumor depending on the clearance of HCV viremia. Kubo et al [210] reported a decreased recurrence after surgical resection independent of clearance of HCV or normalization of serum ALT. Another study demonstrated prevention of HCC recurrence after medical ablation therapy for primary tumors in hepatitis B but not in hepatitis C patients by the use of interferon-alfa [211].

Conclusions

Chronic hepatitis B and C, mostly in the cirrhotic stage, are responsible for the majority of the hepatocellular carcinomas worldwide. The rising incidence in HCC in developed countries during the last two decades is due to the increasing rate of hepatitis C infection and improvement of the clinical management of cirrhosis. Vaccination against hepatitis B seems to protect against the development of HCC.

In patients with chronic hepatitis B or C, interferon alpha treatment in a noncirrhotic stage is protective for HCC development in responders, probably by prevention of cirrhosis development. When cirrhosis is already present, the protective effect is less clear. Further prospective long-term studies should be performed on the new treatments for chronic hepatitis B and C. Some studies also suggested a favourable effect of interferon alpha in the prevention of HCC recurrence in patients with chronic hepatitis B and C after curative resection or ablation.

Competing interests

The author(s) declare that they have no competing interests.

Authors’ contributions

PPM participated in the literature search and was responsible for the redaction of the paper.

SMF participated in the redaction of the manuscript and critical review of the paper.

JLV participated in the literature search and finalizing of the lay-out of the paper.

References

1. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Paglieri L, Colombo M, Rodes J. EASL Panel of Experts on HCC: Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. J Hepatol 2001, 35:421-430.

2. Llovet JM, Beaugrand M: Hepatocellular carcinoma: present status and future prospects. J Hepatol 2003, 38(Suppl 1):136-149.

3. Bosch FX, Ribes J, Borràs J: Epidemiology of primary liver cancer. Semin Liver Dis 1999, 19:271-287.

4. Zaman SN, Melia WM, Johnson RD, Portmann BC, Johnson PJ, Williams R: Risk factors in development of hepatocellular carcinoma in cirrhosis: prospective study of 613 patients. Lancet 1985, 1:1357-1360.

5. Poyntard T, Aubert A, Lazizi Y, Bedossa P, Hamelin B, Terris B, Naveau S, Dubreuil P, Pillet J, Chaput JC: Independent risk factors for hepatocellular carcinoma in French drinkers. Hepatology 1991, 13:896-901.

6. Colomba M, de Franciis R, Del Ninno E, Sangiovanni A, De Fazio C, Tommasini M, Donato MF, Piva A, Di Carlo V, Dioguardi N: Hepatocellular carcinoma in Italian patients with cirrhosis. N Engl J Med 1991, 325:675-680.

7. Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, Nakashishi K, Fujimoto I, Inoue A, Yamazaki H, Kawashima T: Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 1993, 328:1797-1801.

8. Chevret S, Trinchet JC, Mathieu D, Rached AA, Beaugrand M, Chastang C: A new prognostic classification for predicting survival in patients with hepatocellular carcinoma. J Hepatol 1999, 31:133-141.

9. International Agency for Research on Cancer: Hepatitis Viruses. In IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Vol. 59. Lyon: IARC; 1994.

10. Kew MC, Dusheiko G, Hadzijnannis SJ, Paterson A: Does Delta infection play a part in the pathogenesis of Hepatitis B virus related hepatocellular carcinoma? Br Med J 1984, 288:1727.

11. Verme G, Brunetto MR, Oliveri F, Baldi M, Forzani B, Plantino P, Fonziello A, Bonino F: Role of hepatitis delta virus infection in hepatocellular carcinoma. J Hepatol 1987, 5:134-136.

12. EASL Jury: EASL International Consensus Conference on Hepatitis B. 13-14 September, 2002: Geneva, Switzerland. Consensus statement (short version). J Hepatol 2003, 38:533-540.

13. Yotsuyanagi H, Yasuda K, Iino S, Moriya K, Shimotani Y, Fujie H, Tsurumi T, Kimura S, Koike K: Persistent viremia after recovery from self-limited acute hepatitis B. Hepatology 1998, 27:1377-1382.

14. Okada K, Kamiyama I, Inomata M, Imai M, Miyakawa Y: e antigen and anti-e in the serum of asymptomatic carrier mothers as indicators of positive and negative transmission of hepatitis B virus to their infants. N Engl J Med 1976, 294:746-749.

15. Liaw YF, Tai DI, Chu CM, Lin DY, Sheen IS, Chen TJ, Pao CC: Early detection of hepatocellular carcinoma in patients with chronic type B hepatitis. A prospective study. Gastroenterology 1986, 90:263-267.

16. Beasley RP, Hwang LY, Lin CC, Chien CS: Hepatocellular carcinoma and hepatitis B virus, A prospective study of 22707 men in Taiwan. J Hepatol 1981, 1206-1213.

17. Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, Furuta S, Akahane Y, Nishioka K, Pucellin RH, Alter HJ: Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatitis B virus infections on the natural history of compensated cirrhosis: a cohort study of 297 E: Hepatitis B e antigen and the risk of hepatocellular carcinoma. J Hepatol 1993, 27:1392-1397.

18. Realdi G, Fattovich G, Hadziyanannis S, Schalm SW, Almasio P, Sanchez-Tapias J, Christensen E, Giustina G, Noventa F: Survival and prognostic factors in 366 patients with compensated cirrhosis type B: a multicenter study. J Hepatol 1994, 21:656-666.

19. Fattovich G, Giustina G, Schalm SW, Hadziyanannis S, Sanchez-Tapias J, Almasio P, Christensen E, Krosggaard K, Degos F, Carneiro De Moura M, Solinas A, Noventa F, Realdi G: Occurrence of hepatocellular carcinoma and decompenensation in western European patients with cirrhosis type B. Hepatology 1995, 21:77-82.

20. Fattovich G: Progression of hepatitis B and C to hepatocellular carcinoma in western countries. Hepatogastroenterology 1998, 45:1206-1213.

21. Fattovich G, Pantaleoni M, Zagni I, Realdi G, Schalm SW, Christensen E: Effect of hepatitis B and C virus infections on the natural history of compensated cirrhosis: a cohort study of 297 patients. J Gastroenterol 2002, 47:2886-2895.

22. Sakuma K, Saitoh N, Kasi M, Jitsukawa H, Yoshino I, Yamaguchi M, Nobutomo K, Yamumi M, Tsuchida F, Komazawa T: Relative risks of death due to liver disease among Japanese male adults having various statuses for hepatitis B and e antigen/antibody in serum: a prospective study. J Hepatol 1998, 28:1642-1646.

23. Yang HI, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, Hsiao CK, Chen PJ, Chen DS, Chen CJ: Taiwan Community-Based Cancer Screening Project Group: Hepatitis B antigen and the risk of hepatocellular carcinoma. N Engl J Med 2002, 347:168-174.

24. Ohkubo K, Kato Y, Ichikawa T, Kajiy A, Takeda Y, Higashi S, Hamaaki S, Nakao K, Nakata K, Eguchi K: Viral load is a significant prognostic factor for hepatitis B virus-associated hepatocellular carcinoma. Cancer 2002, 94:2663-2668.
25. Yano Y, Yamashita F, Sumie S, Ando E, Fukumori K, Kiyama M, Oyama T, Kuroki S, Kato O, Yamamoto H, Tanaka M, Sata M. Clinical features of hepatocellular carcinoma seronegative for both HBsAg and anti-HCV antibody but positive for anti-HBc antibody in Japan. Am J Gastroenterol 2002; 97:156-161.

26. Okamoto H, Tsudo F, Sakagawa H, Sastrosawiwijoyo R, Imai M, Miyakawa Y, Mayumi M. Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. J Gen Virol 1988, 69:2575-2583.

27. Stuyver L, De Gendt S, Van Geyt C, Zoulim F, Fried M, Schinazi RF, Rossau R: A new type of hepatitis B virus: complete genome and phylogenetic relatedness. J Gen Virol 2000, 81:17-74.

28. Arauz-Ruiz P, Norder H, Robertson BH, Magnius LO: Genotype H: a new American genotype of hepatitis B virus revealed in Central America. J Gen Virol 2002, 83:2059-2073.

29. Norder H, Courouce AM, Magnius LO: Complete genomes, phylogeny, relatedness, and structural protein of six strains of the hepatitis B virus, four of which represent two new genotypes. Virology 1994, 198:489-503.

30. Kao JH, Chen PJ, Lai MY, Chen DS: Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. Gastroenterology 2000, 118:555-569.

31. Ding X, Mizokami M, Yao G, Xu B, Orito E, Ueda R, Nakashima M. Hepatitis B virus genotype distribution among chronic hepatitis B virus carriers in Shanghai, China. Interim virology 2001, 44:43-47.

32. Orito E, Ichida T, Sakagawa H, Sata M, Horikke N, Hino K, Okita K, Okanoue T, Iino S, Tanaka E, Suzuki K, Watanabe H, Hige S, Mizokami M. Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. Hepatology 2001, 34:590-594.

33. Chen JD, Liu CJ, Lee PH, Chen PJ, Lai MY, Chen DS: Hepatitis B genotypes correlate with tumor recurrence after curative resection of hepatocellular carcinoma. Clin Gastroenterol Hepatol 2004, 2:64-71.

34. Kao JH, Chen PJ, Lai MY, Chen DS: Basal core promoter mutations of hepatitis B virus increase the risk of hepatocellular carcinoma in hepatitis B carriers. Gastroenterology 2003, 124:327-334.

35. Suguchi F, Ohno T, Orito E, Sakagawa H, Ichinda T, Komatsu M, Kuramitsuyu T, Ueda RF, Mizokami M. Infection of hepatitis B virus genotypes on the development of preS deletions and advanced liver disease. J Med Virol 2003, 70:537-544.

36. Anon: Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. J Hepatol 1999, 31:635-47.

37. Crowe J, Doyle C, Fielding JW, Holloway H, Keegan M, Kelleher D, Kelly P, Leader M, Little M, McDonald G, McCarthy CF, McWeeney J, O'Keane C, Rajan E: The role of hepatitis C virus infection in patients with chronic hepatitis B and C virus infections causing in hepatocellular carcinoma. Int J Cancer 1999, 86:793-798.

38. Hoofnagle JH, Manns M, Schiff ER, Van Thiel DH: Hepatitis C virus infection and cirrhosis: a prospective study. Hepatology 1991, 14:967-974.

39. van Thiel DH, Manns M, Schiff ER, Van Thiel DH: Hepatitis C virus and cirrhosis: a prospective study. Hepatology 1993, 18:47-53.

40. Benvegnu L, Pontisso P, Cavalletto D, Noventa F, Chemello L, Alberti A: Lack of correlation between hepatitis C virus genotypes and clinical course of hepatitis C virus-related cirrhosis. Hepatology 1997, 25:111-215.

41. Borondi L, Sofia S, Siringo S, Gaiani S, Casali A, Zironi G, Piscaglia F, Gramantieri L, Zanetti M, Sherman M. Surveillance programme of cirrhotic patients for early diagnosis and treatment of hepatocellular carcinoma: a cost effectiveness analysis. Gut 2001, 48:251-259.

42. Tong MJ, El Farra NS, Reikes AR, Co RL: Clinical outcomes after transfusion-associated hepatitis C. N Engl J Med 1995, 332:1463-1466.

43. Anon: Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. J Hepatol 1999, 31:635-47.
infected patients with chronic hepatitis C. Am J Gastroenterol 2001, 96:179-183.

65. Di Bisceglie AM, Klein JL, Woodruff JG, Sjogren MH, Kuo G, Houghton M, Choo QL, Hoofnagle JH: The role of chronic viral hepatitis in hepatocellular carcinoma in the United States. Am J Gastroenterol 1991, 86:335-338.

66. Gadzyniansis S, Tabor E, Kaklamani E, Tzonou A, Suver S, Tassopoulos N, Mueller N, Trichopoulos D: A case-control study of hepatitis B and C virus infections in the etiology of hepatocellular carcinoma. Int J Cancer 1995, 60:627-631.

67. Colombo M, Kuo G, Choo QL, Donato MF, Del Ninno E, Tommasini MA, Dioguardi N, Houghton M: Prevalence of antibodies to hepatitis C virus in patients with hepatocellular carcinoma. Lancet 1989, 2:1006-1008.

68. Nalpas B, Driss F, Pol S, Hamelin B, Houssot C, Brechtol C, Berthelot P: Association between HCV and HBV infection in hepatocellular carcinoma and alcoholic liver disease. J Hepatol 1991, 14:70-74.

69. Van Roey F, Fevery J, Van Steenbergen W: Hepatocellular carcinoma in Belgium: clinical and virological characteristics of 154 consecutive cirrhotic and non-cirrhotic patients. Ann Gastroenterol Hepatol 2000, 12:61-66.

70. Di Bisceglie AM, Klein JL, Woodruff JG, Sjogren MH, Kuo G, Houghton M, Choo QL, Hoofnagle JH: The role of chronic viral hepatitis in hepatocellular carcinoma in the United States. Am J Gastroenterol 1991, 86:335-338.

71. Gadzyniansis S, Tabor E, Kaklamani E, Tzonou A, Suver S, Tassopoulos N, Mueller N, Trichopoulos D: A case-control study of hepatitis B and C virus infections in the etiology of hepatocellular carcinoma. Int J Cancer 1995, 60:627-631.

72. Colombo M, Kuo G, Choo QL, Donato MF, Del Ninno E, Tommasini MA, Dioguardi N, Houghton M: Prevalence of antibodies to hepatitis C virus in patients with hepatocellular carcinoma. Lancet 1989, 2:1006-1008.

73. Nalpas B, Driss F, Pol S, Hamelin B, Houssot C, Brechtol C, Berthelot P: Association between HCV and HBV infection in hepatocellular carcinoma and alcoholic liver disease. J Hepatol 1991, 14:70-74.

74. Van Roey F, Fevery J, Van Steenbergen W: Hepatocellular carcinoma in Belgium: clinical and virological characteristics of 154 consecutive cirrhotic and non-cirrhotic patients. Ann Gastroenterol Hepatol 2000, 12:61-66.

75. Nalpas B, Driss F, Pol S, Hamelin B, Houssot C, Brechtol C, Berthelot P: Association between HCV and HBV infection in hepatocellular carcinoma and alcoholic liver disease. J Hepatol 1991, 14:70-74.

76. Van Roey F, Fevery J, Van Steenbergen W: Hepatocellular carcinoma in Belgium: clinical and virological characteristics of 154 consecutive cirrhotic and non-cirrhotic patients. Ann Gastroenterol Hepatol 2000, 12:61-66.

77. Haydon GH, Jarvis LM, Simmonds P, Harrison DJ, Garden OJ, Hayes PC: Association between chronic hepatitis C infection and hepatocellular carcinoma in a Scottish population. Gut 1997, 40:128-132.

78. Yeh FS, Yu MC, Mo CC, Luo S, Tong MJ, Henderson BE: Hepatitis B virus, aflatoxins, and hepatocellular carcinoma in southern Guangxi, China. Cancer Res 1989, 49:2506-2509.

79. El Serag HB, Mason AC: Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 1999, 340:745-750.

80. El Serag HB, Mason AC: Risk factors for the rising rates of primary liver cancer in the United States. Arch Intern Med 2000, 160:3277-3230.

81. Benhamiche AM, Fairev C, Minello A, Clindar F, Cartier C, Hillon P, Fairev J: Time trends and age-period-cohort effects on the incidence of primary liver cancer in a well-defined French population. 1976-1995. J Hepatol 1998, 29:802-806.

82. Lam MG, Roberts SK, Dore GJ, Kaldor JM: Primary hepatocellular carcinoma in Australia, 1978-1997: increasing incidence and mortality. Med J Aust 2000, 173:403-405.

83. Yoshizawa H: Hepatocellular carcinoma associated with hepatitis C virus infection in Japan: projection to other countries in the foreseeable future. Oncology 2002, 62(Suppl 1):8-17.

84. Kiyosawa K, Furuta S: Clinical aspects and epidemiology of hepatitis B and C viruses in hepatocellular carcinoma. Gastroenterology 1991, 101:1345-1348.

85. Deuffic S, Buffat L, Poynard T, Valleron AJ: The role of chronic hepatitis C in hepatocellular carcinoma: a case control study among Egyptian patients. J Clin Gastroenterol 2001, 33:13-26.

86. Kew MC, Houghton M, Choo QL, Kuo G: Hepatitis C virus antibodies in southern African blacks with hepatocellular carcinoma. Lancet 1990, 335:873-874.

87. Yu MC, Tong MJ, Courasges P, Ross RK, Govindaraj S, Henderson BE: Prevalence of hepatitis B and C viral markers in black and white patients with hepatocellular carcinoma in the United States. J Natl Cancer Inst 1990, 82:1038-1041.
the next two decades. Proc Natl Acad Sci USA 2002, 99:15584-15589.

104. Chang-Tea L, Current management of the complications of cirrhosis and portal hypertension: variceal hemorrhage, ascites, and spontaneous bacterial peritonitis. Gastroenterology 2001, 120:726-748.

105. Solmi L, Primerano AM, Gandolfi L: Ultrasonad follow-up of patients at risk for hepatocellular carcinoma: results of a prospective study on 360 cases. Am J Gastroenterol 1996, 91:1189-1194.

106. Gebo KA, Chander G, Jenckes MW, Gahnem KG, Herlong HF, Torbenson MS, El-Kamary SS, Bass EB. Screening tests for hepatocellular carcinoma in patients with chronic hepatitis C: a systematic review. Hepatology 2002, 36(Suppl 1):84-92.

107. Di Bisceglie AM, Hoofnagle JH: Elevations in serum alpha-feto-protein levels in patients with chronic hepatitis B. Cancer 1989, 64:2117-2120.

108. Izzo F, Cremona F, Ruffolo F, Palaia R, Parisi V, Curley SA: Outcome of 67 patients with hepatocellular cancer detected during screening of 1125 patients with chronic hepatitis. Ann Surg 1998, 227:513-518.

109. Sheu JC, Sung JL, Chen DS, Yang PM, Lai MY, Lee CS, Hsu HC, Chuang CN, Yang TI, Lin TH, Lin JT, Lee CQ: Growth rate of asymptomatic hepatocellular carcinoma and its clinical implications. Gastroenterology 1985, 89:259-266.

110. Collier J, Sherman M: Screening for hepatocellular carcinoma. Hepatology 1998, 27:273-278.

111. Hitomi F, Cremin JA, Sullfo P, Palia R, Parisi V, Curley SA: Outcome of 67 patients with hepatocellular cancer detected during screening of 1125 patients with chronic hepatitis. Ann Surg 1998, 227:513-518.

112. Kim CM, Koike K, Saito I, Miyamura T, Jay G: Adenomatous hyperplasia of the liver as a precancerous lesion. Liver 1993, 13:1-9.

113. Summers J, Smolec JM, Snyder R: A virus similar to human hepatitis B virus associated with hepatitis and hepatoma in woodchucks. Proc Natl Acad Sci USA 1978, 75:4533-4537.

114. Weinberg RA: Oncogenes, anti-oncogenes, and the molecular bases of multistep carcinogenesis. Cancer Res 1989, 49:3731-3732.

115. Idilman R, De Maria N, Colantino A, Van Thiel DH: Pathogenesis of hepatitis B and hepatitis D induced hepatocellular carcinoma. J Viral Hepat 1998, 5:285-298.

116. Nakamura Y, Terada T, Ueda K, Terasaki S, Nonomura A, Matsui O: Adenomatous hyperplasia of the liver as a precancerous lesion. Liver 1993, 13:1-9.

117. These ND: Mouse regenerative (dysplastic) nodules and hepatocarcinogenesis: theoretical and clinical considerations. Semin Liver Dis 1995, 15:360-371.

118. Sh Shibata M, Morizane T, Uchida T, Yamagami T, Onozuka Y, Nakano M, Miura K, Ueno Y: Irregular regeneration of hepatocytes and risk of hepatocellular carcinoma in chronic hepatitis and cirrhosis with hepatitis C virus infection. Lancet 1998, 351:1773-1777.

119. Sugtani S, Sakamoto M, Ichida T, Genda T, Asakura H, Hirohashi S: Hyperplastic foci reflect the risk of multicentric development of human hepatocellular carcinoma. J Hepatol 1998, 28:1045-1053.

120. Lee RG, Tamasand AC, Demetris AJ: Large cell change (liver cell dysplasia) and hepatocellular carcinoma in cirrhosis: matched case-control study, pathological analysis, and pathogenetic hypothesis. Hepatology 1997, 26:1415-1422.

121. Borzio M, Bruno S, Roncalli M, Mels GC, Ramella G, Borzio F, Leandro G, Servida E, Podd M: Liver cell dysplasia is a major risk factor for hepatocellular carcinoma in cirrhosis: a prospective study. Gastroenterology 1995, 108:812-817.

122. Zhao M, Zhang NX, Laissue JA, Zimmermann A: Immunohistochemical analysis of p53 protein overexpression in liver cell dysplasia and in hepatocellular carcinoma. Virchows Arch 1994, 424:613-621.

123. Kew MC: Hepatitis B and C viruses and hepatocellular carcinoma. Clin Lab Med 1996, 16:395-406.

124. Kew MC: Hepatitis viruses and hepatocellular carcinoma. Res Virol 1998, 149:257-262.

125. Chisari FV, Klopchin K, Moriyama T, Pasquinelli C, Dunsford HA, Sell S, Pinkert CA, Brinster RL, Palmiter RD: Molecular pathogenesis of hepatocellular carcinoma in hepatitis B virus transgenic mice. Cell 1989, 59:1145-1156.

126. Chen JW, Ye Q, Forgues M, Choa Y, Budhu A, Sime J, Hofseth LJ, Kaul R, Wang XY: Cancer-associated molecular signature in the tissue samples of patients with cirrhosis. Hepatology 2004, 39:518-527.

127. Lee JS, Chu IS, Hoon J, Calvisi DF, Sun Z, Roskams T, Perumel A, Demetris AJ, Thorgeirsson SS: Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. Hepatology 2004, 40:667-676.

128. Delpeuch O, Trabut JB, Carnot F, Feuillard J, Brechot C, Cremers D: Identification, using cDNA macroarray analysis, of distinct gene expression profiles associated with pathological and virological features of hepatocellular carcinoma. Oncogene 2002, 21:2926-2937.

129. Mann HW, Lee J, Bussard A, Liu C, Jin YR, Guha K, Clayton MM, Ardlie K, Pellini MJ, Feitelson MA: Preneoplastic markers of hepatocellular B virus associated hepatocellular carcinoma. Cancer Res 2004, 64:7329-7335.

130. Chen PJ, Chen DS, Lai MY, Chang MH, Huang GT, Yuan PM, Sheu JC, Lee SC, Hsu HC, Sung JL: Clonal origin of recurrent hepatocellular carcinomas. Gastroenterology 1989, 96(2 Pt 1):527-529.

131. Lugasay C, Berneau J, Thierys Y, Kroskaard K, Defort C, Wantzin P, Schalm SW, Rueff B, Benhamou JP, Tiollais P, Brechot C: Sequences of hepatitis B virus DNA in the serum and liver of patients with acute benign and fulminating hepatitis. J Infect Dis 1987, 155:64-71.

132. Koshy R, Maupas P, Muller R, Hofschenh PH: Detection of hepatitis B virus-specific DNA in the genomes of human hepatocellular carcinoma and liver cirrhosis tissues. J Gen Virol 1981, 57(1):95-102.

133. Rowley JD: Molecular cytogenetics: Rosetta stone for understanding cancer – twenty-ninth G. H. A. Clowes memorial award lecture. Cancer Res 1990, 50:3816-3825.

134. Schimke RT: The search for early genetic events in tumorigenesis: an amplification paradigm. Cancer Cells 1990, 2:149-151.

135. Slagle BL, Zhou YZ, Butel JS: Hepatitis B virus integration event in human chromosome 17p near the p53 gene identifies the region of the chromosome commonly deleted in virus-positive hepatocellular carcinomas. Cancer Res 1991, 51:49-54.

136. Robinson WS: Molecular events in the pathogenesis of hepatitis B virus-associated hepatocellular carcinoma. Annu Rev Med 1994, 45:297-323.

137. Shirakata Y, Kawada M, Fujiki Y, Sano H, Oda M, Yaginuma K, Kobayashi M, Koike K: The X gene of hepatitis B virus induces growth stimulation and tumorigenic transformation of Mouse NIH3T3 cells. Ann J Cancer Res 1989, 80:617-621.

138. Twu JS, Schloemer RH: Transcriptional trans-activating function of hepatitis B virus. J Virol 1987, 61:3448-3453.

139. Paterlini P, Pousain K, Kew M, Franco D, Brechot C: Selective accumulation of the X transcript of hepatitis B virus in patients negative for hepatitis B surface antigen with hepatocellular carcinoma. Hepatology 1995, 21:313-321.

140. Henkler FF, Koshy R: Hepatitis B virus transcriptional activators: mechanisms and possible role in oncogenesis. J Viral Hepat 1996, 3:109-121.

141. Wang XY, Forrester K, Yeh H, Feitelson MA, Gu JR, Harris CC: Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. Proc Natl Acad Sci USA 1994, 91:2230-2234.

142. Tschopp R, Antunovic J, Greenblatt J, Prives C, Cromlish JA: Direct interaction of the hepatitis B virus HBx protein with p53 leads to inhibition by HBx of p53 response element-directed transactivation. J Virol 1995, 69:1851-1859.

143. Zhao M, Ullrich SJ, Gangemi JD, Kappel CA, Ngo L, Feitelson MA, Jay G: Functional inactivation but not structural mutation of p53 causes liver cancer. Nat Genet 1995, 9:41-47.

144. Pasquinelli C, Bhavani K, Chisari FV: Multiple oncogenes and tumor suppressor genes are structurally and functionally intact during hepatocarcinogenesis in hepatitis B virus transgenic mice. Cancer Res 1992, 52:2827-2829.

145. Kekule AS, Lauer U, Meyer M, Caselmann WH, Hofschneider PH: The preS2/S region of integrated hepatitis B virus DNA encodes a transcriptional transactivator. Nature 1990, 343:457-461.
146. Fu XX, Su CY, Lee Y, Hintz R, Biempiaca L, Snyder R, Rogler CE: Insulinlike growth factor II expression and oval cell proliferation associated with hepatocarcinogenesis in woodchuck hepatitis virus carriers. J Virol 1988; 62:2492-3430.

147. Yeh SH, Lin MW, Lu SF, Wu DC, Tsai SF, Tsai CY, Lai MY, Hsu HC, Yano M, Kumada H, Kage M, Ikeda K, Shimamatsu K, Inoue O, Hashimoto G, Bruni CB, Riccio A, Zarrilli R: Determination of intracellular localization and appearance of hepatitis B virus. Hepatology 1999; 30:517-525.

148. Xu Z, Jensen G, Yen TS: Activation of hepatitis B virus promoters by the viral large surface protein via induction of stress in the endoplasmic reticulum. J Virol 1997; 71:7387-7392.

149. Bock CT, Tillmann HL, Manns MP, Trautwein C: The pre-S region determines the intracellular localization and appearance of hepatitis B virus. Hepatology 1999; 30:517-525.

150. Cholakzai JL, Chisari FV, Merlino G: Synergy between transforming growth factor alpha and hepatitis B virus surface antigen in hepatocellular proliferation and cancerization. Cancer Res 1993; 53:110-1113.

151. Shen Z, Jensen G, Yen TS: Activation of hepatitis B virus promoters by the viral large surface protein via induction of stress in the endoplasmic reticulum. J Virol 1997; 71:7387-7392.

152. Takamizawa A, Mori C, Fuke I, Manabe S, Murakami S, Fujita J, Onishi K, Kimura M, Shimada K, Inoue O, Hashimoto G: Activation of hepatitis B virus S promoter by the viral large surface protein via induction of stress in the endoplasmic reticulum. J Virol 1997; 71:7387-7392.

153. Borowski P, Oehlmann K, Heiland M, Laufs R: Determination of intracellular localization and appearance of hepatitis B virus. Hepatology 1999; 30:517-525.

154. Borowski P, Oehlmann K, Heiland M, Laufs R: Determination of intracellular localization and appearance of hepatitis B virus. Hepatology 1999; 30:517-525.

155. Borowski P, Oehlmann K, Heiland M, Laufs R: Determination of intracellular localization and appearance of hepatitis B virus. Hepatology 1999; 30:517-525.

156. Borowski P, Oehlmann K, Heiland M, Laufs R: Determination of intracellular localization and appearance of hepatitis B virus. Hepatology 1999; 30:517-525.

157. Borowski P, Oehlmann K, Heiland M, Laufs R: Determination of intracellular localization and appearance of hepatitis B virus. Hepatology 1999; 30:517-525.

158. Borowski P, Oehlmann K, Heiland M, Laufs R: Determination of intracellular localization and appearance of hepatitis B virus. Hepatology 1999; 30:517-525.
185. Fattovich G, Giustina G, Realdi G, Corrocher R, Schalm SW: Long-term outcome of hepatitis B e antigen-positive patients with compensated cirrhosis treated with interferon alfa. *Hepatology* 1997, 26:1338-1342.

186. Ikeda K, Saito S, Suzuki Y, Kobayashi M, Tsuota A, Fukuda M, Koida I, Arase Y, Chayama K, Murashima N, Kumada H: Interferon decreases hepatocellular carcinogenesis in patients with cirrhosis caused by the hepatitis B virus: a pilot study. *Cancer* 1998, 82:827-835.

187. International Interferon-alpha Hepatocellular Carcinoma Study Group: Effect of interferon-alpha on progression of cirrhosis to hepatocellular carcinoma: a retrospective cohort study. *Lancet* 1998, 351:1535-1539.

188. Benevenga L, Chemello L, Novella F, Fattovich G, Pontisso P, Alberti A: Retrospective analysis of the effect of interferon therapy on the clinical outcome of patients with viral cirrhosis. *Cancer* 1998, 83:901-909.

189. Di Marco V, Lo Iacono O, Camma C, Vaccaro A, Giunta M, Martorana O, Di Marco V, Lo Iacono O, Camma C, Vaccaro A, Giunta M, Martorana O, Di Marco V, Lo Iacono O, Camma C, Vaccaro A, Giunta M, Martorana O, Di Marco V, Lo Iacono O, Camma C, Vaccaro A, Giunta M, Martorana.

190. Camma C, Giunta M, Andreone P, Craxi A: Interferon and preven-tion of hepatocellular carcinoma in viral cirrhosis: an eval-uation. *J Hepatol* 2000, 34:593-602.

191. Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, Tanwande T, Tao QM, Shue K, Keene ON, Dixon JS, Gray DF, Sabbat J. Cirrhosis Asian Lamivudine Multicentre Study Group: Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004, 351:1521-1531.

192. Kasahara A, Hayashi N, Mochizuki K, Takayamagi M, Yoshikawa K, Kakumu S, ljiima A, Urushihara A, Kiyosawa K, Okuda M, Hino K, Okita K: Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. *Hepatitis C* 1998, 27:1394-1402.

193. Camma C, Di Marco V, Lo Iacono O, Almasio P, Giunta M, Fuschi P, Vaccaro A, Fabiano C, Magrini S, Di Stefano R, Bonura C, Pagliaro L, Craxi A: Long-term course of interferon-treated chronic hepatitis C. *J Hepatol* 1998, 28:531-537.

194. Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sato M, Inoue O, Yano M, Tanaka M, Fujimura S, Nishiguchi S, Kuroki T, Imazeki F, Poupon RE, Poupon R: Retrospective analysis of the effect of interferon therapy on the incidence of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach. *J Hepatol* 2000, 33:1636-1638.

195. Heathcote EJ, Shiffman ML, Thomas H, Cooksley WG, Dusheiko GM, Lee SS, Balart L, Reindollar R, Reddy RK, Wright TL, Lin A, Hoffman J, De Pampelis J: Peginterferon alfa-2a in patients with chronic hepatitis C and cirrhosis. *N Engl J Med* 2000, 343:1673-1680.

196. Pagliaro L, Craxi A, Camma C, Tine F, Di Marco V, Lo Iacono O, Almasio P: Interferon-ribavirin for chronic hepatitis C: An analysis of pretreatment clinical predictors of response. *Hepatology* 1994, 19:820-828.

197. Ikeda K, Arase Y, Saioht S, Kobashy M, Suzuki Y, Tsubota A, Chayama K, Murashima N, Kumada H: Interferon beta prevents recurrence of hepatocellular carcinoma after complete resection or ablation of the primary tumor: A prospective randomized study of hepatitis C virus-related liver cancer. *Hepatology* 2000, 32:228-232.

198. Nishiguchi S, Hiroshiki S, Hirashiki K, Tanaka H, Shuto T, Kinoshita H: Randomized clinical trial of long-term outcome after resection of hepatocellular carcinoma related to hepatocellular carcinoma by postoperative interferon therapy. *Br J Surg* 2002, 89:418-422.

199. Long-term outcome of interferon therapy in hepatitis C virus-associated cirrhosis: does IFN prevent development of hepatocellular carcinoma? *Oncol Rep* 1998, 5:205-208.

200. Nishiguchi S, Hiroshiki S, Hirashiki K, Tanaka H, Shuto T, Kinoshita H: Randomized clinical trial of long-term outcome after resection of hepatocellular carcinoma related to hepatocellular carcinoma by postoperative interferon therapy. *Br J Surg* 2002, 89:418-422.

201. Nishiguchi S, Hiroshiki S, Hirashiki K, Tanaka H, Shuto T, Kinoshita H: Randomized clinical trial of long-term outcome after resection of hepatocellular carcinoma related to hepatocellular carcinoma by postoperative interferon therapy. *Br J Surg* 2002, 89:418-422.

202. Nishiguchi S, Hiroshiki S, Hirashiki K, Tanaka H, Shuto T, Kinoshita H: Randomized clinical trial of long-term outcome after resection of hepatocellular carcinoma related to hepatocellular carcinoma by postoperative interferon therapy. *Br J Surg* 2002, 89:418-422.