Screening for Hepatocellular Carcinoma

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Keywords
hepatocellular carcinoma, liver cancer, diagnosis, detection, hepatitis B, hepatitis C

Abstract: Hepatocellular carcinoma (HCC) currently has the fifth highest incidence rate among tumors worldwide, a rate expected to continue to increase over the next several decades. The majority of patients with HCC have cirrhosis of the liver, with chronic hepatitis B and C as the major agents of etiology. Despite advances in technology, the prognosis of patients with HCC has shown little improvement over time, most likely because most patients are diagnosed at advanced stages. HCC meets the criteria established by the World Health Organization for performing surveillance in those at risk for developing this tumor (ie, patients with cirrhosis of the liver). The objective of surveillance is to use a relatively simple and inexpensive examination in a large number of individuals to determine whether or not they are likely to develop cancer, with the overall goal of reducing morbidity and mortality from the cancer. In this article, we evaluate the criteria for performing surveillance for HCC and review the data on the efficacy of current surveillance programs.

The decision to screen an at-risk population for cancer is based on well-established criteria.[1] Although the overall goal is to reduce morbidity and mortality from cancer, the objective of screening is the utilization of a relatively simple and inexpensive examination in a large number of individuals to determine whether or not they are likely to develop the cancer for which they are being screened.[2] Screening is the one-time application of an examination that allows detection of a disease at a stage in which curative intervention may achieve the goal of reducing morbidity and mortality. Surveillance refers to the continuous monitoring of disease occurrence (using the screening examination) within a population to accomplish the same goals of screening.[3] This review evaluates the process of screening and surveillance for hepatocellular carcinoma (HCC).

The following criteria,[4] first promoted by the World Health Organization (WHO), have been developed to assess the benefits of screening for a specific disease: the disease in question should be an important health issue, and its significance may be defined by disease burden, including morbidity and mortality; there should be an identifiable target population; treatment of occult disease (ie, disease diagnosed prior to the appearance of symptoms) should offer advantages compared to the treatment of symptomatic disease; the screening examination should be affordable and provide benefits that justify its cost; the examination must be acceptable to

the target population and healthcare professionals; there must be standardized recall procedures; screening examinations must achieve an acceptable level of accuracy in the population undergoing screening; and surveillance should reduce mortality from the disease. In this article, we evaluate the rationale for the surveillance of patients with HCC based on these criteria.

Disease Burden of Hepatocellular Carcinoma as an Important Health Issue

The incidence rate of HCC is the fifth highest among solid tumors worldwide as is the death rate.[5] In the 2007 annual report to the nation on the status of cancer, liver cancer had the thirteenth highest incidence rate among tumors in the United States and had the largest increase in incidence of all solid tumors from 1995 to 2004.[5] The incidence of HCC has been rising in both Europe and the United States, largely due to the growing prevalence of hepatitis C cirrhosis.[6-9] A molecular clock study indicated that the epidemic of hepatitis C virus (HCV) in the United States started in the 1960s and peaked in the late 1980s.[10] Due to the lag time between the onset of infection and the development of cirrhosis, the authors postulate that the incidence of HCV-related HCC will continue to increase over the next 20 years. HCC is the third most common cause of cancer-related deaths worldwide, resulting in over 500,000 deaths per year. In the United States, HCC is the eighth most common cause of cancer-related death at 8.5 deaths per 100,000 but has the largest increase in mortality of all solid tumors from 1995 to 2004.[5] Despite advances in technology and the available treatments, the 5-year survival rate in 1996 showed little improvement from the 5-year survival rate in 1985 (5% vs 4%).[11,12]

Identification of the Target Population

Cirrhosis has been recognized as the most important risk factor for the development of HCC.[3] HCV and hepatitis B virus (HBV) are the major agents of etiology that lead to the development of HCC.[13,14] HCV-associated cirrhosis is the causative agent largely responsible for the increase in incidence of HCC in the United States. However, HBV is the leading cause of HCC worldwide, particularly in Asia and Africa.[15] Alcoholic cirrhosis is another well-established major etiologic risk factor for the development of HCC. Recently, an association between nonalcoholic liver disease and HCC was made,[16] but there have been no cohort studies evaluating the natural history of nonalcoholic fatty liver disease. Other etiologies of chronic liver disease, such as hemochromatosis, primary biliary cirrhosis, autoimmune hepatitis, and alpha-1 antitrypsin deficiency, are less common causes of chronic liver disease, with prevalence rates of 1–8% in patients with HCC.[17-20] Furthermore, improvements in the survival of patients with cirrhosis due to better specialty care may further increase the number of individuals at risk for developing HCC.[21]

The annual risk of developing HCC among patients with cirrhosis is between 2% and 7% and appears to be a cumulative risk.[22] Among patients with cirrhosis, male gender, older age, alcohol and tobacco consumption, obesity, and diabetes are factors associated with an increased risk of HCC.[23-26] In patients with chronic HBV infection, a baseline HBV DNA level of greater than 100,000 copies/mL increases the risk of HCC 10-fold.[27] This biologic gradient of HCC risk in relation to HBV DNA level suggests that persistent viral replication increases the risk of HCC. A prospective cohort study of patients with cirrhosis found that prothrombin activity less than 75% of baseline, age of more than 55 years, platelet count less than 75 mm^3, and presence of HCV were independent risk factors for developing HCC.[28] When the researchers stratified patients into a high-risk group (presence of these factors) and a low-risk group (absence of risk factors), the 5-year cumulative incidence of HCC was 30% for the high-risk group and 4% for the low-risk group ($P<.0001$). Further studies should be performed to determine whether stratification according to risk factors is beneficial for delineating a subgroup of high-risk patients at whom surveillance can be targeted.

Advantages of Treating Occult Hepatocellular Carcinoma

The effectiveness of HCC treatment depends upon the disease stage at the time of diagnosis. The Barcelona Clinic Liver Cancer (BCLC) staging system has been recommended as the main determinant of prognosis and the treatment guide for patients with HCC.[29] For early-stage tumors (BCLC stage A), surgical resection has provided 5-year survival rates of 70% in carefully selected patients with preserved hepatic function, no evidence of portal hypertension, and single small asymptomatic tumors (<5 cm in maximal diameter).[30] Liver transplantation is the preferred method of treatment for patients not amenable to surgical resection but only for those restricted to the Milan criteria (single nodule <5 cm or <3 nodules each <3 cm in diameter).[31] The 5-year survival rate reported for liver transplantation is 74%.[30] Ablative treatments, specifically percutaneous ethanol injection and radiofrequency ablation, have demonstrated 5-year survival rates of 37% in BCLC stage A patients not amenable to resection or transplantation.[30] It is estimated that approximately 30% of patients with HCC are currently diagnosed at early stages at which these therapies can be administered. Cura-

tive therapies exist for patients with early-stage HCC, and an efficacious surveillance program is critical for the identification of HCC at early stages.

Affordability and Benefits of Hepatocellular Carcinoma Screening

The standard threshold for cost effectiveness has been determined to be a maximal of $50,000 per quality-adjusted life year (QALY). Economic models studying the benefits of surveillance programs in HCC have been analyzed. Surveillance with biannual alpha-fetoprotein (AFP) and ultrasonography in Child-Pugh class A cirrhotics increases the mean life expectancy with cost effectiveness ratios between $26,000 and $55,000 per QALY.[32] When a similar analysis was performed in HCV cirrhotics, the cost-utility ratio was $26,689 per QALY.[33] Another study evaluating the cost effectiveness of biannual AFP and ultrasound in HCV Child-Pugh class A cirrhosis revealed a cost effectiveness ratio of $33,083 per QALY.[34] Therefore, screening with ultrasound and AFP has been demonstrated to be cost effective in compensated cirrhotics.

Acceptance of the Target Population and Healthcare Professionals

Surveillance for HCC appears to be acceptable to patients with cirrhosis. Such data are indirectly derived from cohort studies showing that only approximately 3–18% of cirrhotic patients were noncompliant with ultrasound and AFP surveillance,[3] which compares favorably with the 67% noncompliance rate seen with colonoscopy surveillance for colon cancer screening.[4]

HCC surveillance also appears to be well accepted by physicians. In a national survey of 554 members of the American Association for the Study of Liver Diseases, 84% of respondents indicated that they routinely screen patients with cirrhosis for HCC using AFP and ultrasound.[35]

Standardization of Recall Procedures

A recent consensus conference offered guidelines on how to investigate abnormalities of the commonly used screening examinations AFP and ultrasound in patients with cirrhosis.[29] Computed tomography (CT), magnetic resonance imaging (MRI), and contrast-enhanced ultrasound are the major diagnostic modalities used to establish the diagnosis of HCC without the need for histopathologic examination. The main imaging characteristic for HCC is the finding of arterial enhancement of the lesion followed by washout of contrast in the delayed venous phases.[36] If the screening ultrasound shows a nodule of less than 1 cm in maximal diameter, repeat ultrasound is recommended because of the low probability of having HCC. When the lesion is at least 1 cm on ultrasound or the AFP is more than 20 ng/mL, cross-sectional imaging techniques (CT or MRI) or contrast-enhanced ultrasound should be performed, given the high likelihood of HCC. The guidelines state that HCC can be accurately diagnosed by imaging if characteristic findings are seen on two modalities for lesions less than 2 cm in diameter. However, if the imaging characteristics are atypical for HCC, liver biopsy is recommended in order to establish the diagnosis. For nodules of at least 2 cm in diameter, HCC can be accurately diagnosed if the imaging characteristics are seen on one imaging test. A negative biopsy in these patients does not rule out HCC and sometimes may require repetition. Therefore, appropriate recall modalities do exist to evaluate abnormal surveillance tests.

Achieving An Acceptable Level of Hepatocellular Carcinoma Screening Accuracy

The ideal marker for HCC would be specific for HCC and undetectable in premalignant liver disease (ie, cirrhosis regardless of the etiology). In addition, such a marker would be easily measurable and reproducible, minimally invasive, and acceptable to patients and physicians.[37] Both radiographic and serologic examinations are currently utilized for HCC surveillance.

Ultrasound has been recommended as the primary radiologic screening examination for HCC.[29] It is the least expensive, and it is noninvasive and widely available, which makes it an attractive screening examination. There have been no randomized controlled trials in patients with cirrhosis to date assessing the efficacy of ultrasound as a screening examination. The use of ultrasound has been evaluated primarily in cohort studies, as shown in Table 1. The sensitivity for the detection of early-stage HCC ranges from 29% to 100%, whereas its specificity ranges from 94% to 100%. The high degree of operator dependence, differences in the equipment, and body habitus significantly limit ultrasound from being the best surveillance examination for HCC.

AFP has been the most widely utilized serologic examination for HCC screening. The operating characteristics of AFP are dependent on the cutoff level chosen to support the diagnosis of HCC. At higher cutoff levels, the test is more specific for HCC but at a cost of decreased sensitivity; conversely, at lower cutoff levels, AFP becomes increasingly sensitive but with a higher rate of false-positives.[38] A case-control study of 170 patients with HCC (approximately 60% of whom had advanced HCC) and 170 matched patients without HCC demonstrated that

Table 1. Performance of Alpha-fetoprotein (AFP) and Ultrasound as Surveillance Examinations in Cohort Studies in Patients With Cirrhosis

Study	Cutoff (ng/mL)	No. of HCC cases	Sensitivity, %	Specificity, %
AFP				
Peng YC, et al.[57]	20	205	65	88
Trevisani F, et al.[39]	16	170	60	90
Cedrone A, et al.[58]	100	74	25	95
Soresi M, et al.[59]	30	197	65	89
Lee HS, et al.[60]	200	54	53	79
Nguyen MH, et al.[61]	20	163	63	79
Pateron D, et al.[62]	15	14	50	87
Oka H, et al.[63]	20	55	39	76
Bolondi L, et al.[64]	20	61	41	82
Tong MJ, et al.[65]	11	31	86	89
Study	**Cohort**	**No. of early HCC cases**	**Sensitivity, %**	**Specificity, %**
Ultrasound				
Cottone M, et al.[66]	Child-Pugh class A	5	80	100
Pateron D, et al.[62]	Child-Pugh class A–B	14	29	96
Bolondi L, et al.[64]	Child-Pugh class A–B	61	82	95
Kobayashi K, et al.[67]	Cirrhosis*	8	50	98
Sheu JC, et al.[68]	Cirrhosis*	7	100	100
Oka H, et al.[69]	Cirrhosis*	40	68	NA
Van Thiel DH, et al.[70]	Transplant waiting List	20	55	94

Ultrasound data refer to early-stage hepatocellular carcinoma (HCC), whereas AFP data refer to all patients with HCC, as the performance for early-stage disease was not specified.

*The population of cirrhosis was not further specified.

the optimal cutoff was 20 ng/mL via receiver operating curve analysis.[39] Therefore, a level greater than 20 ng/mL has become the most commonly used cutoff in clinical practice to trigger a recall examination for the diagnosis of HCC, though various other cutoffs have been studied, as seen in Table 1. Even at the optimal cutoff level in this study, the sensitivity was only 60%, whereas the specificity was 90.6%. A recent systematic review of five studies evaluating AFP in patients with HCV cirrhosis showed sensitivities ranging from 41% to 65% and specificity ranging from 80% to 94%.[40] In addition, serum AFP values are frequently elevated among patients with chronic HCV with advanced hepatic fibrosis even in the absence of HCC, with levels declining after antiviral therapy.[41] AFP alone is not sufficient for the surveillance of HCC in patients with cirrhosis. In hepatitis B carriers, the combination of ultrasound and AFP increased the sensitivity of HCC detection, when compared to either examination alone, from 71% with ultrasound alone to 79% when ultrasound and AFP were used together.[42] Chronic elevations of AFP have also been shown to increase the risk of developing HCC among patients with cirrhosis[43] and among hepatitis B carriers.[44] Although better examinations are needed to improve the detection of early-stage HCC, AFP offers benefits in the surveillance of patients with cirrhosis and leads to diagnosis in approximately half of patients with HCC as well as the determination of their risk of developing the tumor.

Other tumor markers have been studied for the detection of HCC. Des-gamma carboxy-prothrombin (DCP) is an abnormal prothrombin protein generated as a result of an acquired defect in the posttranslational

carboxylation of the prothrombin precursor in malignant hepatic cells.[45] Several prospective cohort studies of patients with cirrhosis but without HCC have been performed to determine the performance of DCP.[46-49] The sensitivities for detecting HCC ranged from 23% to 57% for DCP compared to 14% to 71% for AFP. In the largest study with DCP, 734 patients with cirrhosis were followed for a mean of 13 months (range, 7–17), during which HCC was detected in 29 patients. The sensitivity and specificity of DCP at baseline were 41% and 90%, respectively, and 40% and 62% for AFP, respectively. Overall, AFP and DCP had equal sensitivity, but DCP had better specificity.

Several variants of AFP with differences in the sugar chain have been identified. The fucosylated variant has a high affinity of the sugar chain to lens culinaris. This variant, the lens culinaris-agglutinin reactive fraction of AFP (AFP-L3), has been shown to be more specific for HCC than total AFP.[50] Prospective studies in patients with cirrhosis have shown sensitivities for AFP-L3 ranging from 55% to 75% and specificities ranging from 68% to 90%.[51-54] However, two studies included only HCC patients with elevated total AFP at baseline, making it impossible to compare the accuracy of AFP-L3 with total AFP. Even though these two markers have potential, at this time there is no evidence of their efficacy in the surveillance of patients with cirrhosis or evidence that these markers are better than AFP and ultrasound in this capacity. More studies are needed to assess the performance of DCP and AFP-L3 to detect preclinical HCC.

Reducing Mortality From Hepatocellular Carcinoma

The most reliable method of evaluating the efficacy of ultrasound and AFP for HCC surveillance would be conducting a randomized controlled trial. There have been two large randomized controlled trials in China using ultrasound and AFP in patients with chronic HBV.[55,56] In both trials, surveillance was conducted every 6 months and compared to patients who did not receive any routine screening. The first study evaluated 17,920 carriers of HBV randomized to surveillance (n=8,109) or no surveillance (n=9,711) and then followed for an average of 14.4 months.[55] Among the patients randomized to the surveillance group, 38 patients developed HCC, of whom 29 (76.3%) were detected at early stages; in contrast, 18 patients developed HCC in the nonsurveillance group and none of them were detected at an early stage (P<.01). A higher proportion of patients in the surveillance group met the criteria for surgical therapy, with 24 patients having surgical resection in the surveillance group compared to 0 patients in the nonscreening group (P<.05). Accordingly, the 1- and 2-year survival rates for the surveillance group were 88.1% and 77.5%, respectively, compared to a 0% survival rate at 1 year for the nonscreening group. The authors acknowledged that this study was limited by lead-time bias, though it would theoretically account for only a survival difference of 5.4 months. Given that over three fourths of the surveillance population survived for 2 years, whereas no patients survived longer than 1 year in the nonscreening group, the authors concluded that surveillance would reduce HCC-associated mortality rates. The second randomized controlled trial evaluated 19,200 hepatitis B carriers who were randomized to surveillance (n=9,757) or no surveillance (n=9,443).[56] A total of 86 patients developed HCC in the surveillance group, of whom 45% were early stage, compared to 67 patients who developed HCC in the nonsurveillance group, of whom none were early stage. The mortality rate of patients undergoing surveillance was significantly lower than the control group (83.2 vs 131.5 per 100,000; P<.01), with a hazard ratio of 0.63 (95% confidence interval, 0.41–0.98). These results demonstrate that the strategy of surveillance among patients with chronic HBV reduces overall mortality. However, it is unclear whether all the patients in these two studies had the same risk of developing HCC, given the low rate of HCC development seen. These studies did not mention the number of patients who had cirrhosis or evidence of viral replication, and the studies most likely included patients who were asymptomatic carriers and are at a lower risk for developing HCC. Therefore, the results are not generalizable to the majority of patients at risk for developing HCC.

Although randomized controlled trials have been performed in China in patients with chronic HBV, the results cannot be extrapolated to cirrhotic patients, who account for the majority of patients with HCC worldwide. No randomized trials have been performed in a cirrhotic population, so most of the data on surveillance in patients with cirrhosis come from cohort studies. Some studies have shown that patients undergoing surveillance with ultrasound and AFP have a better overall survival when compared to either historical controls or patients with HCC who did not undergo surveillance. Table 2 shows the details of these cohort studies, including the number of HCC and early-stage HCC cases that developed during follow-up. The results of these studies are also fraught with lead-time and length-time biases that limit their generalizability of improvements in survival with surveillance. Therefore, the impact of surveillance on mortality in patients with cirrhosis has been assessed only in nonrandomized trials to date (ie, a level II recommendation consisting of cohort or uncontrolled studies).[29] As shown in Table 2, there has

Table 2. Cohort Studies Evaluating Ultrasound and Alpha-fetoprotein (AFP) for the Detection of Hepatocellular Carcinoma (HCC)

Study	No. of patients	Mean follow-up (months)	Surveillance method	HCC detected, n (%)	Early-stage HCC, n (%)
Cottone M, et al.[66]	147	24	AFP and ultrasound	5 (3)	4 (80)
Pateron D, et al.[62]	118	36	AFP, DCP, and ultrasound	14 (12)	5 (36)
Bolondi L, et al.[64]	313	56	AFP and ultrasound	57 (18)	53 (87)
Velazquez RF, et al.[28]	463	39	AFP and ultrasound	38 (8)	18 (47)
Sangiovanni A, et al.[71]	417	148	AFP and ultrasound	112 (27)	27 (24)
Santagostino E, et al.[72]	66	72	AFP and ultrasound	8 (12)	2 (25)
Henrion J, et al.[73]	94	34	AFP and ultrasound	6 (6)	5 (83)
Zoli M, et al.[74]	164	28	AFP and ultrasound	34 (21)	32 (94)
Tradati F, et al.[75]	40	48	AFP and ultrasound	6 (15)	2 (33)
Kobayashi K, et al.[67]	95	50	AFP, ultrasound, and CT	8 (8)	6 (75)
Sheu JC, et al.[68]	223	17	AFP and ultrasound	7 (3)	7 (100)
Oka H, et al.[69]	140	41	AFP and ultrasound	39 (28)	27 (82)
Van Thiel DH, et al.[70]	100	20	AFP, ultrasound, and triple-phase CT	14 (14)	13 (93)
Imberti D, et al.[76]	228	44	AFP and ultrasound	38 (17)	14 (37)
Colombo M, et al.[77]	417	33	AFP and ultrasound	26 (6)	9 (35)
Cottone M, et al.[78]	147	65	AFP and ultrasound	30 (20)	25 (83)
Oka H, et al.[69]	260	39	AFP and ultrasound	55 (21)	50 (91)
Degos F, et al.[79]	416	68	AFP and ultrasound	60 (14)	37 (62)
Bruno S, et al.[80]	163	68	AFP and ultrasound	22 (13)	16 (73)
Caturelli E, et al.[81]	1599	43	AFP and ultrasound	269 (17)	253 (94)
Tong MJ, et al.[65]	173	35	AFP and ultrasound	31 (18)	18 (58)
Iavarone M, et al.[82]	201	50	AFP and ultrasound	27 (13)	17 (63)

CT=computed tomography; DCP=des-gamma-carboxy prothrombin.

been a significant amount of heterogeneity among these studies pertaining to the sample size (ranging from 66 to 1,599), population studied (Child-Pugh class A, Child-Pugh class A or B, transplant candidates), the incidence of HCC (ranging from 3% to 28%), and the number of early-stage HCC cases detected (ranging from 24% to 100%). Randomized or controlled trials are needed in this area. At the present time, ultrasound with AFP is the recommended strategy for the surveillance of patients with cirrhosis.

Conclusion

HCC meets all of the WHO criteria for establishing a surveillance program. First, it is a disease with increasing incidence in most areas of the world and has significant associated morbidity and mortality rates. Second, HCC is found primarily in an identifiable population, specifically those with underlying liver disease. Third, cost-effective diagnostic tools and treatments are available for those at the earlier stages of the disease. The current strategy of surveillance with AFP and ultrasound has been shown in two large randomized controlled trials to lead to a reduction in overall mortality in patients with chronic HBV. Although the effect of surveillance in patients with cirrhosis has not been evaluated by a randomized controlled trial in cirrhosis, several nonrandomized and uncontrolled cohort studies suggest that surveillance can lead to a portion of patients being diagnosed at early stages of disease. Novel surveillance tests are needed to improve the detec-

tion of HCC at stages at which curative interventions can be applied.

References

1. Cole P, Morrison AS. Basic issues in population screening for cancer. *J Natl Cancer Inst.* 1980;64:1263-1272.
2. Smith RA. Screening fundamentals. *J Natl Cancer Inst Monogr.* 1997:15-19.
3. Collier J, Sherman M. Screening for hepatocellular carcinoma. *Hepatology.* 1998;27:273-278.
4. Meissner HI, Smith RA, Rimer BK, Wilson KM, Rakowski W, et al. Promoting cancer screening: learning from experience. *Cancer.* 2004;101:1107-1117.
5. Espey DK, Wu XC, Swan J, Wiggins C, Jim MA, et al. Annual report to the nation on the status of cancer, 1975-2004, featuring cancer in American Indians and Alaska Natives. *Cancer.* 2007;110:2119-2152.
6. Bosch FX, Ribes J, Borras J. Epidemiology of primary liver cancer. *Semin Liver Dis.* 1999;19:271-285.
7. Bosch FX, Ribes J, Cleries R, Diaz M. Epidemiology of hepatocellular carcinoma. *Clin Liver Dis*. 2005;9:191-211, v.
8. El-Serag HB. Hepatocellular carcinoma: recent trends in the United States. *Gastroenterology*. 2004;127:S27-S34.
9. El-Serag HB, Davila JA, Petersen NJ, McGlynn KA. The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. *Ann Intern Med*. 2003;139:817-823.
10. Tanaka Y, Hanada K, Mizokami M, Yeo AE, Shih JW, et al. Inaugural article: a comparison of the molecular clock of hepatitis C virus in the United States and Japan predicts that hepatocellular carcinoma incidence in the United States will increase over the next two decades. *Proc Natl Acad Sci U S A*. 2002;99:15584-15589.
11. Capocaccia R, Sant M, Berrino F, Simonetti A, Santi V, Trevisani F, EUROCARE Working Group. Hepatocellular carcinoma: trends of incidence and survival in Europe and the United States at the end of the 20th century. *Am J Gastroenterol.* 2007;102:1661-1670; quiz 1660, 1671.
12. El-Serag HB, Mason AC, Key C. Trends in survival of patients with hepatocellular carcinoma between 1977 and 1996 in the United States. *Hepatology.* 2001;33:62-65.
13. Beasley RP, Hwang LY, Lin CC, Chien CS. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22,707 men in Taiwan. *Lancet.* 1981;2:1129-1133.
14. Colombo M, Kuo G, Choo QL, Donato MF, Del Ninno E, et al. Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *Lancet.* 1989;2:1006-1008.
15. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology.* 2007;132:2557-2576.
16. El-Serag HB. Epidemiology of hepatocellular carcinoma. *Clin Liver Dis.* 2001;5:87-107, vi.
17. Deugnier Y, Turlin B. Iron and hepatocellular carcinoma. *J Gastroenterol Hepatol.* 2001;16:491-494.
18. Farinati F, Floreani A, De Maria N, Fagiuoli S, Naccarato R, Chiaramonte M. Hepatocellular carcinoma in primary biliary cirrhosis. *J Hepatol*. 1994;21:315-316.
19. Marrero JA, Fontana RJ, Su GL, Conjeevaram HS, Emick DM, Lok AS. NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. *Hepatology*. 2002;36:1349-1354.
20. Nishiyama R, Kanai T, Abe J, Hara R, Watahiki Y, et al. Hepatocellular carcinoma associated with autoimmune hepatitis. *J Hepatobiliary Pancreat Surg.* 2004;11:215-219.
21. Carbonell N, Pauwels A, Serfaty L, Fourdan O, Levy VG, Poupon R. Improved survival after variceal bleeding in patients with cirrhosis over the past two decades. *Hepatology.* 2004;40:652-659.
22. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology.* 2004;127:S35-S50.
23. 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;348:1625-1638.
24. Donato F, Tagger A, Gelatti U, Parrinello G, Boffetta P, et al. Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. *Am J Epidemiol.* 2002;155:323-331.
25. El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology*. 2004;126:460-468.
26. Kuper H, Tzonou A, Kaklamani E, Hsieh CC, Lagiou P, et al. Tobacco smoking, alcohol consumption and their interaction in the causation of hepatocellular carcinoma. *Int J Cancer.* 2000;85:498-502.
27. Chen CJ, Yang HI, Su J, Jen CL, You SL, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA.* 2006;295:65-73.
28. Velazquez RF, Rodriguez M, Navascues CA, Linares A, Perez R, et al. Prospective analysis of risk factors for hepatocellular carcinoma in patients with liver cirrhosis. *Hepatology.* 2003;37:520-527.
29. Bruix J, Sherman M. Management of hepatocellular carcinoma. *Hepatology.* 2005;42:1208-1236.
30. Llovet JM, Bruix J. Early diagnosis and treatment of hepatocellular carcinoma. *Baillieres Best Pract Res Clin Gastroenterol.* 2000;14:991-1008.
31. Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med.* 1996;334:693-699.
32. Sarasin FP, Giostra E, Hadengue A. Cost-effectiveness of screening for detection of small hepatocellular carcinoma in western patients with Child-Pugh class A cirrhosis. *Am J Med.* 1996;101:422-434.
33. Arguedas MR, Chen VK, Eloubeidi MA, Fallon MB. Screening for hepatocellular carcinoma in patients with hepatitis C cirrhosis: a cost-utility analysis. *Am J Gastroenterol*. 2003;98:679-690.
34. Lin OS, Keeffe EB, Sanders GD, Owens DK. Cost-effectiveness of screening for hepatocellular carcinoma in patients with cirrhosis due to chronic hepatitis C. *Aliment Pharmacol Ther.* 2004;19:1159-1172.
35. Chalasani N, Said A, Ness R, Hoen H, Lumeng L. Screening for hepatocellular carcinoma in patients with cirrhosis in the United States: results of a national survey. *Am J Gastroenterol.* 1999;94:2224-2229.
36. Marrero JA, Hussain HK, Nghiem HV, Umar R, Fontana RJ, Lok AS. Improving the prediction of hepatocellular carcinoma in cirrhotic patients with an arterially-enhancing liver mass. *Liver Transpl.* 2005;11:281-289.
37. Srinivas PR, Kramer BS, Srivastava S. Trends in biomarker research for cancer detection. *Lancet Oncol.* 2001;2:698-704.
38. Colli A, Fraquelli M, Conte D. Alpha-fetoprotein and hepatocellular carcinoma. *Am J Gastroenterol.* 2006;101:1939; author reply 1940-1931.
39. Trevisani F, D'Intino PE, Morselli-Labate AM, Mazzella G, Accogli E, et al. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. *J Hepatol.* 2001;34:570-575.
40. Gupta S, Bent S, Kohlwes J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. A systematic review and critical analysis. *Ann Intern Med.* 2003;139:46-50.
41. Di Bisceglie AM, Sterling RK, Chung RT, Everhart JE, Dienstag JL, et al. Serum alpha-fetoprotein levels in patients with advanced hepatitis C: results from the HALT-C Trial. *J Hepatol.* 2005;43:434-441.
42. Sherman M, Peltekian KM, Lee C. Screening for hepatocellular carcinoma in chronic carriers of hepatitis B virus: incidence and prevalence of hepatocellular carcinoma in a North American urban population. *Hepatology*. 1995;22:432-438.
43. Sangiovanni A, Colombo E, Radaelli F, Bortoli A, Bovo G, et al. Hepatocyte proliferation and risk of hepatocellular carcinoma in cirrhotic patients. *Am J Gastroenterol.* 2001;96:1575-1580.
44. McMahon BJ, Bulkow L, Harpster A, Snowball M, Lanier A, et al. Screening for hepatocellular carcinoma in Alaska natives infected with chronic hepatitis B: a 16-year population-based study. *Hepatology*. 2000;32:842-846.
45. Ono M, Ohta H, Ohhira M, Sekiya C, Namiki M. Measurement of immunoreactive prothrombin precursor and vitamin-K-dependent gamma-carboxylation in human hepatocellular carcinoma tissues: decreased carboxylation of prothrombin precursor as a cause of des-gamma-carboxyprothrombin synthesis. *Tumour Biol.* 1990;11:319-326.
46. Ikoma J, Kaito M, Ishihara T, Nakagawa N, Kamei A, et al. Early diagnosis of hepatocellular carcinoma using a sensitive assay for serum des-gamma-carboxy prothrombin: a prospective study. *Hepatogastroenterology*. 2002;49:235-238.
47. Ishii M, Gama H, Chida N, Ueno Y, Shinzawa H, et al. Simultaneous measurements of serum alpha-fetoprotein and protein induced by vitamin K absence for detecting hepatocellular carcinoma. South Tohoku District Study Group. *Am J Gastroenterol*. 2000;95:1036-1040.
48. Izuno K, Fujiyama S, Yamasaki K, Sato M, Sato T. Early detection of hepatocellular carcinoma associated with cirrhosis by combined assay of des-

gamma-carboxy prothrombin and alpha-fetoprotein: a prospective study. *Hepatogastroenterology*. 1995;42:387-393.
49. Shimauchi Y, Tanaka M, Kuromatsu R, Ogata R, Tateishi Y, et al. A simultaneous monitoring of Lens culinaris agglutinin A-reactive alpha-fetoprotein and des-gamma-carboxy prothrombin as an early diagnosis of hepatocellular carcinoma in the follow-up of cirrhotic patients. *Oncol Rep.* 2000;7:249-256.
50. Taketa K, Sekiya C, Namiki M, Akamatsu K, Ohta Y, et al. Lectin-reactive profiles of alpha-fetoprotein characterizing hepatocellular carcinoma and related conditions. *Gastroenterology*. 1990;99:508-518.
51. Sato Y, Nakata K, Kato Y, Shima M, Ishii N, et al. Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. *N Engl J Med.* 1993;328:1802-1806.
52. Shiraki K, Takase K, Tameda Y, Hamada M, Kosaka Y, Nakano T. A clinical study of lectin-reactive alpha-fetoprotein as an early indicator of hepatocellular carcinoma in the follow-up of cirrhotic patients. *Hepatology.* 1995;22:802-807.
53. Wang SS, Lu RH, Lee FY, Chao Y, Huang YS, et al. Utility of lentil lectin affinity of alpha-fetoprotein in the diagnosis of hepatocellular carcinoma. *J Hepatol*. 1996;25:166-171.
54. Shokar NK, Vernon SW, Weller SC. Cancer and colorectal cancer: knowledge, beliefs, and screening preferences of a diverse patient population. *Fam Med.* 2005;37:341-347.
55. Yang B, Zhang B, Xu Y, Wang W, Shen Y, et al. Prospective study of early detection for primary liver cancer. *J Cancer Res Clin Oncol.* 1997;123:357-360.
56. Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. *J Cancer Res Clin Oncol*. 2004;130:417-422.
57. Peng YC, Chan CS, Chen GH. The effectiveness of serum alpha-fetoprotein level in anti-HCV positive patients for screening hepatocellular carcinoma. *Hepatogastroenterology.* 1999;46:3208-3211.
58. Cedrone A, Covino M, Caturelli E, Pompili M, Lorenzelli G, et al. Utility of alpha-fetoprotein (AFP) in the screening of patients with virus-related chronic liver disease: does different viral etiology influence AFP levels in HCC? A study in 350 western patients. *Hepatogastroenterology.* 2000;47:1654-1658.
59. Soresi M, Magliarisi C, Campagna P, Leto G, Bonfissuto G, et al. Usefulness of alpha-fetoprotein in the diagnosis of hepatocellular carcinoma. *Anticancer Res.* 2003;23:1747-1753.
60. Lee HS, Chung YH, Kim CY. Specificities of serum alpha-fetoprotein in HBsAg+ and HBsAg- patients in the diagnosis of hepatocellular carcinoma. *Hepatology.* 1991;14:68-72.
61. Nguyen MH, Garcia RT, Simpson PW, Wright TL, Keeffe EB. Racial differences in effectiveness of alpha-fetoprotein for diagnosis of hepatocellular carcinoma in hepatitis C virus cirrhosis. *Hepatology.* 2002;36:410-417.
62. Pateron D, Ganne N, Trinchet JC, Aurousseau MH, Mal F, et al. Prospective study of screening for hepatocellular carcinoma in Caucasian patients with cirrhosis. *J Hepatol.* 1994;20:65-71.
63. Oka H, Tamori A, Kuroki T, Kobayashi K, Yamamoto S. Prospective study of alpha-fetoprotein in cirrhotic patients monitored for development of hepatocellular carcinoma. *Hepatology.* 1994;19:61-66.
64. Bolondi L, Sofia S, Siringo S, Gaiani S, Casali A, et al. Surveillance program of cirrhotic patients for early diagnosis and treatment of hepatocellular carcinoma: a cost effectiveness analysis. *Gut*. 2001;48:251-259.
65. Tong MJ, Blatt LM, Kao VW. Surveillance for hepatocellular carcinoma in patients with chronic viral hepatitis in the United States of America. *J Gastroenterol Hepatol.* 2001;16:553-559.
66. Cottone M, Turri M, Caltagirone M, Maringhini A, Sciarrino E, et al. Early detection of hepatocellular carcinoma associated with cirrhosis by ultrasound and alfafetoprotein: a prospective study. *Hepatogastroenterology*. 1988;35:101-103.
67. Kobayashi K, Sugimoto T, Makino H, Kumagai M, Unoura M, et al. Screening methods for early detection of hepatocellular carcinoma. *Hepatology.* 1985;5:1100-1105.
68. Sheu JC, Sung JL, Chen DS, Lai MY, Wang TH, et al. Early detection of hepatocellular carcinoma by real-time ultrasonography. A prospective study. *Cancer.* 1985;56:660-666.
69. Oka H, Kurioka N, Kim K, Kanno T, Kuroki T, et al. Prospective study of early detection of hepatocellular carcinoma in patients with cirrhosis. *Hepatology.* 1990;12:680-687.
70. Van Thiel DH, Yong S, Li SD, Kennedy M, Brems J. The development of de novo hepatocellular carcinoma in patients on a liver transplant list: frequency, size, and assessment of current screening methods. *Liver Transpl.* 2004;10:631-637.
71. Sangiovanni A, Del Ninno E, Fasani P, De Fazio C, Ronchi G, et al. Increased survival of cirrhotic patients with a hepatocellular carcinoma detected during surveillance. *Gastroenterology*. 2004;126:1005-1014.
72. Santagostino E, Colombo M, Rivi M, Rumi MG, Rocino A, et al. A 6-month versus a 12-month surveillance for hepatocellular carcinoma in 559 hemophiliacs infected with the hepatitis C virus. *Blood*. 2003;102:78-82.
73. Henrion J, Libon E, De Maeght S, Schapira M, Ghilain JM, et al. Surveillance for hepatocellular carcinoma: compliance and results according to the aetiology of cirrhosis in a cohort of 141 patients. *Acta Gastroenterol Belg*. 2000;63:5-9.
74. Zoli M, Magalotti D, Bianchi G, Gueli C, Marchesini G, Pisi E. Efficacy of a surveillance program for early detection of hepatocellular carcinoma. *Cancer.* 1996;78:977-985.
75. Tradati F, Colombo M, Mannucci PM, Rumi MG, De Fazio C, et al. A prospective multicenter study of hepatocellular carcinoma in Italian hemophiliacs with chronic hepatitis C. The Study Group of the Association of Italian Hemophilia Centers. *Blood*. 1998;91:1173-1177.
76. Imberti D, Fornari F, Sbolli G, Buscarini E, Squassante L, Buscarini L. Hepatocellular carcinoma in liver cirrhosis. A prospective study. *Scand J Gastroenterol.* 1993;28:540-544.
77. Colombo M, de Franchis R, Del Ninno E, Sangiovanni A, De Fazio C, et al. Hepatocellular carcinoma in Italian patients with cirrhosis. *N Engl J Med*. 1991;325:675-680.
78. Cottone M, Turri M, Caltagirone M, Parisi P, Orlando A, et al. Screening for hepatocellular carcinoma in patients with Child's A cirrhosis: an 8-year prospective study by ultrasound and alphafetoprotein. *J Hepatol*. 1994;21:1029-1034.
79. Degos F, Christidis C, Ganne-Carrie N, Farmachidi JP, Degott C, et al. Hepatitis C virus related cirrhosis: time to occurrence of hepatocellular carcinoma and death. *Gut.* 2000;47:131-136.
80. Bruno S, Silini E, Crosignani A, Borzio F, Leandro G, et al. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: a prospective study. *Hepatology.* 1997;25:754-758.
81. Caturelli E, Bartolucci F, Biasini E, Vigliotti ML, Andriulli A, et al. Diagnosis of liver nodules observed in chronic liver disease patients during ultrasound screening for early detection of hepatocellular carcinoma. *Am J Gastroenterol.* 2002;97:397-405.
82. Iavarone M, Lampertico P, Ronchi G, Del Ninno E, Zanella A, Colombo M. A prospective study of blood alpha-fetoprotein messenger RNA as a predictor of hepatocellular carcinoma in patients with cirrhosis. *J Viral Hepat*. 2003;10:423-426.