RISK OF HEPATOCELLULAR CARCINOMA IN CHRONIC VIRAL HEPATITIS

Lara J Kane[1] and Graeme A Macdonald[1,2]

1. Department of Gastroenterology and Hepatology, Princess Alexandra Hospital, Brisbane, Australia.

2. Diamantina Institute of Cancer Immunology and Metabolic Medicine, University of Queensland, Brisbane, Australia.

Email: g.macdonald@uq.edu.au

Abstract

Chronic viral hepatitis B and C infections are the diseases associated with the highest risk for developing hepatocellular carcinoma. These infections are prevalent worldwide. Many factors modulate the risk of developing hepatocellular carcinoma in chronic viral hepatitis, such that the assessment of an individual patient's risk is a complex consideration. The presence of cirrhosis is the most important risk factor for the development of hepatocellular carcinoma in both hepatitis B and C. Thus, one of the major mechanisms for hepatocarcinogenesis in these infections is mediated in some way through chronic liver injury. In addition, there is evidence to support a direct oncogenic effect of both HBV and HCV, although the evidence is weaker for HCV. Other risk factors for hepatocellular carcinoma in chronic viral hepatitis include: geographical location; whether in a high or low prevalence area; host factors, particularly sex and age; and specific viral factors. In chronic hepatitis B without cirrhosis the risk of hepatocellular carcinoma is 0.5%-0.8% per annum, increasing to 1.4-2.5% in cirrhotic patients. In chronic hepatitis C with cirrhosis the risk is 1.4-2.5% per annum, while in Australia, patients with hepatitis C rarely develop hepatocellular carcinoma in the absence of cirrhosis.

Hepatocellular carcinoma (HCC) is a complication of chronic viral hepatitis and is associated with significant morbidity and mortality. Hepatitis B, C and D (HBV, HCV and HDV) are the viral hepatitides associated with chronic infection and HCC. The worldwide burden of chronic viral hepatitis is significant – approximately 400 million people (7%) with chronic HBV (CHB)and 170 million (3%) with chronic HCV (CHC).[1,2] In Australia, an estimated 91,500 to 163,500 people (0.5% to 0.9%) have CHB and 210,000 (1.1%) CHC.[3,4] These two infections are the most important diseases associated with the development of HCC. Worldwide, more than 50% of registered cases of HCC are associated with CHB and 25% with CHC.[5,6] In developed countries, up to 70% of HCC is attributable to HCV,[7-9] whereas in Asia and Africa, HBV is mainly responsible.[10]

While the viral hepatitides are a major cause of HCC worldwide, determining the risk associated with being infected with these viruses is not straight forward. Other recognised risk factors for HCC include the presence of cirrhosis, viral co-infection, age, sex, alcohol exposure, obesity and diabetes mellitus. Genetic factors also appear to play a role, as well as environmental agents. The interactions between these factors and HCC are complex. In its simplest form, the viral agents: may be directly oncogenic; may contribute to HCC risk by causing chronic liver damage that results in cirrhosis; or may be associated with other risk factors such as diabetes.

Role of cirrhosis

In general, the presence of cirrhosis in both HBV and HCV is the most important risk factor for HCC.[11] In developed countries, 85% of HCC in CHB arises in cirrhotic livers, with the remainder occurring in non-cirrhotic livers.[7,12] In CHC, HCC is uncommon in the absence of cirrhosis, at least in western countries. Risk factors for progression to cirrhosis and the development of HCC in viral hepatitis are well described. In CHB, these include young age at the time of infection, longer duration of infection, elevated serum alanine aminotransferase (ALT) levels, male gender, alcohol excess and viral co-infection with HCV, HDV and/or HIV.[13] Serum HBV DNA concentration is a key predictor of the development of cirrhosis and HCC.[14-16] The importance of other HBV viral factors remains incompletely understood. In HCV, in addition to co-infection with HBV and/or HIV, progression of liver disease is adversely affected by alcohol, smoking, hepatic steatosis and insulin resistance.[17]

Other risk factors for HCC

Men are more likely to develop HCC than women.[18] This is most evident in high prevalence regions, where men are affected 2.1 to 5.7 times more frequently than women (mean 3.7:1). The ratio is lower (mean of 2.4:1) in intermediate prevalence areas, and is lower again in low prevalence regions.[19] The differences in gender distribution may reflect variations in hepatitis carrier states, exposure to environmental toxins, and the trophic effect of androgens. Age >50 year increases risk for HCC 4-fold compared with younger individuals.[20] The effect may be due to age *per se*, or be a consequence of longer duration of infection. Alcohol appears to synergistically increase the risk of developing HCC. The risk in HBsAg positive populations is doubled in those who drink more than 60g/day of alcohol compared to non-drinkers.[21] Smoking is associated with a 1.5-2.0 fold increase in the risk of HCC compared to non-smokers.[22,23] Obesity is another risk

factor for HCC in CHB,[24] especially in combination with alcohol, tobacco and diabetes.[25] Environmental factors also play a role, with the dietary mycotoxin, aflatoxin B1 found on mouldy food, being a major contributor to HCC risk in regions with a high prevalence of HCC such as sub-Saharan Africa.[26,27] While this is not a significant problem in Australia, it is relevant for immigrants from these regions.

Risk of HCC in viral hepatitis compared to other liver diseases

Although there are relatively few studies directly comparing the incidence of HCC in different liver diseases, there appears to be significant variation. Fattovich et al compared the five-year cumulative incidence of HCC in patients with cirrhosis from different aetiologies.[13] In CHC with cirrhosis in western countries this was 17%, and up to 30% in Japan, while for CHB these figures were 10% in the west and 15% in highly endemic regions. This compared with 21% for hereditary haemochromatosis, 8% in alcoholic cirrhotics and 4% in advanced biliary cirrhosis. There is limited data on HCC risk in cirrhosis of other causes. It is tempting to ascribe this to the underlying liver disease, but it may also reflect the disease specific processes that contribute to the development of cirrhosis. For example, cirrhosis from haemochromatosis is more likely to occur in older males, the group at highest risk of developing HCC, while biliary cirrhosis is more likely to occur in younger women, a lower risk group. In an old study of the prevalence of HCC at autopsy in patients with cirrhosis, the proportion of patients in each disease group with HCC was closely related to the proportion that were male.[28] Thus, the variation in HCC risk between different chronic liver diseases does not necessarily reflect direct oncogenic effects of the underlying disease.

Risk of HCC in chronic hepatitis B infection

HBV is a DNA hepadnavirus. It is transmitted by perinatal, parenteral and sexual exposure. In highly endemic areas such as Eastern Asia, China and Africa, approximately 70% of HBV infections are acquired either perinatally or in early childhood.[29] Perinatal exposure leads to chronic infection in 90-95% of cases, while childhood exposure leads to CHB in 50% of cases. The lifetime risk of cirrhosis is 20-30% in perinatal and childhood infections. In low prevalence areas such as Australia, North America and Western Europe, infection mostly occurs in adulthood through sexual contact or injecting drug use. Ninety five per cent of adults acutely infected will clear HBV and become immune.[30,31] HCC develops in 0.5%-0.8% per annum in patients with CHB compared with 1.4-2.5% in those with cirrhosis secondary to CHB.[32,33]

Epidemiological data strongly supports a causal relationship between CHB and HCC. The regional variation in the incidence of HCC worldwide mirrors the prevalence of CHB in the local population.[34,35] In highly endemic countries such as Taiwan with successful immunisation programs, there has been a decline in both the prevalence of CHB and in the incidence of HCC.[36] Experimental data using animal hepadnavirus provides additional support for this relationship. Newborn woodchucks inoculated with woodchuck hepatitis virus (a hepadnavirus used as

a model of human HBV infection) develop chronic viral hepatitis and HCC within three years.[37]

Mechanisms of carcinogenesis in chronic hepatitis B infection

As stated above, in CHB, HCC usually occurs in cirrhotic patients. However, in 20% of cases in the developed world and 40% in sub-Saharan Africa and China, HCC occurs in non-cirrhotic livers.[38] The contribution of hepatocellular injury and fibrosis in non-cirrhotic patients with CHB and HCC is difficult to quantify, but there is evidence that HBV is directly oncogenic. HBV DNA integrates into the host genome leading to alterations in cellular signalling and growth control. Chromosomal alterations are significantly increased in HBV-related tumours compared with tumours associated with other liver diseases.[39,40] Additionally, HBV proteins may enhance genomic instability. The HBV encoded X antigen (HBxAg) produced in chronically infected cells facilitates malignant transformation through several mechanisms. HBxAg has a direct stimulatory effect on cell growth. It binds and inactivates the key tumour suppressor p53 protein and may interfere with DNA repair mechanisms, allowing genomic damage to accumulate.[41,42] The usual site of viral integration into the host DNA is adjacent to the HBx gene, facilitating expression of the associated protein.[41] It is likely that the cellular immune response against infected hepatocytes, combined with long-term toxic effects of viral gene products, trigger chronic necroinflammation with subsequent fibrosis and hepatocyte proliferation, increasing the likelihood of malignant transformation.

Hepatitis B viral factors which modify risk of HCC

Serum HBV DNA levels across a biological gradient appear strongly predictive of the risk of disease progression and the development of HCC, independently of HBeAg status, serum ALT and liver cirrhosis.[14,16] In a community based survey, Taiwanese patients developed HCC at a 10 times greater rate if HBV DNA was persistently >20,000 IU/mL than in those with HBBV DNA <2,000 IU/mL. However, even with a serum HBV DNA titre of 2000 IU/mL, an increased risk for HCC existed.[15]

The importance of HBV e antigen (HBeAg) sero-status, pre-core or core promoter mutants and HBV genotype in relation to HCC risk remains incompletely understood. Several case control studies suggest that HBeAg positivity may be a predictive marker for HCC. HBeAg prevalence is higher among patients with HCC than among matched HBsAg carriers. A large cohort study found that the relative risk of HCC was increased by six-fold among patients who were HBeAg and HBsAg positive, compared with those positive for HBsAg alone.[23]

HBV genotype appears to play a role in Asian studies of genotype B and C HBV.[43,44] Genotype C has been shown to have a more aggressive disease course than genotype B in HBeAg positive patients and is an independent risk factor for HCC, with an adjusted relative risk of 2.8. The relative risk associated with cirrhosis was 10.2.[45] In Western Europe and North America, genotype D is

FORUM

associated with more severe liver disease and higher incidence of HCC, than genotype A.[46]

The prevalence of the T1762/A1764 mutation in the basal core promoter region increases with the progression of liver disease and this mutation is significantly associated with the development of HCC, in both genotypes B and C.[47] The T1762/A1764 mutation can be detected in plasma up to eight years prior to HCC diagnosis and may be a strong predictive biomarker of HCC.[47,48]

Risk of HCC in chronic hepatitis C

HCV is a positive single-stranded RNA flavivirus. Its mode of transmission is predominately parenteral. In Australia, at least 80% of patients became infected through injecting drug use.[49,50] Most people infected with HCV (up to 80%), are unable to spontaneously eliminate the virus and progress to CHC.[51-53] CHC is the causative agent associated with the majority of HCC in developed countries, where up to 70% of patients with HCC have anti-HCV antibodies in serum.[7-9] The risk of HCC in CHC is 1.2-1.7% per annum in patients with underlying chronic hepatitis and 1.4-2.5% per annum in those with cirrhosis.[32,54,55,56]

Mechanisms of carcinogenesis in chronic hepatitis C infection

HCV does not integrate into the host genome as reverse transcription of viral RNA to DNA does not occur.[57] In CHC, HCC almost always arises in the setting of cirrhosis. The likely mechanism of hepatocarcinogenesis is chronic necroinflammation, cellular regeneration and fibrosis which predispose to genomic damage.[51,58] HCC in patients with CHC who are not cirrhotic has been reported.[59,60] However, there is limited evidence supporting a direct carcinogenic role for HCV. Animal models provide support for a direct oncogenic effect of HCV. Transgenic mice expressing the complete HCV core gene at similar levels to that found in human infection develop hepatic steatosis after three months, adenomas after 12 months and eventually HCCs within the adenomas. This was in the absence of significant inflammation or fibrosis. HCC did not develop in mice expressing HCV envelope proteins, suggesting that the oncogenic potential is specific for the HCV core protein.[61] In vitro studies have also shown that HCV core peptide can bind to and influence proteins involved in the regulation of apoptosis and hepatocyte proliferation, including p53, tumour necrosis factor receptor 1, the Fas system, nuclear factor-kappa and the cell cycle regulator, p21WAF1. These interactions may contribute to the development of HCC.

Factors which modify risk of HCC in chronic hepatitis C infection

The risk for patients with CHC developing HCC varies by country of report, length of follow-up and presence of cirrhosis. As occurs in other liver diseases, males are at increased risk of HCC.[56,62] Other risk factors in CHC are longer duration of infection and age greater than 60 years.[32,63] Among patients with HCV cirrhosis the risk of HCC is significantly increased in current smokers and former heavy drinkers, but is not significantly increased in current heavy drinkers.[54] This suggests that alcohol is an important risk factor for progression to cirrhosis, but once cirrhotic, alcohol does not confer any additional risk for HCC than that attributable to cirrhosis alone. Cigarette smoking may have a role in the development of HCC from liver cirrhosis.

Steatosis and insulin resistance are frequently observed in CHC. Steatosis is associated with an increased rate of progression of hepatic fibrosis.[64,65] In a local study, hepatic steatosis was not associated with increased risk of HCC.[66] Among blacks, Hispanic and non-Hispanic whites in Los Angeles with CHC and/or CHB, diabetes was shown to be an independent risk factor for HCC.[67] Synergistic effects on HCC risk of HBV/HCV co-infection, hazardous alcohol consumption and diabetes were demonstrated in this study population.[68]

The role of HCV genotype is undefined. Some reports indicate increased association of HCC with genotype 1b,[32,69] while others have not found this association.[63] Treatment with interferon reduces the incidence of HCC in some studies, but not in others.[33,56,63,70]

Risk of hepatocellular carcinoma in viral co-infection

HBV and HCV co-infection is prevalent, occurring in more than 10% of CHB patients worldwide.[71] Patients with co-infection have more severe liver disease and are more likely to develop cirrhosis with decompensation. In addition, they have a higher risk of developing HCC than individuals with HCV or HBV alone.[7,72,73] After five years of co-infection with CHB and CHC, the cumulative risk of developing HCC is 23%, compared with 10% for CHB and 21% for CHC.[74] By 10 years the risk of HCC in co-infection is as high as 45%, compared with 16% and 28% for CHB and CHC respectively.[74] The risk of HCC in HBV/HCV co-infection has been analysed in a meta-analysis.[75,76] This found a more than additive effect of co-infection with HBV and HCV on the risk of developing HCC. The odds ratio for HCC compared to the non-infected population for HBsAg positive, anti-HCV/HCV RNA negative subjects was 20.4. In HBsAg negative, anti-HCV/HCV RNA positive subjects it was 23.6 and in subjects positive for both markers it was 135.

HDV super-infection in CHB is associated with more severe liver disease and accelerated progression to cirrhosis. The effect of HDV infection on HCC risk was evaluated in the EUROHEP retrospective cohort study of 200 HBsAg positive compensated cirrhotic patients, followed for a mean of 6.6 years. HDV co-infection was present in 20% of the population and was associated with a 3-fold increased risk of HCC.[20,77]

In the context of prolonged survival of HIV patients on highly active antiretroviral therapy, HCV/HIV co-infection results in the accelerated development of cirrhosis, liver failure and HCC.[76,78,79] Likewise, HBV/HIV co-infection leads to increased liver fibrosis, cirrhosis, HCC and liver-related mortality.[80,81] Schistosomiasis is a major health problem in Africa, particularly in Egypt, and is relevant to migrants from these areas. Schistosomiasis in genotype 4 HCV

appears to worsen portal hypertension with accelerated progression to fibrosis and HCC.[82,83]

In summary, in CHB and CHC, host and environmental factors modulate the risk of developing HCC. For both viruses, the presence of cirrhosis is a major contributor to HCC risk. This may be mediated through necrosis and inflammation related to viral infection, resulting in genotoxicity and enhanced hepatocyte proliferation. There is evidence supporting a direct carcinogenic effect for both viruses, although the evidence in support of this appears stronger for HBV. Viral factors may impact upon HCC risk. The role of viral genotype requires further study, as do other viral factors. Longer duration of infection, age of acquisition, serum ALT, viral co-infection, male gender, alcohol excess, cigarette smoking and hepatic steatosis all appear to increase the risk of HCC in patients with chronic hepatitis B or C.

References

1. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat. 2004;11(2):97-107.

2. Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. J Viral Hepat. 1999;6(1):35-47.

3. O'Sullivan BG, Gidding HF, Law M, Kaldor JM, Gilbert GL, Dore GJ. Estimates of chronic hepatitis B virus infection in Australia, 2000. Aust NZ J Public Health. 2004;28(3):212-216.

4. McDonald A. HIV/AIDs, Viral Hepatitis and Sexually Transmissible Infections in Australia Annual Surveillance Report 2008. NCiHEaCR. 2008.

5. Anthony PP. Hepatocellular carcinoma: an overview. Histopathology. 2001;39(2):109-118.

6. Block TM, Mehta AS, Fimmel CJ, Jordan R. Molecular viral oncology of hepatocellular carcinoma. Oncogene. 2003;22(33):5093-5107.

7. Montalto G, Cervello M, Giannitrapani L, Dantona F, Terranova A, Castagnetta LA. Epidemiology, risk factors, and natural history of hepatocellular carcinoma. Ann N Y Acad Sci. 2002;963:13-20.

8. Colombo M, Kuo G, Choo QL, Donato MF, Del Ninno E, Tommasini MA, et al. Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. Lancet. 1989;2(8670):1006-1008.

9. Yano M, Houghton M, Choo QL, Kuo G, Nishioka K, Watanabe J, et al. A high prevalence of antibody to the hepatitis C virus in patients with hepatocellular carcinoma in Japan. Cancer. 1991;67(2):429-433.

10. Pisani P, Parkin DM, Munoz N, Ferlay J. Cancer and infection: estimates of the attributable fraction in 1990. Cancer Epidemiol Biomarkers Prev. 1997;6(6):387-400.

11. Beasley RP. Hepatitis B virus. The major etiology of hepatocellular carcinoma. Cancer. 1988;61(10):1942-1956.

12. Okuda K, Nakashima T, Kojiro M, Kondo Y, Wada K. Hepatocellular carcinoma without cirrhosis in Japanese patients. Gastroenterology. 1989;97(1):140-146.

13. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology. 2004;127(5 Suppl 1):S35-50.

14. Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology. 2006;130(3):678-686.

15. Chen CJ, Iloeje UH, Yang HI. Long-term outcomes in hepatitis B: the REVEAL-HBV study. Clin Liver Dis. 2007;11(4):797-816, viii.

16. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. 2006;295(1):65-73.

17. Wursthorn K, Manns MP, Wedemeyer H. Natural history: the importance of viral load, liver damage and HCC. Best Pract Res Clin Gastroenterol. 2008;22(6):1063-1079.

18. Law MG, Roberts SK, Dore GJ, Kaldor JM. Primary hepatocellular carcinoma in Australia, 1978-1997: increasing incidence and mortality. Med J Aust. 2000;173(8):403-405.

19. Okuda K. Epidemiology of primary liver cancer. In: Primary liver cancer in Japan. Tobe T, editor. Tokyo: Springer-Verlag;1992. p3.

20. Fattovich G, Giustina G, Schalm SW, Hadziyannis S, Sanchez-Tapias J, Almasio P, et al. Occurrence of hepatocellular carcinoma and decompensation in western European patients with cirrhosis type B. The EUROHEP Study Group on Hepatitis B Virus and Cirrhosis. Hepatology. 1995;21(1):77-82.

21. Donato F, Tagger A, Gelatti U, Parrinello G, Boffetta P, Albertini A, et al. Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. Am J Epidemiol. 2002;155(4):323-331.

22. Chen ZM, Liu BQ, Boreham J, Wu YP, Chen JS, Peto R: Smoking and liver cancer in China: case-control comparison of 36,000 liver cancer deaths vs. 17,000 cirrhosis deaths. Int J Cancer. 2003;107(1):106-112.

23. Yang HI, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, et al. Hepatitis B e antigen and the risk of hepatocellular carcinoma. N Engl J Med. 2002;347(3):168-174.

24. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med. 2003;348(17):1625-1638.

25. Donato F, Gelatti U, Limina RM, Fattovich G. Southern Europe as an example of interaction between various environmental factors: a systematic review of the epidemiologic evidence. Oncogene. 2006;25(27):3756-3770.

26. Ross RK, Yuan JM, Yu MC, Wogan GN, Qian GS, Tu JT, et al. Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. Lancet. 1992;339(8799):943-946.

27. Groopman JD, Scholl P, Wang JS. Epidemiology of human aflatoxin exposures and their relationship to liver cancer. Prog Clin Biol Res. 1996;395:211-222.

28. Johnson PJ, Krasner N, Portmann B, Eddleston AL, Williams R. Hepatocellular carcinoma in Great Britain: influence of age, sex, HBsAg status, and aetiology of underlying cirrhosis. Gut. 1978;19(11):1022-1026.

29. Global progress toward universal childhood hepatitis B vaccination, 2003. MMWR Morb Mortal Wkly Rep. 2003;52(36):868-870.

30. Fattovich G. Natural history and prognosis of hepatitis B. Semin Liver Dis. 2003;23(1):47-58.

31. McMahon BJ. The natural history of chronic hepatitis B virus infection. Semin Liver Dis. 2004;24 Suppl 1:17-21.

32. Bruno S, Silini E, Crosignani A, Borzio F, Leandro G, Bono F, et al. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: a prospective study. Hepatology. 1997;25(3):754-758.

33. Fattovich G, Giustina G, Degos F, Diodati G, Tremolada F, Nevens F, et al. Effectiveness of interferon alfa on incidence of hepatocellular carcinoma and decompensation in cirrhosis type C. European Concerted Action on Viral Hepatitis (EUROHEP). J Hepatol. 1997;27(1):201-205.

34. Conjeevaram HS, Lok AS. Management of chronic hepatitis B. J Hepatol. 2003;38 Suppl 1:S90-103.

35. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. Int J Cancer. 2001;94(2):153-156.

36. Chang MH, Chen CJ, Lai MS, Hsu HM, Wu TC, Kong MS, et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. N Engl J Med. 1997;336(26):1855-1859.

37. Popper H, Roth L, Purcell RH, Tennant BC, Gerin JL. Hepatocarcinogenicity of the woodchuck hepatitis virus. Proc Natl Acad Sci USA. 1987;84(3):866-870.

38. Murakami T, Baron RL, Peterson MS, Oliver JH 3rd, Davis PL, Confer SR, et al. Hepatocellular carcinoma: MR imaging with mangafodipir trisodium (Mn-DPDP). Radiology. 1996;200(1):69-77.

39. Marchio A, Pineau P, Meddeb M, Terris B, Tiollais P, Bernheim A, et al. Distinct chromosomal abnormality pattern in primary liver cancer of non-B, non-C patients. Oncogene. 2000;19(33):3733-3738.

40. Laurent-Puig P, Legoix P, Bluteau O, Belghiti J, Franco D, Binot F, et al. Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis. Gastroenterology. 2001;120(7):1763-1773.

41. Feitelson MA, Duan LX. Hepatitis B virus X antigen in the pathogenesis of chronic infections and the development of hepatocellular carcinoma. Am J Pathol. 1997;150(4):1141-1157.

42. Tagger A, Donato F, Ribero ML, Chiesa R, Portera G, Gelatti U, et al. Case-control study on hepatitis C virus (HCV) as a risk factor for hepatocellular carcinoma: the role of HCV genotypes and the synergism with hepatitis B virus and alcohol. Brescia HCC Study. Int J Cancer. 1999;81(5):695-699.

43. Ni YH, Chang MH, Wang KJ, Hsu HY, Chen HL, Kao JH, et al. Clinical relevance of hepatitis B virus genotype in children with chronic infection and hepatocellular carcinoma. Gastroenterology. 2004;127(6):1733-1738.

44. Orito E, Mizokami M. Hepatitis B virus genotypes and hepatocellular carcinoma in Japan. Intervirology. 2003;46(6):408-412.

45. Chan HL, Hui AY, Wong ML, Tse AML, Hung LCT, Wong VWS, et al. Genotype C hepatitis B virus infection is associated with an increased risk of hepatocellular carcinoma. Gut. 2004;53(10):1494-1498.

46. Liu CJ, Kao JH. Hepatitis B virus-related hepatocellular carcinoma: epidemiology and pathogenic role of viral factors. J Chin Med Assoc. 2007;70(4):141-145.

47. 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(2):327-334.

48. Kuang SY, Jackson PE, Wang JB, Lu PX, Muñoz A, Qian GS, et al. Specific mutations of hepatitis B virus in plasma predict liver cancer development. Proc Natl Acad Sci USA. 2004;101(10):3575-3580.

49. Garner JJ, Gaughwin M, Dodding J, Wilson K. Prevalence of hepatitis C infection in pregnant women in South Australia. Med J Aust. 1997;167(9):470-472.

50. Selvey LA, Lush D, Mistry SA, Sheridan JW, Krause V, Passaris I, et al. Investigation of notifications of hepatitis C in 1994: the experience of three health departments. Aust N Z J Public Health. 1996;20(5):525-529.

51. Suruki RY, Mueller N, Hayashi K, Harn D, DeGruttola V, Raker CA, et al. Host immune status and incidence of hepatocellular carcinoma among subjects infected with hepatitis C virus: a nested case-control study in Japan. Cancer Epidemiol Biomarkers Prev. 2006;15(12):2521-2525.

52. Gruner NH, Gerlach TJ, Jung MC, Diepolder HM, Schirren CA, Schraut WW, et al. Association of hepatitis C virus-specific CD8+ T cells with viral clearance in acute hepatitis C. J Infect Dis. 2000;181(5):1528-1536.

53. Tsai SL, Liaw YF, Chen MH, Huang CY, Kuo GC. Detection of type 2-like T-helper cells in hepatitis C virus infection: implications for hepatitis C virus chronicity. Hepatology. 1997;25(2):449-458.

54. Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med. 1993;328(25):1797-1801.

55. Takano S, Yokosuka O, Imazeki F, Tagawa M, Omata M. Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients. Hepatology. 1995;21(3):650-655.

56. Fattovich G, Giustina G, Degos F, Tremolada F, Diodati G, Almasio P, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. Gastroenterology. 1997;112(2):463-472.

57. Gerber MA. Relation of hepatitis C virus to hepatocellular carcinoma. J Hepatol. 1993;17 Suppl 3:S108-111.

58. Ahn J, Flamm SL. Hepatocellular carcinoma. Dis Mon. 2004;50(10):556-573.

59. De Mitri MS, Poussin K, Baccarini P, Pontisso P, D'Errico A, Simon N, et al. HCV-associated liver cancer without cirrhosis. Lancet. 1995;345(8947):413-415.

60. el-Refaie A, Savage K, Bhattacharya S, Khakoo S, Harrison TJ, el-Batanony M, et al. HCV-associated hepatocellular carcinoma without cirrhosis. J Hepatol. 1996;24(3):277-285.

61. Moriya K, Fujie H, Shintani Y, Yotsuyanagi H, Tsutsumi T, Ishibashi K, et al. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. Nat Med. 1998;4(9):1065-1067.

62. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet. 1997;349(9055):825-832.

63. Niederau C, Lange S, Heintges T, Erhardt A, Buschkamp M, Hürter D, et al. Prognosis of chronic hepatitis C: results of a large, prospective cohort study. Hepatology. 1998;28(6):1687-1695.

64. Powell EE, Jonsson JR, Clouston AD. Steatosis: co-factor in other liver diseases. Hepatology. 2005;42(1):5-13.

65. Hourigan LF, Macdonald GA, Purdie D, Whitehall VH, Shorthouse C, Clouston A, et al. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. Hepatology. 1999;29(4):1215-1219.

66. Kumar D, Farrell GC, Kench J, George J. Hepatic steatosis and the risk of hepatocellular carcinoma in chronic hepatitis C. J Gastroenterol Hepatol. 2005;20(9):1395-1400.

67. Yu MC, Tong MJ, Govindarajan S, Henderson BE. Nonviral risk factors for hepatocellular carcinoma in a low-risk population, the non-Asians of Los Angeles County, California. J Natl Cancer Inst. 1991;83(24):1820-1826.

68. Yuan JM, Govindarajan S, Arakawa K, Yu MC. Synergism of alcohol, diabetes, and viral hepatitis on the risk of hepatocellular carcinoma in blacks and whites in the U.S. Cancer. 2004;101(5):1009-1017.

69. Silini E, Bottelli R, Asti M, Bruno S, Candusso ME, Brambilla S, et al. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: a case-control study. Gastroenterology. 1996;111(1):199-205.

70. Nishiguchi S, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima S, et al. Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet. 1995;346(8982):1051-1055.

71. Liaw YF. Role of hepatitis C virus in dual and triple hepatitis virus infection. Hepatology. 1995;22(4 Pt 1):1101-1108.

72. Zarski JP, Bohn B, Bastie A, Pawlotsky JM, Baud M, Bost-Bezeaux F, et al. Characteristics of patients with dual infection by hepatitis B and C viruses. J Hepatol. 1998;28(1):27-33.

73. Benvegnu L, Fattovich G, Noventa F, Tremolada F, Chemello L, Cecchetto A, et al. Concurrent hepatitis B and C virus infection and risk of hepatocellular carcinoma in cirrhosis. A prospective study. Cancer. 1994;74(9):2442-2448.

74. Chiaramonte M, Stroffolini T, Vian A, Stazi MA, Floreani A, Lorenzoni U, et al. Rate of incidence of hepatocellular carcinoma in patients with compensated viral cirrhosis. Cancer. 1999;85(10):2132-2137.

75. Donato F, Boffetta P, Puoti M. A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma. Int J Cancer. 1998;75(3):347-354.

76. Michielsen PP, Francque SM, van Dongen JL. Viral hepatitis and hepatocellular carcinoma. World J Surg Oncol. 2005;3:27.

77. Fattovich G, Giustina G, Christensen E, Pantalena M, Zagni I, Realdi G, et al. Influence of hepatitis delta virus infection on morbidity and mortality in compensated cirrhosis type B. The European Concerted Action on Viral Hepatitis (Eurohep). Gut. 2000;46(3):420-426.

78. Telfer P, Sabin C, Devereux H, Scott F, Dusheiko G, Lee C. The progression of HCV-associated liver disease in a cohort of haemophilic patients. Br J Haematol. 1994;87(3):555-561.

79. Garcia-Samaniego J, Rodriguez M, Berenguer J, Rodríguez-Rosado R, Carbó J, Asensi V, et al. Hepatocellular carcinoma in HIV-infected patients with chronic hepatitis C. Am J Gastroenterol. 2001;96(1):179-183.

80. Thio CL SE, Skolasky Jr R, Phair J, Visscher B, Munoz A, Thomas DL. HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter Cohort Study (MACS). Lancet. 2002;360(9349):1921-1926.

81. Puoti M TC, Bruno R, Filice G, Carosi G. Natural history of chronic hepatitis B in co-infected patients. J Hepatol. 2006;44(Suppl 1):S65-70.

82. el-Zayadi AR, Badran HM, Barakat EM, Attia Mel-D, Shawky S, Mohamed MK, et al. Hepatocellular carcinoma in Egypt: a single center study over a decade. World J Gastroenterol. 2005;11(33):5193-5198.

83. Hassan MM, Zaghloul AS, El-Serag HB, Soliman O, Patt YZ, Chappell CL, et al. The role of hepatitis C in hepatocellular carcinoma: a case control study among Egyptian patients. J Clin Gastroenterol. 2001;33(2):123-126.