Hepatocellular Carcinoma: No Longer an Exotic Disease

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Incidence of Hepatocellular Carcinoma: Past and Present

If some are puzzled by the title chosen for this Medical Grand Rounds, it may be helpful to explain that during the 1970's, when I attended medical school and trained in internal medicine, hepatocellular carcinoma was a disease that was rarely seen by clinicians in the U.S. I do not recall encountering a single case of primary liver cancer until after entering subspecialty training in the early 1980's. The U.S. and European medical literature of the 1970's and 1980's regarded hepatocellular carcinoma as a rare and somewhat exotic disease and most descriptions of its clinical course were based upon the experiences of physicians from Asia or Africa. Despite the relative lack of attention paid to this seemingly "rare" disease by the English language texts of the 1970's and 1980's, hepatocellular carcinoma was actually a relatively common disease worldwide, and in some countries in Asia and Africa it was the most common cause of cancer in men [1,2](see figure 1 below).

Figure 1. Age-adjusted incidence rates of primary liver cancer [3-10].

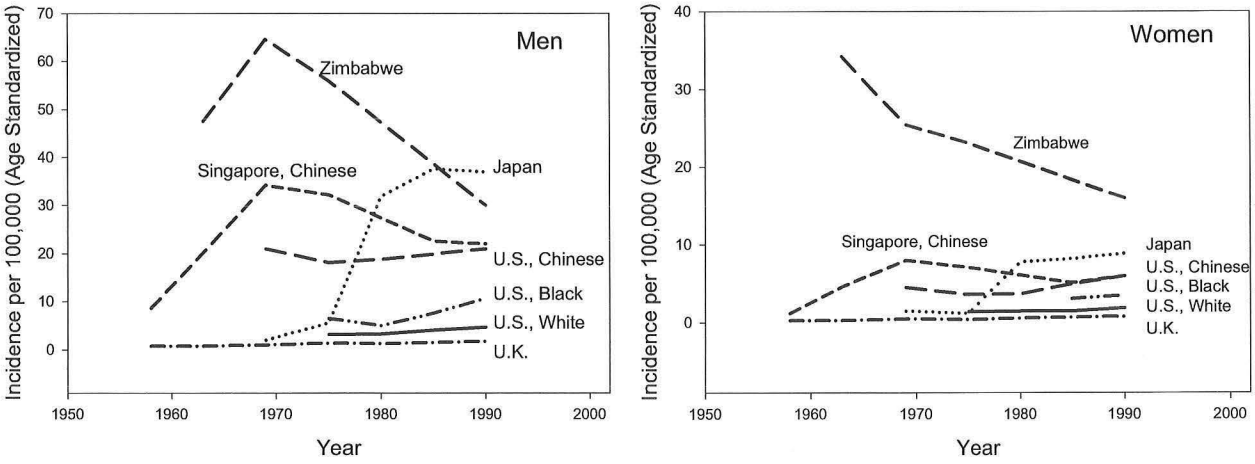
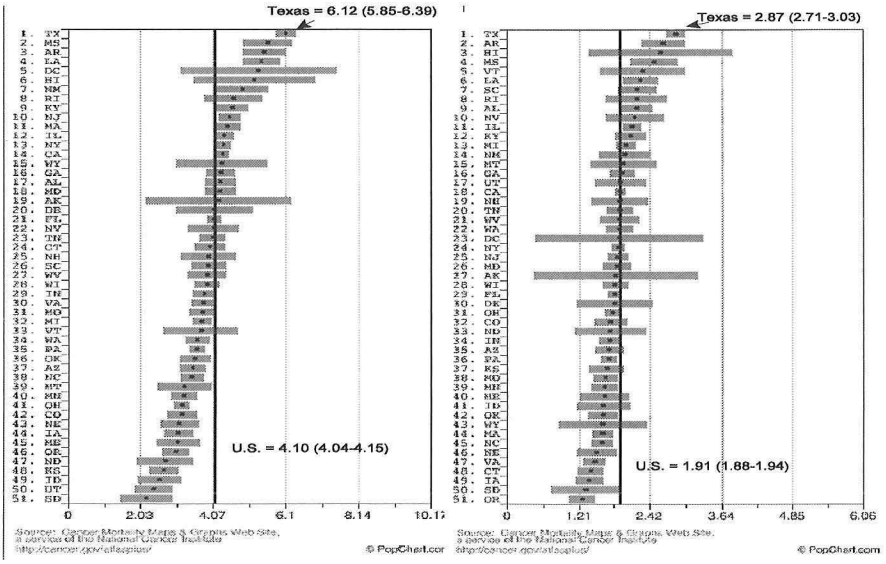


Figure 2. Hepatocellular Carcinoma Mortality Rates per 100,000, 1990-1994
White Males White Females

However, by the early 1990's subspecialists caring for U.S. patients with liver disease began to encounter an increasing frequency of hepatocellular carcinoma that was formally recognized and reported by epidemiologists later in the same decade [11]. This increase in primary liver cancer rates may be especially apparent to local physicians since Texas has become a relatively high incidence state for this disease. Texas currently ranks 6th among the 50



states overall in age adjusted incidence of primary liver malignancies and 1st among the 50 states in mortality rates from liver cancer among both white males and white females [12]. This trend towards an increasing rate of primary liver cancer in the U.S. that was first recognized in the early 1990's is clearly continuing. Data from the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) program indicates that increases in primary liver malignancy rates (+4.3%/yr) during the 1992-1999 time interval were greater than for any other malignancy tracked in the SEER program [10]. Indeed, in contrast to its ranking as only the 22nd most frequent cause of cancer mortality 2 decades ago [1], the most recent U.S. mortality statistics indicate that in 2001, hepatocellular carcinoma had entered the "top ten" by ranking as the 8th commonest cause of cancer death among U.S. males [13].

Figure 3. Estimated Annual Percent Change (EAPC) over time by primary cancer site [10]. * The EAPC is significantly different from zero ($p<0.05$).

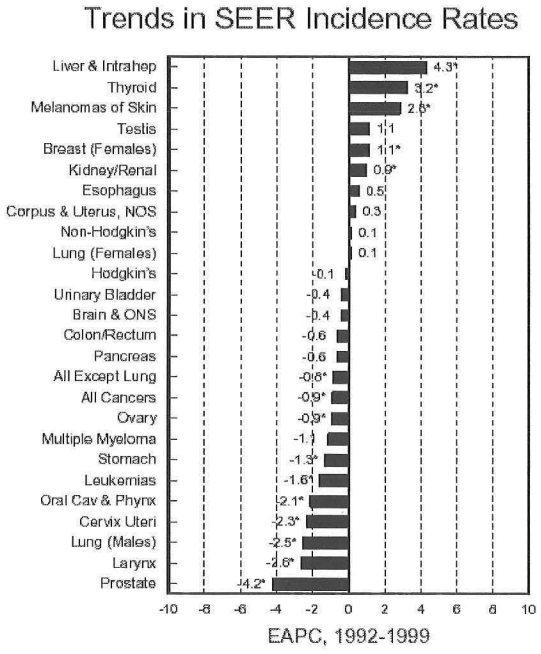
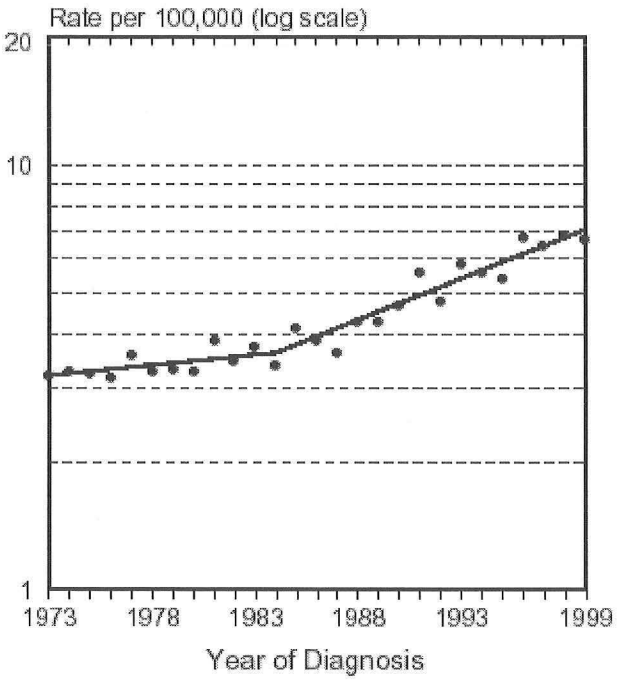


Figure 4. SEER Incidence Rates for Primary Liver Malignancies in White Males [10].



Despite a doubling in U.S. primary liver cancer rates among both men and women during the past 15 years [10], these rates (7.5/100,000 for men, 2.1/100,000 for women, 1995-1999) are still relatively low compared to the 5-fold higher rates of primary liver cancer historically observed in high incidence areas of Africa and Asia (see figure 10 [3-9]. These rates also remain well below the incidence rates for other digestive tract malignancies such as colorectal carcinoma (53.7/100,000, 1995-1999) and pancreatic carcinoma (12.4/100,000 in men, 9.8/100,000 in women, 1995-1999) [10]. However, analysis of the underlying reasons for the changes in rates of hepatocellular carcinoma, suggests that within the next 15 years, hepatocellular carcinoma death rates in the U.S. will continue to increase to the point where this disease becomes one of the "top five" causes of cancer death among male members of the post-WWII "baby boom" generation.

Pathogenesis of Hepatocellular Carcinoma

The pioneering studies of R. Palmer Beasley and colleagues among Chinese male, government employees in Taiwan, demonstrated that chronic hepatitis B virus (HBV) infection was associated with a greatly increased (RR $\approx$ 100) risk of hepatocellular carcinoma among this population [1,2]. As detailed in Table 1, this association between Hepatitis B infection and hepatocellular carcinoma was confirmed in additional epidemiological studies conducted in Asia, Europe and North America [14-17] and historical patterns of geographic variation in incidence of hepatocellular carcinoma were found to correlate closely with prevalence of chronic HBV infection [2].

Table 1. Relative Risk for Hepatocellular Carcinoma in HBV Carriers

Author	Site of Study / Sex	Relative Risk in HBsAg (+)
Beasley [1]	Taiwan / Male	98
Chen, et al [14]	Taiwan / Male	22
Prince, et al [17]	New York City / Male	>10
McMahon, et al [16]	Alaska / M & F	148
Lamont, et al [15]	Scotland / M & F	44(M) / 49 (F)

Concurrent with appreciation for the epidemiologic link between HBV infection and hepatocellular carcinoma, it became known that HBV is an incomplete double stranded DNA virus that encodes a polymerase which serves as a reverse transcriptase. This led to the hypothesis that, as in the case of certain other retroviruses, HBV DNA may integrate into host genes and either, by insertion of a viral promoter into a proto-oncogene, or, by integration events that disrupt function of tumor suppressor genes, induce hepatocellular carcinoma [18]. This hypothesis was initially supported by observations that integration of HBV DNA could be demonstrated in > 80% of such tumors [18, 19]. However, in only rare cases could it be demonstrated that such integration occurred within the domain of known oncogenes [19]. Rather, HBV DNA appears to integrate into random sites within the host genome [20].

It was subsequently appreciated that the usual site at which HBV DNA integration into host DNA is adjacent to the HBx gene which in turn facilitates expression of the X antigen product of this gene[20]. Transgenic mouse lines expressing the entire HBx gene under control by its own regulatory elements develop hepatic adenomas that progress to hepatocellular carcinoma in the absence of any preceding hepatocyte injury or inflammation [21]. Transgenic expression of HBsAg also leads to development of hepatocellular carcinoma but only in transgenic lines expressing very high, apparently toxic levels of this antigen that are associated with chronic hepatocyte injury and inflammation [22]. The X antigen encoded by HBV exhibits promiscuous transactivation functions that have been found to have a direct stimulatory effect on both viral replication and eucaryotic cell growth. In addition, HBx interferes with DNA repair mechanisms and binds to and inactivates the tumor suppressor p53 [20].

Studies of concurrent risks factors for development of this malignancy in developing countries provide additional support for the role of p53 inactivation in pathogenesis of hepatocellular carcinoma . While the geographic variation in incidence of hepatocellular carcinoma as observed in the 1970's and 1980's correlated closely with prevalence of neonatal or early childhood acquired chronic hepatitis B infection [2], the clustering of cases of hepatocellular carcinoma in sub-Saharan Africa and Asia also appeared to correlate with dietary intake of foodstuffs contaminated with the mycotoxin, aflatoxin B1 produced by *Aspergillus flavus* contamination of food grains and groundnuts [23-25]. Two seminal studies published in 1991 identified selective G to T mutations at codon 249 of the p53 gene in hepatocellular carcinomas from patients exposed to aflatoxin B1 in their diet [26, 27]. This mutation is rarely seen in hepatocellular carcinomas from patients without evidence of aflatoxin B1 exposure [28-31] and appears to be induced by aflatoxin 8,9 epoxide a mutagenic intermediate in aflatoxin metabolism that is normally detoxified by microsomal epoxide hydrolase and glutathione S-transferase [32]. Genetic variations in these two aflatoxin B1 detoxification genes that are associated with diminished enzymatic activity have been found to be over represented in individuals with measurable levels of serum aflatoxin B1-albumin adducts [32]. In addition, mutant alleles of epoxide hydrolase were found to be significantly over represented in persons with hepatocellular carcinoma and only hepatocellular carcinomas arising in patients with mutations in one or both of these aflatoxin detoxifying genes were found to have codon 249 mutations in p53 [32]. Of note however, > 50% of aflatoxin exposed hepatocellular carcinoma patients with codon 249 mutations also had evidence of chronic HBV infection [27,28] suggesting a "two hit" mechanism of p53 mutation in pathogenesis of hepatocellular carcinoma in this patient population.

Thus, a significant body of data now indicates that both chronic infection with HBV and aflatoxin B1 exposure in genetically susceptible individuals contribute to disruption of p53 expression and development of hepatocellular carcinoma. A recent report also suggests that iron overload may promote p53 mutations in hepatocytes. In a study performed in the United Kingdom, 10 of 14 (71%) hemochromatosis patients with hepatocellular carcinoma were found to have p53 mutations that clustered around codon 220 of p53 [33]. However, a variety of observations indicate that this p53 mutagenesis model for hepatocellular carcinoma pathogenesis has limited relevance for tumors arising outside of high incidence areas of Asia and sub-Saharan Africa. In a survey of 170 tumor samples in Great Britain, only 29% of hepatocellular carcinomas had any evidence of p53 mutations[33]. Other reports also indicate that most hepatocellular carcinomas in the U.S. and Japan lack codon 249 or other p53 mutations [28, 33].

The most common risk factor associated with hepatocellular carcinoma in Europe is cirrhosis. In autopsy series conducted between 1931 and 1985 in Western Europe, hepatocellular carcinoma was noted in 7-23% of patients with cirrhosis but <0.3% of non-cirrhotics [34-37]. Such autopsy studies also noted that male cirrhotic patients appeared to have a 3-4 fold greater risk for hepatocellular carcinoma than female cirrhotic patients whereas tumors arising in non-cirrhotic livers were evenly divided among men and women [34, 36]. Analysis of the role of liver disease etiology in risk for hepatocellular carcinoma revealed that among British patients with cirrhosis secondary to chronic hepatitis B or hemochromatosis, 42% and 36%, respectively had an associated hepatocellular carcinoma whereas only 3% of patients with primary biliary cirrhosis had an associated malignancy[38]. However, >90% of the patients with chronic hepatitis B or hemochromatosis were

male versus <10% of patients with primary biliary cirrhosis and when gender and age were taken into account, cirrhosis, male gender and age >50 but not etiology of cirrhosis were found to be risk factors for hepatocellular carcinoma. There is a paucity of data regarding the contribution of male gender to hepatic carcinogenesis, but a higher frequency of this form of malignancy among males is also readily apparent in developing counties (see figure 1) and in transgenic animal models of hepatic carcinogenesis [21, 39].

The role of both increasing age and severity of liver disease likely relates to accumulation of genetic damage following an increased number of total hepatocyte divisions. This hypothesis is supported by a number of reports of shorter telomere length in hepatocytes from cirrhotic livers [40] and evidence for chromosomal instability within hepatocellular carcinomas arising in cirrhotic livers. Two studies examining all chromosomes from multiple hepatocellular carcinomas have found significant loss at similar sites including chromosomal regions 1p, 4 q, 6 q, 8p, 13q, 16p, 16q and 17p [41,42]. Of interest, loss of the 4q34-35 region has been found to correlate with alcohol intake and with high grade of differentiation of the tumor [43]. A number of candidate tumor suppressor genes are localized to regions of observed chromosome loss including p53 on 17p, p73 on 1p, insulin-like growth factor 2 receptor (IGF-IIr) on 6q and BRCA2 and RB on 13q. Further analysis of some of these tumor suppressor genes has found evidence for mutation or chromosomal loss of both alleles of IGF-IIR in a significant fraction of hepatocellular carcinomas [44]. Nevertheless, no single, predominant set of mutations has been defined in hepatocellular carcinomas from cirrhotic patients without aflatoxin B1 / HBV exposure suggesting that hepatocellular carcinoma is a heterogeneous disease resulting from diverse underlying molecular events [45, 46].

In contrast to data collected prior to the mid-1970's indicating relatively uniform rates of hepatocellular carcinoma in age and gender matched European patients with diverse causes of cirrhosis [38], autopsy data from Italy in the late 1970's and early 1980's revealed an abrupt increase in incidence of hepatocellular carcinoma that was out of proportion to a more modest rise in incidence of cirrhosis [36]. A similar, abrupt rise in hepatocellular carcinoma rates was also noted during the same era in Japan (See figure 1). As in Italy, the rise in hepatoma rates in Japan during the early 1980's could not be explained by traditional risk factors such as hepatitis B infection [47]. With subsequent discovery of the hepatitis C virus (HCV), the greater than 5 fold increase in incidence of hepatocellular carcinoma observed in areas of these two countries between 1975 and the late 1980's was found to be related to tumors associated with hepatitis C infection [48-50]. Subsequently, increasing rates of hepatocellular carcinoma in Spain, France and most recently the U.S. also have been associated with an increase in hepatitis C associated cases [11, 51-53]. At present, hepatitis C infection and associated liver disease appears to be the predominant risk factor for hepatocellular carcinoma in Japan, Southern Europe and at least some regions of the U.S. [54]. In developed countries, the relative risk for hepatocellular carcinoma in patients with cirrhosis secondary to chronic hepatitis C (RR≈35) appears at least as high as for cirrhosis secondary to chronic hepatitis B (RR≈18) and higher than for cirrhosis secondary to alcohol abuse (RR≈5-6) [55].

As an RNA virus, HCV is unable to integrate into the host genome, but as in the case of HBV, there is evidence that certain hepatitis C viral proteins may play a direct role in hepatic carcinogenesis. Transgenic mice expressing the HCV core protein develop adenomas followed by foci of

hepatocellular carcinoma within adenomatous tissue [39]. HCV core protein has also been demonstrated to bind to and repress p53 activity, impair TNF induced apoptosis, repress p21WAF1 activity and activate nuclear factor-kappa B [56-59]. Each of these activities of HCV core protein could play a potential role in hepatic carcinogenesis via inhibition of apoptosis or modulation of cell proliferation. However, unlike HBV associated hepatocellular carcinomas that not infrequently arise in non-cirrhotic livers [60], Hepatitis C associated hepatocellular carcinomas appear to arise almost exclusively in patients with cirrhosis [46, 60, 61]. Thus, in addition to any direct carcinogenic effect of HCV viral proteins, chronic inflammation, increased numbers of hepatocyte divisions and secondary accumulation of genetic damage also seems to play an important role in hepatic carcinogenesis in patients with HCV infection.

Diagnosis of Hepatocellular Carcinoma

The classic clinical features of hepatocellular carcinoma include right upper quadrant pain and weight loss that may be associated with decompensation of underlying liver disease. Less commonly, this disease may present with intra-abdominal bleeding secondary to rupture of the liver tumor, or with a variety of paraneoplastic manifestations such as hypoglycemia, polycythemia, hypercalcemia or even hypercholesterolemia due to unregulated cholesterol synthesis within malignant hepatocytes [62]. However, increasingly, use of modern imaging techniques and / or serum α-fetoprotein (AFP) screening tests in high risk patients has permitted diagnosis of this malignancy in a pre-symptomatic stage [63].

Table 2. Sensitivity of AFP Testing in Diagnosis of Hepatocellular Carcinoma

Diagnostic Cut-Off	Chinese HCC [64]	Japanese HCC, ≤ 2 cm [65]	All Japanese HCC [66]
> ULN (10 or 20)*	87 %	72 %	65 %
> 100 ng/ml	73 %	33 %	N. A.**
> 400 ng/ml	~ 50 %	17 %	26.1%

* Second study [65] used 10 ng/ml as upper limit of normal (ULN), other studies [64, 66]used 20 ng/ml.
** Data not available, but only 32.9 % with AFP > 200.

AFP is the major protein component of fetal serum but soon after birth, levels fall rapidly and become virtually undetectable (< 10 ng/ml) [64]. In adult life, detectable levels of AFP are usually only observed in patients with malignancy, and prominent levels (> 1000 ng/ml) are observed only in patients with hepatocellular carcinomas or nonseminomatous germ cell tumors [64]. In patients with chronic liver disease, lesser elevations of AFP in the 20-400 ng/ml range, and, in chronic hepatitis B, transient elevations in the 500-1000 ng/ml range or occasionally even higher are observed [62, 64, 67]. Thus, in patients with chronic liver disease, only sustained levels of > 400 ng/ml are believed to be "diagnostic" for hepatocellular carcinoma or other AFP producing malignancy such as germ cell tumors or childhood hepatoblastomas [62, 64, 65]. Unfortunately, while 70-85% of hepatocellular carcinomas are associated with AFP elevations above 10 ng/ml, only about 60% of such tumors in

HBV endemic countries [64] and less than 40% of non-HBV associated hepatocellular carcinomas have AFP elevations of > 400 ng/ml [65, 66]. However, in chronic liver disease patients, the combination of an AFP level > 100 ng/ml and evidence on imaging studies of a liver mass or masses thought to be consistent with hepatocellular carcinoma appears to be sufficient to establish a diagnosis of hepatocellular carcinoma [68]. In patients with chronic HBV infection and normal ALT values, AFP levels of > 100 are also reported to have > 98% specificity (positive predictive value) for hepatocellular carcinoma [67].

While other serum protein markers such as des-γ-carboxy prothrombin (also known as protein induced by vitamin K antagonist II or PIVKA-II) and tumor associated isoenzymes of γ-glutamyl transferase have been found to be selectively elevated in the serum of hepatocellular carcinoma patients, none of these other candidate markers has been found to exhibit sufficient sensitivity or specificity to be used in routine clinical practice [69]. Thus, in the current era, serum AFP and a liver imaging study are the routine initial tests used to evaluate patients for hepatocellular carcinoma. In general, ultrasonography (US) has been recommended as the most cost-effective initial imaging study for this purpose [70]. However, US has only ~45% sensitivity in detecting hepatocellular carcinomas in patients with cirrhotic livers [71]. This low sensitivity is nevertheless equivalent to that of conventional computerized tomography (CT) and greatly superior to that of radioisotope scans conventionally used for this purpose prior to the 1980's. US is also the imaging technique of choice for use in obtaining guided biopsies of intrahepatic masses [72].

US has been superceded by specialized CT and magnetic resonance (MR) techniques as the procedure(s) of choice in many clinical settings where hepatocellular carcinoma is suspected [72, 73]. Hepatocellular carcinomas differ from nonmalignant hepatic tissues in blood supply and the nature of cell lineages that are present while usually retaining many hepatocyte specific functions. These characteristics are the bases for advances in CT and MR techniques that have greatly improved the sensitivity and specificity of imaging approaches in liver tumor evaluation. Unlike the dual arterial and portal venous blood supply in nonmalignant liver tissue, blood inflow into hepatocellular carcinomas derives almost exclusively from arterial sources. For this reason, greatly improved sensitivity and a diminished false positive rate have been achieved when CT scanning is performed with intravenous contrast agents and rapid, spiral imaging techniques that permit image acquisition during both the early arterial phase, when preferential enhancement of hepatocellular carcinomas is observed, and during the later, portal venous phase, when normal hepatic parenchyma is maximally enhanced [74]. Such, triphasic CT scans that collect unenhanced, arterial phase enhanced and portal phase enhanced images have now been reported to have 68-80 % sensitivity and 81-92 % specificity in detecting cirrhotic patients with hepatocellular carcinoma who are evaluated prior to liver transplantation or partial hepatic resections [72, 75-77] .

Despite the greatly improved sensitivity and specificity of triphasic CT scanning, up to a third of cases (patients with hepatocellular carcinoma) and an even higher percentage of individual malignant lesions are missed by this technique. In addition, some patients, such as those with pre-renal azotemia, have contraindications to the use of the intravenous contrast agents employed for these studies. For these reasons, MR imaging techniques have replaced or have been used as an alternative

to triphasic CT scanning in many centers [73]. Three classes of contrast agents have been shown to improve sensitivity and/or specificity of MR imaging techniques for assessment of hepatic masses. These include the gadolinium chelates that produce enhancement of vascular tissues on T1-weighted images and have become the agents of choice for detection of typically hypervascular hepatocellular carcinomas. Gadolinium-enhanced MR imaging achieves levels of sensitivity and specificity equal to or slightly superior to that of triphasic CT scanning [72, 78-80]. In situations where other imaging techniques have already detected a liver mass, MR imaging may prove helpful in distinguishing such lesions from metastatic lesions by use of hepatocyte specific contrast agents such as mangafodipir trisodium (Teslascan) or gadobenate dimeglumine (Multihance) that enhance hepatocellular carcinomas but not metastatic tumors [72, 79]. Alternatively, reticuloendothelial system specific ferumoxides (Feridex) may be used to distinguish hepatocellular carcinomas, which do not contain Kupffer cells from regenerative nodules or areas of focal nodular hyperplasia which usually have normal or even increased numbers of Kupffer cells [79, 81].

Table 3. Imaging Techniques for Detection of Hepatocellular Carcinoma (HCC)

Modality	Sensitivity*	Specificity
US [71]	50 %	98 %
Conventional CT [76]	62 %	63 %
Triphasic Spiral CT [72, 75-77]	68-80 %	81-92 %
MR [72, 78]	77%	N.A.

* Defined as detecting at least one mass in patient with 1 or more HCC nodules.

In practice, most patients with mass lesions suspicious for hepatocellular carcinoma, are eventually evaluated by more than one modality. Although this approach may seem to entail additional expense, it can be argued that this is the most efficient approach towards managing such patients, For instance, it has been observed that after completion of standard clinical, laboratory and radiologic evaluation, a clinical diagnosis of hepatocellular carcinoma is correct in > 96 % of case [68, 82]. Because guided needle biopsies are associated with approximately a 2.5 % bleeding complication rate [83], up to a 5% needle tract implantation / tumor dissemination rate [84] and may yield false negative results that greatly delay appropriate therapy [85], it has become standard of care to ascribe diagnosis and proceed with management of the majority of hepatocellular carcinomas without biopsy or histologic confirmation [68, 73, 86, 87]. It contrast to previous practices of pursuing tissue confirmation in liver mass patients who did not have AFP values of > 400 or 500 ng/ml, with use of modern imaging technologies, it has been recommended that among patients with known risk factors for hepatocellular carcinoma, percutaneous guided tumor biopsies should be reserved only for those with small tumors (< 3 cm) and AFP values < 100 ng/ml [68] or for those with atypical, hypovascular masses and AFP values < 20 ng/ml [87].

Therapy of Hepatocellular Carcinoma

Analysis of U.S. survival data from the SEER database reveals that median survival following

presentation with hepatocellular carcinoma during 1992-1996 was 0.64 years which was only marginally better than a median survival of 0.57 years during the 1977 to 1981 time interval [88]. This apparent slight improvement in survival has been attributed largely to lead-time bias related to improved diagnostic modalities and present 5 year survival rates are estimated to be only ~5% [88]. Thus, current therapies have proven ineffective for most patients with hepatocellular carcinoma.

A wide variety of chemotherapeutic agents have been assessed for efficacy in treating hepatocellular carcinoma and a few have been associated with antitumor responses [89]. However, response rates following systemic chemotherapy are typically < 25% and not associated with meaningful improvements in survival. This dismal response is related, in part, to need for dose reductions in patients with underlying cirrhosis. For these reasons, attempts to systemically administer classic chemotherapeutic agents in this patient population have been largely abandoned. Phase I and phase II trials of newer agents such as capecitabine, thalidomide and anti-angiogenic agents that appear to be better tolerated in cirrhotic patients are in progress [89].

The radiosensitivity of normal liver tissue has prevented use of classic high dose radiotherapy, but the disparity in blood supply between malignant and non-malignant hepatic tissues has been targeted by interventional radiologists who have pursued a variety of regional chemotherapeutic, embolization and combination "chemoembolization" approaches. The most enthusiastically pursued approach has been trans-arterial chemoembolization (TACE) therapy. In TACE therapy, chemotherapeutic drugs such as doxorubicin, cisplatinum and/or mitomycin C are mixed with either water soluble contrast material or lipiodol (iodized poppyseed oil) to form an emulsion which is injected into arteries feeding hepatic tumors. Following this injection, embolization materials such as gelatin sponges (Gelfoam) are injected to compromise hepatic arterial supply to the tumor. Multiple anecdotal reports of TACE induced tumor shrinkage and/or disappearance and apparent prolonged survival compared to "historical" controls have appeared in the literature. However, despite the fact that partial response rates of > 55% are observed following transarterial embolization along (without chemotherapy) [90], two European and one South African prospective, randomized control trials of TACE therapy found no statistically significant survival benefit from this therapy. The authors of these reports suggest that benefits from the frequently observed tumor responses appear to be offset by episodes of TACE induced decompensation of liver function and increased mortality from liver failure. When used as an interim, bridging therapy in patients awaiting liver transplantation, these adverse effects on hepatic function appear to have a lesser impact. Among patients who have received TACE to maintain "resectability" until a donor organ becomes available, the majority (89%) have been able to be transplanted before tumor progression exceeds United Network for Organ Sharing (UNOS) criteria for liver transplant candidacy and one and two year tumor free survivals have been excellent (91% and 84%, respectively) [91]. More recently, a randomized controlled trial from Hong Kong, China has demonstrated improved survival in carefully selected, Chinese patients with predominately HBV associated hepatocellular carcinomas who received TACE therapy. In this group, as in prior trials, increased rates of death from liver failure were observed in the therapeutic group but, unlike experiences in other studies, net mortality benefit was still realized because of greater decreases in tumor-related mortality [92]. It should be noted, however, that the authors attribute their more favorable outcomes to use of this therapy only in patients without evidence of hepatic decompensation, with patent portal veins and with tumors

restricted to the liver. These strict selection criteria excluded >70% of patients with hepatocellular carcinomas at their institution [92].

Because of the limited benefit of systemic chemotherapy and questionable benefits of regional chemotherapy or chemoembolization therapy, a variety of other, localized non-surgical ablative therapies have been devised. These include cryotherapy achieved by placement of cryogenic probes into the tumor, percutaneous ethanol injection (PEI), and radiofrequency ablation (RFA) achieved by insertion of a needle electrode array into the tumor and production of thermal energy by alternating electrical current in radiofrequency ranges of 200-1200 kHz. Because of greater complication rates among cirrhotic patients, cryotherapy has been supplanted by other ablation approaches in cirrhotics [93]. Multiple studies have documented tumor necrosis after PEI and, as the technique is generally well tolerated, multiple sessions can be performed to achieve eventual necrosis of tumors up to 5 cm in diameter. While survival rates in patients subjected to this therapy for solitary tumors < 5 cm in diameter have appeared promising [94], this therapy has not been subjected to prospective, controlled trial except when recently compared to RFA [95]. Although RFA, appears to have a higher rate of local complications than PEI, it has been found to be more effective in achieving total tumor necrosis in fewer sessions than PEI and is now recommended as the ablation therapy of choice [86, 95]. However, ablation therapies are viewed only as applicable to small solitary tumors [89, 93, 96] and are thus only seen as an option in a limited fraction of patients with hepatocellular carcinoma.

The only therapies for hepatocellular carcinoma reliably associated with significant 5 year survival rates are subtotal hepatic resection and liver transplantation. In non-cirrhotic patients with even relatively large tumors, significant 5 and 10 year tumor free survival rates (40% and 26%, respectively) can be achieved following resection of lesions localized to the liver. However, most hepatic malignancies arise in cirrhotic livers. Surgical morbidity and mortality rates are high in cirrhotic patients with limited hepatic reserve and resection is only recommended as the therapy of choice in cirrhotics with excellent liver function as defined by Child's A functional classification, normal bilirubin values and no evidence of portal hypertension [86, 97-99]. When these criteria are applied to U.S. or European patients with hepatocellular carcinoma, only about 5% qualify for attempts at curative resections [100]. However, in such carefully selected cirrhotic patients, 5 year survival rates of up to 50% can be achieved by resection of hepatocellular carcinomas [97].

Because of the lack of applicability and/or success of other therapeutic modalities, orthotopic liver transplantation (OLT) has been viewed as the most efficacious therapeutic approach in patients with hepatocellular carcinomas arising in cirrhotic livers. However, initial outcomes after orthotopic liver transplantation for symptomatic hepatocellular carcinomas were dismal, in large part because of overly enthusiastic attempts to salvage patients with large liver tumors that had already invaded vascular structures [101]. After retrospective analysis of early experiences, it was noted that if OLT was restricted to patients with either isolated < 5 cm tumors or with ≤ 3 tumors of ≤3 cm each and without evidence for spread into adjacent vascular structures or remote metastases, 75% 4 year survival rates were achieved [102]. These survival rates are comparable to survival rates after OLT for other indications [91, 103]. However, organ shortages and prolonged waiting times have progressively limited access to this therapy, even for patients who initially meet these criteria. Therefore, when

possible, resection or ablation therapies are recommended as a first approach for patients who are candidates [100].

Earlier this year, UNOS implemented a new U.S. liver organ allocation system based on objective measures of hepatic function using the Model for End Stage Liver Disease (MELD) scoring system[104]. The MELD score was derived to correlate with probability of patient death within the next 3 months [105].

MELD Score = 0.957 X Log_e(creatinine mg/dL) + 0.378 X Log_e(bilirubin md/dL) + 1.120 X Log_e (INR) + 0.643
where: laboratory values < 1.0 are set to 1.0
creatinine values of > 4.0 are set to 4.0
patients dialyzed ≥2 times per week have creatinine values set to 4.0)

Patients listed for liver transplant for hepatocellular carcinomas < 2.0 cm in diameter (single lesion) are assigned a MELD score equivalent to a 15% probability of death within 3 months whereas those with single lesions 2-5 cm in diameter or ≤3 lesions with largest ≤3 cm diameter are registered at a MELD score equivalent to a 30% probability of death within 3 months [104]. Those with tumor numbers or sizes exceeding these criteria or with spread outside the liver parenchyma are not considered candidates, as in the past. While on the waiting list, hepatocellular carcinoma patients receive additional MELD points equivalent to a 10% increase in pre-transplant mortality every 3 months until they receive a transplant or are determined to be unsuitable candidates for transplantation due to tumor growth, tumor metastasis or other medical conditions. It remains to be determined whether greater or fewer numbers of hepatocellular carcinoma patients will receive liver transplantation under this system.

Table 4. Therapeutic Options for Hepatocellular Carcinoma

	Candidates*		
Modality:	Lesion Number, Size	Function Class	Other Considerations
Partial Hepatectomy	1 lesion < 5 cm or ≤3 lesions, ≤ 3 cm	Child's A, No Portal Hypertension, Nl Bilirubin	No other medical contraindications
Liver Transplantation	1 lesion < 5 cm or ≤3 lesions, ≤ 3 cm	Child's B/C or "unresectable"	No other medical contraindications
RFA	usually ≤ 3 cm	Child's A or B	
PEI	usually ≤ 3 cm	Child's A or B	

* No extrahepatic disease, no portal vein thrombosis

Prevention of Hepatocellular Carcinoma

In light of the high mortality rate and limited therapeutic options for hepatocellular carcinoma, it is readily apparent that prevention of this malignancy should be a high priority. Better understanding of the risk factors for development of hepatocellular carcinoma and some of the pathogenetic mechanisms involved has provided significant guidance for tumor prevention strategies. The well defined mechanisms underlying the role of aflatoxin B1 in hepatic carcinogenesis has motivated development of strategies for limiting fungal contamination of dietary staple foods (groundnuts, maize) by *Aspergillus flavus*, the source of this mycotoxin [106]. Unfortunately, the resources available in countries with endemic hepatitis infection and fungal contamination of foods are often severely limited. Of interest, however, a double-blind phase IIa trial conducted in Qidong, China [107] has demonstrated efficacy in detoxification of aflatoxin by administration of oltipraz, a drug originally marketed as an antischistosomal agent.

After the hepatitis B vaccination was developed in the early 1980's, universal vaccination programs were aggressively pursued in many endemic areas with the goal of preventing future HBV associated malignancies. Progress already has been observed in reducing malignancy rates attributable to chronic HBV infection following institution of universal Hepatitis B vaccination programs in Taiwan in 1984 [108]. The average annual incidence of hepatocellular carcinoma in Taiwanese children 6 to 14 years of age declined from 0.70 per 100,000 children between 1981 and 1986 to 0.36 per 100,000 between 1990 and 1994 ($P<0.01$). The incidence of hepatocellular carcinoma in children 6 to 9 years of age declined from 0.52 for those born between 1974 and 1984 to 0.13 for those born between 1984 and 1986 ($P<0.001$).The corresponding rates of mortality from hepatocellular carcinoma also decreased. It is anticipated that, as this vaccinated cohort reaches adulthood, even greater declines in liver malignancy rates will be appreciated.

Unfortunately, there is no immediate prospect for effective vaccination against hepatitis C, the commonest viral risk factor for hepatocellular carcinoma in industrialized countries. Fortunately, unlike hepatitis B, hepatitis C is much less readily transmitted among household members and much progress has already been made in reducing the most important routes of transmission via blood products and injection drug use [109]. Post-transfusion hepatitis C infection has become an exceedingly rare event following institution of blood donor screening and a decrease in incidence of new cases of hepatitis C among injection drug users has been observed since the late 1980's [109,110]. Clearly, such efforts at prevention of new infections need to be continued.

Nevertheless, the bulk of U.S. residents at risk for Hepatitis C associated hepatocellular carcinoma in the decades to come have already been infected [109] and thus prevention efforts have focused on antiviral or other therapies that might lower the risk of carcinogenesis. There is already a growing body of literature suggesting that sustained clearance of HCV RNA following a course of interferon-α based therapy [111-113] or even interferon-α therapy not associated with sustained viral clearance [114] may decrease future risk of hepatocellular carcinoma. However, such conclusions are largely derived from retrospective, cohort analysis or from very small, randomized trials [114] with short periods of follow-up. These results must be viewed with some skepticism since during non-randomized selection of patients for interferon-α therapy, patients with more advanced liver disease are less

likely to be selected for treatment because of concern about thrombocytopenia or leukopenias related to hypersplenism. Even in randomized, prospective trials, patients with more advanced disease tend to be less tolerant of full dose therapy and have lower sustained response rates [115,116]. Thus, sustained responders to interferon-α therapy also tend to be patients with less advanced disease at time of entry to therapy and it can be argued that they likely would have achieved a lower initial incidence of hepatocellular carcinoma and other liver disease complications even if therapy had never been administered. For these reasons, large, prospectively, randomized trials assessing the benefit of interferon-α therapy for prevention of hepatocellular carcinoma in HCV infected patients with advanced, fibrotic liver disease are in progress [117]. Nevertheless, all logic argues that if hepatocellular carcinoma only develops after the onset of cirrhosis and if HCV infection can be resolved prior to development of cirrhosis by antiviral therapy, then rates of liver cancer development should be reduced. Furthermore, unlike DNA viruses like HBV that can integrate potentially carcinogenic "genes" into the host genome, HCV is an RNA virus with no known mechanism for integration of viral genes into the host genome. Thus, more so than in the case of chronic HBV infection, there is optimism for significant reduction in hepatocellular carcinoma risk if successful anti-viral therapy can be administered.

In addition, to specific antiviral therapy, other personal habits, especially ethanol consumption and tobacco use appear to increase risk for hepatocellular carcinoma in subjects with underlying chronic viral hepatitis [14, 55, 118]. The most dramatic additive risk is observed in patients with chronic viral hepatitis who consume more than 40 alcoholic beverages per week and smoke cigarettes (RR ≈ 11) [118]. Thus, patients with chronic viral hepatitis should be advised to discontinue smoking and ethanol consumption.

With respect to other, rarer causes of cirrhosis and associated hepatocellular carcinomas, it also seems reasonable to focus preventive measures on use of effective therapies when available. In the case of hereditary hemochromatosis, it has been noted, that, as in the case for hepatitis C and other common Western liver diseases, hepatocellular carcinoma almost invariably develops in cirrhotic livers. The risk for hepatocellular carcinoma appears to remain low for patients in whom therapy is instituted prior to development of cirrhosis but remains elevated for those with established cirrhosis prior to onset of therapy [119].

Screening for Hepatocellular Carcinoma

Perhaps the most controversial aspect of hepatocellular carcinoma management, is the question of cancer screening. As the only "curative" therapies appear to be surgical approaches that are limited to patients with small, localized tumors, it seems logical that early detection ought to improve patient outcomes. Indeed, screening for hepatocellular carcinoma among patients with chronic hepatitis B infection or established cirrhosis has already been widely recommended despite acknowledgement that efficacy and cost effectiveness have yet to be demonstrated [115, 120]. Nevertheless, it has yet to be established that hepatocellular carcinoma meets the widely accepted criteria for diseases in which screening programs are traditionally advocated (see Table 5) [121].

Table 5. Criteria for Establishing a Screening Programs

Criteria:	Satisfied in HCC
Disease must be common with significant morbidity / mortality	Yes
Target population can be identified	Yes
Screening test(s) safe, sensitive and specific	Yes
Must be standardized recall system for positive screen	Yes
Screening test must be acceptable to the target population	Yes
Must be acceptable and effective therapy for "screen positive"	No*

* Therapy effective in only a minority of cases detected by screening

Traditional levels of hepatocellular carcinoma incidence would argue that this is a disease too rare to be considered for screening by primary care physicians in the U.S.. However, as outlined in the introduction to this Grand Rounds there is reason to believe that the incidence of this disease is and will continue to rise to a level where it will inevitably begin to be seen on a more frequent basis by generalists. All analyses of trends towards increasing incidence of hepatocellular carcinoma in developed countries suggests that this phenomenon is related to the effects of a historically recent "HCV Epidemic" [52, 53, 109, 110, 122-125]. Following initial isolation of the Hepatitis C virus in the late 1980's there has been a explosion of knowledge about this virus and its epidemiology. Analysis of sequence diversity among specimens collected worldwide suggests that the 6 major genotypes of HCV diverged from a common ancestor 500-2000 years ago [126]. Genotype 1b variants presently common in Japan, Europe and the U.S. appear to have diverged 70-80 years ago [126]. Sequences of genotype1b isolates from Japan, where it is the most prevalent genotype, appear to have diversified from a common source at a point in time estimated to be between 1943 and1949 [127]. Similarly, sequences of U.S. isolates of genotype 1a, a genotype commonly found only in the U.S., appear to have diverged more recently from a common source with estimated time of initial divergence being 1966-1970 [127].

These time estimates derived by molecular virologists for recent dissemination of HCV correlate well with estimates derived by epidemiologists from age related prevalence of HCV infection in the U.S., Japan and Europe [50, 109, 110, 123, 128]. In all industrialized countries, hepatitis C infection has been found to be predominately a disease of adults who acquire the infection due to parenteral risk factors uncommon during childhood [109, 110], In Japan, the birth cohort born between 1925 and 1935 [50] has the highest prevalence of HCV infection. The high prevalence of HCV infection in this cohort is ascribed by Japanese epidemiologists to use of methamphetamine by the military late in World War II to "improve the fighting spirits of the soldiers" [127] and to an ongoing intravenous methamphetamine epidemic during the immediate post-World War II era [128] that led to contamination of the Japanese blood supply and additional spread to the general population. Folk medicine therapies in Japan that involve skin breakage may have also contributed to the dissemination of this viral infection [128]. Analysis of the HCV epidemic in Italy and France also yields an estimated start date in the 1940's or 1950's [123, 129]. As in Japan, the peak prevalence of HCV

infection in Italy is among individuals born prior to 1940. In contrast to Japan and Southern Europe, the U.S. HCV epidemic appears to be much more recent in onset with peak prevalence of infection in the birth cohort born between ~1948 and 1958 [109]. This U.S. birth cohort represents the generation that reached adulthood during the Vietnam War and immediate post-Vietnam War era during which injection drug use became much more common. Epidemiologic models of the U.S. HCV epidemic indicate a large increase in the incidence of new HCV infections from the late 1960s to the early 1980s, a plateau in incidence during the 1980's and then a sharp decline in new cases after 1989 [109].

Thus, both virologic sequence data and epidemiologic data indicate that both the onset (late 1960's) and peaks (1980's) of the U.S. HCV epidemic [109] lag significantly behind such dates for the HCV epidemic in Japan (onset ~1945, peak ~1960's, [128]) or Southern Europe (onset ~1945, peak 1970-1980, [123]). Recently, median time from new HCV infections in young adults to death from hepatocellular carcinoma has been estimated to be as long as 41 years [123]. For these reasons, it appears likely that increases in HCV associated hepatocellular carcinoma observed in the U.S. in the 1990's [53] represent the "leading edge" of a liver cancer epidemic that will continue to evolve during the first third of the 21st century with peak incidence yet to be appreciated [109]. In Italy and Japan, prevalence of HCV infection appears to vary significantly among different regions with rates as low as 2 and 3% in Fukuoka, Japan and Campogalliano / Cormons, Northern Italy, respectively to as high as 26 and 28% in Castellana, Southern Italy and Yamagata, Japan [129]. Thus, it is difficult to compare prevalence in these countries to that in the U.S. where overall prevalence figures (1.8%) are commonly quoted for the total population [130]. Nevertheless, estimates for lifetime prevalence of HCV infection for the U.S. birth cohort with peak prevalence (~4%, [109]) are about 2-4 fold lower than estimates for prevalence among Italians and Japanese in the peak age cohort (~7.5 - 15% [110, 128, 129]). However, in the U.S., prevalence among men, the gender with the greatest incidence for hepatocellular carcinoma, is 2-fold greater than among women resulting in a 5.5-6% lifetime

Table 6. Features of Japanese and U.S. HCV "Epidemics"

	Japan	U.S.
Time of Onset	circa 1945	circa 1968
Mode of Transmission	IDU, then transfusions, folk medicine	IDU, then transfusions
Birth Cohort with Peak Prevalence (% Male Infected)	1925-1935 (7.5-15%)	1948-1958 (~5.5%)
Male HCC incidence (per 10^5),1961-1965	1.3 (Miyagi)	1.6 (Nevada)
Time of initial doubling of HCC rate	~1968→1978	1985→1999
Time of Peak HCC incidence	1985-present	?
Peak Male HCC incidence (per 10^5)	20-35	?

prevalence for men in the peak age cohort. In Italy [129] and Japan [131] the prevalence of HCV infection

is higher in women than among men resulting in estimates of prevalence of HCV infection among men in the Japanese peak age cohort that is only about 1.2-2.5 fold higher than in the U.S. Most areas of Japan experienced a ≥10-fold increase in hepatocellular carcinoma rates between the mid-1960's and 1980 [4, 8] with incidence rates among men exceeding 35 per 100,000 by 1990 [3, 124]. By extrapolation from Japanese experiences and from estimates of numbers of U.S. residents infected with HCV for > 20 years [109], it seems likely that another several fold increase in HCV associated hepatocellular carcinoma will be noted by 2015 as the U.S. male, 1948-1958 birth cohort ages.

Thus, hepatitis C associated hepatocellular carcinoma prevalence in the U.S., if not already considered a sufficiently common disease, will almost certainly rise to a level deemed worthy of consideration for screening. In addition, among selected ethnic groups in the U.S. with historically high levels of chronic HBV infection (see figure 1), persistently high rates of this disease are likely to continue for many years until the benefits of HBV vaccination are appreciated in the > 50 year old age group with highest risk for hepatic malignancy. Thus, target populations for hepatocellular carcinoma screening with high risk for the disease are clearly identified. These include all individuals with cirrhosis secondary to chronic hepatitis B or hepatitis C infection and pre-cirrhotic HBV carriers from endemic populations. Some would also add patients with cirrhosis secondary to hemochromatosis to this list because of an incidence of hepatic malignancy comparable to that in chronic viral hepatitis [119]. Screening tests (AFP, US) with satisfactory sensitivity and specificity have been developed and generally found satisfactory with respect to patient acceptance [61, 70, 132]. However, as pointed in reviews on this topic, widely different recall procedures have been utilized that make cost analyses difficult [70]. In screening studies published thus far, prospective detection rates of 1-7% per year have been achieved using AFP and US as screening techniques among cirrhotic patients with predominately hepatitis C or Hepatitis B related liver disease [133]. It appears that such testing must be performed at least every 6 months among cirrhotics if hepatocellular carcinoma is to be detected at a single < 3cm nodule stage [70, 133]. When HBV carriers without known cirrhosis are subjected to prospective screening, only 0.3-0.5%/yr detection rates are achieved [16, 70]. However, in two studies prospectively screening HBV carriers, 50-75 % of tumors were discovered at a "resectable" stage and 20-40% long-term tumor free survivals appeared to be achieved among patients with hepatocellular carcinomas detected by screening [16, 70, 134].

In contrast, outcomes of therapy for hepatocellular carcinomas detected by screening cirrhotic patients have been much less satisfying with surgical resection rates of only 7 - 54% being reported and recurrence rates of 60% noted among those resected [70, 133]. Some authors have reported improved survival among patients with hepatocellular carcinomas detected by screening versus unscreened hepatocellular carcinoma patients [132, 135], but lead time bias may explain much of this apparent prolongation of survival. Thus, because of modest improvements in outcomes among patients with tumors detected by screening, there is little evidence to suggest that this approach is likely to be cost effective in the high risk population at large [70, 132].

Table 7. Screening for Hepatocellular Carcinoma: High and Low Risk Liver Disease Groups

Risk Level	Examples
Very Low, <<0.5% detection when screened	American/European HBV carrier, nl ALT[136, 137] Chronic HCV without cirrhosis [60] Women with autoimmune liver disease [38]
High Risk, 0.5-4%/yr HCC detection when screened	chronic Hepatitis B, endemic population [16] Chronic Hepatitis C with cirrhosis [70, 133] Chronic Hepatitis B with cirrhosis [70, 133] Hemochromatosis with cirrhosis [119]
Very High Risk	Any high risk + male gender, age > 45 [16, 70, 138] Chronic HBV + HCV [139] Chronic HBV or HCV + Alcohol abuse [55] Initial AFP > 50 ng/ml [140]

Nevertheless, some analyses have argued that when conducted in patients being evaluated for liver transplantation, screening for hepatocellular carcinoma is cost effective [125, 141]. Certainly, in this setting, there are other societal benefits to evaluating and monitoring patients for hepatocellular carcinoma so that patients with advanced liver disease and associated hepatocellular carcinomas might receive organs while tumors are still small and localized while, on the other hand, donor organs are not allocated to patients with unsuspected, inoperable tumors. Thus, screening, or at least thorough initial diagnostic evaluation for hepatocellular carcinoma among cirrhotic patients viewed as liver transplant candidates is a widely accepted practice. Other populations recommended as suitable candidates for hepatocellular carcinoma screening are patients with Child's A cirrhosis who are still viewed as resection candidates [140] and HBV carriers from endemic populations in which tumors are not uncommonly discovered in non-cirrhotic patients who are better surgical candidates [16, 70]. It has also been proposed that screening might become more cost-effective if focused on patients at the very highest risks as defined by factors outlined in Table 7. Clearly, however, patients who are neither resection nor transplantation candidates are unlikely to benefit from screening irrespective of level of risk.

Summary

Hepatocellular carcinoma is becoming an increasingly common malignancy in the U.S., especially among patients with cirrhosis due to chronic hepatitis C or those with chronic hepatitis B infection. Diagnostic modalities have steadily improved to the point where a diagnosis can be made by clinical, radiologic and serologic criteria in the majority of patients. In patients with risk factors for hepatocellular carcinoma who present with either specific symptoms (right upper quadrant pain, weight loss) or any decompensation of chronic liver disease, investigation with AFP testing and either triphasic CT scanning or MR imaging techniques will have high diagnostic yield and discovery of a tumor will often change management approaches. Less expensive screening strategies using AFP testing +/- US will also have a significant yield when applied to asymptomatic patients with active chronic hepatitis B infection or cirrhosis secondary to chronic hepatitis C. Unfortunately, most

patients found to have hepatocellular carcinoma will not achieve curative therapy. For this reason, screening of all at high risk for this disease cannot be recommended as cost-effective.

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