HEPATOCELLULAR CARCINOMA

New Insights Into Pathogenesis and Management

Internal Medicine Grand Rounds

October 1, 1992

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INTRODUCTION

Hepatocellular carcinoma is regarded by most American physicians as a rare, rapidly progressive malignancy that is almost uniformly fatal within a matter of weeks or months after diagnosis. In actuality, hepatocellular carcinoma is probably the most common malignancy of men worldwide with an annual incidence of approximately 1,000,000 cases per year (1). The median doubling time of hepatocellular carcinomas has been shown to not be dramatically different from that of other moderately slow growing adenocarcinomas such as breast or colon cancer (1,2). In addition, while most series report median survivals of < 6 months following clinical presentation of this malignancy (3,4), when hepatocellular carcinoma is diagnosed by screening studies in an earlier, asymptomatic phase, a 1 year survival rate in excess of 90% in untreated patients has been noted in at least one study (5). Moreover, when diagnosed in a "resectable", usually asymptomatic stage, 5 year survival rates in the range of 30-60% have been reported after surgical therapy (6-8).

This apparently confusing clinical spectrum is related in part to the fact that there are dramatic geographic differences in both prevalence and apparent etiology of hepatocellular carcinoma. In countries where this malignancy is common and linked to well characterized epidemiologic risk factors, early diagnosis is vigorously pursued through implementation of serologic and radiologic screening programs and a high percentage of patients receive either surgical or medical therapy. In contrast in the U.S. and Europe, hepatocellular carcinoma is most commonly diagnosed in a symptomatic stage or at autopsy in patients with advanced cirrhosis and only a small minority of patients receive specific therapy. However, new insights into the pathogenesis of hepatocellular carcinoma, availability of reliable diagnostic modalities and development of an increasing array of therapies for both hepatocellular carcinoma and associated liver diseases has led many American and European physicians to question whether it is time to discard current biases regarding the "incurable" nature of this disease and begin to join our Asian counterparts in pursuit of more aggressive diagnostic and therapeutic approaches.

EPIDEMIOLOGY AND PATHOGENESIS

The marked disparities among hepatocellular prevalence rates in various human populations is shown in figure 1. In some countries, such as Taiwan and Mozambique hepatocellular carcinoma is an extremely common disease. This tumor is also common among virtually all indigenous human populations in countries bordering the Pacific Ocean with the exception of the Amerindian populations of the Americas. Hepatocellular carcinoma also has a high prevalence in many areas of Sub-Saharan Africa and intermediate prevalence in Southern and East-Central Europe while it is quite rare in Northwestern Europe and most of the populations resident in North and South America. In all areas of the world, this malignancy occurs with greater frequency in males with male:female ratios ranging from 2-3:1 in areas of low prevalence to ratios of 4-5:1 in areas of high incidence. While, in Africa and Taiwan, hepatocellular carcinoma frequently develops in patients who have no prior clinical manifestations of liver disease, in these countries as well as in those where this malignancy is less common, 80-90% of hepatocellular carcinomas are found at autopsy or surgery to have arisen in a cirrhotic liver.

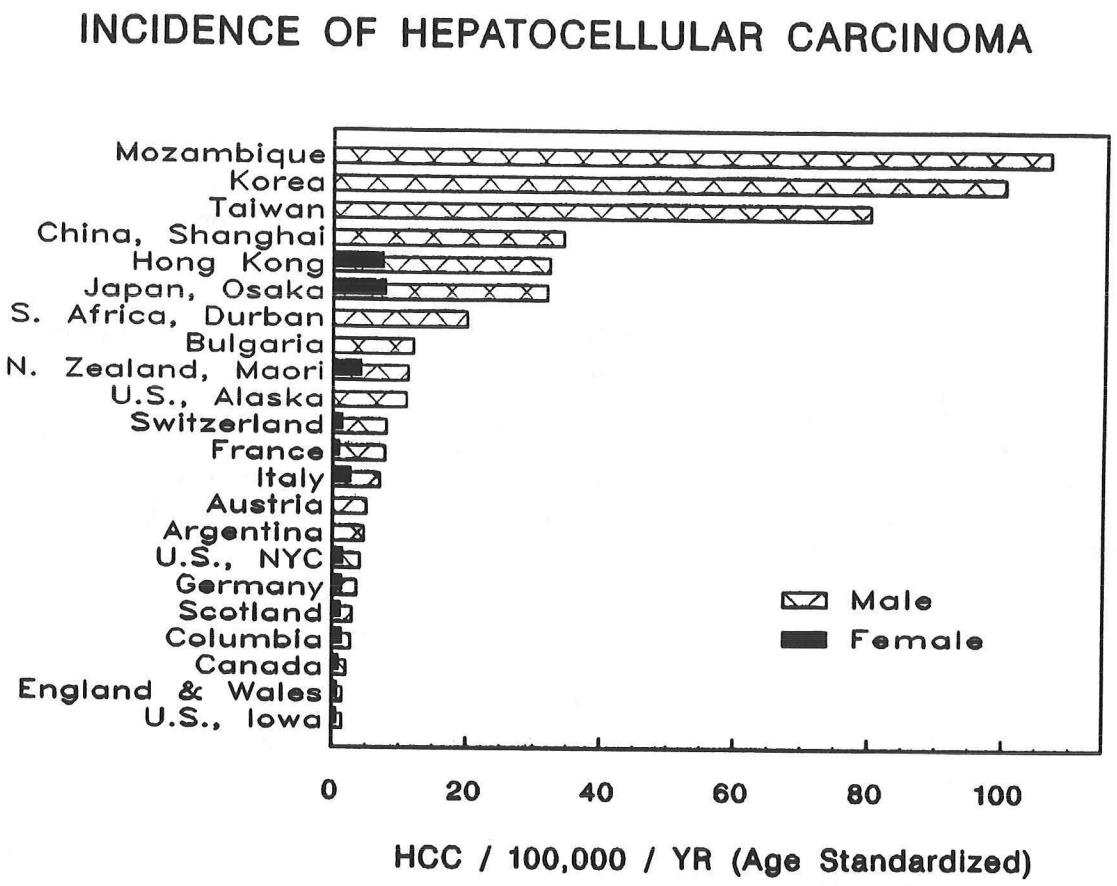

Figure 1. Data obtained from references 9 and 10.

ROLE OF HEPATITIS B IN HEPATOCELLULAR CARCINOMA

The frequency of underlying liver disease and the similarity between prevalence rates for hepatocellular carcinoma and chronic hepatitis B led to speculation regarding the relationship between these two diseases. Populations with high hepatocellular carcinoma prevalence such as Sub-Saharan Africans and most populations in Southern and Eastern Asia were noted to have rates of chronic HBV carrier status between 5 and 15% while areas of very low incidence of hepatocellular prevalence generally were known to have HBsAg (+) rates of < 1%. The landmark study conducted by R. Palmer Beasley in Taiwan firmly established that chronic HBV infection is a risk factor for development of hepatocellular carcinoma. In this study, 22,707 male government workers were evaluated for the presence of HBV serologic markers and followed prospectively for development of hepatocellular carcinoma(11,12). At time of enrollment, 81.6% were between ages 40 and 59. Most subjects (93%) had emigrated from 16 of China's 35 provinces where the prevalence of HBsAg carrier status varied from 4.7-20.1%. As summarized in Table 1, after 202,000 man years of follow-up, the rate of development of hepatocellular carcinoma in HBsAg(+) subjects was 98 fold higher than in HBsAg(-) subjects. Relative risk ratios associated with HBsAg carrier status did not differ among patients born in different areas of China. In addition, risk of death from cirrhosis in the HBsAg (+) men was 24-fold higher than in HBsAg (-) subjects. During the course of this study patients who were HBsAg (+) at entry lost this marker at a rate of 1% per year. Of note, 9 cases of hepatocellular carcinoma

developed in HBsAg (-) subjects who had antibodies to HBc and/or HBs. These observations suggested that some patients with prior active HBV infection may have presented clinically with tumors after loss of HBsAg. However, the rate of hepatocellular carcinoma in this antibody positive, HBsAg (-) population (9/17,818) was not statistically different than the 0/1272 rate in subjects with no HBV markers.

Table 1

Hepatocellular Carcinoma (HCC) Incidence and Relative Risk from

Time of Enrollment (11/3/75-12/31/75) and December 31, 1986

Recruitment Status	HCC	Population at Risk	HCC/100,000/yr	Relative Risk
HBsAg (+)	152	3454	495	98.4
HBsAg (-), ALL	9	19,253	5	
HBsAg (-), anti-HBc (+), anti-HBs (+)	7	15,570	5	
HBsAg (-), anti-HBc (+), anti-HBs (-)	2	2248	10	
HBsAg (-), anti-HBc (-)	0	1272	0	
Unknown	0	163	0	
Total	161	22,707	80	

Values from reference 12.

Following the studies of Beasley, numerous other epidemiologic studies based on a variety of statistical strategies examined the relative risk of hepatocellular carcinoma in HBsAg carriers in areas of high or low prevalence of this malignancy. As shown by the results of representative studies summarized in Table II, an increased risk for development of this malignancy was found in association with HBV infection in either sex in all such populations. Of note, the relative risk ascribed to HBsAg carrier status varied widely from 10-148 in these studies. Each study used somewhat different strategies to identify control populations for calculation of relative risk ratios. The age ranges of HBsAg (+) subjects and/or hepatocellular carcinoma patients studied also varied widely. The fact that the second study from Taiwan listed in Table II (and other studies from Taiwan, not shown) estimated a relative risk ratio much lower than that of Beasley, suggested that such differences in methodology may have accounted for much of these differences in relative risk ratios. In addition, the study by Prince, et al

estimated risk ratios by attempting to match names of known chronic HBsAg carriers with death certificates and is acknowledged to provide only a minimum estimate of death rate from hepatocellular carcinoma (14). Other characteristics of the study populations also suggest other reasons why relative risk for development of hepatocellular carcinoma appears higher in some populations than in others. As summarized by the data detailed in Table III, risk for development of hepatoma is even higher for Chinese men whose mothers are HBsAg (+) thus suggesting that neonatal acquisition of infection results in higher risk of malignancy than does infection in early adulthood which is more common in the U.S. and Western Europe.

Table II

Relative Risk for Development of Hepatocellular Carcinoma in Various HBsAg (+) Populations

Author	Site of Study / Sex	Relative Risk in HBsAg (+)
Beasley (12)	Taiwan / Male	98
Chen, et al (13)	Taiwan / Male	22
Prince, et al (14)	New York City / Male	> 10
McMahon, et al (15)	Alaskan Natives / M & F	148
Lamont, et al (16)	Scotland / M & F	44 (M) / 49 (F)

Table III

Association Between Maternal HBsAg Status and Hepatocellular Carcinoma

	Mothers	Fathers
	(% HBsAg+)	(% HBsAg+)
Hepatocellular Ca	86% *	18%
Control HBsAg (+)	35%	21%

* $p < 0.05$, data from reference 12.

MECHANISMS OF CARCINOGENESIS IN CHRONIC HBV INFECTION

Concurrent with appreciation of the epidemiologic association between HBV infection and hepatocellular carcinoma, it became known that HBV is an incomplete, double stranded DNA virus that contains an enzyme that serves as a reverse transcriptase. These observations suggested the hypothesis that as in the case of certain retroviruses, HBV DNA may become integrated in host DNA during chronic infection and via a promotor insertion mechanism activate

protooncogenes or disrupt function of tumor suppressor genes and thereby induce hepatocellular carcinomas (reviewed in reference 17). This hypothesis received a good deal of support from observations indicating that integration of HBV DNA could be demonstrated in at least 80% of tumor DNA from HBsAg (+) patients. In addition, related Hepadna viruses were isolated from the American woodchuck (*Marmota marmosa*), the Pekin duck (*Pata domestica*) and the California ground squirrel (*Spermophilus beechev*) and have been found to be associated with varying frequencies of hepatocellular carcinoma in infected animals. The most striking association between Hepadna virus infection and hepatic malignancies has been noted in captive populations of woodchucks where hepatocellular carcinoma develops in >90% of infected animals with no tumors observed in non-infected controls (18). Integration of woodchuck hepatitis virus DNA in hepatic tumors also has been observed. However, despite this impressive amount of circumstantial evidence, a direct role of HBV DNA integration events in carcinogenesis has been difficult to demonstrate in all but a very few patients with evidence of HBV integration within the domain of known oncogenes (19). Rather an ever increasing body of evidence including (1) lack of demonstrable DNA integration in 10-15% of HBV associated tumors, (2) the discovery of viral integrations at different chromosomal DNA sites in different tumors or even within different tumor isolates from the same individual and (3) the frequent lack of integration within domains of known protooncogenes, has suggested that HBV DNA integration does not commonly promote tumorigenesis via a cis-acting mechanism (19).

At least two other likely mechanisms for tumor generation in conjunction with chronic HBV infection have been proposed. It has been noted repeatedly that hepatocellular carcinoma tends to develop relatively late in the course of chronic HBV infection after cirrhosis has already developed. In Chinese men the risk for development of this malignancy is 1000-fold increased in HBsAg (+) subjects with clinically apparent cirrhosis (12). During the course of chronic hepatitis that precedes fibrosis and cirrhosis, increased rates of hepatocyte death and regeneration are observed. The relative risk for development of hepatocellular carcinoma has been found to be associated with the degree of increase in rates of hepatocyte DNA synthesis (20). These observations have suggested that in the liver as in other organs afflicted by chronic inflammatory diseases, elevated rates of cell turnover increase the likelihood of random DNA mutations or mutations secondary to inflammatory mediators and therefore increase the likelihood of protooncogene activation. The observation that hepatocellular carcinoma develops in other forms of cirrhosis has also supported this mechanism of carcinogenesis.

Generation of transgenic mice expressing different HBV genes has both provided some support for the chronic cellular injury/secondary neoplastic transformation model of HBV associated hepatic carcinogenesis and for an additional, novel mechanism of tumorigenesis. In most transgenic mice expressing HBV gene products neither liver disease or carcinoma has been observed (17,19,21). However, in studies reported by Chisari, et al (21), lineages of transgenic mice expressing very high levels of HBsAg were observed to develop modest evidence of hepatocyte injury. Over time, in transgenic mouse lineages with evidence of hepatocyte injury but not in those without evidence of increased hepatocyte turnover, regenerative hyperplasia, nuclear aneuploidy, benign hepatic adenomas and finally multi-focal hepatocellular carcinomas were observed to develop. While the results of such early transgenic studies argued for a predominate role for chronic cell injury in HBV associated carcinogenesis, more recent studies (22) of transgenic mice expressing the HBx gene product have supported an additional

mechanism whereby HBV gene products might play a direct role in the molecular events leading to hepatic carcinogenesis. The HBx protein appears to transactivate the enhancer of the HBV genome, thereby upregulating the expression of HBV genes and other heterologous viruses (23). When the entire HBx gene under its own regulatory elements was placed directly into the germline of mice, transgenic animals harboring this gene were noted to sequentially develop multifocal areas of altered hepatocytes, then hepatic adenomas and finally malignant carcinoma (22). In this model as well as in that of Chisari, et al (21), tumors developed more frequently and at an earlier age in male mice suggesting that hormonal influences played a role in either mechanism of carcinogenesis. Thus HBV infection may contribute to development of hepatic malignancies by any of several mechanisms. However, the preponderance of clinical evidence in humans indicates that prolonged periods of active hepatitis with associated cellular injury and regeneration are closely associated with this process.

AFLATOXIN B_1 AND HEPATOCELLULAR CARCINOMA

Epidemiologists interested in the high prevalence of hepatocellular carcinoma in Africa and Asia have long speculated on the role of non-infectious environmental factors. Much of such speculation has centered on the potential causative or co-carcinogenic role of the mycotoxin, aflatoxin B_1. Aflatoxin B_1 has been shown to be a potent carcinogen in animal models and in particular to mutate DNA by inducing G→T transversions (24). Contamination of food with aflatoxin has been well documented in areas of Africa, China and Southeastern Asia (25-27). However, until recently the only evidence for a role for this agent in human liver cancer was based on crude correlations between levels of aflatoxin ingestion incidence and hepatocellular carcinoma in selected locales in Africa and Asia (25-27). However, within the last two years a number of reports of selective G to T mutations in the p53 gene in hepatocellular carcinomas from patients residing in areas of high aflatoxin exposure have provided much more persuasive evidence for a role of aflatoxin in generation of hepatic malignancies.

The p53 tumor suppressor gene has been shown to be mutated in a significant number of colon, lung, breast and other human cancers (28). As shown in figure 2, such mutations have been localized to multiple sites in this gene though clustering of mutations in "hotspots" around codons 175, 248, 273 and 282 have been previously demonstrated in human colon, lung and breast cancers. As detailed in Table IV, sequencing of p53 genes from hepatocellular carcinoma specimens has demonstrated frequent mutations in codon 249 of genes isolated from specimens collected in areas of high aflatoxin exposure but not in specimens form areas of low aflatoxin exposure risk (29-32). Two additional p53 mutations at codons 157 and 286 have also been noted in hepatic tumors. The vast majority of p53 mutations in hepatocellular carcinoma represent G→T transformation events. Virtually all hepatocellular carcinoma specimens from Mozambique or China with p53 mutations came from HBsAg (+) individuals, although no p53 mutations were found in HBsAg (+) South African specimens. This is not entirely unexpected as areas of high HBV prevalence and aflatoxin exposure overlap. Such mutations were absent in surrounding normal hepatic tissue indicating that they were somatically acquired. In addition in most tumors, expression of homozygous mutant p53 was noted. This latter observation is the anticipated finding in tumors generated after loss of normal suppressor gene activity via mutation and loss of heterozygosity. While these findings do not conclusively demonstrate that such mutations are sufficient to trigger neoplastic transformation, they do suggest that aflatoxin B_1 exposure (or other environmental agents in these locales) appear to cause somatic mutations likely to contribute to generation of hepatic malignancies.

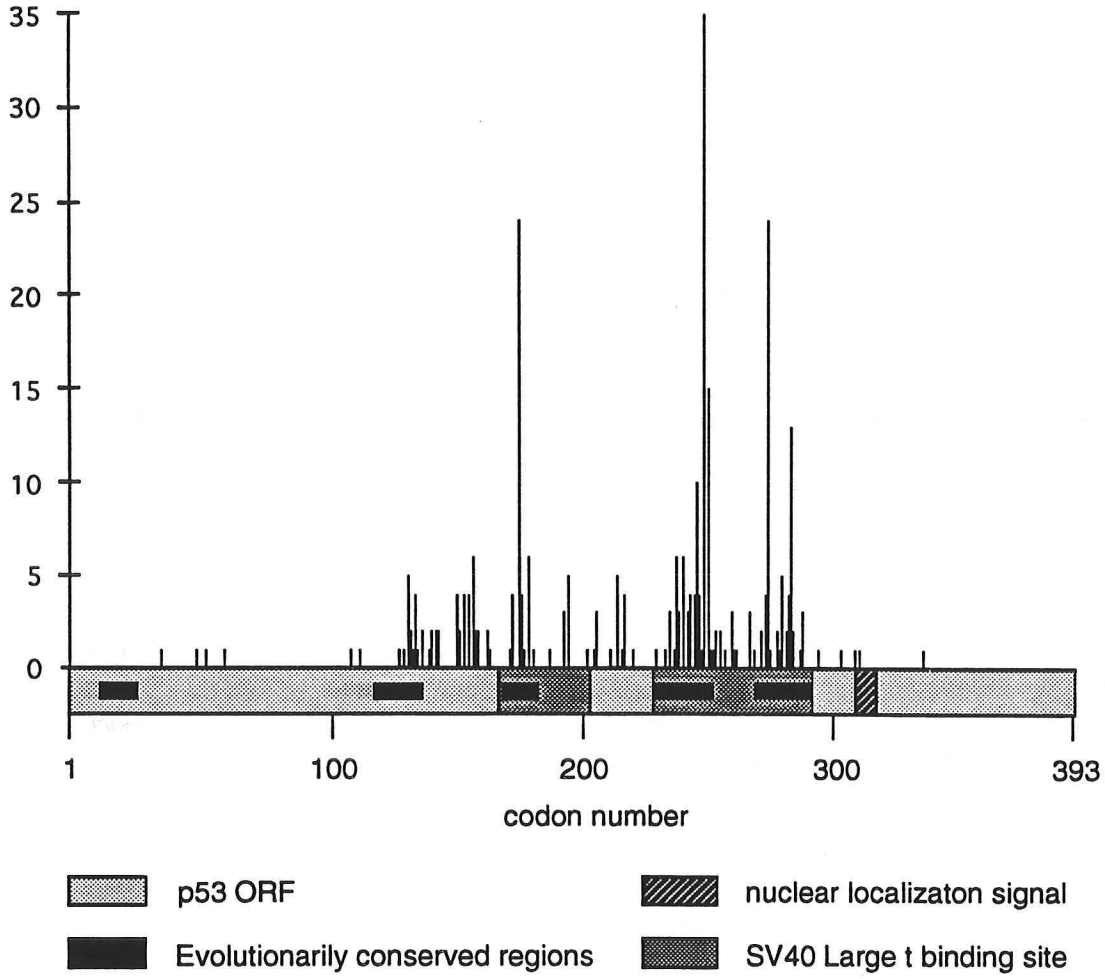

Figure 2. Schematic diagram of the p53 open reading frame and locations of mutations in diverse tumor types. Data and figure provided by Dr. David Carbone, Department of Internal Medicine, UT Southwestern Medical Center.

Table IV

Frequency of p53, Codon 249 Mutations in Hepatocellular Carcinoma

Author	Country of Origin	Dietary Aflatoxin Intake	Frequency of p53, Codon 249 Mutations
Hsu (29)	China, Qidong	High	8/16
Hosono (30)	Taiwan, China	Variable	0/18
Ozturk (31)	Mozambique	High	8/15
	China/Vietnam/Korea	Variable	3/38
	South Africa	Low	1/24
	US/Europe/Middle East	Low	0/78
Patel (32)	Europe	Low	1/36

ROLE OF HBV IN HBsAg (-) HEPATOCELLULAR CARCINOMAS

While much epidemiologic evidence has linked HBV infection and/or aflatoxin exposure to the high prevalence of hepatocellular carcinoma in much of Africa and Asia, most U.S., European and Japanese patients with hepatocellular carcinoma are HBsAg (-) and appear not to be exposed to significant amounts of dietary aflatoxin. In the U.S., only 7-26% of patients with this malignancy have been found to be HBsAg (+) (33-35). As this HBV infection rate is far higher than the $<1\%$ HBsAg carrier rate in the general population, presence of HBsAg appears to be a true risk factor in such patients. Nevertheless, as this risk factor appears to be present in only a small fraction of cases in the U.S., Europe and Japan, it has long been suspected that other forms of chronic liver disease and cirrhosis were the major associated risk factor for development of liver cancer. In the U.S. and Europe a high percentage of hepatocellular carcinoma patients appear to have underlying alcoholic cirrhosis and in France the relative risk of developing hepatocellular carcinoma in the setting of chronic HBsAg (+) liver disease is only ~ 2.5 fold higher than the risk in patients with cirrhosis apparently related to long-standing alcohol abuse (36). Thus while HBV infection appears to be a strong risk factor for development of this malignancy, in countries where alcoholism and other factors are much more frequent causes of chronic liver disease, the majority of such tumors have appeared to be unrelated to HBV.

This concept has been challenged by a group of investigators who extracted DNA from a series of hepatocellular carcinomas from French patients with apparent alcoholic liver disease and reported, initially, that by direct hybridization techniques HBV DNA could be detected in all such tumors (37). This finding was all the more astonishing because many such patients not only lacked serum HBsAg but were also negative for all antibody markers of previous HBV infection. However, similar analysis of hepatocellular carcinomas from HBsAg (-) patients performed by multiple investigators around the world yielded quite different results with HBV DNA being detected in the vast majority of HBsAg (+) patients but only rarely in hepatomas from HBsAg (-) patients (38-42). Moreover, such HBV DNA (+), serum HBsAg (-) patients have generally been found to be anti-HBc (+) +/- anti-HBs (+). Such patients likely represent patients chronically infected with HBV who have lost HBsAg late in their clinical course.

However, Brechot and co-investigators from France have published additional follow-up studies suggesting that very sensitive PCR based analyses indeed detect HBV DNA in some but not all tumors from HBsAg (-) patients (43-45). These follow-up reports seem not only to tacitly admit to inability to reproduce initial direct hybridization HBV DNA results, but again raise questions regarding potential for false positive results. These investigators detail multiple laboratory controls including sequencing of PCR products obtained in separate experiments from the same tumor to demonstrate both that true HBV DNA is present and that patient HBV DNA sequences are reproducible but different from other laboratory HBV DNA isolates (44). However, scant details regarding controls at site of collection or criteria for selection of hepatomas for analysis have been provided. HBV viral particles are present at extremely high titers in the sera of infected patients (46) and HBV DNA is quite stable even after sera or tissues are removed from the host. Thus even seemingly infinitesimal contamination of hepatocellular carcinoma specimens with sera or tissue from another HBsAg (+) patient processed in the same hospital laboratory could account for subsequent repeated detection of HBV DNA in tumor tissue by PCR based assays.

A second group of investigators from Spain has recently reported the results of HBV DNA PCR assays performed in sera collected from a series of 70 consecutive patients with hepatocellular carcinoma (47). Again no controls to assure lack of sample contamination were provided. In addition to finding HBV DNA in all HBsAg (+) patients (12/12), these investigators also found HBV DNA in 12/58 HBsAg (-) samples. Of note, such HBV DNA (+),

HBsAg (-) results were equally distributed among anti- HBc and/or anti-HBs (+) patients (7/23 HBV DNA+) and anti-HBc and anti-HBs (-) patients (5/21 HBV DNA+), a pattern that again appears inconsistent with expected correlations between HBV infection and antibody responses and raises questions about specificity of PCR assays for HBV DNA.

Some reviewers have interpreted these reports of HBV DNA (+), HBsAg (-), anti-HBc (-), anti-HBs (-) liver disease as suggesting the presence of a Mediterranean HBV variant virus that causes chronic liver disease and eventually hepatocellular carcinoma without producing readily detectable levels of serum HBsAg or eliciting a sustained host antibody response (44). Such hypotheses propose pathogenetic mechanisms quite different from many well documented features of HBV related liver disease. Thus, even in individuals with only acute resolving HBV infection the host anti-HBc response is long-lived. Greater than 30 years after a World War II epidemic of hepatitis B related to a contaminated yellow vaccine, >97% of individuals with clinically apparent illness were found to be anti-HBc (+) (48). Moreover, in individuals with acute or chronic HBV infection, hepatocellular injury appears to be mediated largely if not entirely by host immune responses directed at virally infected cells with host T cell responses to HBc epitopes appearing to play a prominent role (49). Finally, in the course of chronic HBV infections there appears to be an inverse correlation between levels of circulating viral particles and levels of host immune response and hepatocellular injury. Thus, in immunocompromised individuals titers of serum HBV DNA and HBsAg are very high while only when a significant host immune response is mounted does level of HBV DNA appear to fall (49,50). For all of these reasons, an HBV variant virus that not only evades all apparent host immune response but also does not proliferate above the threshold of levels detected by conventional HBV DNA and HBsAg assays yet causes chronic liver disease leading to both cirrhosis and hepatocellular carcinoma seemingly contradicts current understanding of pathogenesis of HBV related liver disease. Until such time as assays are performed in parallel in both hepatocellular carcinoma samples and appropriate clinical controls, the significance of isolated low level HBV DNA in individuals without conventional markers of HBV infection remains of questionable significance.

HCV AND HEPATOCELLULAR CARCINOMA

In addition to skepticism regarding the role of HBV in the majority of U.S., European and Japanese hepatocellular carcinomas, a variety of epidemiologic observations made during the late 1970's and early to mid-1980's suggested that a major additional cause of hepatocellular carcinoma was becoming apparent in Italy and Japan. As shown by the results detailed in figure 3 on the next page, a major increase in the frequency of hepatocellular carcinoma was noted in autopsies performed at the University of Florence between 1958 and 1982 (51). This increase could not be attributed entirely to somewhat more modest increases in the rate of cirrhosis at autopsy. As summarized in figure 4, between 1976 and 1987 the number of cases of hepatocellular carcinoma referred to the National Cancer Center Hospital in Tokyo, Japan steadily increased with the increase appearing to composed entirely of HBsAg (-) patients (38). HBV DNA analysis of 53 such HBsAg (-) hepatocellular carcinoma patients revealed HBV DNA in only 4 cases despite the fact that 65% were anti-HBc (+). As summarized in figure 5, rates of hepatocellular carcinoma in various geographic regions of Japan prior to 1978 were quite low and comparable to that of white residents of the San Francisco Bay Area (52-54). However, the incidence of hepatocellular carcinoma seen between 1978 and 1982 rose ~4-fold to rates that in some areas were comparable to those seen in Chinese populations. Shown in figure 5 are the incidence rates for the prefectures with the highest (Osaka) and lowest (Miyagi) reported incidence rates during this interval. Similar increases were seen in hepatocellular carcinoma rates in other areas of Japan with those in Nagasaki and Hiroshima intermediate to those shown for Osaka and Miyagi. Of note, incidence of this form of malignancy did not change among

AUTOPSIES, UNIVERSITY OF FLORENCE, ITALY

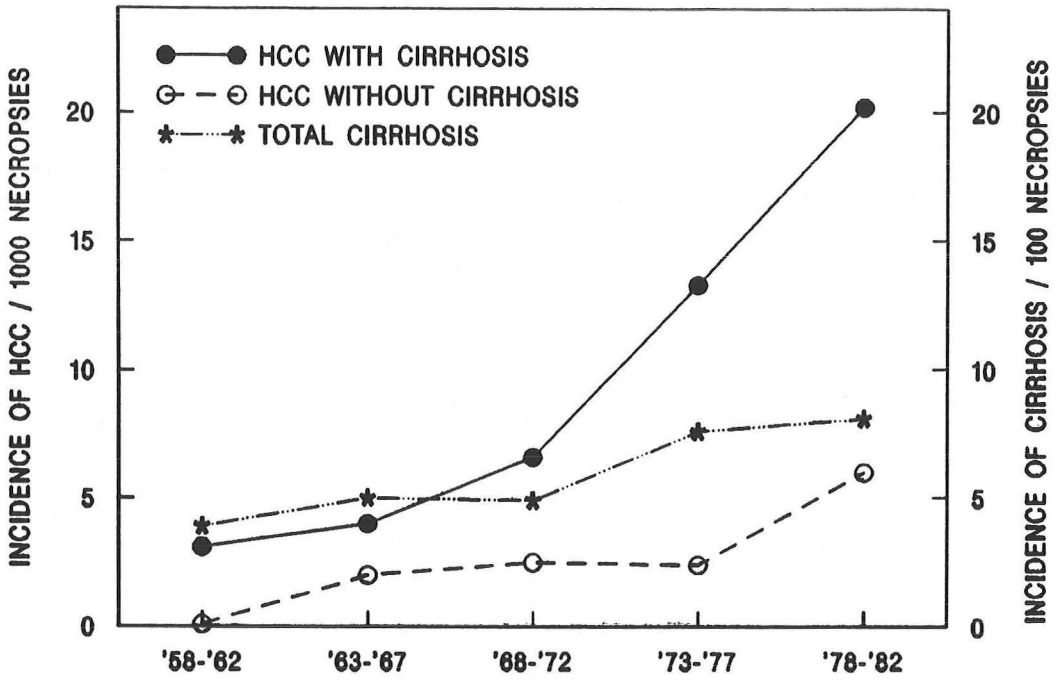

FIGURE 3

NEW HCC PATIENTS, NCCH, TOKYO, JAPAN

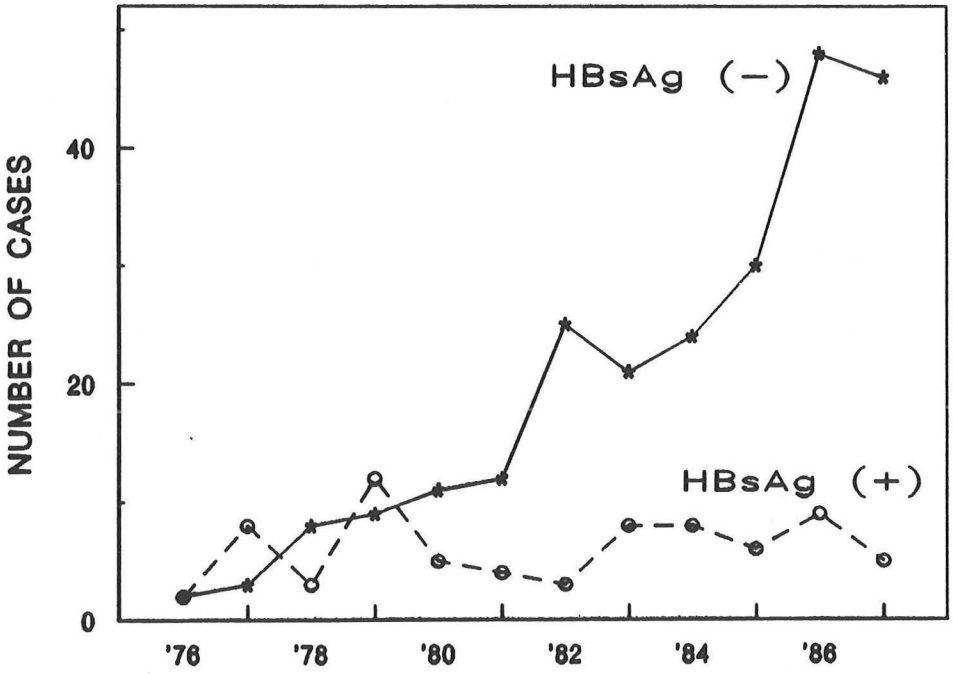

FIGURE 4

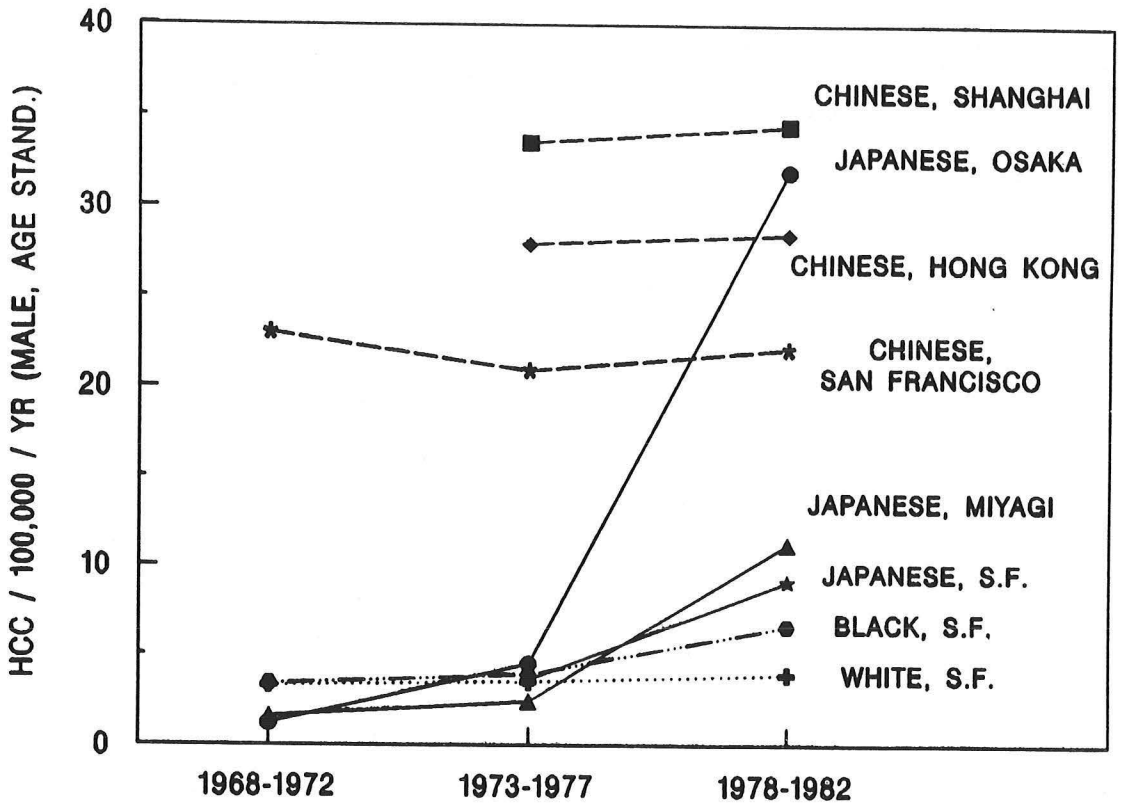

FIGURE 5

individuals of Chinese descent residing in Shanghai, Hong Kong or San Francisco during this same time period (figure 5). While hepatocellular carcinoma rates remained stable in white residents of San Francisco during this time interval and through the period of 1983-1989 as well (personal communication, Angela Harras, Surveillance, Epidemiology and End Results (SEER) Program, NCI, Bethesda, Maryland) modest increases in rates among Black and Japanese residents of the San Francisco Bay area were noted between 1973-1977 and 1978-1982. Rates among black residents of the San Francisco Bay area continued to increase by an additional 25% during the 1983-1989 interval (data not shown). No additional data is available on more recent rates of hepatocellular carcinoma in San Francisco among residents of Japanese descent.

The majority of hepatocellular carcinomas seen in Japan in the late 1970's and 1980's continued to occur in cirrhotic patients. The only risk factor for liver disease that was increasing among these patients appeared to be an increasingly frequent history of prior blood transfusions in patients who developed hepatocellular carcinoma (55,56). When the hepatitis C virus was isolated in the late 1980's and tests for antibodies to HCV antigens were developed, a number of epidemiologic studies conducted in Japan and Italy demonstrated an association between presence of antibodies to this virus and hepatocellular carcinoma (57-59). Because first generation anti-HCV tests had significant problems with both sensitivity and specificity (60) there was some initial question regarding specificity of such testing in patients with advanced malignancies. However, with development of second generation confirmatory assays that test for reactivity to multiple, independent HCV antigens and include appropriate specificity controls, the association between HCV was actually strengthened as an even higher percentage of

hepatocellular carcinoma patients were found to be anti-HCV positive (58). In addition, while there is no HCV equivalent of the HBsAg antigen test to assess for evidence of viral proteins in the serum, when PCR assays for HCV RNA have been used to screen sera of hepatocellular carcinoma patients (47), a very close correlation between presence of antibodies to HCV and presence of HCV RNA has been found. Finally in all epidemiologic studies using serodiagnostic tests for both HCV and HBV, both presence of HBsAg and a positive anti-HCV test have proven to be independent risk factors for hepatocellular carcinoma with the presence of both markers conferring addition risk (35,59,61). Surprisingly, in both Italy and Taiwan the relative risk associated with evidence of HCV infection has been found to be higher than the risk associated with being HBsAg (+) (59,61).

Table V

Frequency of HBsAg and Anti-HCV in Patients with Hepatocellular Carcinoma

Country of Origin	HBsAg (+) Anti-HCV (-)	HBsAg (+) anti-HCV (+)	HBsAg (-) anti-HCV (+)	HBsAg (-) anti-HCV (-)
Japan (58)	22/125 (**18%**)	1/125(**1%**)	85/125 (**68%**)	17/125 (**14%**)
Italy (59)	15/212 (**7%**)	18/212 (**8%**)	133/212 (**63%**)	46/212 (**22%**)
Taiwan (61)	87/128 (**68%**)	12/128 (**9%**)	13/128 (**10%**)	16/128 (**13%**)
U.S., Johns Hopkins (35)	6/99 (**6%**)	1/99 (**1%**)	12/99 (**12%**)	70/99 (**71%**)
U.S., PMH (see Table VI)	2/9 (**22%**)	0/9 (**0%**)	4/9 (**44%**)	3/9 (**33%**)

The frequency of HCV and HBV markers in series of hepatocellular carcinoma patients from various areas of the world is summarized in Table V. Of note, in Italy, Spain and Japan >60% of hepatocellular carcinomas occur in anti-HCV (+) patients, 15-20% in patients who are HBsAg (+) and only 14-22% of cases in patients who are HBsAg and anti-HCV (-). In Taiwan, 77% of hepatocellular carcinoma patients are HBsAg (+), 19% anti-HCV (+) and only 13% negative for either marker. Only in analysis of a recent series of U.S. hepatocellular carcinoma patients referred to Johns Hopkins (35) has the majority of patients proven to be negative for both HBsAg and anti-HCV. Of note, as detailed in Tables V and VI, recent experience in Parkland Memorial Hospital (PMH) is somewhat different. Since the onset of clinical trials that have provided access to a second generation, confirmatory anti-HCV assay (Matrix HCV, Abbott Laboratories), 9 consecutive patients with hepatocellular carcinoma seen at PMH between May, 1991 and July, 1992 by the Liver consult service have been assessed for presence of markers of HBV or HCV infection. The majority had markers of either ongoing HBV infection (2/9 were HBsAg+) or presumed HCV infection (4/9 were anti-HCV+ by a confirmatory assay) while only a third (3/9) lacked evidence of either HBV or HCV infection. Dr. Y. Patt at M.D. Anderson Cancer Center in Houston has also indicated (personal

communication) that >50% of patients recently referred to him for therapy of hepatocellular carcinoma (52 total patients) have had markers of HBV and/or HCV infection. Thus the current spectrum of hepatocellular carcinoma seen in Texas appears somewhat different than that referred to Johns Hopkins. Recent hepatocellular carcinoma patients seen at PMH resemble Southern European patients not only in relative frequency of chronic viral hepatitis but also with respect to frequency of history of alcohol abuse (see Table VI).

Table VI

Patients with Hepatocellular Carcinoma Evaluated by the

Liver Consult Service at PMH from May, 1992 thru July, 1992

Patient	Age	Sex	HBsAg	Anti-HCV	Lifestyle Risks	Presenting Complaint	Mean Diameter	α-FP (ng/ml)
1	63	M	+	-	↑ EtOH X 30 yr	↑ Ascites	2.5 cm	11,143
2	50	M	-	+	IVDA X 25 yr	Variceal Bleeding	N.M.	77
3	84	M	-	-	none	wt loss	15 cm	< 3
4	57	F	-	+	Transfusion, 22yr PTA	Ascites	10 cm	390,000
5	52	M	-	-	↑ EtOH X 35 yr	Pain	5 cm (multiple)	< 3
6	48	M	-	+	Penitentiary X 10 yr, ↑ EtOH	Variceal Bleeding	10 cm	3264
7	52	M	-	-	↑ EtOH X 25 yr	ascites	7 cm	4538
8	19	M	+	-	none	Pain	19 cm	4824
9	55	F	-	+	Transfusion, 23 yr PTA	Variceal Bleeding	3 cm (multiple)	9

N.M. = not measured, tumor found in portal vein at time of surgery

OTHER LIVER DISEASES AND HEPATOCELLULAR CARCINOMA

Development of hepatocellular carcinoma in the setting of chronic alcoholism and cirrhosis has been observed frequently and has generally been listed as the most common predisposing risk factor for hepatocellular carcinoma in the U.S. and Europe. However, alcoholics frequently require blood transfusions following traumatic injuries or episodes of gastrointestinal bleeding and/or indulge in mixed patterns of alcohol and intravenous drug abuse. Perhaps for these reasons, the incidence of antibodies to HCV is much increased in U.S. and Southern European patients with alcoholic liver disease (59, 62-62) and the prevalence is even higher in alcoholics with hepatocellular carcinoma (59,61). Multiple epidemiologic analyses have

shown that alcoholism or the presence of cirrhosis secondary to alcoholic liver disease are risk factors for hepatocellular carcinoma (1,13,36). However, as such analyses have been performed prior to the availability of reliable anti-HCV tests, it is difficult to determine whether alcoholism is a truly independent risk factor though it seems likely that this is the case (59).

Rare cases of hepatocellular carcinoma have been reported in virtually every form of cirrhosis. However, thorough screening for chronic viral co-infection is absent from most such reports. Nevertheless, hepatologists have been impressed with the relative rarity of hepatic malignancies in patients with certain forms of liver disease such as Wilson's disease, primary biliary cirrhosis, or autoimmune chronic active hepatitis, while this complication is commonly observed in diseases such as hereditary tyrosinemia (65) and genetic hemochromatosis (66). In hemochromatosis, the relative risk for development of hepatocellular carcinoma ($\sim$220 fold increase) is even higher than for either chronic HBV or HCV infection (66). It therefore appears that multiple forms of chronic liver injury can predispose to development of this malignancy. Nevertheless, the most common risk factors for this malignancy in developed countries appears to be chronic viral hepatitis and alcoholic liver disease.

DIAGNOSIS

When hepatocellular carcinoma has reached an advanced, symptomatic stage, diagnosis is rarely difficult once the possibility of a malignancy is considered. Right upper quadrant pain or epigastric pain is the most common initial symptom. Other common presentations include the development of new complications of liver dysfunction such as ascites, encephalopathy, variceal hemorrhage or abrupt worsening of jaundice (67,68). As most patients have underlying cirrhosis, it is easy to dismiss these latter problems as simple progression of chronic liver disease unless one maintains a high level of suspicion for this diagnosis in patients with unexplained acute deterioration of liver function in the setting of chronic, stable liver disease. Finally, up to 10% of patients may present with an acute abdomen secondary to abrupt rupture of a tumor or with one of the multiple paraneoplastic manifestations of this malignancy such as hypoglycemia, fever, hypercalcemia, erythrocytosis, dysfibrinogenemias or hemolysis(69). Most patients at this stage of the disease have a mass apparent by any of a number of imaging techniques and have elevated levels of α-fetoprotein or abnormal forms of alkaline phosphatase, novel γ-glutamyltranspeptidase isoenzymes, or abnormal forms of prothrombin (des-γ-carboxy prothrombin, 70,71).

Of these serum protein markers, α-fetoprotein abnormalities have been most widely studied and this test is the most widely available. Normal adult serum α-fetoprotein levels are usually either undetectable or below 10 or 20 ng/ml. In chronic liver disease, elevations of α-fetoprotein are seen in 10-30% of patients, but these elevations are usually in the 10-100 ng/ml range and those $>$ 100 ng/ml are often transient and may correlate with flares of disease activity (1, 70-73). Sustained levels of α-fetoprotein above 400 or any progressive exponential increase in this marker in patients with chronic liver disease strongly suggests the diagnosis of hepatocellular carcinoma (70-73). Pregnancy and malignancies of the ovaries or testes appear

to be the only other causes of sustained α-fetoprotein elevations of this degree (72,73). While most large symptomatic hepatocellular carcinomas are said to have α-fetoprotein values > 500 or 1000 ng/ml (1), this is not invariably the case (see Table VI) tumor marker is far less sensitive in patients with small often asymptomatic tumors (see Table VII). As shown in Table VII, the frequency and degree of α-fetoprotein elevation varies with size of lesion and in tumors between 2 and 5 cm in diameter, only 50% of patients have α-fetoprotein levels above 100 ng/ml and only 25-40% have values > 400ng/ml.

Table VII

Correlation between Tumor Size and α-Fetoprotein Levels in Japanese Patients with Hepatocellular Carcinoma

Size (Diameter)	α-Fetoprotein (ng/ml)			
	> 10	> 100	> 400	> 1000
≤ 2 cm	72%	33%	17%	6%
2-3 cm	81%	50%	25%	15%
3-5 cm	83%	51%	39%	22%
Liver disease controls	29%	7%	2%	<1%

Data from Japanese patients detailed in reference 70.

In patients with either symptomatic tumors or in asymptomatic patients with elevated α-fetoprotein levels, ultrasonography has ≥ 90% sensitivity. However, in patients with small hepatocellular carcinomas, all non-invasive imaging techniques have lesser degrees of sensitivity although >75% of 1-3 cm lesions can still be detected by ultrasonography alone (see Table VIII, 74,75). In diagnosis of progressively smaller lesions, only angiographic techniques have significant yield. As ultrasonography is in general a more sensitive technique than more expensive computerized tomography (CT) or magnetic resonance imaging (74,75), this technique is routinely recommended as the initial imaging study. In addition to standard CT scanning or angiographic techniques, Japanese physicians have noted that following injection of lipiodol, an iodized oil contrast media (ethyl ester of the fatty acid of poppyseed oil, 38% iodine by weight), into the hepatic artery, this agent is initially evenly distributed in the liver but then fairly rapidly cleared by normal liver parenchyma while being retained in primary or secondary malignant lesions (76). The selective retention of lipiodol in neoplastic tissue is thought to be related to the absence of normal Kupffer cell and/or lymphatic function which is an especially prominent feature of hepatocellular carcinoma. When follow-up CT scans are performed 7-10 days after injection, retention in hepatocellular carcinomas but not regenerative nodules or benign tumors is observed. This lipiodol-CT scanning technique has been found in some but not all studies to be more sensitive than angiographic or other imaging modalities in detection of small

hepatocellular carcinomas (74,75). However, lipiodol, which was first used as a lymphangiographic dye, is not FDA approved for intra-arterial use in the U.S. and use of this diagnostic technique has largely been confined to Japan. Intra-arterial lipiodol injections commonly are associated with fever and variable degrees of abdominal discomfort (77).

Table VIII

Sensitivity of Imaging Techniques in Hepatocellular Carcinoma

Technique	Percent of Lesions Detected	
	≤ 1 cm	1-3 cm
Ultrasound	8%	77%
CT	8%	59%
Angiography	29%	71%
Lipiodol CT	100%	89%
MRI	4%	78%

Results obtained from combined data presented in references 74 and 75.

In patients with hepatic mass lesion and normal or equivocal α-fetoprotein levels, biopsy of the lesion is required to confirm the diagnosis. However, in patients with sustained elevations of α-fetoprotein above 400 ng/ml, a compatible mass lesion by imaging techniques and no other cause for ↑↑ α-fetoprotein (i.e. not pregnant and no evidence of gonadal malignancy) the diagnosis of hepatocellular carcinoma is a virtual certainty. As hepatocellular carcinomas are vascular tumors associated with an risk of bleeding at time of biopsy and have been reported to metastasize along needle tracks (78,79), biopsy is felt not to be necessary in such cases and to be relatively contraindicated in patients who appear to be candidates for curative resections (1).

THERAPY OF HEPATOCELLULAR CARCINOMA

SURGICAL THERAPY

Only surgical therapies of hepatocellular carcinoma are thought to be potentially curative. However, both the frequency of associated severe liver disease and the advanced stage of most clinically apparent hepatocellular carcinoma has traditionally resulted in only a small percentage of patients being judged as candidates for curative resections. In a survey of primary liver cancers evaluated and treated by the Liver Cancer Study Group of Japan between January 1, 1968 and December 31, 1977, resections could be performed in only 361 of 4031 cases (9%), and operative mortality was 27.5% (80). Moreover, survival for greater than 3 years was observed in only 19.6% of patients following resection of hepatocellular carcinoma. More than

half of long term survivors were among the small minority of patients (16.5% in this series) who presented with hepatocellular carcinomas in non-cirrhotic livers.

However, over the next decade major advances in both diagnostic and surgical techniques led to an increasing fraction of patients who presented with resectable lesions and to dramatically lower operative mortality rates. By the eighth report of the Liver cancer Study Group of Japan (81,82) citing 7,320 patients treated in 1984-1985, 1217 (16.6%) patients received hepatectomies with an overall operative mortality rate of 3.4%. Even more impressive was an improvement in 5 year survival from 11.8 to 28.5% with many more cirrhotic patients receiving successful resections. Comparable operative mortality rates have been reported by surgeons in China (2.3%, 6) and Europe (8 and 6.9%, 5,83). As detailed in Table IX, a number of surgical series from Asia, Europe and the U.S. have now documented 3 yr survivals between 39 and 83% and 5 year survivals between 28 and 67%. These results are especially impressive as most series were composed of largely cirrhotic patients. However, several of the series were composed of small highly selected groups of patients. Only the report from the Japanese Liver cancer Study group would appear to comprise all hepatic resections performed in multiple hospitals. Of note the survival rates achieved by Tang, et al appears distinctly different from that of all other groups, perhaps because fully 75% of these cases were detected in an early, asymptomatic stage through an α-fetoprotein/ultrasonography survey (6). The fact that 10 year survival in this study (53.4%) was also >50% suggests that these excellent results were not merely the result of lead time bias. While no randomized prospective trials of surgical vs. non-surgical therapy of this disease have been reported, survival of patients presenting with symptomatic unresectable, tumors has generally been <5% at 3-5 years irrespective of modality of non-surgical therapy employed (85). Surgical candidates of course tend to have less advanced disease and better preserved hepatic function. Series of patients with small, asymptomatic tumors who did not receive any therapy have been reported to have one year survivals between 30-96% (5,85). However, in the series with 96% one year survival in untreated patients, half of the patients died in the second year and no longer term follow-up is provided. In a larger compilation of patients with untreated hepatocellular carcinoma presenting at various stages of disease progression, even initially asymptomatic patients had only ~10% 2.5 year survival and no patients survived beyond 3.5 years (85). Thus any therapy that achieves >25% 5 year survival in this disease likely provides significant overall prolongation of survival.

Table IX

Survival after Resection of Hepatocellular Carcinoma

Authors	# of Patients	% cirrhosis	Survival		
			1 yr	3 yr	5 yr
Tang, et al (6)	132	90%	94%	83%	67%
LCSGJ (7)	2478	74%	67%	40%	29%
Paquet, et al (8)	23	100%	77%	62%	49%
Franco, et al (83)	72	100%	68%	51%	-
Iwatsuki, et al (84)	76	22%	71%	47%	33%

Resectability of hepatocellular carcinoma is limited not only by extension of tumor beyond the confines of the liver parenchyma or involvement of both lobes or central vasculature, but also by the extent of underlying liver disease. Successful resection with acceptable operative mortality rates has been achieved only in series composed largely of patients with Child's A liver function and smaller numbers of patients with of Child's B liver function (6,7,8,72,76). Especially in the U.S. and Europe, hepatocellular carcinomas tend to present with more advanced underlying liver disease. Even if patients survive a curative resection, progression of underlying liver disease may lead to mortality over the ensuing several years or patients may develop a second focus of hepatocellular carcinoma.

For all of these reasons it was anticipated that in patients with hepatocellular carcinoma and underlying cirrhosis, liver transplantation would lead to significantly improved long term survival. However, early experience with liver transplantation in such patients was very disappointing. Compilation of U.S. transplant experience by the Cincinnati (previously Denver) Transplant Tumor Registry (87) revealed that patients with typical symptomatic hepatocellular carcinomas ("usual" hepatomas in Table X) had a 39% recurrence rate after transplantation and overall survival of only 30% at 2 years and 18% at 5 years. However, as shown by the results detailed in Table X, two groups of hepatocellular carcinoma, those with small tumors discovered only incidentally at time of transplantation for liver failure and those with the fibrolamellar histologic variant had significantly better long term survival. Some of the poor survival in this complete listing of U.S. hepatocellular carcinoma patients may reflect inclusion of some patients from the pre-cyclosporine era as a recent report of 76 hepatocellular carcinoma patients transplanted at the University of Pittsburgh since 1980 had a somewhat better long term survival (84). However, this series contained a somewhat higher percentage of noncirrhotic patients (32%) and 6/13 patients surviving for more than five year were pediatric cases. A report of the European Liver Transplant Registry in 1987 revealed a two year survival of only 31 % in patients undergoing transplantation for hepatocellular carcinoma (88).

Table X

Survival After Liver Transplantation for Hepatocellular Carcinoma

Series	Type of Tumor	Recurrence	Survival	
			2 yr	5 yr
U.S. Registry	Usual*	39%	30%	18%
(87)	Fibrolamellar	39%	60%	55%
	Incidental	13%	57%	57%
	All	37%	~34%	~24%
Pittsburgh	Fibrolamellar	N.S.	70%	38%
1980-1989 (84)	All	43%	49%	36%**
European Registry (88)	All	N.S.	14%	--

* Usual hepatomas = symptomatic, non-fibrolamellar; ** Comprised of 10% fibrolamellar and unstated fraction of incidental hepatocellular carcinomas; N.S. = Not Stated

Analysis of cases treated by both resection and transplantation has revealed a number of factors that significantly affected survival (87,89,90). Presence of lymph node metastases or other gross tumor outside resection boundaries was associated with 0% 4-5 year survival in all patients treated with either resection or transplantation. Survival was also negatively influenced by presence of cirrhosis, HBV infection, presence of tumors > 5 cm in diameter (or > 2.2 cm when only small tumors analyzed), the absence of a macroscopic capsule around the tumor or tumor infiltration into the capsule, and the presence of tumor in the portal trunk or the first or second branches of the portal vein (7,89,90). Upon multivariate analysis, factors predictive of recurrence after hepatic resection were location deep in the liver, fibrous capsular formation around tumor and macroscopic portal vein involvement. Analysis of patients transplanted at the University of Pittsburgh (89) revealed that those patients with any of three characteristics, infiltrative tumors (not enclosed by capsule), macroscopic involvement of portal vein or other vasculature, or tumor outside of resection margins had 0% 3 year survival. The finding conferring the highest risk of tumor recurrence was macroscopic involvement of vascular structures.

NONSURGICAL THERAPY

While resection and/or transplantation appear to offer some chance of cure, most patients are not candidates for curative resection and because of the poor overall results following transplantation, many centers have greatly restricted transplants in patients with malignancy because of limited supplies of transplant organs and other resources and the large numbers of patients with better post-transplant prognosis awaiting transplantation. A number of non-surgical therapies have been tried over the years. Therapies such as irradiation, hepatic artery ligation or embolization have been largely discarded because of limited response in the face of significant complications (85). Use of multiple forms of oral, intravenous, and intra-arterial chemotherapy have been employed with little success other than response rates of 11-25% for intravenous (or intra-arterial) doxorubicin (1). While responses to intravenous cisplatin have been disappointing, use of this agent in conjunction with irradiation in a multimodality Phase I,II trial has been associated with a 36% overall response rate (91). Nevertheless, duration of response to intravenous chemotherapy tends to be short and as this therapy is associated with significant morbidity, there has been little enthusiasm for its use in most patients.

More recently, Japanese investigators have reported promising results with doxorubicin or cisplatin that is targeted to hepatocellular carcinoma by suspension of the drug in lipiodol prior to intra-arterial injection. There is no binding of chemotherapeutic agent to lipiodol. Rather, this agent merely serves as a vehicle for drug delivery. The manner in which drug and lipiodol are mixed appears to influence final drug delivery. Doxorubicin suspended in methanol prior to mixing with lecithin and lipiodol prior to intra-arterial injection has been shown to sustained high levels of the drug in tumor tissue while mere mixing of aqueous drug solutions with lipiodol prior to injection has not been shown to be associated with significant targeting of drug to tumor tissue (92). Perhaps for these reasons, a trial of intra-arterial injection of aqueous doxorubicin/lipiodol emulsions performed in the United Kingdom showed no difference in systemic drug levels of response rates when compared to historical controls receiving intra-arterial doxorubicin (93), while multiple other trials performed in Japan, Europe and the U.S. have shown response rates somewhat higher than previously reported for intravenous or intra-

arterial doxorubicin (92,94,95). Other investigators have reported similar response rates using mitomycin C or cisplatin suspended in lipiodol. Intra-arterial lipiodol chemotherapy has often been used in combination with embolization of the hepatic artery with gelfoam or autologous clot to induce additional stasis of blood flow and hopefully prolonged retention of chemotherapeutic agent in tumor tissue (94-99). Other investigators have taken advantage of the high iodine content of lipiodol and have linked ^{131}I to this agent prior to intra-arterial injection (100). Finally, a number of institutions have reported use of direct injections of ethanol into hepatocellular carcinoma tumor masses either in conjunction with chemoembolization protocols or as a sole form of palliative therapy (101, 102).

The results of selected trials are detailed in Table XI. As patients with greatly different tumor sizes and underlying liver function were enrolled in different trials it is difficult to compare responses to different regimens. Most though not all investigators (93) have reported better response rates and/or survival than "historical controls" treated with conventional chemotherapy or no therapy. As in Japanese series there have been trends towards earlier discovery of hepatic malignancies and improved survival, it is difficult to exclude lead time bias in these results. Unfortunately, no prospective, randomized comparisons of therapies has been performed. Nevertheless, as no series of untreated controls has demonstrated significant survival beyond 36-42 months after diagnosis, and one chemoembolization protocol actually achieved survival comparable to that achieved with surgical resection (97), there is reason for optimism regarding these results. However, again results appear far superior when these therapies are applied to Japanese patients with smaller, often circumscribed lesions than in typical Western patients with more advanced disease. In addition these "oily chemoembolization" protocols are not without side effects. Common side effects include fever, pain, transient worsening of liver function and gastric and duodenal ulceration related to embolization of lipiodol/chemotherapy into the gastroduodenal arteries (92-100). In addition, patients with Child's C cirrhosis and those with tumor involvement of the main portal trunk are at risk of early fatal complications (95) and are excluded from most trials.

Table XI

Response Rates and Survival Rates in Patients Treated by Intra-Arterial Injection With Chemotherapeutic or Radiotherapeutic Agents Suspended in Lipiodol

Authors	Agent Suspended in Lipiodol	Embolization	Response Rate	Survival				
				1 yr	2 yr	3 yr	4 yr	5 yr
Vetter, et al (95)	Doxorubicin	Gelatin Cubes	58%	59%	30%	-	-	-
	nil	nil	--	0%	0%	-	-	-
Gunji, et al (96)	Mitomycin C	Blood Clot	N.S.	100%	100%	-	-	-
	Mitomycin C	Gelatin	N.S.	89%	72%	36%	22%	-
Yoshimi(97)	Mitomycin C	gelatin	N.S.	A-78%* B-90%	A-65% B-72%	A-65% B-43%	A-65% B-22%	A-65% B-10%
	Surgical Resection		-	A-78% B-79%	A-61% B-55%	A-47% B-37%	A-43% B-28%	A-28% B-18%
Shibata, et al (98)	Cisplatin	None	47%	55%	32%	-	-	-
	Neocarzinastatin	None	N.S.	29%	12%	-	-	-
Beppu, et al (99)	Cisplatin	ACR/μsphere	50%	81%	64%	51%	-	-
	nil	ACR/μsphere	N.S.	50%	18%	9%	-	-
Raoul, et al (100)	^{131} I	nil	57%	N.S.	-	-	-	-

COMBINED CHEMOTHERAPEUTIC AND SURGICAL PROTOCOLS

Several groups of investigators have reported the results of combined chemotherapeutic and surgical approaches to therapy of hepatocellular carcinoma. Bismuth, et al from France have reported the use of arterial chemoembolization prior to hepatic resection or liver transplantation and report 2 year survival figures of 49% in Child's A patients, 29% in Child's B, and 9% in Child's C that appear better than historical controls (103). Nonami, et al from Japan report the use of adjuvant arterial lipiodol chemoembolization therapy post-operatively in patients with risk factors for recurrence and after 12-24 months of follow-up report better survival than in matched historical controls (104). Finally, Stone, et al from Baylor University Medical Center in Dallas report the results of a neoadjuvant chemotherapy and liver transplantation protocol in which 20 patients received pre-, intra-, and post-operative intravenous doxorubicin therapy with achievement of 59% actuarial survival at three years. These results are especially impressive since most patients had risk factors such as large tumors (17/20 > 5 cm), underlying cirrhosis or chronic hepatitis in 12/20 patients and HBsAg in 8/20 patients with no patients having fibrolamellar tumor variants. Thus, the overall 3 year survival rate achieved with this protocol appears superior than any previous series of patients receiving liver transplantation as sole therapy for hepatocellular carcinoma. However, it should be noted that lack of tumor involvement of the portal vein was a criteria for enrollment and thus even after surgery, only 1/20 patients was found to have a thrombus in the portal vein secondary to tumor involvement of the portal vein. As macroscopic portal vein involvement has in multiple analyses proven to be a major predictor of tumor recurrence (7,89,90), it may be that this selection criteria rather than use of doxorubicin was the essential component of this trial that afforded an excellent outcome.

SCREENING STRATEGIES FOR HEPATOCELLULAR CARCINOMA

One of the major obstacles to achieving a better outcome in patients with hepatocellular carcinoma continues to be the fact that most such patients only become symptomatic with advanced malignancies that respond poorly to all therapeutic modalities. Results of screening programs for detection of early hepatocellular carcinoma in Chinese and Alaskan Eskimos have been reported (73,106). In both cases, HBsAg (+) individuals in populations where HBV infection is commonly acquired in childhood were targeted and success ,was reported in both detecting asymptomatic malignancies and detecting these lesions at a stage where they were resectable in 7/8 (73) and 4/9 (106) of patients respectively. In part this high resectability rate was related to a high frequency of tumor detection in non-cirrhotic livers. Results of a recent study in Italy have cast doubt on the efficacy of prospective screening of cirrhotic patients (107). When 447 Italians with Child's A or B cirrhosis were enrolled in a screening program, 30 patients had tumors detected at baseline evaluation of which 13 were resectable. However, while 29 additional patients had tumors detected during the prospective phase of the study, only 3 were resectable. Of note, however, many of the patients who developed ultrasonographically apparent tumors during the prospective phase of this study had increased α-fetoprotein at baseline. Thus it is not clear whether more aggressive diagnostic evaluation earlier in the course would have increased the resectability rate. In addition, 14% of the patients progressed to Child's C cirrhosis during the course of the study and this may have also influenced the resectability rate.

In both China, where screening is targeted to HBsAg (+) males, and Japan, where screening is focused on patients with predominately anti-HCV (+) liver disease, aggressive screening programs employ α-fetoprotein tests and ultrasonography every 3-6 months. The timing of these tests are based on estimates of the doubling time of hepatocellular carcinomas that predict that the most rapidly growing tumors will increase from 1 to 3 cm diameter in 4.5 months. While Japanese physicians estimate that the cost of detecting one hepatocellular carcinoma by this strategy to be $8,000 (106) in the U.S., where the estimated risk of hepatocellular carcinoma detection is lower and testing more expensive, it has been estimated that detection of a single hepatocellular carcinoma by this aggressive screening strategy may be as high as $270,000 (107).

Rationales for prospective screening are heavily dependent upon conclusions regarding efficacy of therapy. Thus, patients who are neither candidates for surgical resection or transplantation will receive little benefit from prospective screening. It may be for this reason that reported success of screening programs have been most impressive when applied to patients without clinically apparent cirrhosis. In addition, the persistence of low incidence of this tumor in the U.S. have led many to question the yield in the U.S. of screening programs. In this regard, however, it should be noted that the risk for development of this malignancy in subsets of the U.S. population who are chronic carriers of HBV has been well documented (14,15). In addition, the demographics of HCV infection in this country may explain both a low current incidence of hepatocellular carcinoma and predict an increased frequency in the future. At present and the frequency of anti-HCV (+) donors in U.S. blood banks in large metropolitan areas is not that dissimilar from that recorded in Japan (109). In Japanese patients with chronic HCV infection preceding hepatocellular carcinoma mean interval between infection and presentation with cancer is $\sim$30 years (57). As shown in figure 6, in New York blood donors anti-HCV reactivity reported in 1990 (109) is heavily concentrated in 30-39 year old individuals while anti-HBc reactivity is more typical of a stable endemic disease with seropositivity increasing with age through all age brackets. A similar peak in anti-HCV reactivity was reported among 35-44 year old males in a study of Johns Hopkins emergency room patients performed several years after the New York blood donor study (110). As HCV infection is uncommonly acquired before young adulthood (110), it seems possible that a cluster of patients with < 25 years of HCV infection is poised to enter their peak years of hepatocellular carcinoma risk.

An NIH conference on hepatocellular carcinoma held in 1986 (when risks related to HCV were not known) recommended that "high risk" HBsAg (+) individuals (adult males with childhood infection) be screened at 3-4 month intervals with α-fetoprotein levels and every 4-6 months with liver ultrasound while only 3-4 monthly α-fetoprotein tests were recommended for other HBsAg (+) individuals (1). Subsequent reviewers have suggested that because of cost considerations, testing intervals for α-fetoprotein test be extended to 6 month intervals and ultrasound exams to 12 month intervals (107). While the cost-effectiveness of screening strategies has yet to be proven in the general U.S. population, it appears prudent to begin to at least use this modified screening strategy in adults, and especially adult males, who appear to have been infected with HBsAg or HCV for 1-2 decades or longer and are otherwise in good health and candidates for surgical therapy of any hepatocellular carcinoma that is discovered in a resectable stage.

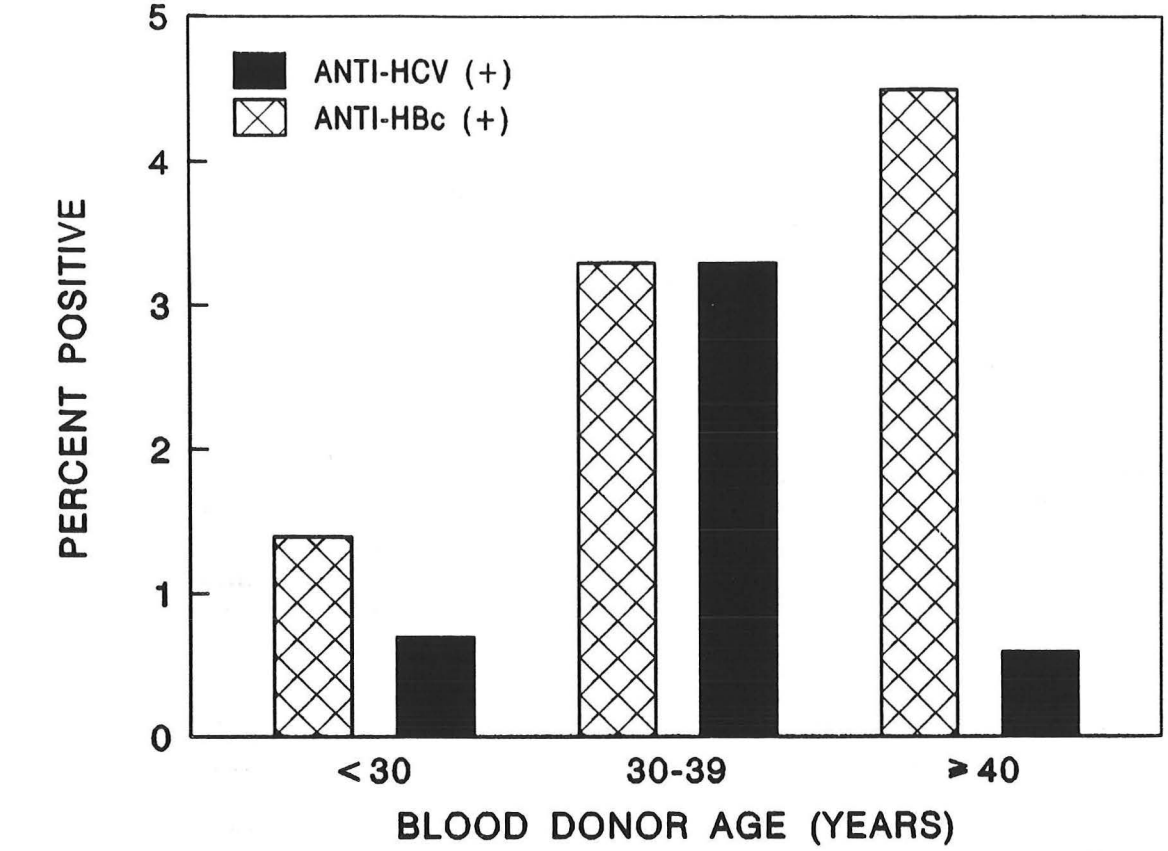

Figure 6

References

1. DiBisceglie AM, Rustgi VK, Hoofnagle JH, et al. Hepatocellular carcinoma. Ann Intern Med 108:390, 1988.
2. Sheu J-C, Sung J-L, Chen D-S, et al. Growth rate of asymptomatic hepatocellular carcinoma and its clinical implications. Gastroenterology 89:259, 1985.
3. Falkson G, Cnaan A, Schutt AJ, et al. Prognosis factors for survival in hepatocellular carcinoma. Cancer Res 48:7314, 1988.
4. Okuda K, Obata H, Nakajima Y, et al. Prognosis of primary hepatocellular carcinoma. Hepatology 41:3S, 1984.
5. Cottone M, Virdone R, Fusco G, et al. Asymptomatic hepatocellular carcinoma in Child's A cirrhosis. A comparison of natural history and surgical treatment. Gastroenterology 96:1566, 1989.
6. Tang Z-Y, Yu Y-Z, Zhou X-D, et al. Surgery of small hepatocellular carcinoma. Analysis of 144 cases. Cancer 64:536, 1989.
7. The Liver Cancer Study Group of Japan. Primary liver cancer in Japan. Clinicopathologic features and results of surgical treatment. Ann Surg 211:277, 1990.
8. Pasquet K-J, Koussouris P, Mercado MA, et al. Limited hepatic resection for selected cirrhotic patients with hepatocellular or cholangiocellular carcinoma: a prospective study. Br J Surg 78:459, 1991.
9. Cancer Incidence in Five Continents, Vol. V. Ed: Muir C, Waterhouse J, Mack T, Powell J, Whelan S. International Agency for Research on Cancer, Lyon, France, 1987.
10. Simonetti RG, Camma C, Fiorello F, et al. Hepatocellular carcinoma. A worldwide problem and the major

risk factors. Dig Dis Sci 36:962, 1991.
11. Beasley RP, Hwang L-Y, Lin C-C, et al. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22,707 men in Taiwan. Lancet ii:1129, 1981.
12. Beasley RP. Hepatitis B virus. The major etiology of hepatocellular carcinoma. Cancer 61:1942, 1988.
13. Chen C-J, Liang K-Y, Chang A-S, et al. Effects of hepatitis B virus, alcohol drinking, cigarette smoking and familial tendency on hepatocellular carcinoma. Hepatology 13:398, 1991.
14. Prince AM, Alcabes P. The risk of development of hepatocellular carcinoma in hepatitis B virus carriers in New York. A preliminary estimate using death-records matching. Hepatology 2:15S, 1982.
15. McMahon BJ, Alberts SR, Wainwright RB, et al. Hepatitis B-related sequalae. Prospective study in 1400 hepatitis B surface antigen-positive Alaska native carriers. Arch Intern Med 150:1051, 1990.
16. Lamont DW, Buchan KA, Gillis, CR, et al. Primary hepatocellular carcinoma in an area of low incidence: Evidence for a viral aetiology from routinely collected data. Int J Epidemiol 20:60, 1991.
17. Robinson WS. Hepadnaviruses and hepatocellular carcinoma (HCC). Cancer Detection and Prevention 14:245, 1989.
18. Popper H, Roth L, Purcell RH, et al. Hepatocarcinogenicity of the woodchuck hepatitis virus. Proc Natl Acad Sci USA 84:866, 1987.
19. Robinson WS, Klote L, Aoki N. Hepadnaviruses in cirrhotic liver and hepatocellular carcinoma. J Med Virol 31:18, 1990.
20. Tarao K, Shimizu A, Ohkawa S, et al. Development of hepatocellular carcinoma associated with increases in DNA synthesis in the surrounding cirrhosis. Gastroenterology 103:595, 1992.
21. Chisari FV, Klopchin K, Moriyama T, et al. Molecular pathogenesis of hepatocellular carcinoma in hepatitis B virus transgenic mice. Cell 59:1145, 1989.
22. Kim C-M, Koike K, Saito I, et al. HBs gene of hepatitis B virus induces liver cancer in transgenic mice. Nature 351:317, 1991.
23. Koshy R, Hofschneider PH. Transactivation by hepatitis B virus may contribute to hepatocarcinogenesis. Current Topics in Microbiol, Immunobiol 144:265, 1989.
24. Benasutti M, Ejadi S, Whitlow MD, et al. Mapping of the binding site of aflatoxin B1 in DNA: systematic analysis of the reactivity of aflatoxin B1 with guanines in different DNA sequences. Biochemistry 27:472, 1988.
25. Shank RC, Gordon JE, Wogan GN, et al. Dietary aflatoxins and human liver cancer. III, Field survey of rural Thai families for ingested aflatoxins. Food Cosmet Toxicol 10:71, 1972.
26. Van Rosenburg SJ, Cook-Mozaffari P, Van Schalkwyk DJ, et al. Hepatocellular carcinoma and dietary aflatoxin in Mozambique and Transkei. Br J Cancer 51:713, 1985.
27. Yeh FS, Yu MC, Mo CC, et al. Hepatitis B virus, aflatoxins and hepatocellular carcinoma in southern Guanxi, China. Cancer Res 49:2506, 1989.
28. Carbone D. Focusing in on P53 in hepatocellular carcinoma. Hepatology 14:742, 1991.
29. Hsu IC, Metcalf RA, Sun T, et al. Mutation hotspot in the p53 gene in human hepatocellular carcinomas. Nature 350:427, 1991.
30. Hosono S, Lee C-S, Chou M-J, et al. Molecular analysis of the p53 alleles in primary hepatocellular carcinomas and cell lines. Oncogene 6:237, 1991.
31. Ozturk M and collaborators. p53 mutation in hepatocellular carcinoma after aflatoxin exposure. Lancet 338:1356, 1991.
32. Patel P, Stephenson J, Scheuer PJ, et al. p53 codon 249^{ser} mutations in hepatocellular carcinoma patients with low aflatoxin exposure. Lancet 338:881, 1992.
33. Hadziyannis SJ. Hepatocellular carcinoma and type B hepatitis. Clin Gastroenterol 9:117, 1980.
34. Yarrish RL, Werner BG, Blumberg BS. Association of hepatitis B virus infection with hepatocellular carcinoma in American patients. Int J Cancer 26:711, 1980.
35. DiBisceglie AM, Order SE, Klein JL, et al. The role of chronic viral hepatitis in hepatocellular carcinoma in the United States. Am J Gastroenterol 86:335, 1991.
36. Hadengue A, N'Dri N, Benhamou J-P. Relative risk of hepatocellular carcinoma in HBsAg positive vs alcoholic cirrhosis. A cross-sectional study. Liver 10:147, 1990.
37. Brechot C, Nalpas B, Courouce AM, et al. Evidence that hepatitis B virus has a role in liver-cell carcinoma in alcoholic liver disease. New Engl J Med 306:1384, 1982.
38. Sakamoto S, Hirohashi S, Tsuda T, et al. Increasing incidence of hepatocellular carcinoma possibly

associated with non-A, non-B hepatitis in Japan, disclosed by hepatitis B virus DNA analysis of surgically resected cases. Cancer Res 48:7294, 1988.

39. Fong TL, Govarindarajan S, Valinluck B, et al. Status of hepatitis B virus DNA in alcoholic liver disease: a study of a large urban population in the United States. Hepatology 8:1602, 1988.
40. Pontisso P, Stenico D, Diodati G, et al. HBV-DNA sequences are rarely detected in the liver of patients with HBsAg-negative chronic active liver disease and with hepatocellular carcinoma in Italy. Liver 7:211-215, 1987.
41. Walter E, Blum HE, Meier P, et al. Hepatocellular carcinoma in alcoholic liver disease: No evidence for a pathogenetic role of hepatitis B virus infection. Hepatology 8:745, 1988.
42. Hino O, Kitagawa T, Sugano H. Relationship between serum and histochemical markers for hepatitis B virus and rate of viral integration in hepatocellular carcinoma in Japan. Int J Cancer 35:5, 1985.
43. Dazza MC, Meneses LV, Girard PM, et al. Polymerase chain reaction for detection of hepatitis B virus DNA in HBsAg seronegative patients with hepatocellular carcinoma from Mozambique. Ann Trop Med Parsitol 85:277, 1991.
44. Paterlini P, Brechot C. The detection of hepatitis B virus (HBV) in HBsAg negative individuals with primary liver cancer. Dig Dis Sci 36:1122, 1991.
45. Paterline P, Gerken G, Nakajima E, et al. Polymerase chain reaction to detect hepatitis B virus DNA and RNA sequences in primary liver cancers from patients negative for hepatitis B surface antigen. New Engl J Med 323:80, 1990.
46. Jenison SA, Lemon SM, Baker LN, et al. Quantitative analysis of hepatitis B virus DNA in saliva and semen of chronically infected homosexual men. J Infect Dis 156:299-307, 1987.
47. Ruiz J, Sangro B, Cuende JI, et al. Hepatitis B and C viral infections in patients with hepatocellular carcinoma. Hepatology 16:637, 1992.
48. Seeff LB, Beebe GW, Hoofnagle JH, et al. A serologic follow-up of the 1942 epidemic of post-vaccination hepatitis in the United States Army. New Engl J Med 316:965, 1987.
49. Thomas HC, Jacyna M, Waters J, et al. Virus-host interaction in chronic hepatitis B virus infection. Sem Liver Dis 8:342, 1988.
50. Scullard GH, Smith CI, Merigan TC, et al. Effects of immunosuppressive therapy on viral markers in chronic viral hepatitis B. Gastroenterology 81:987, 1981.
51. Bartolini St. Omer F, Giannini A, Napoli P. Hepatocellular carcinoma and cirrhosis: A review of their relative incidence in a 25-year period in the Florence area. Hepato-gastroenterol 31:215, 1984.
52. Cancer Incidence in Five Continents, Vol. III. Ed. Waterhouse J, Muir C, Correa P, Powell J, Davis W. International Agency for Research on Cancer, Lyon, France, 1976.
53. Cancer Incidence in Five Continents, Vol. IV. Ed. Waterhouse J, Muir C, Shanmugaratnam K, Powell J, Peacham D, Whelan S, Davis W. International Agency for Research on Cancer, Lyon, France, 1982.
54. Cancer Incidence in Five Continents, Vol. V. Ed. Muir C, Waterhouse J, Mack T, Powell J, Whelan S. International Agency for Research on Cancer, Lyon, France, 1987.
55. Okuda F, Fujimoto I, Hanai A, et al. Changing incidence of hepatocellular carcinoma in Japan. Cancer Res 47:4967, 1987.
56. Kiyosawa K, Akahane Y, Nagata A, et al. The significance of blood transfusion in non-A, non-B chronic liver disease in Japan. Vox Sang 43:45-52, 1982.
57. Kiyosawa K, Sodeyama T, Tanaka E, et al. Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: Analysis by detection of antibody to hepatitis C virus. Hepatology 12:671, 1990.
58. Watanabe Y, Harada S, Saito I, et al. Prevalence of antibody against the core protein of hepatitis C virus in patients with hepatocellular carcinoma. Int J Cancer 48:340, 1991.
59. Simonetti RG, Camma C, Fiorello F, et al. Hepatitis C virus infection as a risk factor for hepatocellular carcinoma in patients with cirrhosis. A case-control study. Ann Intern Med 116:97, 1992.
60. Alter HJ. New kit on the block: evaluation of second-generation assays for detection of antibody to the hepatitis C virus. Hepatology 15:350, 1992.
61. Chuang W-L, Chang W-Y, Lu S-N, et al. The role of hepatitis B and C viruses in hepatocellular carcinoma in a hepatitis B endemic area. A case-control study. Cancer 69:2052, 1992.
62. Caporaso N, Romano M, Marmo R, et al. Hepatitis C virus infection is an additive risk factor for development of hepatocellular carcinoma in patients with cirrhosis. J Hepatology 12:367, 1991.

63. Esteban JI, Esteban R, Viladominu L, et al. Hepatitis C virus antibodies among risk groups in Spain. Lancet 2:294-296, 1989.
64. Mendenhall CL, Seeff L, Diehl AM, et al. Antibodies to hepatitis B virus and hepatitis C virus in alcoholic hepatitis and cirrhosis: Their prevalence and clinical relevance. Hepatology 14:581, 1991.
65. Weinberg AG, et al. The occurrence of hepatoma in the chronic form of hereditary tyrosinemia. J Pediatr 88:454, 1976.
66. Niederau C, Fischer R, Sonnenberg A, et al. Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. New Engl J Med 313:1256, 1985.
67. Okuda K. The Liver Cancer Study Group of Japan. Primary liver cancer in Japan. Cancer 45:2663, 1980.
68. Okuda K. Clinical aspects of hepatocellular carcinoma - analysis of 134 cases. In: Okuda K, Peters RL, eds.: Hepatocellular Carcinoma. New York, John Wiley & Sons, 1976.
69. Edmonson HA, Craig JR. Neoplasms of the liver. In: Schiff L, Schiff ER, eds.: Diseases of the Liver. Philadelphia, J. B. Lippincott Company, 1987.
70. Sawabu N, Ohta H, Motoo Y, et al. Immuno-biochemical diagnosis of liver cancer. In: Okuda K, Tobe T, Kitagawa T, eds.: Early Detection and Treatment of Liver Cancer. Tokyo, Japan Scientific Societies Press, 1991.
71. Jones DB, Koorey DJ. Screening studies and markers. Gastroenterology Clinics of North America 16:563, 1987.
72. Wepsic HT, Kirkpatrick A. Alpha-fetoprotein and its relevance to human disease. Gastroenterology 77:787, 1979.
73. Liaw Y-F, Tai D-I, Chu C-M, et al. Early detection of hepatocellular carcinoma in patients with chronic type B hepatitis. A prospective study. Gastroenterology 90:263, 1986.
74. Utsunomiya T, Matsumata T, Adachi E, et al. Limitations of current preoperative liver imaging techniques for intrahepatic metastatic nodules of hepatocellular carcinoma. Hepatology 16:694, 1992.
75. DeSantis M, Romagnoli R, Cristiani A, et al. MRI of small hepatocellular carcinoma: Comparison with US, CT, DSA, and lipiodol-CT. J Computer Assisted Tomography 16:189, 1992.
76. Yumoto Y, Jinno K, Tokuyama K, et al. Hepatocellular carcinoma detected by iodized oil. Radiology 154:19, 1985.
77. Nonami T, Isshiki K, Katoh H, et al. The poatential of postoperative hepatic artery chemotherapy in patiens with high-risk hepatomas. Ann. Surg. 213:22, 1991.
78. Cedrone A, Rapaccini G, Pompili M, et al. Neoplastic seeding complicating percutaneous ethanol injection for treatment of hepatocellular carcinoma. Radiology 183:787, 1992.
79. Goletti O, De Negri F, Pucciarelli M, et al. Subcutaneous seeding after percutaneous ethanol injection of liver metastasis. Radiology 183:785, 1992.
80. Okuda K. and the Liver Cancer Study Group. Primary liver cancers in Japan. Cancer 45:2663, 1980.
81. Liver Cancer Study Group of Japan. Survey and follow up study of primary liver cancer in Japan-Report 8. Acta Hepatolog. Jpn. 29:1619, 1988.
82. Makuuchi M, Kosuge T, Takayama T, et al. Recent technical advancements in segmentectomy, subsegmentectomy, and limited resection in patients with small hepatocellular carcinoma and liver cirrhosis with special reference to intraoperative sonography. Gann Monograph on Cancer Research 38:179, 1991.
83. Franco D, Capussotti L, Smadja C, et al. Resection of Hepatocellular carcinomas. Results in 72 European patients with cirrhosis. Gastro. 98:733, 1990.
84. Iwatsuki S, Starzl T, Sheahan D, et al. Hepatic resection versus liver transplantation for hepatocellular cirrhosis. Ann. Surg. 214:221, 1991.
85. Lai E, Choi T, Tong S, et al. Treatment of unresectable hepatocellular carcinoma: results of a randomized control trial. World J. Surg. 10:501, 1986.
86. Okuda K, Ohtsuki T, Obata H, et al. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Cancer 56: 918, 1985.
87. Penn I. Hepatic transplantation for primary and metastatic cancers of the liver. Surgery 110:726, 1991.
88. Bismuth H, Castaing D, Ericzon B, et al. Hepatic transplantation in Europe: first report of the European Liver Transplant Registry. Lancet 2:674, 1987.
89. Yokayama I, Sheahan D, Carr S, et al Clinicopathologic factors affecting patient survival and tumor recurrence after orthotopic liver transplantation for hepatocellular carcinoma. Trans. Proc. 23:2194, 1991.
90. Shirabe K, Kanematsu T, Matsumata T, et al. Factors linked to early recurrence of small hepatocellular

carcinoma after hepatectomy: Univariate and Multivariate analyses. Hepatology 14:802, 1991.
91. Epstein B, Ettinger D. Leichner P, et al. Multimodality cisplatin treatment in nonresectable α-fetoprotein positive hepatoma. Cancer 67:896, 1991.
92. Konno T. Targeting cancer chemotherapeutic agents by use of lipiodol contrast medium.l Cancer 66:1897, 1990.
93. Kalayci C, Johnson P, Raby N, et al. Intraarterial adriamycin and lipiodol for inoperable hepatocellular carcinoma: a comparison with intravenous adriamycin. J. Hepatol. 11:349, 1990.
94. Kuroda C, Sakurai M, Monden M, et al. Limitation of transcatheter arterial chemoembolization using iodized oil for small hepatocellular carcinomas. Cancer 67:81, 1990.
95. Vetter D, Wenger J-J, Bergier J-M, et al. Transcatheter oily chemoembolization in the management of advanced hepatocellular carcinoma in cirrhosis: Results of a western comparative study in 60 patients. Hepatology 13:427, 1991.
96. Gunji T, Kawauchi N, Ohnishi S, et al. Treatment o hepatocellular carcinoma associated with advanced cirrhosis by transcatheter arterial chemoembolization using autologous blood clot: A preliminary report. Hepatology 15:252, 1992.
97. Yoshimi F, Nagoa T, Inoue, S, et al. Comparison of hepatectomy and transcatheter arterial chemoembolization for the treatment of hepatocellular carcinoma: Necessity for prospective trials. Hepatology 16:702, 1992.
98. Shibata J, Fujiyama S, Sato T, et al. Hepatic arterial injection chemotherapy with cisplatin suspended in oily lymphographic agent for hepatocellular carcinoma. Cancer 64:1586, 1989.
99. Beppu T, Ohara C, Yamaguchi Y, et al. A new approach to chemoembolization for unresectable hepatocellular carcinoma using aclarubicin microspheres in combination with cisplatin suspended in iodized oil. Cancer 68:2555, 1991.
100. Raoul J, Bretagne J, Caucanas J, et al. Internal radiation therapy for hepatocellular carcinoma. Results of a French multicenter Phase II trial of Transarterial injection of iodine 133-labeled lipiodol. Cancer 69:346, 1992.
101. Tanaka K, Okazaki H, Nakamura S, et al. Hepatocellular carcinoma: Treatment with a combination of transcatheter arterial embolization and percutaneous ethanol injection. Radiology 179:713, 191.
102. Livraghi T, Salmi A, Blondi L, et al. Small hepatocellular carcinoma: percutaneous ethanol injection-results in 23 patients. Radiology 168:313, 1988.
103. Bismuth H, Morino M, Sherlock D, et al. Primary treatment of hepatocellular carcinoma by arterial chemoembolization. Amer. J. of Surg. 163:387, 1992.
104. Nonami T, Isshiki K, Katoh H, et al. The potential role of postoperative hepatic artery chemotherapy in patients with high-risk hepatomas. Ann. Surg. 213:222, 1991.
105. Stone M, Klintmalm G, Polter D, et al. Neoadjuvant chemotherapy and liver transplantation for hepatocellular carcinoma: Results in 20 patients. Gastroenterology, In Press.
106. Okuda K. Advances in detection and treatment of liver cancer. Gann Monograph on Cancer Research 38:3, 1991.
107. Regan L. Screening for hepatocellular carcinoma in high-risk individuals. A clinical review. Arch. Intern. Med. 149:1741, 1989.
108. Colombo M, De Franchis R, Del Ninno E, et al. Hepatocellular carcinoma in Italian patients with cirrhosis. N. Eng. J. Med. 325:675, 1991.
109. Stevens C, Taylor P, Pindyck J, et al. Epidemiology of hepatitis C virus. A preliminary study of volunteer blood donors. JAMA 263:49, 1990.
110. Kelen G, Green G, Purcell R, et al. Hepatitis B and C in emergency department patients. N. Eng. J. Med. 326:1399, 1992.