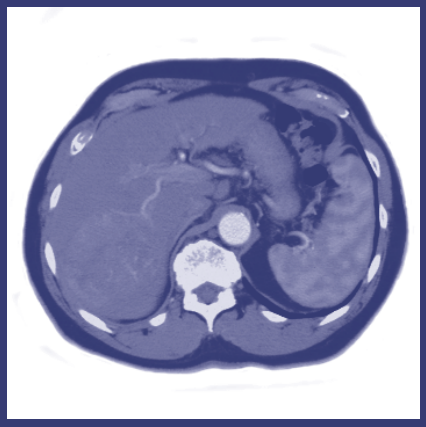
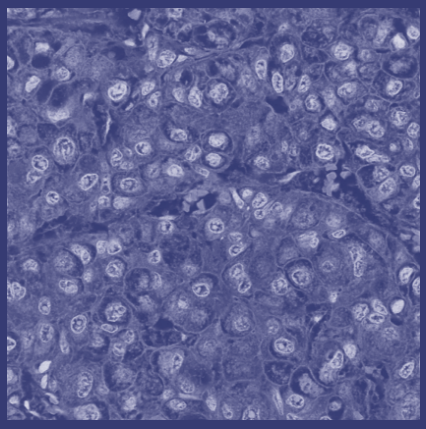
Hepatocellular Carcinoma

Screening, Diagnosis, and Management





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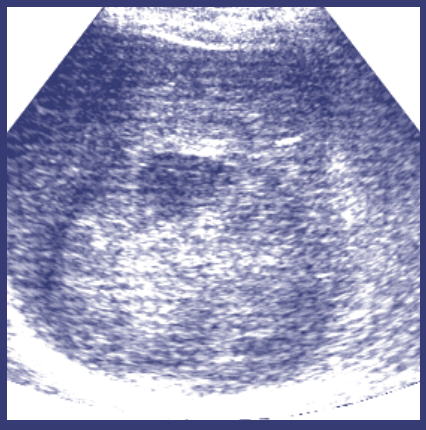

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Hepatocellular Carcinoma

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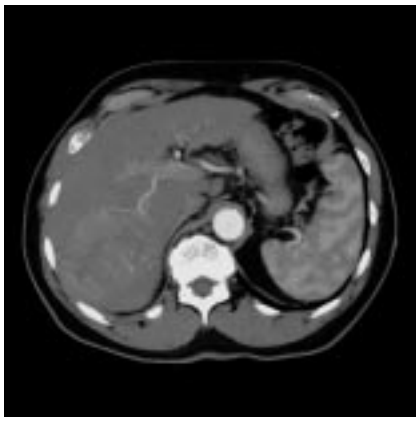

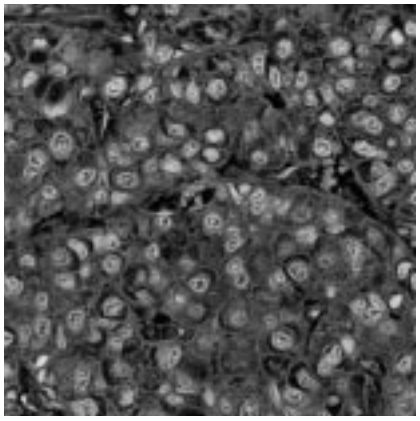

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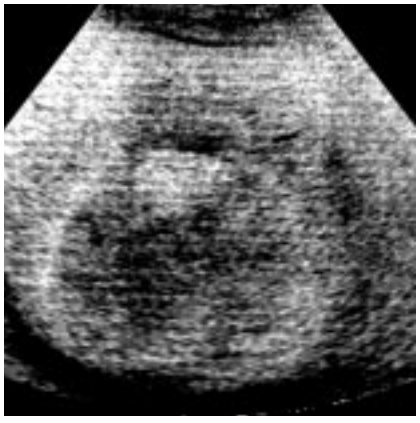

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Contents

Hepatocellular Carcinoma: *Screening, Diagnosis, and Management*

Agenda

Hepatocellular Carcinoma: *Screening, Diagnosis, and Management*

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9:00 am	Recent Trends of Hepatocellular Carcinoma in Japan *Kendo Kiyosawa, Shinshu University School of Medicine, Japan*
9:20 am	HCC: Recent Epidemiological Trends in the United States *Hashem El Serag, VA Medical Center, Houston, TX*
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Speaker Abstracts

The Worldwide Epidemiology of Primary Liver Cancer

F. Xavier Bosch, MD, MPH

Institut Català d'Oncologia, Barcelona, Spain

Primary liver cancer (PLC) remains one of the most common malignancies in the world and the most common in men in many developing countries. It is also the first human cancer largely amenable to prevention using hepatitis B virus (HBV) vaccines and screening of blood and blood products for the hepatitis C virus (HCV). It has been estimated that worldwide some 564,000 new cases (398,000 in men and 166,000 in women) occurred in 2000. PLC accounts for 5.6% of all human cancers (7.5% among men and 3.5% among women). LC is a tumor with high lethality: the fatality ratio (LC AAMRs/LC AAIRs) is around or greater than 1, indicating that most cases do not survive one year. In Europe, for the 1985-1989 period and in North America, for 1983-1988, the 5-year relative survival rate (mortality from LC adjusted for mortality from competing causes) was 5% and 6%, respectively. In developing countries LC is inevitably fatal.

The geographic areas at highest risk are located in Eastern Asia, Middle Africa and some countries of Western Africa. Low-risk areas include Northern Europe, Australia, New Zealand, and the Caucasian populations in North and Latin America. In high-risk countries, incidence rates are typically 2 to 3-fold higher than those in developed countries.

An excess of LC incidence among men compared to women has been well documented (range of the sex ratios in the AAIRs is 1.4 to 3.3). In high risk countries, sex ratios tend to be higher, and the male excess is more pronounced around 40-50 years of age. In populations with low incidence, the highest sex ratios occur later, around 60-70 years of age. The correlation between AAIRs LC in men and women is extremely high (Correlation Coefficient: 0.953, $p<0.001$) suggesting similarities in the relevant risk factors.

Time trends analyses have reported significant increasing rates for the Black, White and Hispanic populations in the US. The interpretation made by the authors suggest that HCV exposure in the relevant generations in the period 1960-70 may explain most of the cases observed. In Japan, increasing LC incidence and mortality trends since the early 1970s have been largely attributed to increasing consumption of alcohol, to massive exposure of the population to HCV through blood transfusion or contaminated needles in vaccination campaigns against tuberculosis after World War II and to illegal intravenous drug abuse. Other registries that have suggested increasing trends in LC incidence among men included Australia, India, Israel, Canada, Italy, Spain and Finland. Decreasing incidence trends are observed in several registries in Scandinavia, parts of China, and among Japanese populations in the US.

The predominant role of environmental factors in the etiology of LC is strongly suggested by: a) the variations in LC incidence among different populations living in the same geographical area; and b) by the trends in PLC incidence in migrant populations which tend to adopt the incidence rates of their host populations in their second and subsequent generations. The distribution of LC incidence rates between countries and within countries is largely explained by the distribution of the prevalence of the Hepatitis B and C viruses. The Attributable Risk estimates for the combined effects of these infections account for well over 80% of LC cases worldwide. Co-infections with HBV and HCV or with HBV and the defective Delta virus (HDV) further increase the risk of progressive liver cirrhosis and PLC. Other documented risk factors such as alcohol consumption, cigarette smoking, Aflatoxin exposure in diets and use of oral contraceptives may explain the residual variation between and within countries.

Reduction of LC burden in most developing countries should give priority to HBV vaccination campaigns and other interventions to reduce exposure to HBV and HCV. This implies reinforcing control of blood and the use of sterile medical equipment.

HBV chronic carries may benefit from reductions in Aflatoxin exposure in their diets. If achieved, AF reduction may also offer some protection to HCV carriers. In low risk populations, alcohol consumption may account for the majority of the LC cases that do not show viral markers.

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Recent Trends of Hepatocellular Carcinoma in Japan

Kendo Kiyosawa, MD, Tetsuya Ichijo, Akihiro Matsumoto, Kaname Yoshizawa, Eiji Tanaka

Shinshu University School of Medicine, Japan

Annual deaths due to liver cancer including hepatocellular carcinoma (HCC) and cholangiocarcinoma was under 10,000 and the death rate (deaths/100,000 population) was under 10 by 1975 in Japan. However, thereafter annual death and the death rate of liver cancer increased year by year. According to the VITAL STATISTICS OF JAPAN 2001, the number of deaths due to liver cancer was 34,311, with males representing 63% of this total. The death rate of HCC in 2001 was 27.3, which ranked fourth among all cancer-caused deaths in total, and third and fourth among men and women, respectively. According to the report of the 15th follow-up survey of primary liver cancer in 1998 and 1999 conducted by the Liver Cancer Study Group of Japan, 94.9% patients were HCC, and of them 72.3% and 16.8% patients were positive for antibody to hepatitis C virus (anti-HCV) and hepatitis B surface antigen (HBsAg), respectively.

In cross-sectional studies we conducted in 1982, 1990, and 2003, HBsAg positive patients with HCC represented 55 (51%) of 108, 29(34%) of 83, and 27(19%) of 145, respectively. Although anti-HCV test was not available in 1982, non-B or anti-HCV positive HCC patients in 1982, 1990, and 2003 were 53 (49%), 51(61%), and 107 (74%), respectively. The proportion of hepatitis B virus (HBV) related-HCC to hepatitis C virus (HCV)-related HCC has changed dramatically over the past 20 years, with a dramatic increase in HCV-related HCC. This indicates that the increase in patients with HCC is due to the spread of HCV infection. Mean ages of patients with HCV-related HCC in 1982, 1990, and 1993 were 61.6, 63.1, and 67.8 years, respectively. To the contrary, mean ages of HBV-related HCC in each year were 55.4, 54.8 and 53.8 years, respectively. This indicates that mean age of patients with HCV-related HCC has elevated past 20 years, but not in patients with HBV-related HCC. Prevalences of history of blood transfusion in patients with non-B/anti-HCV positive HCC

were 13%, 42%, and 26% in 1982, 1990 and 2003, respectively. The intervals between time of blood transfusion and date of diagnosis of HCV-related HCC were 23.4, 29.0, and 36.6 years, in 1982, 1990, and 2003, respectively. As one of the explanations for prolongation of interval between blood transfusion and detection of HCC in patients with HCV-related HCC, antiviral therapy and anti-inflammatory therapy might be considered.

It is well known that there are several significant differences in clinicopathological features between HCV- and HBV-related HCC patients. The age of patients with HBV-related HCC is significantly lower than that of HCV-related HCC patients. A family history of clustering liver diseases is seen in HBV-related HCC patients, and a history of blood transfusion or surgical operation is seen in HCV-related HCC patients. Liver cirrhosis usually accompanies HCV-related HCC, though not in all cases. The multifocal occurrence of HCC is seen frequently in HCV-related HCC, but is rare in HBV-related HCC. Intrahepatic metastasis is frequent in HCV-related HCC. These differences influence survival after treatment for HCC. The five-year survival rate in patients with HBV-related HCC is better than that in patients with HCV-related HCC.

Though Japan is a small country, distribution of HCC clearly differs among regions. The report of the Japanese Ministry of Health and Welfare on the incidence of deaths as a result of HCC in its 50 prefectures shows an increasing gradient along the axis of Japan from east to west. Prefectures showing over 30 deaths/100,000 population of HCC were biased toward the west. This inclination of death rate is coincident with an incline in the positive rates of anti-HCV and HBsAg in the adult population.

The Japan Society of Hepatology published 'THE LIVER CANCER WHITE PAPER' in 1999 for the purpose of attempting to eradicate liver cancer from Japan. It proposed several recommendations, including improvement in sanitary conditions in community; intensifying the individual's concern for prevention of hepatitis virus infection; promoting scientific activity to establish active immunization for HCV; introducing new treatment against chronic hepatitis to inhibit progression to cirrhosis of the liver and HCC; and establishing close communication between physicians and hepatologists to detect small HCC and to treat HCC earlier. Furthermore, because HCV carriers and HBV carriers are considered to be candidates for HCC, it is strongly recommended to find asymptomatic hepatitis virus carriers and to establish follow-up system over the long term. Nationwide screening for anti-HCV and HBsAg in the population over 40 started in 2002 in Japan. Interferon and ribavirin combination therapy for chronic hepatitis C and lamivudine therapy for chronic hepatitis B are being applied. It has been demonstrated that these therapies suppress the occurrence of HCC significantly.

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HCC: Recent Epidemiological Trends in the United States

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VA Medical Center, Houston, TX

Incidence of HCC in the United States

Hepatocellular carcinoma (HCC) constitutes approximately 90% of primary liver cancer in the United States. A progressive increase in the incidence of HCC was observed in the United States starting in the mid 1980s. The best incidence data has been derived from information collected by population-based registries of the Surveillance, Epidemiology, and End Results (SEER). In nine SEER registries representing approximately 10% of the US population, the overall age-adjusted incidence rates of HCC increased from 1.4 per 100,000 in 1975-77 to 3.0 per 100,000 in 1996-98. There was a 25% increase during the last 3 years of the study (1996-98) compared to the preceding 3 years (1993-95). The increase affected most age groups above 40, but the greatest increase occurred between ages 45 and 49. White men had the greatest increase (31%) in the last time period (1996-98) as compared to 1993-95. The two-fold increase in HCC has been confirmed, adjusting for changes in the demographic features (age, gender, race, geographic region). The figures stated above probably underestimate the true incidence of HCC by 15 to 20% as they represent only cases with confirmatory histological, or cytological evidence of HCC. Importantly however, the proportion of HCC cases confirmed using any of these methods has remained relatively stable between 1981 and 1998, making it less likely for the observed increase to be attributed to a diagnostic bias[1].

The recent rise in HCC mortality in the US is a direct result of the rising incidence rate of HCC during the same time period; the five-year survival has remained dismal (5%)[2]. According to the US vital statistics, the overall age-adjusted mortality rate for primary liver cancer has risen significantly from 1.7 per 100,000 during 1981 to 1995 to 2.4 per 100,000 during 1991 to 1995 representing a 45% increase[3].

Demographic Risk Factors for HCC

Caucasians are two to three times less affected than African Americans, who in turn are two to three times less affected than Asians, Pacific Islanders, or Native Americans. Men are two to three time more affected than women. Asians such as Chinese, Korean, Filipino, and Japanese men have the highest age-adjusted incidence rates (up to 23 per 100,000). However, all ethnic groups and both men and women have been affected to varying degrees by the recent increase in incidence. The reasons of these ethnic and gender variations probably relate to the prevalence and time of acquisition of the major HCC risk factors (HCV, HBV, and alcohol). It is known that the prevalence of HCV, HBV, and alcoholic cirrhosis is two to three folds higher in African Americans and Hispanics than whites. Native American Eskimos, and Asians particularly recent immigrants from China, Taiwan, Korea and Vietnam have high prevalence rates of HBV similar to those in their original countries. There are significant geographic variations in HCC (irrespective of the demographic differences between these regions): For example, among the 9 SEER registries, Hawaii had the highest age-adjusted incidence rate (4.6/100,000), followed by San Francisco-Oakland (3.2/100,000), New Mexico (2.0/100,000), whereas Iowa and Utah lowest rates of approximately 1.0/100,000[4]. HCC is very rare before age 40, increases progressively with older age and peaks in incidence around ages 70 to 75. However, concomitant with the rising rates of HCC, there was a shift of incidence from typically elderly patients to relatively younger patients between ages 40 to 60[1,3].

The Cause(s) of the Rising HCC in the United States

Due to the essential role of cirrhosis in the development of HCC in the majority of cases, an increase in the number of persons living with cirrhosis is the likely explanation of the rising incidence of HCC. Once cirrhosis is established, HCC develops at an annual rate of 1% to 5%. *There are no prospective studies examining the distribution of the underlying risk factors among patients with HCC in the US, or the temporal changes in these risk factors. Therefore the specific reason for the*

increase in HCC, as well as the forecast for future cases remains uncertain. There have been four retrospective non-population based studies (two published studies, and two in abstract form) that examined temporal changes in risk factors among patients with HCC; all studies indicate an increase in HCV-related HCC. In the first study, the computerized records (VA Patient Treatment File) of 1,605 patients who were hospitalized with a first time diagnosis of primary liver cancer between 1993 and 1998 were searched for known risk factors for HCC among these patients. There was a 3-fold increase in the age-adjusted rates for primary liver cancer associated with HCV from 2.3 per 100,000 between 1993 and 1995 to 7.0 per 100,000 between 1996 and 1998. HCV infection accounted for at least half of the increase in the number of HCC among US veterans. During the same time periods, age-adjusted rates for primary liver cancer with either hepatitis B virus (2.2 versus 3.1 per 100,000) or alcoholic cirrhosis (8.4 versus 9.1 per 100,000) remained stable. The rates for primary liver cancer without risk factors have also remained without a statistically significant change from 17.5 between 1993 and 1995 to 19.0 per 100,000 between 1996 and 1998[5]. Similar trends have been observed from the large referral setting of MD Andersen Medical Center where we recently reviewed the medical records of all patients residing in the United States who received a pathological diagnosis of HCC during 1993-1998; all patients were tested for HCV and HBV. The number of patients referred with HCC steadily increased from 143 in 1993–1995 to 216 in 1996-1998; of those, 26 patients (18%) and 66 patients (31%) were HCV positive during 1993 to 1995 and 1996 to 1998, respectively (P=0.01)[6].

In studies with the best-documented time of infection onset, there is an average incidence of 1% per year cirrhosis and 0.05% HCC (20% and 1% at 20 years, respectively) in patients with chronic HCV infection. Using SEER-Medicare linked data, we conducted a population-based study to examine temporal changes in risk factors for patients 65 years and older diagnosed with HCC between 1993-1999. We identified 2,584 patients with continuous Medicare enrollment 2 years before and up to 2 years following the HCC diagnosis. The proportion of HCV-related HCC increased from 11% during 1/1993-6/1996 to 21% during 7/1996-12/1999, while HBV-related HCC increased from

6% to 11% (p<0.0001). In multiple logistic regression analyses that adjusted for age, gender, race, and geographic region, the risk of HCV-related HCC and HBV-related HCC increased by 226% and 67%, respectively. No significant changes over time were observed for alcoholic liver disease, or non-specific cirrhosis[7].

A recent cross sectional retrospective survey was conducted at liver transplantation centers in the US (7/1997 to 7/1999) in which 691 patients were described of whom 15.4% were positive for HBsAg, 46.5% had antibodies to HCV, 4.7% had both HBsAg and anti-HCV, and 33.1% had neither marker present. Anti-HCV positivity was the most frequent risk factor in both blacks and whites, whereas HBsAg positivity was the most frequent etiological factor in Asians with HCC[8].

"Idiopathic" HCC. Previous studies failed to identify specific risk factors in 15% and 50% of HCC cases[9,10]. These findings might indicate a limitation of the data source. Recent studies have implicated diabetes and non-alcoholic fatty liver disease as risk factors for at least a proportion of these "idiopathic" cases. Diabetes mellitus has been associated with non-alcoholic fatty liver disease including its most severe form nonalcoholic steatohepatitis (NASH). Earlier epidemiological studies showed no association between diabetes and HCC, while several more recent case control studies some of which were conducted in samples from the US[11] indicate a significant statistical association between HCC and diabetes. The difficulty in interpreting these studies is that diabetes could be a result (rather than a cause) of end stage liver disease in general, or some specific causes such as HCV. However, a recent large cohort study confirmed this association and showed a plausible temporal association where diabetes preceded HCC by several years. The study cohort comprised 173,643 patients with diabetes and 650,620 patients without diabetes with no liver disease recorded at baseline. HCC was increased (incidence rate: 2.39 vs. 0.87 per 10,000 person-years). Diabetes was associated with a hazard ratio of 2.16 (95 CI: 1.86 to 2.52, p<0.0001) of HCC. Diabetes carried the highest risk among patients with >10 years of follow-up[12].

The future of HCC in the US. Due to the large pool of HCV-infected persons, it is likely that the rising incidence of HCC will continue over the next several years and a crude estimate of 200-300,000 HCV-related HCC 1-6% of all HCV-infected patients will develop HCC, it is likely that the rising incidence of HCC will continue over the next several years. In addition, the influx of recent immigrants from China, Taiwan, Korea and Vietnam with high HBV prevalence is likely to continue and further contribute to the rising incidence of HCC. To the extent that obesity and diabetes increase the risk of HCC, these may also contribute to the future toll of HCC. Prospective population-based studies are required to accurately describe the current and future distribution of risk factors among HCC patients; these estimates are required to better anticipate future trends and to design appropriate preventive measures.

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Cirrhosis & Hepatocellular Carcinoma: Incidence & Factors Related to Hepatocellular Development

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Cirrhosis from any cause predisposes to hepatocellular carcinoma (HCC) and can be considered as a premalignant condition. The present review highlights the burden of HCC in cirrhosis, the incidence rates of HCC according to aetiology of cirrhosis and the role of host, viral and environmental factors in HCC occurrence, with emphasis on hepatitis C virus (HCV)- and hepatitis B virus (HBV)-related cirrhosis.

The Burden of HCC in Cirrhosis

HCC represents nowadays the major cause of liver-related death (up to 80%) among cirrhotic patients. Opposite trends of increasing mortality for HCC and declining mortality for liver cirrhosis due to non HCC complications have been recently observed in Europe and the United States (1, 2). As markers of HCV infection are found in 27-75% of HCC cases in the same areas, the current reservoirs of HCV infection in the general population of Europe and the United States raises concern about the prospect of an increasing incidence of cirrhosis and HCC in the coming decades.

HCC With and Without Underlying Cirrhosis

The prevalence of cirrhosis in HCC is about 80-90% in autopsied series worldwide, thus HCC develops on non-cirrhotic liver in 10-20% of cases. However, a very small proportion of patients with HCC in non-cirrhotic liver has a normal liver histology, whereas the majority of them show fibrosis, necroinflammation, steatosis and liver cell dysplasia (3). Few recent European studies investigated HCC aetiology with respect to the presence or absence of cirrhosis. In HCC cases with cirrhosis, HCV infection was found in 27-73%, HBV infection in 12-55%, heavy alcohol intake in 6-29%, and hereditary hemochromatosis (HH) and other causes in 2-6%, leaving 4-6% of total cases without an identified agent. In HCC without underlying cirrhosis,

HCV infection was found in 3-44 %, HBV infection in 12-29%, heavy alcohol intake in 12-28%, and less common factors in 1-5% of the cases; in a variable proportion of HCC cases the aetiology was unknown.

Incidence Rates and Risk Factors of HCC According to Aetiology of Cirrhosis

In order to estimate the incidence (absolute risk) of HCC according to aetiology of cirrhosis we selected published studies according to the following inclusion criteria: 1) longitudinal studies; 2) studies including patients with histological proven liver disease; alternatively diagnosis of cirrhosis was accepted when based on well-defined clinical criteria; 3) studies including patients with compensated cirrhosis; 4) patients untreated for HCV or HBV. We estimated a summary measure of the incidence rates for each aetiology and the 5-year cumulative incidence as an immediate assessment of the patient's absolute risk. The risk of HCC in patients with chronic HBV or HCV infection or the presence of alcoholism according to the clinical setting and geographic area is shown in Table 1 *(page 32)*.

HCV-related cirrhosis. Overall, the HCC incidence rate in patients with HCV-related cirrhosis is 3.5 per 100 patients per year in Europe and the United States. In Japan, the HCC incidence is 1.7 per 100 patients per year in subjects with chronic hepatitis C without cirrhosis at diagnosis and 7.6 in compensated cirrhosis, thus providing a 4.5 fold higher risk of HCC for cirrhosis than chronic hepatitis. The 5-year cumulative risk for HCC in patients with cirrhosis is 16% in Europe and the United States and 32% in Japan. Major factors affecting progression to HCC are older age at infection, older age at diagnosis of cirrhosis, male sex and stage of compensated cirrhosis at presentation (4). Additional prognostic factors are HBV coinfection (2-6-fold increased risk) and heavy alcohol intake (2-4- fold increased risk). There is growing evidence that occult HBV coinfection and HIV coinfection as well as liver steatosis may increase the HCC risk. No conclusions can be drawn on the role of HCV genotype in HCC risk.

HBV-related cirrhosis. In areas at high HBV endemicity the HCC incidence rate is 0.2 per 100 patients per year in inactive carriers, 1.0 in chronic hepatitis B without preexisting cirrhosis at diagnosis and 3.2 in Chinese with compensated cirrhosis, with a 5-year HCC cumulative incidence of 15% in cirrhotics. In Western countries with low or intermediate endemicity for the infection, the HCC incidence is 0.02 per 100 patients per year in inactive carriers, 0.07 in chronic hepatitis B without cirrhosis at diagnosis and 2.1 in compensated cirrhosis, with a 5-year HCC cumulative incidence of 10% in cirrhotics. Therefore, in the West, the cirrhotic has an about 28-fold higher risk for HCC than the patient with chronic hepatitis without cirrhosis and a 100-fold higher HCC risk than the inactive carrier. Major risk factors for HCC in HBV-related cirrhosis include older age at diagnosis of cirrhosis, male gender, severity of compensated cirrhosis at presentation, hepatitis delta virus coinfection (3-fold increased risk), HCV coinfection (2-6-fold increased risk) and heavy alcohol intake (about 2-fold increased risk) (4). Sustained reduction of HBV replication, ALT normalization and eventually HBsAg loss are associated with a very low risk of HCC. The role of HBV genotype and of HIV coinfection on the risk of liver tumor requires more research. Dietary carcinogens, such as aflatoxin, may be relevant in HBV endemic regions.

Alcoholic Cirrhosis. The HCC incidence is 0.009 per 100 patients per year in alcoholics and 1.8 in patients with HBsAg- and anti-HCV negative alcoholic cirrhosis, thus providing a 212-fold increased risk of HCC for cirrhosis than alcoholism without cirrhosis.

Other etiologies. In one study of cirrhotic patients with HH, the HCC incidence is 5 per 100 patients per year; however, the majority of cases had HCV infection, HBV infection or alcohol abuse. In primary biliary cirrhosis without viral infection, HCC almost exclusively develops in patients with advanced stage III-IV with an incidence of 0.9 per 100 patients per year. Very limited, if any, data on HCC incidence are available for cirrhosis due to other causes, due to the rare occurrence of this complication.

Predictors of HCC in Cirrhosis

Age and gender. Older age and male sex are important prognostic factors independent of aetiology of cirrhosis. ***Stage of cirrhosis.*** Patients with worsening Child-Pugh grades are at higher risk of liver tumor. ***Activity of liver disease.*** Longitudinal studies of patients with cirrhosis of different causes have indicated that sustained high serum ALT levels are associated with a significant higher risk of HCC occurrence. ***Alfafetoprotein.*** The prognostic role of alfafetoprotein elevation at baseline is still controversial. ***Histologic assessment.*** Large cell change and nucleolar hypertrophy may predict HCC risk in HBV-related cirrhosis. Macronodules characterize a subgroup of cirrhotics with high risk of HCC and the risk is further increased in the presence of morphologic features of high-grade dysplasic nodule.

Conclusions

1. The burden of HCC in cirrhosis is rising in most developed countries, possibly due to improved medical management of non-HCC complications of cirrhosis, leading to longer survival of cirrhotic patients. 2. In Western countries, HCV infection, HBV infection and heavy alcohol intake are found in almost all HCC cases in cirrhosis; the same factors are evident in a variable proportion of HCC cases without cirrhosis, though the aetiology of some of them is still unknown. Hereditary hemochromatosis and other causes account for only a small number of the total liver cancer. 3. Irrespective of the geographical area and of aetiology, the cirrhotic patient has an increased risk of HCC than the non cirrhotic, thus suggesting that cirrhosis *per se* is the major risk factor for HCC development. 4. In HCV-related cirrhosis the risk of HCC appears about 2 fold higher in Japan than in Western countries. Irrespective of the severity of the underlying liver disease, the risk of HCC in HBV infection is higher in areas at high endemicity than in Western countries, possibly because of earlier acquisition of the virus, greater duration of disease and differences in environmental toxin exposure. 5. Several factors and cofactors influence the risk for HCC in the cirrhotic patients and better knowledge of individual risk factors for HCC is the basis for disease management and for designing better prevention strategies.

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Table 1. Overall hepatocellular carcinoma (HCC) incidence rates according to aetiologies, clinical setting and geographic area in longitudinal studies

Aetiology	Clinical setting	Geographic area	No. Studies	No. patients	Mean Follow-up (yrs)	HCC Incidence[1]	95% Confidence Interval
HCV	Chronic hepatitis[2]	Europe	1	329	4.2	0	-
		Japan	5	1315	5.9	1.68	1.39-1.97
	Compensated cirrhosis[3]	Europe & United States	10	1041	4.8	3.55	3.03-4.08
		Japan	4	458	4.9	7.64	6.50-8.78
HBV	Asymptomatic carrier	North America	2[4]	1804	16	0.11	0.72-0.14
		Taiwan	2[4]	4920	8.3	0.38	0.32-0.44
	Inactive carrier[5]	Europe	3	410	23.6	0.02	0-0.04
		Taiwan	1	189	8	0.19	0-0.42
	Chronic hepatitis[2]	Europe	5	440	6.2	0.07	0-0.17
		Taiwan	2	461	2.3	0.96	0.36-1.56
		Japan	2	737	4.4	0.76	0.46-1.06
	Compensated cirrhosis[3]	Europe	2	227	5.9	2.09	1.32-2.87
		Taiwan & Singapore	3	278	2.7	3.25	1.94-4.55
		Japan	2	306	6.3	4.32	3.4-5.25
Alcohol	Alcoholism	Europe	2[6]	173389	10.6	0.0088	0.0074-0.01
	Compensated cirrhosis	Europe	2	239	4.9	1.95	1.15-2.74
		Japan	2	174	4.5	1.78	0.84-2.71

[1]Incidence per 100 patients per year. [2]Patients with cirrhosis at entry and patients treated with interferon were excluded from the analysis. [3]Patients treated with interferon were excluded. [4]One population based study. [5]Repeatedly normal alanine aminotransferase levels and absence of hepatitis B e antigen (HBeAg) with presence of anti-HBe. [6]Population based series.

Molecular Pathogenesis of Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world, accounting for an estimated half million deaths annually[1]. While HCC is prevalent in Southeast Asia and sub-Sahara Africa, the incidence of HCC has doubled in the United States over the past 25 years and the incidence and mortality rates of HCC are likely to double over the next 10 to 20 years[2]. Although much is known about both the cellular changes that lead to HCC and the etiological agents (i.e. HBV, HCV infection, and alcohol) responsible for the majority of HCC, the molecular pathogenesis of HCC is not well understood[3]. However, we do know that hepatocarcinogenesis in humans is a slowly evolving process during which genomic changes progressively alter the hepatocellular phenotype to produce cellular intermediates that gradually progress into hepatocellular carcinoma. During the long preneoplastic stage, in which the liver is usually the site of chronic hepatitis and/or cirrhosis, hepatocyte cycling is accelerated by upregulation of mitogenic pathways in part, through epigenetic mechanisms, leading to the production of monoclonal populations of hepatocytes. Phenotypically aberrant and dysplastic hepatocytes develop in these preneoplastic cell populations in association with telomere erosion and telomerase re-expression, microsatellite instability in some instances, and occasional structural aberrations in genes and chromosomes. Development of dysplastic hepatocytes in foci and nodules and emergence of hepatocellular carcinoma are associated with the accumulation of irreversible structural alterations in genes and chromosomes. The genomic basis of the malignant phenotype in hepatocytes is heterogeneous. The malignant hepatocyte phenotype may be produced by aberrant function of multiple genes that in combination disrupt different regulatory pathways, resulting in several molecular variants of hepatocellular carcinoma.

Considerable efforts have been devoted to establishing a prognostic model for HCC by using clinical information and pathological classification in order to provide information at diagnosis on both survival and treatment options[4-10]. Although much progress has been achieved (reviewd in[11]), a number of issues still remains unresolved. For example, a staging system that reliably separates patients with early as well as intermediate and advanced HCC into homogeneous groups with respect to prognosis does not exist. This is particularly important since the natural course of early HCC is unknown and the natural progression of intermediate and advanced HCC are known to be quite heterogeneous[12]. It therefore appears axiomatic that improving the classification of HCC patients into groups with homogeneous prognosis would at minimum improve the application of currently available treatment modalities and at best provide new treatment strategies.

Recently, microarray technologies have been successfully used to predict clinical outcome and survival as well as classify different types of cancer[13-15]. The microarray technologies have also been applied in a number of studies to define the global gene expression patterns in primary human HCC as well as HCC derived cell lines[16] in an attempt to gain insight into the mechanism(s) of hepatocarcinogenesis. The results from these studies have identified subgroups of HCC that differ according to etiological factors[17], mutations of tumor suppressor genes[18], rate of recurrence[19], and intra-hepatic metastasis[20], as well as novel molecular markers for HCC diagnosis[21]. However, most of these studies have identified genes that are associated with limited aspects of the tumor pathogenesis, and thus failed to create molecular prognostic indices that could be applied to the HCC patient population in general.

We recently investigated the possibility that variations in gene-expression in HCC obtained at diagnosis would permit the identification of distinct subclasses of HCC patients with different prognoses. The results reveal two subclasses of HCC patients characterized by significant differences in the length of survival. We also identified expression profiles of a limited number of genes that

accurately predicted the length of survival. Thus, our data indicate that it is possible to use gene expression patterns to accurately predict the clinical outcome of HCC at the time of diagnosis.

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Hepatitis B and HCC

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Hepatitis B (HBV) and C (HCV) chronic viral infections are key risk factors for the occurrence of hepatocellular carcinoma (HCC). A large number of studies have dissected the mechanisms of HBV-and HCV-related liver carcinogenesis. There is strong evidence that chronic inflammation and cirrhosis induced by the viral infections are key elements of HCC development. Yet, it is striking that the pattern of genetic changes present in the HCC tumor cells clearly differ in HBV-and HCV-related HCCs; along the same line, studies based on micro-and macro arrays technologies have also shown a clear different pattern of gene expression in HBV-and HCV-linked HCCs. A large number of investigations have provided support to these findings; they showed that both HBV and HCV proteins are capable of interfering with a number of major signaling pathways controlling cell proliferation, viability and important metabolic networks. In addition, integration of HBV DNA into the host genome contributes to the deregulation of several key cellular gene expression.

Our group has been tackling these issues by combining in vivo studies, based on the direct analyses of HCC tumors, and experimental *in vitro* and *in vivo* models.

We will present recent findings which demonstrate: 1. That integration of HBV DNA into or in the vicinity of cellular genes controlling major metabolic pathways is, in contrast with current views, a frequent finding; in particular, we will report on the identification of "hot spots" for HBV DNA insertion into the human telomerase- and calcium homeostasis-regulators-encoding genes. 2. That HCV core protein, encoded by HCV natural variants isolated from HCC tumor cells, downregulates TGFβ-dependent signaling, a key factor for cell

viability and proliferation control. Our results also show that this property is specifically related to tumor-and not non tumor-derived HCV cores, supporting an important biological impact of those mutations shown in HCC-derived HCV core sequences.

Collectively, such results show that, beside the major benefits which can be expected for public health by preventing and efficiently treating such viral infections, HBV and HCV can be viewed as molecular "probes", complementary to large genetic studies, to dissect the general mechanisms which drive cell clonal expansion during liver carcinogenesis.

Pathogenesis of Hepatitis C-associated Hepatocellular Carcinoma

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Hepatitis C virus infection, in addition to being a major cause of chronic liver disease, is a principal etiological agent of liver cancer in the world. HCV is one of the few human viruses, including hepatitis B virus, human papillomavirus and HTLV-I that have been clearly linked to human cancers. Therefore it is appropriate to consider it as a human oncogenic virus. However the mechanisms of HCV-related oncogenesis remain largely elusive, mostly because of the lack of convenient and suitable tissue culture and animal models and the long duration from infection to cancer development (more than 20 years). The pathogenesis of HCV-associated HCC can be viewed in two ways: viral factors and host related events, which are nevertheless closely linked.

HCV encodes 10 viral gene products that are functionally divided into structural and nonstructural proteins. Several viral proteins including core, NS3 and NS5A have been associated with diverse pleiotropic functions including transcriptional activation, signal transduction, in vitro transformation that could be linked to cancer development. Whether any of these putative functions has biological relevance has not been established in vivo. However it is clear that HCV does not encode a classical oncogene from the point of view of acute transforming oncogenic viruses, because the process of hepatocarcinogenesis takes many years to emerge. Core and NS5A have also been shown to induce the production of reactive oxygen species (ROS) in tissue culture and transgenic animal model. The production of ROS can predispose hepatocytes to DNA damage, which could lead to cumulative mutational events resulting in malignant transformation. Indeed, studies have shown increased chromosomal mutations in cell lines infected with HCV. HCV as a RNA virus, unlike the retroviruses and hepatitis B virus, does not integrate its genome into the host chromosome. Therefore, insertional mutagenesis is not operational in the

development of HCV-associated HCC. On the other hand, HCV replication can induce profound epigenetic changes in infected cells that may set the stage for subsequent malignant transformation.

Other than virus-specific mechanisms that may contribute to carcinogenesis, the widely held view of many of these conditions associated with liver cancer is the chronic "injury and regeneration" model. This model provides a potentially unifying pathway of hepatocarcinogenesis in many of these conditions, including chronic viral hepatitis, autoimmune liver disease, toxic (alcohol) and metabolic liver diseases. In any of the above conditions, the chronic inflammatory changes render a highly carcinogenic environment for the hepatocytes. The production of inflammatory cytokines with generation of reactive oxygen species can induce chromosomal mutations. In addition, the liver is the major organ of detoxifying xenobiotics and toxic by-products derived from either the environment or body metabolism. The condition of chronic hepatitis can result in aberrant processing and accumulation of these compounds that are often potent DNA mutagens. As a large number of infected hepatocytes die from host immune response-as in the case of chronic hepatitis C, new hepatocytes are generated and the liver becomes an actively dividing organ in which the turn-over rate reaches 100-1000 times more than the resting state. The proliferating hepatocytes, in a mutagenic environment, accumulate mutations and eventually become transformed.

Studies have been conducted to identify specific genetic alterations associated with HCC in an attempt to develop a model for the multistage process of hepatocarcinogenesis. Several common loci of chromosomal aberrations have been identified in HCC but none of them have been specifically attributed to HCV infection, again reinforcing the notion that HCV does no target specific host genes per se, but rather causes general random genetic alterations as a result of the recurrent "injury and regeneration" model.

Animal model to study the oncogenic potential of HCV has been limited to the transgenic mouse model, in which a part or the whole HCV genome is expressed in the liver of the mouse. These studies have been

controversial regarding the in vivo oncogenic potential of any of the viral gene products. Even in models where liver cancers have been reported, the process usually takes more than a year to occur. This supports the above notion that the virus does not encode proteins that are potent oncogene, despite the numerous observations in vitro about the transforming ability of various HCV gene products in tissue culture system. Furthermore, the constitutive expression of the transgenes in most of these transgenic models may not reflect the true biological effects of these viral proteins in a naturally infected host. Epigenetic and compensatory changes associated with the constitutive expression of transgene can mask the true biological functions of these genes, making the interpretation of the exhibited phenotype difficult. The recent development of conditional and targeted expression of transgene may obviate some of these problems.

As we understand more about the biology and pathogenesis of the virus, we are gaining insights into the molecular mechanisms of malignant transformation of hepatocytes as related to chronic hepatitis C. From this incremental knowledge, we may garner valuable information to develop better diagnostic, preventive and therapeutic means.

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Environmental Factors and Risk of HCC

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Although relatively rare in North America and Western Europe, primary liver cancer is a common malignancy worldwide. It ranks as the 5th and 8th most common cancer among men and women respectively, accounting for 4% of all newly diagnosed cancers in both sexes. The dominant form of primary liver cancer is hepatocellular carcinoma (HCC). Most other primary liver cancers are cholangiocarcinomas, which are histologically and etiologically distinct from HCC. In the United States, HCC constitutes 70 to 75% of cases of primary liver cancer. In most high-risk regions (East and Southeast Asia, and sub-Sahara Africa), well over 90% of primary liver cancers are HCC. Exceptions are areas with high infection rates of liver flukes, which are established etiological agents of cholangiocarcinoma. In an area in northeast Thailand where such infections are endemic, 90% of primary liver cancers are cholangiocarcinomas (Yu et al, 2000).

Chronic infection by the hepatitis B virus (HBV) is by far the most important risk factor for HCC in humans, and is the primary cause of HCC in high-risk areas including China and Africa. Chronic infection by the hepatitis C virus (HCV) is another viral risk factor for HCC development. While HCV seems to play a relatively minor role in the development of HCC in Africa and China, it is playing an increasingly prominent role in the development of HCC in the United States and Japan. There is strong evidence that co-infection with HBV and HCV exerts a synergistic effect on HCC risk in both low- and high-risk populations. In a meta-analysis of 32 studies conducted in diverse populations and comprising roughly 4500 cases of HCC and 7000 control subjects, the summary relative risk of HCC for co-infection was 135 (95% confidence interval; 80-242) while the corresponding figures for HBV alone and HCV alone were 20 (18-23) and 24 (20-28), respectively (Donato et al, 1998).

Several dietary factors have been implicated in the enhancement or attenuation of HCC risk in HBV/HCV infected individuals. Aflatoxins are potent hepatocarcinogens in animals, and humans are exposed to these mycotoxins through ingestion of moldy foods, a consequence of poor storage of susceptible grains. Highly exposed populations are primarily those residing in sub-Sahara Africa and East and Southeast Asia. Using a urinary biomarker of exposure, a study in China provided compelling evidence that aflatoxin exposure is a risk factor for HCC, especially in the presence of HBV infection. While the relative risk of HCC for aflatoxin exposure in the absence of HBV infection was around 3, the corresponding figure in the presence of HBV was 59 (95% confidence interval, 17-212). Subsequent studies in Chinese highlight the importance of aflatoxin detoxifying genotypes as co-determinants of risk in aflatoxin-exposed individuals (Yu et al, 2000).

Limited data have implicated a protective role for dietary antioxidants in modifying the HBV-HCC association. A cohort study of Chinese men in Taiwan examining serum levels of retinol at baseline (i.e., prior to cancer diagnosis) in relation to subsequent risk of HCC showed a dose-dependent decrease in risk with increasing level of serum retinol (Yu et al, 1995). In a separate cohort study, the same group of investigators showed that plasma level of selenium at baseline inversely predicted risk of HCC, especially among subjects exhibiting low levels of serum retinol (Yu et al, 1999b).

Clinical studies strongly suggested that excessive alcohol intake was an important contributor to HCC in North America and Western Europe long before epidemiological data confirmed this exposure-cancer relationship. It should be noted that a clear excess in risk exists only among heavy, long term drinkers (60 drink-years or more, one drink-year is defined as one drink per day for a year); there is no evidence that moderate drinking (1-3 drinks per day) is related to an increase in HCC risk (Yu et al, 2000). A cohort study using blood-based biomarkers of antioxidants suggested that dietary antioxidants could attenuate the carcinogenic potential of heavy alcohol intake (Yu et al, 1999a).

Multiple chemical components of cigarette smoke are hepatic carcinogens in animals. While results are not totally consistent, a number of case-control and cohort studies have suggested cigarette smoking as an independent risk factor for HCC, especially in low-risk regions (North America, Western Europe). However, given the strong positive correlation between use of tobacco and alcohol in most populations and particularly in the West where both exposures are relatively prevalent, these interview-based epidemiological data are considered by many to be inconclusive in establishing a causal role for tobacco in HCC development. A case-control study measuring DNA adducts of 4-aminobiphenyl, a hepatic carcinogen in animals and a constituent of cigarette smoke, in liver tissues of study subjects showed a statistically significant increase in HCC risk with increasing levels of adducts (Wang et al, 1998). Since cigarette smoking is considered the primary source of exposure to 4-aminobiphenyl in humans, this molecular epidemiologic study has strengthened the notion that tobacco smoke is a hepatic carcinogen in humans.

A number of case-control and cohort studies have implicated diabetes as a risk factor for HCC development (Yu et al, 2000; Hassan et al, 2002). Obesity is the most important risk factor for diabetes, and the two conditions are highly related events. Although the precise mechanism by which obesity/diabetes leads to HCC is unknown, hepatic inflammation leading to oxidative stress/lipid peroxidation, which can cause hepatic injury, fibrosis, and eventual cirrhosis is one possible pathway. Obesity/diabetes is likely to play an increasingly important role in the development of HCC in the United States, given that an increasing number of Americans approaching the high-risk age range for HCC are positive for diabetes.

Several studies have provided evidence that viral hepatitis, alcohol, and diabetes interact synergistically (more than sum of their individual effects) in the development of HCC (Donato et al, 2002; Hassan et al, 2002).

Estrogens, including those in oral contraceptive formulations, are powerful promoters of hepatocarcinogenesis in animals. A number of case-control studies conducted in US white and European women have consistently observed a duration dependent risk of HCC among users of oral contraceptives. On the other hand, studies conducted in high-risk Asian and African women uniformly yielded null results. These seemingly disparate observations can be explained if the combined effect of viral (HBV) and hormonal (estrogen) factors on HCC are not synergistic, but simply the sum of their individual effects. Under this assumption, a sample size many times larger than those used in the Asian and African studies would be required to detect the additional risk in oral contraceptive users (about 2-3 fold) against the very high background risk (at least 20 fold) in HBV carriers.

Epidemiologic studies have linked HCC development in special populations in Taiwan, Chile, Argentina, and Inner Mongolia, China to the high contents of inorganic arsenic in their drinking water supply. Subjects exposed to higher levels of arsenic showed raised levels of lipid peroxides in serum, suggesting oxidative stress as one possible explanation for the hepatocarcinogenicity of inorganic arsenic (Pi et al, 2002).

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Hepatocellular Carcinoma in Hereditary Hemochromatosis

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Hereditary hemochromatosis is associated with a homozygous C282Y mutation in the HFE gene. The specific mechanism whereby this mutation leads to iron overload is known. However, clinical features of this disorder have been recognized for several decades. Increased intestinal absorption of iron from a normal diet can lead to deposition of this metal in the parenchymal cells of the liver, heart, pancreas, joints and brain. Hepatic iron overload can lead to cirrhosis and hepatocellular carcinoma. Hepatocellular carcinoma is an important cause of mortality in cirrhotic patients with hemochromatosis. The risk of hepatocellular carcinoma in patients with hemochromatosis is estimated to be up to 200-fold elevated. Recent studies suggest that the risk of hepatocellular carcinoma in patients with HFE-associated hemochromatosis may be lower but still significantly elevated, with an estimated odds ratio of 20-30. In addition, first degree relatives of patients with HFE-associated hemochromatosis also appear to be at increased risk of hemochromatosis. The mechanism whereby iron overload leads to hepatic carcinogeneis is not entirely clear. It is clear that the presence of cirrhosis is important, as almost all patients with hepatocellular carcinoma in the setting of hemochromatosis have cirrhosis, with only a rare collection of case reports describing hepatocellular carcinoma among patients without cirrhosis. Iron deposition in the liver has been implicated as a risk factor for hepatocellular carcinoma not only in patients with hemochromatosis but also among patients with African iron overload, alcoholic liver disease and hepatitis C. Therefore, excess parenchymal iron deposition is probably associated increased activity of carcinogenic pathways among patients who are already predisposed to liver cancer because of underlying cirrhosis. Several mechanisms have been proposed for

the contribution of iron to the development of hepatocellular carcinoma. These include acceleration of fibrogenesis, intense lipid peroxidation and generation of free radicals due to a chemical reaction with iron, as well as effects on oncogenes such as c-myc or tumor suppressor genes such as p53.

There is a high prevalence of hepatocellular carcinoma among patients with hepatic iron overload referred for orthotopic liver transplantation. Among such patients, the prevalence of incidental primary liver cancer (found at the time of liver transplant but not previously suspected may be as high as 20%, compared to a rate of 2-6% among the general population of patients with end-stage liver disease.

There is ongoing debate as to whether moderate iron overload with or without heterozygous mutations in the HFE gene contribute to the development of hepatocellular carcinoma in the setting of hepatitis C and alcoholic liver disease. At this time, it is unclear whether hepatic iron deposition contributes to hepatocellular carcinoma in these diseases.

Treatment of hepatocellular carcinoma among patients with hereditary hemochromatosis is problematic since these patients are at increased risk of poor outcomes following liver transplantation. Therefore, every effort must be made to screen for hepatocellular carcinoma among patients with hemochromatosis and cirrhosis to allow early diagnosis and treatment by iron depletion using phlebotomy to avoid having to perform liver transplantation in the setting of uncontrolled iron overload.

Continued

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Alcohol and Hepatocellular Carcinoma

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Alcohol (ethanol) use is usually measured in grams of ethanol per day and the number of years of chronic use. As a general approximation, most standard drinks contain approximately 12 grams of ethanol, although the Europeans have often used a more practical number of 10 grams of ethanol per "drink." Using this conversions, 60 grams of ethanol per day, a level thought to be injurious to the liver, would correspond to 5 or 6 "drinks" per day.

Alcohol use varies between countries and within countries. Per capita alcohol intake is higher in Western Europe than in the US, and higher in the US than in Asia. Within countries, alcohol use varies by age, gender, race and population studied (e.g., population based studies vs. hospitalized patients). Forty-five percent (45%) of adult Americans are current alcohol users (12 or more drinks/year) and 21% are former alcohol users. Approximately 17% of current alcohol users have an alcohol use disorder (abuse or dependence)[1]. Five times (5-fold) more people have an alcohol use disorder than have chronic hepatitis C.

Alcohol can be considered both as a primary cause of hepatocellular carcinoma (HCC) as well as a co-factor for the development of HCC. For the purpose of this review, publications were included if they also evaluated hepatitis C infection. However, most studies evaluating the interaction of ethanol with hepatitis B were performed prior to the discovery of hepatitis C virus, somewhat limiting the ability to determine the effect of ethanol on HBsAg and HCC.

Alcohol as a Primary Cause of Hepatocellular Carcinoma

Cross-sectional, case-control and longitudinal studies report an association between chronic ethanol use and hepatocellular carcinoma. Alcoholic cirrhosis was the most common underlying cause of HCC (alcohol 12.9 cases per 100 000 hospitalizations; hepatitis C

7.2 cases per 100 000 hospitalizations) in a study of patients admitted to VA Hospitals between 1993 and 1998[2]. The risk for HCC increases when ethanol intake exceeds 60 grams per day for more than 10 years (Bresica study)[3]. Furthermore, the risk of HCC is greater in patients who have stopped drinking for 1-10 years (as compared with current drinkers), although this may be due to cessation of ethanol intake in patients with advanced cirrhosis, or longer survival in patients who stop drinking as compared with patients who continue drinking. A population based longitudinal study of 12,008 Taiwanese males aged 30-64 found that alcohol use (defined as "drinks alcohol") was associated with HCC (odds ration 1.6 as compared with non-drinkers, 95% CI 1.0-2.6)[4]. The risk of developing HCC is approximately 1% per year in male patients with decompensted alcoholic cirrhosis (Morgan, unpublished).

Alcohol and Hepatitis C

Case-control studies and longitudinal studies suggest that chronic ethanol consumption in patients with hepatitis C increases the risk for HCC. The odds ratio for HCC in the Bresica study was 55 in patients with hepatitis C who do not drink alcohol (as compared with patients without hepatitis C and who do not drink) and was 109 for patients with hepatitis C who drank more than 80 grams/day[3]. A case-control study in the United States also found the odds ratio for HCC increased significantly in patients with viral hepatitis (mostly hepatitis C) who drank more than 80 grams/ethanol per day[5]. In both of these studies, the interaction between alcohol and hepatitis C was more than additive (S-statistic 1.7 and 2.7), suggesting that alcohol and hepatitis C may interact with each other in the development in HCC. In a longitudinal study of 252 patients with hepatitis C cirrhosis, Ikeda found that age, AFP level and alcohol use were the only independent predictors of development of HCC[6].

Alcohol and Hepatitis B

Most of the studies of alcohol and hepatitis B were conducted in Asia in the 1980's prior to the discovery of the hepatitis C virus. A 6-year follow-up study of 341 HBsAg positive healthy Japanese blood donors found that chronic alcohol consumption of more than 27 grams per

day (one little bottle ["go"] of sake) increased the risk of HCC more than five-fold[7]. Other studies also suggest that alcohol consumption in patients with hepatitis B is associated with increased risk for HCC and a younger age of onset of the HCC.

Other Issues

Diabetes has recently been shown to increase the risk for hepatocellular carcinoma. A US study reported that alcohol and diabetes significantly increased the risk for HCC, with a suggestion of an "interaction" between alcohol and diabetes in risk for HCC[5]. Although most patients with develop HCC in the setting of alcoholic cirrhosis, HCC can present in patients without cirrhosis.

Population Attributable Risk

Population attributable risk measures the contribution of each etiology to the total number of HCC in a population. In Italy, chronic alcohol use accounts for approximately 45% of HCC, chronic hepatitis C accounts for 36% and hepatitis B plays a major role in 22%[3]. In the US, alcohol is estimated to account for 32%, with HCV, HBV and diabetes accounting for 22%, 16% and 20% respectively[5].

Pathogenesis of Hepatocellular Carcinoma with Alcohol Use

The carcinogenic effects of ethanol in hepatocellular carcinoma have been reviewed recently[8]. The pathogenesis remains speculative. Alcohol promotes the development of cirrhosis, a pre-cancerous state. Potential pathogenic mechanisms include direct chromosomal damage as well as ingestion of dietary or environmental carcinogens in alcoholic beverages. Alcohol metabolism produces acetaldehyde and reactive oxygen species that are capable of damaging proteins, lipids and DNA. Cytochrome P450 2E1, an enzyme that is induced with chronic alcohol consumption, may activate xenobiotic carcinogens. Chronic alcohol injury may lead to a change in methylation of DNA, and reduced hepatic retinoic acid levels may increase cell proliferation.

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Hepatocellular Cancer and Obesity

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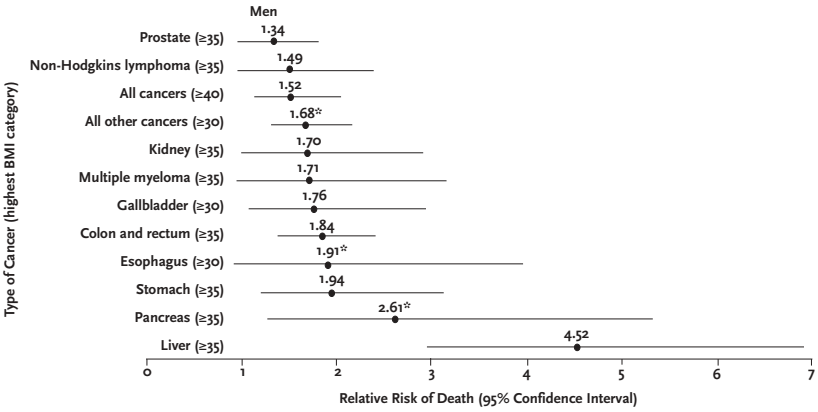
The rising incidence of hepatocellular cancer (HCC) in the US has been largely attributed to the ongoing hepatitis C epidemic and associated cirrhosis.[1,2] However, this marked change in the epidemiology of HCC has also paralleled an epidemic of obesity and type two diabetes and an associated high prevalence of nonalcoholic fatty liver disease (NAFLD) now recognized as one of the most common of all liver diseases. Although there is probably substantial ethnic variation, it is estimated that 90% of people with obesity (BMI>30) have some form of fatty liver ranging from simple steatosis to more severe forms of NASH.[3] An increasing number of publications point toward underlying NAFLD as the key link between obesity and HCC.

Hepatocellular Cancer in Obesity and Diabetes Mellitus

An increased risk of cancer mortality in general has long been associated with obesity.[4] Similar results were noted in a more recent US study which also revealed an increased incidence of HCC among obese subjects followed prospectively for 16 years.[5] This study included 404,576 men and 495, 477 women age 30 or more and with a BMI> 18.5 at enrollment. Overall and site-specific cancer related deaths were assessed over the study period and stratified based on the BMI at the time of study initiation in 1982. Although histologic data was not available, the relative risk of dying from liver cancer was 1.68 times higher among women with baseline BMI ≥ 35 and 4.52 times higher for similar males compared to the reference groups with baseline BMI of 18.5 to 24.9. Among the male group, liver cancer had the highest relative risk increase of all of the cancers studied.

Figure 1. From Calle et al. Relative risk of cancer based on baseline BMI followed for 16 years



Similar data have indicated an increased risk of HCC among diabetic patients[6,7]–a condition closely associated with obesity and with NAFLD. In one of these recent studies, diabetes was associated with an increased risk only among patients with HCV, HBV or alcohol-related cirrhosis. In the other, diabetes was an independent risk for HCC but neither study clearly identified the type of diabetes although in both the majority were felt to represent type 2. Neither of these studies was able to report data on BMI and the above mentioned obesity study did not report the prevalence of diabetes.

Nair et al examined the role obesity as an independent risk factor for HCC in 19,271 cirrhosis patients who underwent transplantation in the US between 1991 and 2000.[8] The patients were stratified by into three groups based on BMI at the time of listing: <25, 25.1–30 (overweight) and ≥ 30.1 (obese). The overall incidence of HCC was 3.5% (n=659). Multivariate analysis revealed obesity to be a statistically significant independent risk for HCC among patients with alcoholic (OR=3.2) and cryptogenic (OR=11.1) cirrhosis but not in patients with HCV, HBV, PBC and autoimmune disease. The authors conjectured that the role of antecedent steatosis in both alcoholic liver disease and many patients with cryptogenic cirrhosis provided a common thread which explained the apparent disease-specific risk associated with obesity.

Prevalence of NAFLD in Obesity

Although it is unproven, it seems very likely that the association of obesity with HCC occurs primarily in the setting of NAFLD with cirrhosis. This is reasonable based on several established relationships. Foremost is the very high prevalence of NAFLD in obese patients. From past studies, it can be estimated that roughly 90% of obese people have some form of fatty liver. Some interpretation of the existing literature allows this to be broken down into subsets. About 60% of obese patients have simple steatosis or steatosis with only mild inflammation. Roughly 25-30% have NASH (nonalcoholic steatohepatitis) characterized mostly by some degree of fibrosis. A few percent have unrecognized and undiagnosed cirrhosis and, at the other extreme, only about 5-10% have a normal liver. Thus, HCC in an obese patient is very likely to occur in association with some form of NAFLD and, because HCC is rare without cirrhosis except in HBV patients, it is likely that most such patients have underlying cirrhosis albeit without overt complications of portal hypertension in some. This relationship likely also extends to many patients with cryptogenic cirrhosis (see below) many of whom are in a late stage of NAFLD. Likewise, it is conservatively estimated that 70% of type 2 diabetes patients have some form of NAFLD.

As noted above, ethnic variation has been observed in HCC, obesity, diabetes and NAFLD. Thus some additional considerations of these relationships are warranted. African Americans, a group with a high prevalence of obesity and diabetes, also have a relatively high risk of HCC compared to people of primarily European descent. However, while it remains to be established, there does appear to be a lower than expected prevalence of NAFLD in people of African American descent based on the high prevalence of obesity and diabetes in this population. This paradoxical relationship likely relates to genetically determined distribution of body fat.

No data that we are aware of has revealed the relationships between HCC, obesity and ethnicity but, based on the relationships noted above, it seems likely that the relatively high prevalence of HCC in African Americans is due to factors other than NAFLD. Rather, the

high incidence of HCC in obese patients is probably more significant among people of primarily non-African descent. These relationships have yet to be adequately investigated and one could reasonably ask several relevant questions. For example, if the prevalence of NAFLD is low in African Americans while the prevalence of obesity is high, is there some other factor involved besides steatosis which promotes HCC?

HCC in NASH and Cryptogenic Cirrhosis

The Natural progression of NAFLD? Powell et al published one of the first reports on HCC developing in a patient with a history of long-standing NASH proven on prior biopsy.[9] In that study on the long term natural history of NASH, the authors described the development of multi-focal HCC five years after the diagnosis of NASH with cirrhosis by biopsy. More recently, Cotrim et al described a 62 year old, obese and diabetic male with previously undiagnosed NASH and cirrhosis at presentation with variceal bleeding.[10] Four years after the initial diagnosis he was found to have hepatocellular cancer treated locally but with subsequent multi-focal HCC. Another case report described a 62 years old female with NASH by biopsy and extensive workup who subsequently developed multi-nodular HCC approximately 10 years after the initial diagnosis of NASH.[11]

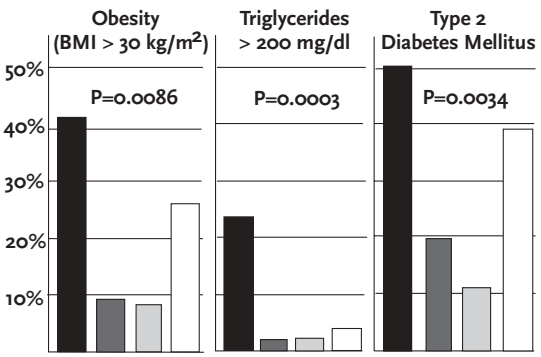
Although cryptogenic cirrhosis, or cirrhosis of uncertain cause, is probably not one disease, NAFLD appears to be the underlying disorder in a substantial portion if not most of such patients. A number of recent publications have further explored this association by examining the relationships between NAFLD, cryptogenic cirrhosis and HCC. Marrero et al examined a series of 105 consecutive patients presenting to a single center with HCC.[12] The mean age was 59 and 69% were male. All of the patients had chronic liver disease and 90% had cirrhosis by biopsy and/or clinical exam.

Not surprisingly, hepatitis C was the single most common underlying disorder seen in 51% of the patients. However, cryptogenic cirrhosis was the second most common single diagnosis seen in 29% of the cases. Moreover, one-half of the patients with cryptogenic cirrhosis

had histological or clinical features associated with NASH. Six of the patients in the cryptogenic group had prior biopsy confirming NASH a mean of 4.6 years prior to the diagnosis of HCC and all had cirrhosis at the time of the diagnosis of HCC. HCC in the cryptogenic group were less often detected by surveillance and were significantly larger than the other patients.

Bugianesi et al compared 23 patients with HCC in the setting of cryptogenic cirrhosis and compared these patients to 115 viral and alcoholic cirrhosis case controls with HCC matched for age (+/- 5 years), gender and time from diagnosis of HCC. Obesity (defined as the BMI prior to the diagnosis of cirrhosis), hyperlipidemia and type 2 diabetes mellitus were significantly more common among the cryptogenic cirrhosis patients. There were no differences between the cases and controls in terms of the number or size of the HCC. Overall, the incidence of HCC was 6.9% from the entire population of cryptogenic cirrhosis patients from which the study group was derived. As noted in an accompanying editorial, this figure suggests an incidence lower than that associated with alcohol or viral hepatitis but higher than that associated with PBC.[13]

Figure 2. from Bugianesi et al. The prevalence of obesity, hypertriglyceridemia and type 2 diabetes in cryptogenic cirrhosis patients with HCC versus case controls (viral and alcohol-related cirrhosis with HCC).



To further characterize the possible etiology and associated natural history of cryptogenic cirrhosis, Ratziu et al compared 27 cryptogenic cirrhosis with a history of overweight (BMI>25) within 10 years of the diagnosis of cryptogenic cirrhosis and compared the course with 10 similar lean patients (BMI<25 prior to the diagnosis of cirrhosis) and 391 hepatitis C patients with cirrhosis.[14] Older age, glucose intolerance and/or diabetes and hyperlipidemia were all significantly more common on the overweight group compared to the other two groups consistent with suspected NASH as the underlying etiology in the overweight group. Overall disease severity and survival was as bad or worse in the overweight cryptogenic group compared to the HCV group. Moreover, HCC was eventually detected in significantly more overweight cryptogenic cirrhosis patients compared to HCV group suggesting that obesity-related cryptogenic carries a risk of HCC on a scale that rivals HCV cirrhosis.

Potential Mechanisms of HCC in NAFLD

Although much data and clinical experience indicates an increased risk of HCC in obesity and much, if not all, of this risk appears to be mediated by the development of NASH and subsequent progression of this disorder to cirrhosis, the possible mechanism of this association remains to be defined. Increased risk of HCC in animal models of NAFLD including the Ob-Ob mouse (see below) and the FLS mouse[15] support progression of NAFLD as the most likely explanation for the increased prevalence of HCC in obese patients. It is noteworthy that markers of apoptosis are often increased in human and animal NAFLD.[16] This observation needs to be reconciled with the anti-neoplastic effects of apoptosis and the observation that actual apoptotic bodies are relatively infrequent in light microscopic specimens. Up-regulation of anti-apoptotic pathways may explain this situation. Thus, the actual cancer promoting effect of NAFLD may lie in the anti-apoptotic counter-regulatory pathways.

Consistent with such a process, Yang et al from Diehl's group at Johns Hopkins' has reported hepatocellular hyperplasia in the leptin deficient Ob-Ob mouse model of NAFLD.[17] This same group has further

reported that to a large extent, such proliferation, seen in both nonalcoholic and alcoholic fatty liver, involves expansion of small oval progenitor cells.[18] This process has further been correlated to mitochondrial production of H_2O_2. Increased production of H_2O_2 likely represents the effects of excessive fatty acid accumulation. The over-abundant fatty acids then produce secondary effects on the mitochondria either as an adaptive process to the presence of steatosis or as a result of excessive lipid peroxidation or both. Lipid peroxidation may also be a source of mutagens from reactive oxygen species (ROS) which promote development of cancer promoting mutations such as p53 tumor suppressor genes–the latter have been implicated in some HCC.[19] Fatty acid accumulation may also alter this process indirectly through effects on prostaglandin metabolism as increased cyclooxygenase-2 has been reported in HCC and alters apoptosis in vitro cell lines.[20]

Histologically, a role of abnormal fatty acid metabolism is further suggested by the common occurrence of fat droplets and even NASH within HCC. A strong link to fatty acid metabolism is also suggested by the common use of lipiodol (a poppy-derived fatty acid) as a marker for HCC. The prominent role of mitochondria in fat metabolism, lipid peroxidation, NAFLD and cancer itself is of interest and offers a possibly fertile area of research into pathogenesis and treatment of HCC.

Therapeutic Implications

Based on the suspected pathogenesis of HCC in obesity (NAFLD, NASH, fibrosis, cirrhosis and HCC), treating NAFLD may also alter the risk of HCC. Thiazolidinediones (TZD) appear to almost uniformly deplete fat from the liver probably through alterations of fatty acid metabolism in mitochondria and peroxisomes.[21] Such an effect is likely to reduce the risk of cancer by decreasing inflammation and lipid peroxidation. Associated alterations trophic factors may also be of benefit. Much has yet to be learned however about the effects of these agents and other forms of therapy such as metformin and even dietary manipulations such as consumption of PUFA (polyunsaturat-

ed fatty acids) which could alter prostaglandin metabolism. Given the expression of COX-2 in human HCC (see above), the use of prophylactic COX-2 inhibitors also deserves consideration although the potential adverse effects of these agents such as fluid retention and bleeding risk warrant caution.

On a practical level, it is also important to note that the risk of HCC appears to be substantial in patients with NAFLD with cirrhosis and in overweight patients with cryptogenic cirrhosis. This raises the issue of screening. There is insufficient data to formally recommend screening such patients at this time but it seems reasonable to have some discussion with the cirrhotic NAFLD patient and to at least consider some form of regular imaging of the liver. Whether screening results in meaningful changes in longevity or quality of life however remains to be seen.

Summary

Several large epidemiologic studies support obesity as a substantial risk factor for hepatocellular carcinoma. Progression of underlying NAFLD to cirrhosis is the most likely explanation for this association and HCC appears to be well within the natural history of NAFLD. A number of case reports have documented this progression and also have observed multi-focal HCC as a common presentation although this does not appear to be as common in larger series. The mechanism most likely involves hepatic hyperplasia and increased progenitor (small oval) cells. Growth factors associated with type 2 diabetes and DNA mutations as a result of lipid peroxidation probably also play a significant role. Whether or not therapy aimed at NAFLD reduces the risk of HCC remains to be seen. Prophylactic measures and the role of tumor screening have not been adequately investigated but current evidence supports a risk of HCC in NAFLD-related cirrhosis that rivals the risk of HCC in HCV-related cirrhosis.

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Issues in Screening for Hepatocellular Carcinoma

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Patients at risk for hepatocellular carcinoma (HCC) include those with cirrhosis, chronic viral hepatitis and certain metabolic diseases such as hemochromatosis and alpha-1-antitrypsin deficiency. Because of the poor prognosis of HCC when it is diagnosed at an advanced stage, early detection is most desirable. Small HCC is amenable to potentially curative treatment by liver transplantation, resection or local ablation by alcohol injection or radiofrequency ablation. Early diagnosis of HCC is possible using imaging techniques such as ultrasound examination or CT, combined with regular measurement of serum alpha-fetoprotein levels. What is not established is whether initial screening or surveillance for HCC among patients with liver disease is associated with prolonged patient survival and whether this approach is cost effective. There is little data available on the benefits of screening from randomized controlled trials. In fact, Sherman et al found that such a trial was not practical in North America and most of the available data comes from cohort or retrospective studies [1]. For example, McMahon and colleagues found that serial measurement of AFP has been valuable in diagnosing HCC among native Alaskans with chronic hepatitis B virus infection[2]. Tong et al screened 173 patients with cirrhosis due to hepatitis C and found 31 who developed HCC[3]. Unfortunately, only 18 of these tumors were single and of a size potentially amenable to treatment at the time of diagnosis. Potentially curative treatments were possible in only 12 of this group (39%) (resection in 4, liver transplantation in 8). There have been several studies of the cost-effectiveness of screening for HCC. Estimates for the cost per tumor detected have ranged between $11,000 and $25,000 and costs per year of life saved between $26,000 and $112,996[4-6]. This compares to an estimate of $25,000 per year of life saved by screening for colo-rectal cancer. A survey of practicing hepatologists in the United States indicated that 83% conduct some form of screening or surveillance for HCC in patients at

risk, most frequently using the combination of ultrasound examination and AFP but only about one third consider screening to be cost-effective in a non-transplant population[7]. Thus, screening for HCC appears to have become standard practice in selected patients, despite the absence of proof of its value. This practice is unlikely to change without new data or denying its value.

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Alfa Fetoprotein and Ultrasonography Screening

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Hepatocellular carcinoma (HCC) is an important public health problem and has an identifiable latent disease stage that fulfils the relevant criteria for screening. The major risk factors for HCC are chronic HBV infection, chronic HCV infection when associated with advanced fibrosis or cirrhosis, and cirrhosis regardless of etiology[1]. The annual incidence of HCC once cirrhosis is established is 2% to 6% per year[2]. Therefore, screening should focus on patients with chronic HBV infection and those with cirrhosis regardless of etiology. In addition, the patients who should undergo screening should be those cirrhotics who could receive a treatment (surgical resection, liver transplantation and percutaneous ablation) if diagnosed with HCC. Therefore, the ideal target population is Child-Pugh's class A cirrhotic patients without any severe associated condition. The surveillance tools are alfa fetoprotein (AFP) concentration and ultrasonography (US)[3]. No prospective randomized trial comparing the outcome of patients with HCC diagnosed during or outside of a screening program has been done and it is highly unlikely that it will be ever done mainly for practical reasons.

Alfa Fetoprotein

AFP has been used as a serum marker for HCC in human for many years and has a sensitivity of 39-64%, a specificity of 76-91% and a positive predictive value of 9-32%[4,5]. Specificity and sensitivity depend on the AFP cut-off level chosen for the diagnosis. This is important since patients with chronic viral hepatitis with reactivation may present increased AFP values without HCC. In a case-control study the higher the AFP cut-off level, the higher the specificity and the lower the sensitivity[6]. Values above 400 ng/ml are generally considered diagnostic of HCC, although hardly are these values observed in patients with HCC detected during screening. In addition, there is no indication in the literature of a specific AFP cut-off level that calls

for additional diagnostic investigations. A progressive increase in AFP levels over time, even with absolute values well below 400, may be considered suspicious and require a diagnostic work-up. However, it seems that the detection rate of HCC is not improved by increased AFP level if US cannot identify a nodule in the liver[7]. Based on the above findings, it has been claimed that AFP determination can be dismissed as a screening test for HCC[5].

Ultrasonography

Ultrasonography became available for identifying hepatic lesions in the early 1980. The reported sensitivity of US imaging in detecting HCC tumor nodules has been quite variable, ranging from 35% to 84%[8] depending on the expertise of the operator as well as on the US equipment available (more sophisticated machines produce better quality images and detect smaller tumors). Two studies using liver ultrasonography as screening tools showed that the sensitivity was as high as 71% and 78%, specificity 93% and positive predictive value 14% and 73%, respectively[9,10].

Combination of Alfa Fetoprotein and Ultrasonography

Sherman et al.[10] compared the combination of AFP+US with AFP in chronic hepatitis B patients. Unfortunately the results indicated that the sample size was not adequate to compare the two screening methods. The combination of AFP and US has been credited with a sensitivity of 100% in one study[11], while Kang et al., using a mathematical model, showed that the combination of AFP and US increases the sensitivity of screening by 5-10% compared to US alone[12].

Screening Interval

Based on the estimated HCC growth rate, the suggested interval for surveillance in patients with cirrhosis has been set at 6 months[13]. However, a number of studies suggest that a longer screening interval (one year) is as effective as the 6-month interval[12,14,15].

Cost Effectiveness

According to a decision analysis model, the cost–effectiveness ratio of screening European patients with only Child–Pugh class A cirrhosis with serum AFP and liver US every 6 months ranged between $48,000 to $284,000 for each additional life–year gained[16]. However, in a group of patients with excellent expected cirrhosis-related survival, the cost–effectiveness ratio ranged between $26,000 to $55,000. In 313 Italian patients with cirrhosis undergoing serum AFP and liver US every 6 months the cost per treatable HCC was $17,934, and the cost per year of life saved was $112,993[7]. In the United States, the cost for each quality-adjusted life–year gained through surveillance is estimated to range from $35,000 to $45,000[17]. The cost-effectiveness of screening should increase in areas where HCC prevalence is high and in high-risk groups.

Conclusions

As for any cancer screening, the important measure of HCC screening should be all cause mortality and mortality from HCC, not merely the number of HCC patients detected or apparent increased survival. This is because of the risk of misclassification of cancer related deaths and because of the risk of death from other causes, which may be associated with the screening performed (e.g., suicides due to anxiety and fear from cancer). Unfortunately, there are not enough quality trials to support or refute screening for HCC. The data currently available come mainly from non randomized studies and there is evidence that cohort studies may reach intervention effects that differ widely from those reached in randomized trials by -78 to +400%[18]. Therefore, while screening with AFP+US seems to detect significantly more HCC compared with no screening and despite the current recommendation to screen subjects at moderate and high risk for HCC every 6 months[3], we do not yet know with certainty if screening is able to reduce all-cause mortality or HCC mortality, which modality of screening should be used (no screening, AFP, US, or AFP+US), or how frequent screening should be offered. It is possible that HCC screening may be effective, but also that harm caused by screening may outweigh any gain.

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Newer Markers for Hepatocellular Carcinoma

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Liver cirrhosis is the strongest risk factor for the development of hepatocellular carcinoma (HCC), and therefore, it is recommended that patients with cirrhosis undergo surveillance with alpha-fetoprotein (AFP) and hepatic ultrasonography. However, AFP has poor sensitivity and specificity for HCC, and the accuracy of ultrasound is operator-dependent and the ability to differentiate HCC from non-neoplastic lesions such as regenerative nodules is limited[1]. Therefore, newer methods for the early detection of HCC are welcomed. These newer methods include biomarkers (from tissue, serum, plasma, or urine) or radiological tests. This review will focus on biomarkers.

There have been many reports of novel biomarkers for HCC. When searching PubMed with a combination of the key words "hepatocellular carcinoma" and "biomarkers", a total of 2697 reports were identified between 1995 and 2004. The recent developments of gene-expression microarrays, proteomics and tumor immunology offer new approaches for cancer screening. In order to define a formal structure to guide the process of biomarker evaluation and development, a 5-phase program is utilized by the Early Detection Research Network of the National Cancer Institute[2]. The 5 phases are: 1) pre-clinical exploratory studies, 2) clinical assay development for clinical disease, 3) retrospective longitudinal repository studies, 4) prospective screening studies, and 5) cancer control studies. These phases help establish criteria to determine the current status of biomarkers in literature, how close to clinical application these biomarkers are, and potentially serve as a guide for future biomarker development. Based on these phases of biomarker development, a summary of the current status of newer HCC markers will be presented.

Phase 1: Preclinical Exploratory studies

These studies are exploratory in nature aimed to identify characteristics unique to HCC that may lead to assays for future clinical use. For each biomarker studied, the key question is how well it can distinguish between cases and controls. The assay should be reliable and reproducible. If the biomarker is measured in a binary scale, the true positive rate (TPR) and the false positive rate (FPR) should be determined to summarize its ability to discriminate between disease and non-disease. If the assay involves continuous variables, then a receiver operating characteristic (ROC) curve should be used[3]. The actual threshold for the selection of a biomarker is unknown, but there is data to suggest the use of the area under the ROC curve for ranking[4]. With regards to HCC, one potential threshold is to compare with AFP and ultrasound. At this phase serum, plasma, tissue or urine may be used for discovery. An example of markers identified at this phase is serum gyplican-3[5]. Gene microarrays and proteomics will play an important role in the discovery phase because of their ability to screen thousands of genes and proteins[6].

Phase 2: Clinical Assay and Validation

One important aspect of this phase is that the specimen be obtained noninvasively if diagnosis of early stage HCC is the goal. Therefore, studies involving tissue would not be practical for biomarkers ultimately targeted for early detection. The aims of this stage are to estimate the TPR and FPR (binary variables) or ROC curves (continuous variables) for the clinical assay, and to assess its ability to distinguish subjects with cancers from subjects without cancer. The biomarker assay should be simple, and its intra- and interassay variability reported. It is important at this phase to correlate the biomarker assay with demographics, etiology of liver disease, environmental exposures, family history of HCC, etc. The design of these studies should be case-controlled studies. The selection of cases with early stage is important to determine the diagnostic capability of the biomarker for early stage HCC. The controls should be patients with cirrhosis without known HCC. Combination of markers can also be evaluated at this stage. The sample size at this phase

should have sufficient power to allow for the random variation of the biomarker assay and for confidence in the results. Examples of Biomarkers identified at this phase are des-gamma carboxyprothrombin[7] and monosialylated AFP[8].

Phase 3: Retrospective Longitudinal Studies

This phase relies on the identification of patients with HCC from a cohort of cirrhotic patients longitudinally followed. It involves the collection of specimens from HCC cases prior to the diagnosis of the tumor and compare it with those who have not developed HCC (controls). The aim is to evaluate the capacity of the biomarker(s) to detect preclinical disease. In addition, it is important to identify variables that affect the discriminatory ability of the biomarker before clinical diagnosis. These variables include demographics, exposure to alcohol and tobacco, family history, etiology of liver disease, prior treatment of underlying liver disease, etc. Other aims include the comparison of biomarkers to determine the performance of a combination of markers, and to help determine the surveillance interval. The data analysis proposed has been to determine the ROC curves at different time intervals (every 6 or 12 months prior to the diagnosis of HCC) in order to describe the capacity of a biomarker to distinguish those destined to develop cancer[9]. Example of a biomarker identified at this phase is insulin-like growth factor-1[10].

Phase 4: Prospective screening studies

Screening with the selected biomarker is applied to an at-risk population and compared to the standard of care. Phase 3 studies are able to determine whether tumors can be detected pre-clinically with a biomarker, but does not establish prospectively the characteristics of the tumors detected. The aim of this phase is to determine the operating characteristics of the biomarker-based surveillance in the relevant population by determining the cancer detection rate and the false-referral rate. Other aims would be to describe the characteristics of the tumors detected, to assess the feasibility of implementing the screening/surveillance program, to determine the costs of screening/surveillance, and to monitor for tumors that may be missed. Ethical considerations play a larger

role at this stage, as well as adequate planning and piloting of the biomarker. Because incidence rate of HCC in cirrhotics is relatively low (1-5% per year), a large sample size is usually required. There are no HCC biomarkers identified at this stage.

Phase 5: Cancer control studies

This phase concentrates on whether surveillance of at risk population using the new biomarker reduces the cancer burden. There are no biomarkers identified at this stage.

In summary, the existing literature on new markers of HCC has several limitations. First, the sample size (i.e., power) and analysis of the results have been heterogeneous. Second, analysis of demographics and etiology of liver disease as covariates in the expression of these markers has been limited. Third, the majority of the markers identified in phase 1 and 2 studies have not progressed for further validation. Fourth, the reporting of the assay variability has also been poor. Lastly, there is a scarcity of longitudinal studies for HCC biomarker development. Formal guidelines are needed in order to prioritize biomarker development so that precious specimen resources and funding can be allocated in a sensible manner. These 5 phases are intended to be a guideline on the assessment of the current literature and for the future development of biomarkers. Several new markers appear to be promising, but further validation of biomarkers in a structured setting is critical to determine future clinical applicability.

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Proteomics for Diagnosis/Screening

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Research discoveries aimed at improving means of early detection for HCC are urgently needed. There is substantial interest in applying proteomics to cancer marker identification. Proteomics promises the discovery of biomarkers and tumor markers for early detection and diagnosis and novel protein-based drug targets for anticancer therapy. Approaches to that effect include comparative analysis of protein expression in normal and disease tissues to identify aberrantly expressed proteins that may represent novel markers and direct serum protein profiling to uncover potential markers. Today, two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), multidimensional chromatography, and protein biochips in combination with mass spectrometry are among the proteomic tools that are available for biomarker and drug target discovery. Our group has embarked on an effort to integrate transcriptomics and proteomics for the profiling of HCC. We have analyzed and compared neoplastic and non-neoplastic liver tissues from the same patients at 2 levels: 1-transcriptomic, by DNA microarrays and 2-proteomic, by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) and mass spectrometry. The combination of genomic and proteomic based profiling uniquely allows delineation of global changes in expression patterns resulting from transcriptional and post-transcriptional control, post-translational modifications and shifts in proteins between cellular compartments. The parallel transcriptomic and proteomic analysis also permits to compare mRNA and protein levels in the same tumors. We observed a clear separation between the neoplastic and non-neoplastic tissues and an association between the etiology of the underlying liver disease and patterns of HCC development. In particular, the HCV positive tumors were largely separable from the HBV positive tumors, the greatest variability being seen within the HBV positive tumors. Our 2-D PAGE and DNA microarray analyses have offered new insights into genes/proteins that are

potentially important in the development and progression of hepatic carcinoma, as well as a potential for identifying new markers for early HCC diagnosis. Among the genes and proteins that have a distinct pattern in HCC, of particular interest are those that encode for surface membrane or secreted proteins. An approach for their assay in tissues and biological fluids, notably serum, is through the use of microarrays that contain corresponding antibodies or other capture agents. The use of microarrays to determine their level in sera and their utility for diagnosing HCC provides a high throughput high sensitivity and low serum volume requirement and therefore is highly advantageous. Protein biochips technology is in its infancy being limited by the availability of suitable binding molecules that can cope effectively with protein diversity: comprehensive proteomic liver profiling and availability of antibodies remain major challenges for biomarker discovery. An organized public effort for the study of liver using proteomics has been engaged, under the Human Proteome Organization (HUPO) initiative (*www.hupo.org*). The scientific objectives include: (i) Comprehensive analysis of liver protein constituents in health and disease, with an initial focus on liver cancer, (ii) Bridging the liver proteome and the plasma proteome projects for biomarker discovery, (iii) Production of antibodies against liver proteins.

The identification of panels of tumor antigens that elicit a humoral response may also have utility in cancer screening and diagnosis. We have recently implemented a proteomic-based approach for the identification of circulating antibodies to tumor antigens in HCC patients. In contrast to approaches for identification of tumor antigens, based on the analysis of recombinant proteins, the proteomic approach we have utilized allows identification of autoantibodies to proteins in lysates prepared from tumors and tumor cell lines and thus may more readily uncover antigenicity associated with post-translational modification. We uncovered a distinct repertoire of autoantibodies that characterize the humoral response in HCC. The first approach involved protein separation by 2-D PAGE followed by Western-blotting using serum obtained from HCC patients. The second approach combines liquid phase protein separations with microarray technology. The strategy of using liquid-based multi-dimensional procedures to separate proteins

in lysates prepared from tissues or cell lines, allows distinct protein containing fractions to be arrayed and interrogated using various types of probes. Microarrays containing the repertoire of proteins expressed in tumor cells should substantially accelerate the pace of discovery of tumor antigens and could provide a molecular signature for immune responses in HCC.

New technologies are emerging that facilitate the identification of diagnostic tumor markers and cancer diagnosis may benefit from a complementation between gene profiling and quantitative proteomics. Refinements of serologic markers and screening of patients at high risk for developing HCC, such as those with chronic hepatitis C infection and cirrhosis or advanced fibrosis, may lead to better HCC detection, earlier intervention, and successful treatment, improving long-term outcomes.

Diagnosis and Staging of Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is the third leading cause of cancer related death worldwide. Rising incidence rates in western countries including the United States have recently been observed. Despite it well described clinical epidemiology, a number of controversial issues remain associated with the diagnosis and staging of HCC.

Diagnosis of HCC

The diagnosis of HCC has gained considerable interest based on the availability of novel therapies, which may reduce morbidity and improve survival in selected patients. To organize the various approaches available, a consensus statement sponsored by the European Association for the Study of the Liver (EASL) has been presented. Histologic confirmation as well as non-invasive features in patients with cirrhosis have been recognized as diagnostic criteria.

For nodules >2 cm, identified on two imaging techniques, the presence of arterial hypervascularization (i.e., contrast enhancement on the arterial phase of an MRI or CT scan) is sufficient to confidently establish the diagnosis of HCC by radiological criteria alone. The combination of one imaging technique demonstrating a mass lesion with arterial hypervascularization and a serum alpha-fetoprotein (AFP) level >400 ng/mL is also diagnostic of HCC. Advantages for using these criteria in lesions >2 cm include avoidance of a 10-20% false negative rate from biopsy and the small but important risk for tumor seeding. Limitations associated with non-invasive criteria include the misclassification of vascular dysplastic nodules and primary hepatic lymphomas as HCC.

For nodules <2 cm, a number of recall procedures have also been outlined by the consensus statement. The presence of malignancy in nodules <1 cm occurs in less than 50% of cases. As a result, serial

ultrasound examinations every three months are recommended until growth beyond 1 cm is established. For nodules between 1-2 cm in size, the use of fine needle aspiration with biopsy is recommended for diagnosis according to consensus guidelines. Limitations with this approach, however, include a 30%-40% false negative rate for diagnosis by fine needle aspiration and the risk of tumor seeding. The diagnostic accuracy of radiologic and combined criteria for lesions between 1-2 cm remains uncertain. In clinical practice, serum AFP levels are often <20 ng/mL which further reduces the usefulness of this surrogate marker. Similarly, the sensitivity of cross-sectional imaging modalities decreases further when nodules of this size range are sought. Given the inadequate test performance of invasive biopsy, further strategies need to be developed for improving the diagnosis in lesions <2 cm.

Role of Individual Cross-Sectional Imaging for Diagnosis of HCC

Earlier studies attempting to document the diagnostic accuracy of imaging techniques were limited by retrospective study design and problems related to verification bias. More recent studies, however, have used explant histology as a comparative gold standard. Sensitivity rates for the detection of HCC by ultrasound (US), contrast-enhanced computed tomography (CT), and magnetic resonance imaging (MRI), however, remain poor with values between 53%-65% for all modalities. In the context of clinical evaluations for possible liver transplantation, improved sensitivity rates between 79%-89% for detecting primary lesions appear related to an increased proportion of larger tumors in this patient subgroup. Comparative studies between CT and MRI are noted only for marginal increases in sensitivity and specificity. MRI angiography, however, was recently described having increased sensitivity (84%) for lesions between 1-2 cm. For all modalities, the failure to detect HCC lesions <1 cm occurs in 30%-40% of cases. Positron emission tomography (PET) is not an effective technique for the detection of HCC in studies reported to date.

Role of Individual Cross-Sectional Imaging for Screening and Surveillance of HCC

The diagnostic test performance of imaging techniques for HCC have been extrapolated to justify the performance of screening and surveillance programs in patients with cirrhosis. Based its cost and availability, however, US remains the modality of choice in the absence of a recognized superior technique. For patients with serum AFP level increases of ≥ 20 ng/ml above baseline and negative US examination, the use of contrast-enhanced CT is recommended to exclude occult lesions. MRI remains a confirmatory tool given its increased cost and limited availability.

Current limitations of HCC surveillance include the failure to distinguish macroregenerative and low-grade dysplastic nodules from true HCC by US examination. In addition, these lesions are often <5 mm yet have a low risk for malignant transformation. High-grade dysplastic nodules, which are usually <1 cm in size and also missed by US, develop into HCC in approximately 30% of cases. Very early HCC lesions, defined histologically as carcinoma in situ, can be identified by US yet appear hypovascular on CT. Local vascular invasion has been observed with very early HCC lesions <2 cm in size. Very early HCC lesions, therefore, are not identical to high-grade dysplastic nodules yet no agreement on diagnostic histologic criteria separating both entities exists to date. The occurrence of atypical cavernous– hemangiomas, which is uncommon in the setting of cirrhosis, can pose a diagnostic dilemma when lesions between <2 cm are present. The ability to exclude multicentric HCC, which occurs in 20%-40% of cases, also severely limits the effectiveness of current HCC surveillance methods.

Staging of Hepatocellular Carcinoma

A number of staging systems have been developed to stratify patients into appropriate risk groups where information about prognosis and eligibility for treatment can be provided. Both tumor node metastasis (TNM) and Okuda classification systems are limited by poor accuracy when applied in clinical practice. The exclusion of variables representing underlying liver function remains a major limitation of the TMN system. Use of the Okuda classification, however,

has been described as potentially appropriate for individuals with advanced HCC. Recently, a number of new classification systems have been developed yet external validation of these criteria has not been performed. The Barcelona-Clinic Liver Cancer staging classification is unique based on its translation into recommendations for particular treatment strategies based on HCC stage. However, a single consensus classification system for HCC is needed. Both overstaging and understaging occurs in approximately 20% of individuals awaiting liver transplantation , respectively, based on current diagnostic testing.

Four publications to date are noted for an increased detection rate of small unifocal HCC lesions within surveillance programs. An estimated 75% of individuals can be identified with solitary lesions <3 cm diameter compared to 15% of non-screened patients. Similar results, however, are not observed with any imaging technique or serum tumor marker for the detection of synchronous or multifocal lesions. As a result, the detection of index lesions in surveillance programs is not considered equivalent to staging based on these limitations.

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Diagnosis of HCC: Ultrasonography

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Conventional US is usually the first imaging modality employed for the screening of hepatocellular carcinoma (HCC). This is particularly the case in countries with high incidences of HCC (usually related to chronic hepatitis and cirrhosis), where the large 'at risk' population requires more extensive screening and strict follow-up. Nowadays, with the increasing diffusion of contrast-enhanced sonography, the role of sonography for detection and characterization of HCC will likely increase significantly.

B-Mode Sonography

The detection rate of HCC with B-mode sonography is related to the size, location and echotexture of the lesions, as well as to the ultrasound technology employed and the experience of the operator. Currently it is believed that 0.5 cm is the minimum lesion size for accurate detectability under ideal conditions. The reported detection rate of HCCs measuring less than 2 cm in diameter varies widely, from 46% to 95% whereas for HCCs between 2 and 3 cm it ranges between 82% and 93%. Considering only HCCs smaller than 1 cm, US is reported to have a detection rate ranging between 13% and 37%. Accurate detection and assessment of such "difficult" lesions is therefore heavily dependent on operator expertise.

Tumor echogenicity (level of echoes, homogeneity etc.) and detectability are closely related, especially for small nodules. A higher success rate with US is noted for HCCs with well demarcated margins, a perilesional halo (due to a fibrous capsule or peritumoral liver cell compression) and a hypoechoic pattern (due purely to cellular mass, without fatty or necrotic changes). Conversely, infiltrative and/or isohyperechoic HCCs without peripheral halos, as well as HCCs with

internal septa ("mosaic pattern") or posterior echo enhancement and lateral shadows are more difficult to detect with US, with reported sensitivities as low as 58.9-68.6%.

Diffuse HCCs are very difficult to identify and characterize with US, since the diffuse parenchymal inhomogeneity can be misinterpreted as being due to cirrhosis. On the other hand, the US detection of portal vessel and/or hepatic vein invasion (occurring in as many as 60-65% and 25-30 % of cases, respectively) can be used to detect the presence of HCCs not previously identified.

Multifocal tumors and daughter nodules are two common features of HCCs. Both non-intraoperative US and other imaging modalities tend to underestimate the number of multicentric nodules and daughter HCCs. This is particularly evident in studies in which end-stage resected cirrhotic livers with pathologic examination following transplantation are compared to previous radiologic findings.

With B-mode US cases of false positive diagnoses can also be detected. Typically, small hypoechoic well marginated HCCs cannot be distinguished from non-malignant (or pre-malignant) "dysplastic" nodules which show the same structural pattern.

Doppler Sonography

Doppler studies of liver parenchyma in patients at risk of developing HCC have not yet been shown to affect sensitivity and specificity for the detection of HCCs. Nevertheless, the detection with pulsed and/or color-power Doppler of arterial flow signals, both around ("basket pattern") and inside a liver mass, with Doppler shifts of 4.5 KHz or more, is thought to be a highly reliable sign of HCC, since it represents high pressure gradients caused by arterovenous shunting. However, in either small or extensively necrotic HCCs, poor vascularity can account for a high rate of false negative diagnoses with Doppler studies.

Contrast-enhanced Sonography

Contrast-enhanced sonography (CEUS) has recently led to a dramatic increase of sensitivity and specificity for both detection and characterization of HCCs, compared with unenhanced B-mode or Doppler sonography.

The initial method for amplifying sonographic signals from blood vessels was to destroy intravascular air-filled microbubbles (i.e., so called first generation contrast agents) at high ultrasound output (above mechanical index of 1.0) using fundamental color/power Doppler modes. However, this technique did not significantly increase the diagnostic accuracy of B-mode and Doppler US because it allowed only an improved depiction of vessel morphology which is not a reliable sign for the diagnosis of HCC, and unavoidable artifacts (motion artifacts, blooming effect, etc.) were frequently encountered.

Subsequently, the development of different types of contrast-specific software capable of selectively detecting the harmonic frequencies produced by contrast microbubbles hit by US waves or the amplitude bands generated by microbubbles within the range of the fundamental frequency represented a clear step forward for CEUS. When this technology is used with first-generation contrast agents and, moreover, with second-generation agents, made of stabilized microbubbles with elastic shell and gases of low solubility in water, employing very low mechanical index, complete survey scans of the whole liver in the arterial, early portal and full portal phases of contrast enhancement can be performed, allowing to increasingly detect and characterize both hyper- and hypovascular lesions. CEUS allows to visualize both macro- and microvascularity, to detect the characteristic hypervascularity of HCCs in arterial phase and the quick wash-out in portal phase, leading to hypoechoic pattern. Furthermore, as for characterization, with CEUS pre-cancerous primary liver lesions (macroregenerative nodules, dysplastic nodules) can be differentiated from early HCCs, thanks to the lack of hypervascularity in arterial phase. Daughter nodules and neoplastic portal thrombi (hypervascular in arterial phase) can be detected and differentiated from nodules of different origin and non-neoplastic portal thrombi.

Currently, with CEUS and second generation contrast agents (combined with clinical data), HCCs larger than 15 mm can be successfully characterized in 90-92% of cases, markedly reducing the need of performing aspiration biopsy, which has to be limited only to cases with atypical sonographic presentation.

Local intrahepatic staging of the disease cannot be reliably achieved with CEUS, due to the very short duration of the arterial phase which does not always allow to scan the entire liver in two different planes within few seconds: for this purpose, multislice CT is the most valuable imaging method.

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CT Imaging of Hepatocellular Carcinoma

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Overview

The ability of CT to detect and characterize liver lesions has been one of the most studied and evolving issues in radiology over the past twenty years. Technological advances, combined with increased knowledge about the pathophysiology of these tumors and the liver has resulted in dramatically increased abilities to detect and characterize HCC. Nonetheless, despite substantial increases in the abilities of imaging to detect early stages of HCC, imaging of early and small lesions remains a difficult task, despite what some authors claim. It is our intent to review the imaging appearances of HCC at CT, and to discuss the sensitivity and specificity of CT imaging in screening patients with cirrhosis for HCC. At the end of this talk the participant should understand key concepts about imaging detection of HCC to understand why older, prehelical CT literature reported higher sensitivities for tumor detection than current state-of-the-art multi-slice helical CT.

Large Tumors

In the western population cirrhosis and/or chronic hepatitis is present in 80-90% of patients who develop HCC. In patients without cirrhosis, HCC is usually large (>4cm) at presentation, due to the silent, asymptomatic course. Prior to the advent of fast scan techniques for CT and MR, even in cirrhotic patients who may have undergone liver imaging screening these tumors were large when first detected due to the difficulties in imaging the cirrhotic liver—distortions of liver parenchyma, and inability of portal venous phase contrast imaging (most common contrast phase utilized) to be effective in cirrhotic patients. Not unexpected then that most reports prior to the mid 1990s described HCC as large tumors, often encapsulated.

The heterogeneous appearance so typical of large HCC has a characteristic organization pattern that has been termed a mosaic pattern[1, 2]. The mosaic appearance of large tumors represents a tumor composed of multiple internal regions of differing appearances separated by often enhancing fibrous septations. Pathologic correlation has shown that these varying tumoral regions reflect changes of tumoral hemorrhage and necrosis, fatty metamorphosis and fibrous elements. The areas of necrosis typically appear as slightly less than liver parenchyma on unenhanced images, and substantially less than enhanced liver parenchyma, but still of higher attenuation than water seen in cysts. The appearance of the fat within the tumors will vary depending on how homogeneous these areas are; usually they are mixed with fibrous tissue and other cellular material, and thus are of low attenuation, but not substantially into the negative Hounsfield Unit range. Occasionally the fat regions will be very homogenous and appear of homogenous low attenuation within the tumor, with Hounsfield numbers of–50 or less. These fat containing zones usually occupy only a portion of the tumor and do not replace the entire tumor, aiding in differentiating these lesions from the total homogeneous appearance of benign focal fatty infiltration of the liver. Calcification is reported in the literature to be present in approximately 5% of HCC cases[3], and an even higher percentage (28%) in noncirrhotic patients with large tumors[4] although recent experience where imaging is detecting smaller tumors in cirrhotics, it is present less often than 5%.

Tumor encapsulation is another characteristic finding, seen in approximately one-third to one-half of cases of large tumors in the United States[1, 4]. Small or early tumors rarely demonstrate a capsule[5]. The capsule is usually nonenhancing on early dynamic contrast enhanced imaging, but typical of fibrous tissue, will retain contrast and appear enhanced on delay or equilibrium phase imaging[6]. CT imaging does not display the capsule as often as MR imaging. The capsule can be a helpful differentiating feature as it is not present with other common lesions such as hemangiomas, focal nodular hyperplasia, or metastases, although it is often seen in liver cell adenoma.

Evidence of advanced tumoral invasion, often seen with HCC, is often seen on CT. These findings include associated satellite tumor nodules, vascular and biliary invasion. These findings are uncommon with metastases to the liver as well as benign lesions and can be an aid in characterizing HCC, as well as stage the tumor. Portal or hepatic vein tumor thrombus can be seen as a solid tissue within the vascular lumen. Enhancement characteristics on arterial phase imaging can show either tumor neovascularity within the thrombus, or diffuse enhancement of the thrombus, both of which confirm the malignant nature of the thrombus[7]. The cross sectional diameter of the thrombus has also been reported to be helpful in differentiating benign from malignant thrombus 23 mm or more for the main portal vein having a sensitivity and specificity of 62% and 100% respectively).

Small Tumors and screening cirrhosis for HCC

The advent of fast CT scanning with helical CT in the mid 1990s afforded the first real opportunity for CT to detect early or small HCC. Detection of these lesions prior to helical CT was exceptionally difficult due to the distortion of the liver by the pathologic process of cirrhosis which can obscure small lesions and at the same time simulate tumor. With the ability to image the liver during the arterial phase of contrast delivery to the liver at 20–40 seconds following initiation of contrast material infusion, liver scanning could be completed before portal venous delivery of contrast material to liver parenchyma (at 60–70 seconds)[8, 9]. Because the majority of blood flow to the liver parenchyma is via the portal venous supply, imaging during the arterial phase will not enhance liver parenchyma substantially, while small HCC due to tumor neovascularity will enhance and be visible. Subsequent tumor washout during more delayed imaging combined with enhancement of liver parenchyma during portal venous phase makes these small lesions isoattenuating with liver and not visible during these later phases.

Key to achieving maximal success with arterial phase contrast CT imaging is the rapid delivery of a large of volume of contrast material to HCC lesions, requiring contrast injection rates of 4–5 ml/sec. Coordination of timing between administration of contrast material

and obtaining images during the arterial phase can be critical to optimally image the liver. Earlier arterial phase images (~18–22 seconds) optimally visualize the arterial anatomy of the liver which is essential when planning surgical or interventional treatments. However, late arterial phase imaging (~35–45 seconds) is a more optimal time to allow contrast enhancement in tumors to be visualized[10, 11]. While some authors have suggested a double arterial imaging sequence that would enable fast multidetector CT scanners to acquire images during both of these time frames[11], this would necessitate 3 passes through the liver (to include parenchymal and venous vascular enhancement which would require a third portal venous phase), with increased radiation exposure and cost. It is generally accepted that one would choose either an early arterial phase for vascular assessment or a later arterial phase for tumor detection in most instances, allowing for a rare case when both would be needed[10].

The delivery of contrast material can be further optimized when needed by delivering the contrast infusion through a hepatic artery catheter (termed CT-Angiography)[12, 13]. This technique has been reported to increase small nodule detection by 60% or more over arterial phase helical CT imaging[14]. This can be a helpful tool when treating patients with chemoembolization, and catheter placement is already utilized.

While arterial phase contrast imaging has the highest tumor detection sensitivity, delayed equilibrium phase imaging can be very helpful, and more sensitive than portal venous phase imaging alone[15, 16]. The visibility of tumors at this time is due to several factors. Delayed enhancement persisting in fibrous portions of the tumor has already been mentioned, notably in a fibrous capsule. Some very vascular tumors will demonstrate a substantial washout of contrast in delay phases, making the lesion very conspicuous as hypoattenuating compared with the still mildly enhanced liver parenchyma during equi librium. Finally, approximately 10% of small tumors will appear hypovascular on CT[9] even in arterial phase imaging, and may persist as such in delayed phase imaging allowing for their detection.

Marked variability has been reported for the ability of CT in screening for HCC. Studies using strong pathologic correlation from screening populations have reported CT sensitivity in detecting patients with HCC of 44–68% of patients[17, 18]. The detection rate for specific tumor nodule burden, always lower than the rate of patient detection, has been reported from 29% to 50%[17, 18] and even as high as 92%[19, 20]. The higher rates are reported from studies using screening populations and strong pathologic correlation after transplantation. The higher reported rates generally are from series with patients referred to institutions with a known diagnosis of HCC and then imaged as a consecutive series of referred patients.

Dysplastic Nodules

Dysplastic nodules are not generally seen on CT. Rare large lesions may be seen on unenhanced CT images with a high attenuation appearance, speculated to be due to iron accumulation or increased glycogen content. No published studies on the ability of CT to detect these lesions has been reported, but in the author's experience, it is very unusual for these lesions to be detected as appearing different than background liver regenerative nodules.

The progression of pathologic changes in liver regenerating nodules from benign regenerative nodule to dysplastic nodule to frank HCC reflects the continuous development of pathologic changes such as tumor neovascularity that are subjective to the pathologist interpretation. This continuous progression can result in some early enhancement of dysplastic nodules at CT that can simulate frank HCC[21, 22].

Specificity of CT findings of HCC

Most benign lesions such as cysts and hemangiomas will not be mistaken for HCC. In a noncirrhotic liver, focal nodular hyperplasia can enhance vividly during arterial phase imaging and rapidly become isoattenuating with liver on later images, simulating HCC. Characteristics of FNH, such as a fibrous central scar, not present in HCC, can be an aid in differentiating these lesions. Without the central scar, these lesions can be difficult in the noncirrhotic patient to differentiate from HCC.

Flash filling small hemangiomas can enhance vividly during arterial phase imaging, but will retain blood pool delayed enhancement characteristics, unlike HCC which will demonstrate washout of contrast to levels less than blood pool[12, 22]. Other vascular lesions such as small arteriovenous malformations following liver biopsy or hepatic peliosis can similarly show arterial phase enhancement, but will again demonstrated blood pool characteristics on delayed images.

Rare dysplastic nodules will show arterial phase contrast enhancement and can simulate the washout characteristics of HCC, as mentioned earlier. Similarly, particularly in Budd-Chiari patients, nodular regenerative hyperplasia lesions will show similar enhancement characteristics[23]. Such patients will often show multiple lesions (often more than 10) which can be an indication of the benign nature of these lesions.

Focal confluent fibrosis in scarred regions of the liver can simulate both hypovascular and vascular tumor in cirrhosis[24, 25]. While most often there are characteristic associated findings (wedge shaped radiating from the hilus to the capsule; atrophy evidenced by overlying capsular retractions), this can occasionally be difficult to distinguish from HCC.

In screening a large cirrhosis pretransplant population, Brancatelli et al reported an 8% false-positive diagnosis for HCC using helical CT[22]. Most of these lesions were hyoattenuating, such as focal confluent fibrosis, but vascular enhancing lesions such as flash filling hemangiomas and enhancing confluent fibrosis were problematic lesions.

Summary

Detecting HCC in the cirrhotic liver is a challenging process. Recent advances in CT technology and understanding of how to optimize contrast material delivery have resulted in increasing abilities for CT to detect HCC. Despite what some studies in the literature report, however, CT sensitivity in detecting both patients and extent of tumor remains less than optimal. An understanding of the limitations of all imaging modalities in screening cirrhotic patients for HCC is important for patient management.

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Magnetic Resonance Imaging of Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is the most common primary liver neoplasm, the fifth most common cancer in the world and is responsible for up to 1 million deaths annually worldwide[1]. The 5-year survival rate for untreated, symptomatic HCC is less than 5%. In contrast, the 5-year survival rate in patients with cirrhosis following transplantation of small [<2 cm] HCC is 80%[2]. The detection of small HCC is clearly critical to patient outcome.

Morphologic distinction of HCC from other liver nodules can be difficult. Cirrhotic livers frequently contain numerous regenerative nodules that are benign. Small HCC, and their precursors, premalignant dysplastic nodules (DN) can both be histologically distinguished from background cirrhotic regenerative nodules by cellular atypia at pathology. However, by noninvasive imaging tools, the distinction between malignant and benign nodules is limited and relies primarily on their different vascular supply[3]. Regenerative nodules, like underlying liver parenchyma, have a portal venous blood supply, while high-grade DN and HCC demonstrate neoarteriogenesis with an increased number of non-triadal or unpaired arteries[4-6].

While many MR studies have reported high diagnostic accuracy for HCC[7-11] and DN in patients with cirrhosis, *most of these have been limited by study design, incomplete pathologic correlation and suboptimal imaging techniques.*

- Most studies have been performed using biopsy or surgical correlation of detected nodules[7-11]. Because HCC and DN are frequently multifocal in the setting of cirrhosis, whole

liver explant studies are required to determine the true accuracy of diagnostic tools. *The lack of whole liver explant correlation has led to an overestimation of the sensitivity of imaging tests.* Therefore, the sensitivity for the detection of HCC and DN cannot be determined without whole explant correlation

- Most studies have been performed in a retrospective manner introducing a bias towards positive results.

- Technological differences regarding spatial and temporal resolution for MR imaging vary widely among published studies with explant correlation. The optimal technique would include 2 mm thin section contrast-enhanced 3D MRI with imaging in the hepatic arterial dominant and portal venous phase.

- Authors of some previously published studies using explant correlation have chosen to exclude consideration of all lesions <1 cm. Exclusion of these lesions results in ineffective transplantation in patients with the miliary, diffuse variant of HCC and those with small satellite lesions and/or intrahepatic metastasis[12].

- Some studies do not evaluate DN. This is problematic as these lesions must have been present at explantation. To make matters more confusing, the criteria used to diagnose DN varied widely among both CT and MR studies.

- Even with explant studies, the mean interval between imaging and pathologic evaluation has often been too long [>60 days], decreasing the accuracy of radiological-pathological correlation, especially when chemoembolization or ablative therapy has been performed in the interval

- In most studies with whole explant pathologic correlation, the explanted liver was sectioned at 0.8-1 cm intervals for correlation. To ensure that even small lesions, measuring 0.8 cm or less, are detected, some authors advocate 0.3 cm sections[13].

MR Imaging with gadolinium-based contrast agents

While many studies have been published regarding the sensitivity of MR imaging for detection of HCC[7-11] and DN only a small number have had whole explant correlation within 90 days of imaging. For MR imaging, a dual or triple-phase contrast-enhanced approach is typically used, where the first acquisition is timed for the hepatic arterial dominant phase. Using this method in 71 patients with end-stage cirrhosis requiring transplantation [but without known HCC], we demonstrated a sensitivity of 55% for detection of HCC and 15% for DN respectively[14]. Rode et al.[15] demonstrated better results in 43 patients with a sensitivity of 77% for detection of HCC and 42% of DN. A smaller study of 34 patients also found slightly better results with a sensitivity of 61% and 27% for HCC and DN respectively[16]. In 26 patients transplanted for cirrhosis, Libbrecht et al.[13] detected 70% of HCC and 27% of DN.

Among 24 patients transplanted specifically for HCC [and cirrhosis], dynamic gadolinium-enhanced MR imaging detected only 39 of 118 HCC for a sensitivity of 33%[12]. The lower sensitivity reflected smaller coexisting lesions that were diagnosed only by careful histopathologic sampling of the entire liver explant. When stratified by lesion size, *only 4% of HCC measuring less than 1 cm were detected*[12]. Additionally, of the nine who had diffuse HCC (all nodules <1 cm), none were detected prior to transplantation and eight showed poor outcomes subsequently[12].

Double-Contrast MR imaging

Using both gadolinium and ferrumoxide MR agents, Bhartia et al.[17] demonstrated a 78% sensitivity for detection of HCC in 31 patients transplanted between 3 and 245 days after MR imaging. The sensitivity dropped to 38% for lesions ≤ 1cm. The authors did not evaluate DN[17].

In conclusion, when the gold standard of imaging HCC and DN is whole explant correlation, MRI appears to be suboptimal for detection of small <2 cm lesions. Further improvements in hardware, software and novel contrast agents will likely result in a higher

sensitivity for lesion detection. Perfusion and diffusion MR imaging will likely also increase the accuracy of MRI by reliably differentiating pseudolesions (usually arterioportal shunts) from HCC. In addition, increased worldwide utilization of live-liver donation, with imaging and histopathologic correlation within 24 hours, will surely advance the science of radiology.

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Molecular Imaging

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Molecular Imaging : What is it and what is different about it?

The term "Molecular Imaging" has been coined only in recent years and there is no consensus regarding what the term really means. One of the better published definitions of the term was by Luker and Piwnica-Worms [1] who defined it as, "The characterization and measurement of biologic processes in living animals, model systems, and humans at the cellular and molecular level by using remote imaging detectors." Although "Molecular Imaging" is a relatively recent term, many of the concepts have been practiced in nuclear medicine and positron emission tomography (PET) for years. Many of the newer concepts such as using reporter gene-probe pairs for imaging gene expression in vivo are derivatives of methods that have been used in cellular imaging. The most widely used strategies in "Molecular Imaging" can be broadly classified as direct, indirect, and surrogate. Direct imaging approach involves direct interaction between the imaging probe and the molecular target. The interaction can be a probe-receptor interaction, a probe trapped in cells by activity of an enzyme or a probe transported into cells by activity of a specific transporter. Good examples of probe-receptor interaction include radionuclide-labeled monoclonal antibody imaging and imaging of glucose utilization with ^{18}F-fluorodeoxyglucose using PET. Indirect imaging strategies employ methods with multiple components and many of the methods used today are adaptations of methods that have been used in cellular imaging. The most commonly used indirect imaging method employs reporter gene-probe pairs such as using herpes simplex virus type 1 thymidine kinase gene HSV-tk as the reporter gene and ^{18}F-labeled 9-[4-fluoro-3-(hydoxymethyl)butyl] guanine (FHBG) as the reporter probe. Cells transfected with the HSV-tk, which is more efficient as compared to mammalian thymidine kinase in phosphorating FHBG, will trap more of the probe

in the cell. As a result, the HSV-tk gene can be used to report on activities of genes linked to it. Since ^{18}F activity can be detected in vivo in whole organisms using PET, HSV-tk activity can be tracked in vivo in a spatially and temporally resolved manner. Surrogate imaging involves using imaging probes to detect effects that are more downstream of the molecular or genetic processes of interest. For example, FDG PET has been used to monitor various tumors before and after different treatments, many of which are not targeted to the glycolytic pathway. It is easy to see from this brief discussion that "Molecular Imaging" techniques aim to provide information about molecular and cellular events in vivo which can supplement the "morphologic" information that can be gained from more conventional medical imaging techniques.

Molecular Imaging in Diagnosis and Treatment Monitoring of Hepatocellular Carcinoma

Many "Molecular Imaging" techniques that have been successfully applied to other solid tumors have been evaluated in patients and preclinical animal models of hepatocellular carcinoma (HCC). Many of these techniques are surrogate imaging techniques that are used for monitoring processes not unique to HCC. ^{18}F-FDG has been studied by multiple groups in patients with HCC and preliminary results showed that about one third of HCCs do not show increased accumulation of ^{18}F-FDG as compared to the surrounding liver [2]. This is because differentiated hepatocytes normally have a relatively high glucose-6-phosphatase activity and HCCs have variable glucose-6-phosphatase activity. ^{11}C-Acetate is a metabolic substrate of β-oxidation and precursors of amino acid and sterol. Preliminary results using an imaging cocktail of ^{18}F-FDG and ^{11}C-Acetate demonstrated that poorly differentiated HCCs are better detected by ^{18}F-FDG and well differentiated HCCs are better detected by ^{11}C-Acetate [3]. The combined sensitivity using both tracers was 100% in the detection of the 39 HCCs in the study. All 16 non-HCC malignant liver lesions were negative for ^{11}C-Acetate uptake and focal nodular hyperplasia (FNH) was the only benign lesions that showed mild ^{11}C-Acetate uptake. So, this combined imaging approach may be more accurate than using either tracer alone. Other tracers that have been used in HCC include

Technetium-99m methoxyisobutylisonitrile (Tc-99m MIBI) [4] and Technetium-99m Tetrofsomin (Tc-TF) for detecting multidrug resistance [5], ^{18}F-EF5 {2-(2-nitro-1 [H]-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl)-acetamide}for detecting tumor hypoxia [6], Tc-99m galactosyl-human serum albumin (GSA-LV) for determining functional hepatic volume [7], and Oxygen-15 for evaluating blood flow [8]. A preliminary report of using technetium-99m labeled anti-hepatoma monoclonal antibody fragment for detecting HCC in animal model was published in 2000 without any further reports [9]. All these reported results are very preliminary and it is difficult to know which of these methods will be clinically useful in the future.

Future applications of Molecular Imaging and Image Guided Tissue Analysis in Studying HCC

The difficulty in developing specific direct imaging methods for HCC is the lack of obvious specific molecular targets and general issues of drug delivery and target-to-background and contrast-to-noise ratio considerations. Since liver is normally metabolically active and is designed to sequester particles of various sizes nonspecific background activity of contrast agents in the liver is a major hurdle to overcome. One way to identify potential molecular targets is the use of image guided tissue procurement. Using imaging to characterize HCC in vivo prior to tissue procurement can add unique information that is impossible to obtain without the imaging information. For example, by looking at regions of HCC with higher angiogenic activity as compared to regions of the same tumor with less angiogenic activity, one can gain insight as to the genes that are upregulated in the angiogenic areas as compared to the less angiogenic areas. These molecular targets can then be used for guiding development of new imaging and therapeutic agents. Once new molecular imaging probes are available they can be used to characterize HCC in vivo and for identifying new molecular targets and pathways that are modulated at the same time as the imaged targets. Using this type of iterative approach, new insights into the molecular characteristics of HCC may be gained. Molecular Imaging and image guided tissue analysis are useful tools for understanding system biology and should definitely be applied for further understanding HCC [10,11].

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Chemical Injection

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In Japan, approximately 30,000 patients died of hepatocellular carcinoma (HCC) last year. Of those, 11% and 83% of the patients with the cancer were positive for HBV (Hepatitis B Virus) and for HCV (Hepatitis C Virus), respectively, indicating that 94% of our patients with HCC are currently infected with either of two hepatitis viruses. In contrast, only 3% (1.5% for HBV and 1.5% for HCV) of general population are infected with the viruses.

Strategy of ours to treat cancer nodules is by PEIT (Percutaneous Ethanol Injection Therapy), PMCT (Percutaneous Microwave Coagulation Therapy) and RFA (Radiofrequency Ablation). Approximately 90% of our patients who are admitted to our Department of Gastroenterology, University of Tokyo, were treated with one of the percutaneous methods. Of our 2000 treated patients, 3-year survival were 65% and 5-year survival were 42%. Our Japanese registry of surgically resected 16,723 HCC cases indicate that 3-year survival were 63% and 5-year survival were 45%, indicating that 3- and 5-year survival between our experiences of our cases, and of surgically resected cases are similar.

Recently, we have completed a prospective control trial of PEIT and RFA for HCC. Percutaneous RFA is a recently introduced treatment for HCC, while ethanol injection is now a standard therapy (1). Two hundred and thirty-two patients with HCC who had three or fewer lesions, each 3 cm or less in diameter were entered onto a randomized controlled trial. The primary endpoint was survival, and the secondary endpoints were overall and local recurrences. One hundred and eighteen patients were assigned to RFA and 114 to ethanol injection. The number of treatment sessions was 2.1 times in RFA while it was 6.4 times in ethanol injection ($P<0.001$). Required hospitalization was 10.8 days in RFA whereas it was 26.1 days in ethanol injection ($P<0.001$). Four-year survival rate was 74% in RFA and 57%

in ethanol injection. RFA lowered the risk of death by 46%, and had a 43% smaller risk of overall recurrence and an 88 % smaller risk of local recurrence than ethanol injection. The incidence of adverse events was not different between the two therapies. This prospective control study indicated that RFA is superior to ethanol injection in the treatment of small HCC. Thus, further improvement of prognosis of our patients are expected, because RFA had just become procedure to be reimbursed by public government insurance in Japan.

In addition to these means to control cancer locally, C-viral HCC often develop with the background of advanced fibrosis and/or cirrhosis, whereas B-viral HCC often without. Therefore, surgical resection or complete ablation of nodules not necessary leads to complete cure of HCC. Recently, it has been shown that interferon treatment results in the attenuation of fibrosis (2), decrease of the incidence of HCC (3) and of overall mortality (4). It is clear that you need the treatment both for backgrounds and tumor nodules. Otherwise, the recurrence of cancer from background could reach 80% within 5 years.

We have initiated a prospective controlled study for the patients who had HCC, treated by percutaneous injection therapy and interferon. The result indicates that 21 patients treated by ablation and interferon which induced good response, 5-year survival were 83% (5). Untreated by interferon (n=31), or treated but failed to respond to the drug response (n=22), 5-year survival of 45% and 50%, respectively.

If you have expertise to treat tumor nodules by medical ablation and eradicate HCV, patients life and survival rate could be drastically improved.

Continued

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Radiofrequency Thermal Ablation of Hepatocellular Carcinoma

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Introduction: Radiofrequency Ablation

Surgical resection or hepatic transplantation are considered the only potentially curative therapies for hepatocellular carcinoma (HCC). However, because of advanced disease, unfavorable location, or impaired clinical condition, only a minority (7-15%) of patients are eligible for surgical intervention. Furthermore, the results of surgical resection are suboptimal, with a 5-year survival rate of 35-50% and a high risk of recurrent intra-hepatic HCC (only 30% of patients remain tumor free at five years). These factors have led to the development of multiple minimally invasive forms of therapy including intra-arterial chemoembolization, injection of ethanol, and thermal ablative techniques. Thermal ablative techniques include radiofrequency (RF) ablation, microwave ablation, and interstitial laser therapy. The most thoroughly studied of these is RF ablation, and it is now a widely accepted modality for the treatment of HCC.

RF Mechanism

RF ablation uses alternating electrical current in the radiofrequency range to create focal thermal lesions. Special needle electrodes and ground pads act as conductors for the alternating current. The current agitates the ions in the tissue adjacent to the needle electrodes, thus creating frictional heat. The heat starts in a glove-like configuration around the electrodes then expands by conduction to form a thermal sphere. Temperatures in excess of 50°C produce coagulative necrosis. The actual size of the zone of coagulative necrosis produced by the RF ablation device is dependent on multiple variables with the most significant being the average sustained core temperature during the ablation, the amount of time at core temperature, and

the vascularity of the tissue being ablated. The single greatest factor limiting the size of an ablation in the liver is portal venous blood flow. The high rate of blood flow delivered via the portal vein serves to cool the ablation process or to create a "heat sink" effect. This effect limits the size of ablation that can be achieved with standard (unassisted) ablation devices to approximately 3.5 cm. Larger ablations can be achieved in large avascular tumors, with modulation of the portal venous and hepatic arterial blood flow, or with the use of adjuvant ionic or chemotherapeutic solutions.

Equipment

There are several designs of RF ablation equipment, which all operate on a similar principle. Three RF systems are in wide use, which are powered by 150-200 watt alternating electric current generators, and which operate at 460-500 kHz. The ground pads (2-4) are large adhesive dispersive electrodes that are placed on the skin over the low back muscles and/or thigh. The electrodes are 14-17 gauge needles that are insulated except for the distal tip. Cooled tip electrodes are straight 17-gauge needles with two internal channels through which chilled water is circulated. Multi-array electrodes have a plunger in the needle hub that advances seven to ten curved prongs from the tip. When deployed, the prongs and the needle resemble an open umbrella or Christmas tree. The diameter of the extended electrodes ranges from 2.5-7 cm. Two of the devices have thermocouples in the tips of the electrodes that can be used to measure local tissue temperature. One of the devices has the ability to infuse hypertonic saline into the ablation site during the ablation.

Procedure

Different institutions have developed slightly different protocols for the RF ablation procedure. At our institution, patients are treated on an outpatient basis. Each procedure takes between 1-3 hours, depending on how many ablations are performed. After the procedure, patients are observed for 4-6 hours then released. All of the procedures are performed using sonographic guidance with local anesthesia and conscious sedation. The

needle electrode is inserted percutaneously and advanced until the tip is positioned in the desired portion of a tumor. If a multi-array probe is used, the plunger in the hub of the needle is advanced, causing the curved electrodes to be deployed into the tumor. The power is initiated and the ablation begun. Each of the three main ablation devices is operated with a different ablation algorithm, based on either impedance, power, or temperature. The typical RF ablation lasts 12 minutes. After each ablation, the needle is repositioned as necessary to heat the entire tumor. The goal in RF thermal ablation is to kill the target tumor as well as a 5-10 mm circumferential cuff of adjacent normal hepatic parenchyma. The number of punctures of the liver capsule is kept to a minimum. In an effort to minimize the risk of bleeding, our procedure is to cauterize the needle tract just below the liver capsule prior to removing the needle electrode.

Evaluation of Treatment Outcome

Institutions have developed their own methods for follow-up. We rely on follow-up blood work and abdominal CT scans, which are scheduled at 2 hours after the procedure and then every three months. If there is no evidence of tumor recurrence, the inter-scan interval is lengthened progressively to 1 year. We judge the success of the ablation by the appearance of the treated tumor on the follow-up CT scans and alpha fetoprotein levels.

Current Results

Multiple clinical series have been published on percutaneous RF ablation of hepatic tumors. The results for local tumor kill rates vary from 45% to 98%. Local success is clearly related to the size of the tumor, with the best results achieved with tumors less than 3 cm in diameter. There has not been any proof to date that tumor debulking alters survival. However, there is a good chance that patients with a minimal hepatic tumor burden will achieve survival rates equal to that seen with surgical resection.

Complications

Worldwide, thousands of patients have been treated by RF ablation. Overall, the complication rate has been very low (<5%). The most common complications are bleeding, tumor seeding along the needle tract, ground pad burns, thermal burns of adjacent viscera, and delayed infection of ablated tissue. A handful of deaths have been attributed to RF ablations; the causes have been bleeding or infection. Approximately 1/3 of patients will experience post-ablation flu-like symptoms that begin 3-5 days after the procedure and last approximately the same period of time. These symptoms are due to an inflammatory reaction to the ablated tissue and typically seen in patients with more extensive ablations. These patients are not infected and can be treated conservatively.

Future Directions

Two interrelated goals for the future of thermal ablation therapy are creating larger ablation zones and increasing overall success rates. Percutaneous microwave ablation therapy is a promising, still-developing modality that is hoped will produce larger zones of ablation than those achievable by current RF equipment. Trials comparing the two are currently under way. Increased overall success rates will likely achieved by combination therapy of thermal ablation with the latest techniques in chemotherapy and radiotherapy. Studies of the effectiveness of such combination therapies have already produced some promising results.

Conclusion

Based on current publications and our own experience, RF ablation is a quick, safe, and highly effective technique for the treatment of small HCC. In addition, microwave ablation and combination therapies hold promise for treating a wider range of HCC.

Chemoembolization for Hepatocellular Carcinoma

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Surveillance of patients with liver cirrhosis aims to detect hepatocellular carcinoma (HCC) at an early stage when curative therapies can be successfully applied and provide a benefit in survival. However, even in expert hands, most of the patients with HCC are diagnosed at a stage when treatments such as resection, transplantation and percutaneous ablation are not indicated [1,2]. The unfeasibility to apply these effective options is due to their advanced clinical stage at diagnosis as reflected by an excessive tumor burden exceeding the conventional definition of early stage (this includes solitary tumors, frequently up to 5 cm, or multifocal HCC with ≤ 3 nodules less than 3 cm in size) and/or by the presence of severe liver failure (Child-Pugh B and C)[3]. Accordingly, these patients are usually evaluated as potential candidates for any of the several palliative options that are available. Unfortunately, for most of them there is no evidence of their beneficial impact in survival, as the number of randomised clinical trials is reduced[4]. It is important to stress, that the aim of palliation is to increase the life expectancy of the patients. Thus, the scientific acceptance of the benefits of any palliative option should be based in the unequivocal demonstration of a survival benefit[2]. In a recent systematic review followed by a meta-analytical assessment the only palliative option that has been demonstrated to provide a survival benefit in patients with unresectable HCC is chemoembolization, while other options with potential significant antitumoral activity have not yet been shown the unequivocally improve in survival[4].

Transarterial embolization associated or not to chemotherapy has been extensively assessed both in phase II studies and in randomised controlled trials. The blood supply to tumor sites is mostly provided through vessels arising from the hepatic artery branches and thus, the obstruction of the arterial vessels feeding the tumor results in

ischemic tumor necrosis. On the contrary, the non-tumor liver is nourished mostly by the portal vein and thus, the obstruction of the hepatic artery blood flow is well tolerated. Of course, if the portal blood flow is obstructed by tumoral invasion or reversed by the presence of advanced cirrhosis, the tolerance to arterial obstruction is reduced and the risk of associated mortality is increased.

Arterial obstruction can be achieved by the injection of gelfoam cubes, polyvinyl alcohol, blood clots or microspheres. Obstruction can also be produced by the placement of metallic coils, but this may reduce the potential for repeated treatment sessions. In fact, objective tumor response is registered in around 50% of the patients, but during follow-up the tumor recovers its vascularization, tumor growth reappears and at that time, new treatments sessions may be considered. For this reason, most authors perform treatment at regular periods of time, but there is no proof to establish that this prospective schedule is better that to repeat treatment according to tumor response and evolution.

The survival benefits of transarterial embolization alone or combined with chemotherapy have been very controversial until recently[3]. The trials published until 2002 reported inconclusive or negative results[5-9], but the publication of two trials coming from Hong-Kong[10] and Barcelona[11] reported a significant survival benefit for patients treated with chemoembolization. This was further confirmed by a meta-analysis combining all the available randomised studies[4]. It has to be pointed out that the published studies are rather heterogeneous in terms of treatment schedule–interval between sessions, chemotherapy used–but in all of them treatment is associated with a marked antitumoral affect[5-11], delay in tumor progression and even prevention of vascular involvement[7,11]. All in all, these data reinforce the usefulness of chemoembolization for palliation in patients with HCC. However, the beneficial effect is restricted to patients with preserved liver function (Child-Pugh A) without cancer-related symptoms and thus, only a minor proportion of individuals (around 10-15% of

the whole HCC population) will be thus optimal candidates for this treatment. Arterial embolization without associated chemotherapy has a similar antitumoral effect but still there is no evidence of its positive impact in survival[4].

A different scenario where transarterial chemoembolization has been tested is in the waiting list for liver transplantation or prior to conventional surgical resection[12]. Despite some positive suggestions, there is no benefit of treatment prior to resection and the absence of randomised controlled trials in patients waiting for transplantation prevent the achievement of robust statements.

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Proton Beam Radiotherapy for Unresectable Hepatocellular Carcinoma

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There is a clear need for more effective therapies for patients with localized but unresectable Hepatocellular Carcinoma (HCC). While conventional radiotherapy techniques (x-rays) play a significant role in many malignancies, there is no defined role in HCC. The main obstacle to delivering effective doses of radiotherapy to patients with HCC lies in the need limit the dose delivered to the non-tumor portions of the liver. Radiation induced liver disease (RILD) is frequently seen when large portions of the liver receive doses above 30 Gy. For carcinomas arising in other parts of the body, a dose of at least 70 Gy is required to provide any hope of long-term local tumor control. Thus the challenge to the to the Radiation Oncologist is to deliver high dose tumor irradiation while limiting the dose to normal liver tissue which surrounds the tumor. This is further complicated by limited hepatic reserve from frequent underlying cirrhosis. Conventional radiotherapy has a limited ability to deliver this type of treatment because inherent physical properties of x-rays do not allow the dose to be conformed to the target in a 3-dimentional fashion. Thus the total dose that can be administered to targets within the liver with these techniques is limited and frequently ineffective. Proton beams, however, have physical properties that differ from x-rays. Protons, being a charged particle, have a limited range in tissue and release nearly all of their energy at the end of their path (Bragg Peak). This high-dose stopping area can be made to occur any depth to correspond to the target region. Protons, being relatively heavy, also have minimal side-scatter upon entering the body. These physical properties are unique to charged particles (i.e. protons) and are a marked contrast to x-ray beams, which have no stopping ability and tend to scatter when entering tissues.

Based on these principles, physicians in Japan have investigated the use of proton therapy in patients with localized HCC. They have recently reported results in 163 patients treated with a median dose of 72 Gy in 16 fractions with or without additional TAE or ethanol injection. The overall 5-year survival rate was 25% for all patients and 44% in patients with minimal signs of cirrhosis. The 5 year local tumor control rate was 83%, however, new HCC's developed in 85% of patients. Reported hepatic side-effects were limited to transient transaminase elevations.

At the Proton Treatment Center at Loma Linda University we initiated a phase II trial to evaluate the role of proton beam therapy in patients with unresectable HCC. Patients with a diagnosis of cirrhosis were eligible if they had compensated liver disease, Child-Pugh Class A or B. Eligible patients included those with T_1-T_3 hepatocellular carcinoma and selected T_4 patients. Patients with lymph node or distant metastases were ineligible. Daily proton beam radiotherapy was directed to the liver tumor with an additional 1 to 2 cm margin to allow for subclinical disease extension and/or tumor motion during treatment. The total dose was 63 CGE, administered in 15 divided fractions over 3 weeks (biologically equivalent to 75Gy). Following treatment patients were monitored with clinical examinations, blood analyses, and CT scanning of the chest and abdomen. Acute toxicity during treatment included skin inflammation, abdominal discomfort, and in some cases, nausea and diarrhea. Post-treatment toxicity included a small but significant decline in mean albumin levels (3.3 to 2.8) and increased total bilirubin (1.2 to 1.7). Three patients experienced significant duodenal or colonic bleeding when bowel was immediately adjacent to the treated tumor; seven experienced a new onset of ascites following treatment. Alphafetoprotein (AFP) levels were monitored in all patients following treatment.

Twenty-seven of 34 patients had elevated AFP at the time of study entry with a mean AFP of 1602. Twenty-three of twenty-seven patients (85%) with elevated pre-treatment AFP levels had a declining value post treatment; the mean AFP nadir was 35. Most patients

achieved an AFP nadir between three and six months following completion of treatment. Four patients showed no decline or persistent elevation of AFP following treatment.

Local tumor control was defined as a stable or declining AFP and radiographic evaluation showing the treated liver tumor to be either stable or smaller in size. Local failure was assumed to be present if AFP was elevating without evidence of metastatic disease despite imaging showing a stable primary tumor. Using these criteria, the observed local tumor control rate was 75% at two years. The rate of tumor recurrence within the liver outside of the primary tumor area was 35% at two years. Metastatic disease outside of the liver was seen in two patients (lung and adrenal gland) representing a 10% distant failure rate at two years. The two-year actuarial survival rate for our patients was 55%.

Following completion of proton therapy, six patients underwent successful liver transplantation. All transplants were completed at Loma Linda University Medical Center and no unusual difficulties were encountered related to proton radiotherapy. Two of the six explanted livers showed no viable residual tumor within the treated region. One liver showed only microscopic evidence of residual HCC. Three patients had gross residual tumor in the explanted specimen.

We conclude that proton radiation therapy can be safely administered to patients who have localized HCC despite pre-existing mild to moderate clinical cirrhosis. Most patients with either declining AFP and/or reduced tumor volumes on imaging. Tumor control and survival was reasonably good in patients in this population and compare favorably to reports in the literature of other non-surgical therapies. In at least in some patients, HCC can be eradicated completely, as indicated by pathologic complete responses seen at the time of liver transplantation. It is our hope that, by modifying target localization procedures or by increasing the dose administered, we can improve upon these results in the future.

Yttrium-90 Microspheres for the Treatment of Unresectable Hepatocellular Carcinoma

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Unresectable hepatocellular carcinoma (HCC) is a highly lethal and difficult to treat condition, whose treatment is often influenced and limited by underlying cirrhosis. Available therapies are palliative, and typically associated with significant side effects and morbidity. The use of intra-arterially delivered yttrium-90 (^{90}Y) microspheres (TheraSphere®) represents a new treatment that shows promising results in objective tumor response, survival and toxicity profile. TheraSphere® is comprised of 20-30 micron sized microspheres incorporating ^{90}Y as an integral constituent of an insoluble glass matrix. The ^{90}Y microspheres, with a physical half-life of 2.7 days, emit pure beta radiation with an average range of 2.5 mm and maximum penetration of approximately 1.0 cm. The microspheres delivery principle relies on hepatic tumors receiving most of their blood supply from the hepatic artery, while normal liver parenchyma receives most of its blood supply from the portal vein. The microspheres, when injected into the hepatic arterial system, flows preferentially to the tumor where they are entrapped in the tumor arterioles, effecting tumor kill while minimizing exposure of normal tissue.

An initial dose escalating study in patients presenting with HCC and metastatic liver disease was conducted, with doses ranging from 50 to 150 Gy. The majority of patients had failed prior chemotherapy regimens. Most patients were treated with a single infusion of ^{90}Y microspheres. Other than transient (few days to 2 weeks) elevation in liver enzymes and mild fever and fatigue, no dose limiting hematologic, hepatic or pulmonary toxicity was observed. However, gastritis and duodenal ulceration was observed in several cases, with biopsy evidence of microsphere deposition in one case. All gastrointestinal events resolved with medical therapy and were attributed to inappropriate catheter placement, which resulted in inadvertent deposition of ^{90}Y microspheres into the gastroduodenal or right gastric arteries.

Tumor response was encouraging, with most patients (70%) experiencing either stabilization or reduction of disease at 4 months (via Computed Tomography). No patient deaths were attributed to treatment. This study indicated that ^{90}Y microspheres had a favorable toxicity profile and illustrated potential for a therapeutic effect in patients with HCC.

Given the encouraging results obtained from the dose escalation study, a fixed dose study, with a targeted dose of 100 Gy, was conducted in 20 patients presenting with unresectable HCC. The purpose of this study was to assess treatment response, patient survival and toxicities after intra-hepatic arterial injection of ^{90}Y microspheres, administered as a single injection. The median dose delivered was 104 Gy. Four of 20 patients (20%) showed a tumor response (WHO criteria), with one complete response. The median duration of response was 127 weeks, the median time to progression was 44 weeks, and the median survival was 54 weeks. There was a trend for increased survival in patients receiving more than 104 Gy (the median dose) compared with those receiving doses of 104 Gy or less (p=0.06), and in patients with Okuda stage I disease compared to those with Okuda stage II disease (p=0.07). As reported in this study, the most common side effects were transitory nausea and fatigue. The most common toxicities were transitory elevation of liver enzymes and bilirubin level (1 week), and gastric ulceration due to inadvertent deposition of ^{90}Y microspheres into the gastric vascular bed. One patient consented to treatment after being informed of the risk of excessive lung shunting, which resulted in the patient receiving 56 Gy to the lungs. The patient died of radiation pneumonitis. The results of this study confirmed the findings of the earlier study and suggested that tumor growth could be stabilized with an apparent improvement in survival. The favorable toxicity profile suggested the possibility of performing ^{90}Y therapy on an outpatient basis, particularly since beta radiation does not require medical confinement of patients for radiation protection.

Based on clinical results obtained in the dose-ranging and fixed does studies, the United States Food and Drug Administration (FDA) granted approval to TheraSphere® for use under a Humanitarian Device Exemption (HDE) in 1999. Following approval, a multi-center single arm study was undertaken at three institutions in August 2000. A total of 85 patients presenting with unresectable HCC were treated at doses ranging from 135-150 Gy. The dose increase (from 100 Gy to 135-150 Gy) was based on the trend toward improved survival observed for patients treated at higher doses in the 100 Gy study. The treatment approach for the 135-150 Gy study was also modified to address the gastrointestinal side effects observed in the prior investigations. ^{90}Y microspheres were administered in a lobar manner by placing the delivery catheter in the right or left hepatic arteries distal to the gastroduodenal and right gastric branches. These vessels were coil-embolized in cases where variant anatomy presented any risk of collateral flow to non-target hepatic tumors. Patients were followed to assess treatment response, survival and toxicities up to two years post-treatment.

The data presented here are the combined toxicity and survival data for 108 HCC patients, including 22 patients from the 100 Gy study and 86 patients from the 100-135 Gy study described above. Survival data were adjusted for Okuda stage using Kaplan-Meier product limit analysis. Toxicities were classified according to the Southwestern Oncology Group (SWOG) criteria. The highest toxicity grade (severe, life threatening, or fatal) observed for a given SWOG body system are reported.

Patient Characteristics: The median age was 67 years (range 28-92 yr), with 79 males (75%). The majority of patients were Caucasian (73%); Black (12%), Hispanic (4%) and Asian (11%). Patients presented with risk factors for HCC due to cirrhosis (74%), IV drug use (16%), alcohol abuse (43%), Hepatitis B positive (32%) and Hepatitis C positive (39%). **Toxicities:** Thirty-six (34%) patients experienced at least one liver toxicity of at least grade 3; including elevated bilirubin (n=23 or 22%), ascites (n=9 or 9%), elevated SGOT/SGPT, elevated alkaline phosphatase (n=9 or 9%), hepatic encephalopathy

(n=3 or 3%), liver failure (n=2 or 2%), hepatic decompensation (n=1 or 1%), hepatitis (n=1or 1%), and radiation hepatitis (n=1 or 1%). Other toxicities occurring with less frequency included **pain** (n=12 or 11%), **gastrointestinal events** [ulcer (n=4 or 4%); cholecystitis (n=2 or 2%); nausea (n=2 or 2%)], **neuro (logic/central)** [fatigue (n=2 or 2%); malaise (n=2 or 2%)], **circulatory** [n=3 or 3%], clotting [n=3 or 3%], **lung** [pleural effusion (n=1 or 1%); aspiration pneumonia (n=1 or 1%); radiation pneumonitis (n=1 or 1%)], **miscellaneous** (n=3 or 3%), **death, NOS** (n=2 or 2%) and one event each (1%) of decreased platelets, hemorrhage, NOS, allergic reaction, bacterial sepsis and hepatorenal failure. Due to the complex presentation of HCC, including patients' underlying liver compromise due to cirrhosis, the occurrence of liver toxicities is not unexpected. Liver related events including ascites, liver failure, hepatic encephalopathy or decompensation typically occurred in patients whose functional liver reserve was compromised at the time of treatment (e.g. bilirubin >2.0 mg/dL; albumin <3.0 mg/dL; infiltrative tumor; or tumors representing at least 70% of liver volume). Post-treatment elevations in bilirubin typically occurred in patients treated with a lobar approach during the 100-135 Gy study. In many cases, patients presented with bi-lobar disease. The one case of radiation hepatitis attributed to ^{90}Y microsphere treatment could not be confirmed pathologically, but could not be ruled out. Transitory post-treatment elevations in liver function tests (transaminases & alkaline phosphatase) were likely due to radiation effect on tumor, and resolved without medical intervention. Serious gastrointestinal events, including gastric and duodenal ulceration (n=4 or 4.0%) all occurred during the 100 Gy study, when the delivery catheter was placed in the proper hepatic artery. No serious GI complications were observed during the 100-135 Gy study, when the delivery catheter was placed distal to collateral vessels. Two cases of cholecystitis reported during the 100-135 Gy study were associated with the development of gallstones. The one case of fatal radiation pneumonitis was due to a patient receiving 56 Gy to the lungs, which was predicted by Tc 99m MAA scanning prior to treatment.

Tumor Response and Survival: Of 50 patients receiving ^{90}Y microsphere treatment for whom tumor response data are available, the majority (70%) exhibited stable disease for a median of 10 months. Median survivals from treatment for Okuda Stages I and II patients were 15 months (95% CI=11-32 months) and 8 months (95% CI= 5-13 months), respectively. Corresponding one year survivals were 63% and 39%, respectively.

Conclusions: TheraSphere® treatment represents a promising new therapy in the armamentarium against primary liver cancer. It can deliver high doses of beta radiation to liver tumors while limiting exposure to surrounding liver parenchyma. The survival data presented here for treating unresectable HCC are encouraging when compared to survival expected with other treatment modalites. Based on TheraSphere®'s lower toxicity profile and encouraging survival, this treatment appears to offer enhanced clinical benefit to patients with this difficult to treat condition.

IMRT and Image-Guided Targeting

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External beam radiation therapy for hepatocellular carcinoma as primary treatment modality or as adjuvant treatment has been relatively uncommon in the US. Growing evidence from predominantly Asian institutions suggests that EBRT may produce objective partial and complete tumor response in up to two-thirds of patients treated. Tumor response appears to be related with radiation dose delivered, with doses in excess of 50 Gy yielding promise for further improvements of tumor response rates. Doses higher than 70 Gy have resulted in median survival rates approaching those of curative surgical resection. The limited radiation tolerance of the normal liver tissue necessitates three-dimensional conformal radiation therapy (3D-CRT) techniques to enable delivery of these high radiation doses.

Intensity-modulated radiotherapy with associated inverse radiation treatment planning is the most recent technical advance in external beam photon radiation therapy. The ability to break a "large" radiation field into smaller field segments or pencil beams of radiation and to modulate the radiation intensity over a target volume with added capabilities to predefine the allowed radiation exposure of normal liver tissue and other organs at risk has enabled the delivery of high radiation doses while maintaining or even reducing the risk for normal liver toxicity.

Radiation target volumes for HCC radiotherapy treatment are defined as gross tumor volume (GTV), clinical target volume (CTV) which encompasses the GTV with additional margins to include assumed subclincial tumor extent and/or nodal drainage areas and a planning target volume (PTV), adding normal tissue margins accounting for inter-fraction patient and target setup uncertainties and intra-fraction organ motion. Daily image-guidance to optimize target volume setup on the linear accelerator treatment couch holds promise to

reduce the component of the PTV safety margins accounting for patient and target setup uncertainties. Deep-inspiration breath-hold radiation or respiratory gating techniques may enable further safety margin reduction, limiting the amount of normal liver tissue exposed to potentially harmful radiation doses.

This presentation will review in brief the dose and normal liver volume dependent probability for development of radiation-induced liver disease (RILD), the impact of radiation dose escalation on HCC treatment response and survival, and present techniques to compute IMRT plans using an inverse treatment planning approach and means of static field or tomotherapy IMRT delivery. Also, techniques for image guidance (CT/cone beam based and ultrasound based) will be presented and discussed.

Clinical examples and preliminary outcomes from a series of patients treated at the Dept. of Radiation Oncology, The University of Texas Health Science Center at San Antonio by sequential tomotherapeutic IMRT using daily ultrasound-based image guidance will be presented. Also, IMRT delivery under stereotactic conditions to treat small HCC nodules in few high-dose radiation fractions (stereotactic body radiation therapy, SBRT) and respective early outcomes will be presented.

Hepatocellular Carcinoma: Systemic Chemotherapy

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The use of systemic chemotherapy in the management of hepatocellular carcinoma (HCC) is complicated by the fact that most patients have both cancer and underlying liver disease. HCC is often diagnosed in patients with deteriorating liver function who are poor medical risks. When the liver function is still normal or nearly normal, however, there are numerous systemic chemotherapies that may be tried.

Confusing the assessment of the efficacy of chemotherapy is the variable natural histories of patients with HCC. The etiology and extent of underlying liver disease have bearing on patient outcomes. For example, results with a therapy studied in China–where hepatitis B is almost always the etiology of the HCC–may not generalize to patients in Japan, where hepatitis C virus is the dominant underlying etiology for liver cirrhosis. Similarly, there may be substantial inter-patient pharmacokinetic variability amongst patients with HCC and liver dysfunction. And finally, because regional treatments such as chemoembolization are generally offered to the fittest HCC patients, systemic agents are typically studied in the more advanced patients, making outcomes predictably worse.

For these reasons, despite the fact that numerous systemic treatments have been tested in HCC patients, there is no data to support a "standard" systemic chemotherapy. In fact, in the United States, there is NO approved systemic treatment for HCC. The systemic treatments that have been studied inconclusively range from systemic chemotherapy, single agent and combination, to interferons and hormones. Of these, only doxorubicin can be considered a mainstream therapy.

For these reasons, new agents with new mechanisms and perhaps different methods of administration need to be developed and tested in patients with HCC.

Cell Specific Targeting for Gene Therapy of Hepatocellular Carcinoma

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Primary hepatocellular carcinoma (HCC) is a common tumor worldwide with a dismal five year survival rate of less than 5 percent. The goals of our research are to develop a high affinity and high stability antibodies and fragments thereof for targeting tumor specific antigens in an attempt to develop new therapeutic agents. Tumor-associated antigens are excellent targets for drug and gene delivery, and offers the advantage of high cellular specificity. We have focused on the use of a monoclonal antibody (mAb) AF-20 raised against a human hepatoma cell line (FOCUS). This antibody binds to a 180 kDa homodimeric cell surface glycoprotein with an apparent association constant (KD) of about 1.4 x 10^{-9} molar. The antigen is uniformly expressed in HCC derived cell lines and human tumors including those with distant metastasis. There is minimal expression in non-tumor tissues, and none detectable in normal liver. Because the AF-20 antigen antibody interactions on the cell surface is rapidly internalized at 37°, there is an opportunity to deliver cytotoxic agents to tumor cells and not adjacent un-involved liver with high specificity. In this research, we have created high affinity single-chain monoclonal antibody fragments (scFv) using a novel yeast display system. In addition, we have prepared a "humanized" intact AF-20 mAb for gene targeting of HCC both in vitro and in vivo using animal model systems. Three types of antitumor agents were targeted: 1) adenovirus containing the E. coli purine nucleoside phosphorylase (PNP/fludarabine) suicidal gene system, 2) methotrexate conjugated directly to the chimeric mAb and 3) high affinity scFv antibody fragments linked as a fusion protein to gelonin, a bacterial-derived glycoprotein that inactivates 28S ribosomal subunits but lacks a cell surface binding domain. Surprisingly, the scFv AF-20 mAb was internalized although more slowly than the intact mAb, and capable of specific delivery of nanoparticles to HCC cells. The AF-20 scFv immunotoxin gelonin conjugate was also internalized and substan-

tially improved HCC cell killing compared to gelonin alone. A "human" chimerization of the AF-20 antibody did not affect its ability to become rapidly internalized. In this context, methotrexate alone, which had no effect on HCC cell killing at any concentration employed, showed delayed, but striking, anti-tumor effects at 144 hrs at low μM concentrations. Finally, specific targeting by AF-20 of adenovirus containing the PNP suicide gene in vivo using a nude mouse model exhibited substantial reduction in growth of established tumors. These studies demonstrate that it is possible to generate high affinity scFv antibody fragments of AF-20 as well as 'humanized' chimeric constructs that will allow specific targeting of adenoviruses containing suicide genes, chemotherapeutic agents such as methotrexate and cytotoxic bacterial derived peptides to produce enhanced antitumor effects both in vitro and in vivo. These studies suggest that specific antibody targeting of cytotoxic "payloads" to tumor cells has the potential for therapeutic application in this devastating disease.

Immunotherapy for HCC

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The only potentially curative treatments for HCC are surgery and liver transplantation, however, only a minority of patients are eligible for these treatments. Therefore, there is a continuing need for innovative, alternative strategies to impact this disease. Immunotherapy of cancer is attractive because of the exquisite specificity of the immune response and the power of the immune system to eliminate infection from anywhere in the body. The goal of immunotherapy for HCC is to activate an HCC-specific immune response targeting the differences between healthy liver and HCC. This can be accomplished by activating an immune response to previously identified and characterized tumor-associated antigens (for example: AFP, MAGE-A family, NY-ESO). Recent gene array studies may quickly and efficiently add to the current list of HCC specific gene products which can be targeted. Alternatively, the immune response can be targeted against hepatitis viral antigens in those patients infected with HBV or HCV, although such an immune response would target any infected cell, not only those cells which progressed to HCC. Uncharacterized antigens can also be targeted with whole tumor cell or tumor lysate-based immunization strategies or with vectors coding for genes which make the tumor more immunogenic. Lastly, the immune system can be activated in a non-specific way, allowing the immune system to naturally evolve specificity against the most immunogenic target antigens expressed by the tumor. Many strategies for activating the immune system exist. Recent animal data supports activation of a CD8 killer T cell response as being central to antitumor effects in the majority of models and tumor types.

Strategies currently being investigated in animal models include: stimulating an immune response by targeting cytokines or costimulatory molecules to tumor; blocking FasL activity; immunizing with activated B cells fused to tumor cells (Guo ’94); immunization with

tumor lysate-pulsed DC; adoptive transfer of viral antigen-specific T cells; and targeting AFP expressing HCC cells by DNA immunization, plasmid prime/adenovirus boost, peptide in adjuvant and dendritic cell strategies. Many of these strategies have shown potent antitumor effects in terms of protection of mice from a challenge with a model HCC tumor. Fewer have shown efficacy against previously established tumor or in orthotopic models.

Strategies which have progressed to human clinical trials include: adoptive transfer of IL-2-activated or IL-2+anti-CD3-activated lymphocytes (Takayama, '00); autologous tumor-pulsed DC (Iwashita, '03) as well as AFP-based strategies: AFP-derived peptides in Montanide (Butterfield '03), and AFP peptides pulsed onto autologous DC. These trials, testing novel immune-based interventions in HCC subjects, have resulted in positive immunological and clinical responses.

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The Potential of Non-Invasive Thermal Ablation of Hepatocellular Carcinoma with MRI-guided Focused Ultrasound

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The therapeutic use of acoustic energy has been investigated for a long time. It has been known several decades ago that focused ultrasound (FUS) beams can be applied as an ablative surgical technique to treat deep lying tumors. Unlike thermal energy deposition by radiofrequency, laser and cryoprobes FUS sonications last only a few seconds with a relatively narrow temperature gradient that peaks between 60-80 ^{C}o. The sharp temperature gradients result in less variable thermal effects in the tissue and the rapid deposition of thermal energy leads to a peak temperature rise that is independent of cooling by blood flow. This is especially important for treating tumors in which the vascular pattern is unpredictable

Without temperature sensitive imaging virtually impossible to predict the location of the focused ultrasound beam, to monitor the temperature changes and to control the deposited thermal dose. In the past, these major constraints held back the development of focused ultrasound as a non-invasive surgical technique. In recent years, integration of FUS with magnetic resonance imaging (MRI) that allows high sensitivity tumor detection and ability to monitor the temperature in real time, has renewed the interest in FUS ablative therapy and opened the way for several, potentially groundbreaking clinical application[1]. The integration of FUS with MRI resulted in a non-invasive therapy delivery system that is used for planning, guiding, monitoring and controlling the therapy for a wide range of applications in the body. Currently clinical trials were completed for the treatment of breast fibroadenoma, breast cancer and uterine fibroid. These successful applications and large number of systematic studies with experimental animal tumor models clearly suggest that MRI-guided FUS has significant potential. Among the promising future applications the non-invasive thermal ablation of hepatocellular carcinoma is particularly exciting.

There have been several experimental and clinical investigations of non-invasive FUS treatment of liver tumors. In animals both tumor bearing and normal liver lobes were treated with high intensity focused beam ultrasound and histopathologic changes consistently showed sharply demarcated homogeneous coagulative necrosis with an irreversible tumor cell death and severe damage to tumor blood vessels at the level of microvasculature within the targeted region. Currently clinical trials were reported with somewhat encouraging results using only diagnostic ultrasound targeting without temperature monitoring[2,3]. Handheld FUS probes are being developed for the intraoperative treatment of liver cancer during an open procedure.

Using MRI based temperature monitoring a 256-element, continuous-wave large scale ultrasonic phased array has been used to thermally coagulate deep-seated liver tissue[4]. Focal lesion volumes greater than 0.5 cm3 in kidney and 2 cm3 in liver were formed from a single 20-s sonication. Most MRI monitoring for temperature in the liver has been done with T1 In the last couple of years, there have been suggestions testing water proton resonance frequency shift -based temperature measurements in liver[5]. This technique is very sensitive to organ motion and requires relatively long breathholding and/or general anesthesia with respiratory pauses. More recently McDannold tested a temperature-activated contrast agent[6].

Experimental work on pigs using the fully integrated commercial MRI-guided FUS system (ExAblate 2000, Insightec Ltd., Israel Haifa) demonstrated that MRI-guided FUS appears to provide safe treatment of liver tumors. The increased accuracy of treatment with thermal mapping combined with the cost savings of ambulatory treatment may lead to significant changes in the treatment of these common malignant tumors.

MR-guided FUS appears to provide targeted destruction in breast tumors and uterine leiomyomas with an excellent safety profile. FUS is the first potential non-invasive surgical therapy for hepatocellular cancer. The prevalence of these tumors and the significant impairment in quality of life associated with them, suggests that the future of liver tumor treatment may be very different if effective non-invasive therapy is possible. The MRI-based thermal mapping not only enhances safety but also should give enhanced efficacy of thermal ablations. Further studies will be important to document the feasibility and cost effectiveness of this new therapy modality.

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Research support has been provided by Insightech Ltd.

Surgical Resection for Hepatocellular Carcinoma

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Hepatocellular carcinoma is one of the most common malignancies worldwide, resulting in death of nearly one million individuals yearly. In patients with no associated cirrhosis, surgical resection can be performed with operative mortalities well less than five percent and result in long-term survival and potential cure in one-third of patients with resected tumor. In patients with associated cirrhosis, operative mortality as recently as a decade ago was reported to be as high as 10-20%.

In recent years, the safety and long-term results of surgical resection of cancers in cirrhotics have improved tremendously. This is due both to improvements in patient selection, as well as improvements in anesthetic and surgical techniques. Advances in methods of assessing functional hepatic capacity has helped in selecting patients who will tolerate such procedures. Advances in radiologic imaging allow for precise staging of patients and detailed technical planning of the surgical procedure. Pre-operative portal vein embolization is now used in many centers to enhance growth of the liver prior to resection to improve perioperative recovery.

A number of major clinical questions are under active investigation. In parallel with the improvements in outcome of hepatic resection for cancer, there have also been major recent improvements in ablative therapy for hepatic cancers and in liver transplantation, The most immediately relevant clinical investigative efforts are those attempting to define the relative efficacies of surgical resection of cancer versus ablation of tumors. Also increasingly relevant is the debate regarding the relative merits of resection and liver transplantation in the treatment of cancer.

Active investigation is also ongoing to find an effective adjuvant therapy for liver cancer that often recur after surgical resection. Studies are sorely needed to define the optimal follow-up strategy after treatment of liver malignancies, including the choice of serum markers and radiologic examinations.

Many interesting biologic questions also deserve consideration. What is the effect of liver growth on hepatitis viral proliferation? Will control of inflammation and viral proliferation post-operatively improve recovery and decrease subsequent recurrence?

Liver Transplantation for Hepatocellular Cancer: The Impact of the MELD Allocation Policy

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The incidence of hepatocellular cancer (HCC) in North America has increased from 1.4 to 2.4 cases per 100,000 between 1976 to 1995. During this same period, HCC related mortality increased 41% in part related to the increase incidence of hepatitis C, which is predicted to further increase during the next decade as the HCV epidemic evolves. Furthermore, recent data demonstrating a five-year survival rate of greater than 75% in select HCC patients (stage 1-2 disease) undergoing liver transplant, has added new enthusiasm as an option for these patients. This new demand has put an additional strain on our all ready short supply of donor liver organs. Indeed, up to 45% of patients on the UNOS liver transplant list wait for over two years before an acceptable deceased donor can be found. Not unexpectedly, this event have led to increased waiting list mortality or in the case of patients with HCC, has led to the development of a non-transplantable condition (beyond stage 2). These recent developments have focused on the need to improve prioritization for the allocation for deceased donor livers, which is particularly pertinent to the HCC patient. Several studies have documented that HCC patients with stage 2 disease have approximately a 50% chance of becoming non-transplantable (advanced beyond stage 2) after 12 months on the waiting list. Thus the increasing shortage of donor organs, increased waiting time, and increased number of patients dropping out from the waiting list because of death/too sick, have recently focused our attention on the need to improve our liver allocation policy, particularly as it pertains to HCC patients.

The previous allocation policy was based on the Child-Turcotte-Pugh (CTP) score and categorized patients with chronic liver disease in the three subgroups. In this system waiting time became the major determinant for liver allocation. The model for end stage liver disease (MELD) has since emerged as a useful tool for estimating mortality

in patients with chronic liver disease and cirrhosis. Originally, MELD was developed to predict the outcome of patients undergoing a TIPS procedure. However, more recently, MELD has been validated both retrospectively and prospectively, as an accurate predictor of mortality for patients with end stage liver disease. Indeed, MELD is able to reasonably rank order patients based on the risk of death over time.

MELD is based on three biochemical variables, namely serum bilirubin, serum creatinine, and the international ratios of prothrombin time, which all are standardized biochemical tests that are readily available and reproducible throughout the country. MELD is independent of liver disease etiology as well as specific complications of portal hypertension such as ascites, variceal bleeding, encephalopathy, and spontaneous bacterial peritonitis. Therefore, these factors do not have to be added to the model to estimate survival. Furthermore, MELD has been found to be superior to the CTP score for predicting short- and medium-term survival. The quandary for patients with HCC is that they often have low MELD scores at a time the diagnosis of HCC is made despite the fatal nature of their disease. Thus the end point for patients with HCC is time to becoming non-transplantable rather than time to death. To rectify this dilemma, patients with HCC and cirrhosis were arbitrarily assigned a MELD score thought to be sufficient to provide adequate timing of liver transplantation. While precious little data was available to support this conclusion, additional data was to be collected as part of the new allocation policy to allow more appropriate prioritization of HCC patients in the future. For patients with HCC, tumor progression beyond stage-2 disease was to be equated with death on the liver transplant waiting list since such patients would lose their additional MELD points and would not receive priority in the UNOS allocation scheme. The goal is to equate the risk of tumor progression beyond stage-2 with the risk of death in non-HCC patients with chronic liver disease on the UNOS waiting list over the same period of time.

Initially, stage-1 tumors (≤ 2 cm) were assigned a MELD score of 24 equal to a 15% probability of becoming non-transplantable within three months. Patients with stage-2 disease (1 lesion >2 cm and ≤ 5

cm or three lesions all ≤ 3 cm) were assigned a priority score of 29 equal to a 30% chance of becoming non-transplantable in a three-month period of time. These estimates were based on tumor doubling time reported by Barber, et al. In addition, after each three-month period of waiting time a 10% increase in the probability of becoming non-transplantable was assigned to HCC patients until the patient was transplanted, became a non-candidate, or died.

Initial results of the new MELD policy indicated an increase in the number of HCC patients undergoing liver transplantation from 167 patients in the year pre-MELD initiation to 408 patients in the year post-MELD ($P<0.001$). In addition, the rate of deceased donor liver transplantation increased from 0.439 per person year in the pre-MELD era to 1.454 per person year in the post-MELD era. Furthermore, the time to deceased donor liver transplant decreased from 2.28 years in the pre-MELD era to 0.69 years in the post-MELD era ($P<0.001$). Additional findings included the five-month waiting list dropout decreased from 16.5% in the pre-MELD era to 8.5% in the post-MELD era ($P<0.001$). This equated with an increase in the five-month candidate transplantable survival rate on the UNOS list, from 90.3% in the pre-MELD era to 95.7% in the post-MELD era. The five-month patient survival following deceased donor liver transplantation was unchanged at 89% in both the pre-and post-transplant MELD eras.

In the MELD era, 86% of T-1 lesions and 91% of T-2 lesions were transplanted within ninety days of entering the UNOS waiting list. Center-specific data revealed that the dropout rate on the waiting list for patients with T-1 lesions was less than 10% at one year. However, patients with T-2 lesions had a 50% dropout rate indicating that T-2 lesions should be given priority. One-year data also indicated that the number of patients with HCC, who became non-transplantable, was significantly less than the number of patients dying on the waiting list with a similar MELD score. Because of these findings, a modification was made in April 2003, at which time the T-1 lesions was given 20 MELD points or 8% chance of becoming non-transplantable within three months; and in T-2 lesions, the MELD score was reduced to

24% or a 15% chance of becoming non-transplantable within three months. A 10% increase in the probability of becoming non-transplantable at three-month intervals on the waiting list was maintained. The impact of the total percent of patients transplanted for HCC increased from 8% in the pre-MELD era to 21% in the first year post-MELD, to presently 14% with the modifications made last April.

In assessing explant pathology, one of the major findings was that over 30% of patients undergoing liver transplant for stage-1 lesions were misdiagnosed on transplant assessment with no evidence of a tumor being found in the explanted liver. In patients diagnosed with stage-2 tumors, 10% were without evidence of tumor on explant. On the basis of these findings, a more recent recommendation has been made that the T-1 lesions do not receive any additional priority; and that the T-2 lesions continue to get 24 MELD points or a 15% chance of becoming non-transplantable within three months with the addition of a 10% probability at three-month intervals. Presently this proposal is out for public comment and will be reviewed by the UNOS Board.

In summary, the MELD allocation has had a marked advantage for HCC patients by increasing the numbers of HCC patients transplanted and decreasing the waiting time for deceased donor transplant. The allocation system has also helped in better defining the natural history of both T-1 and T-2 lesions so that in the future appropriate priority can be given to these patients. While the initial survival rates at one year remain excellent for HCC patients and are similar to patients which chronic liver disease, questions remain. One lingering question is did waiting time select out more biologically favorable tumors and will we now have an increase in the recurrence rate of HCC using our present system? Clearly, 3 to 4 years of data will be needed before this can be fairly assessed. In the end, equitable liver allocation continues to be an evolving process for all diagnoses based on improved understanding of the natural progression of all liver diseases. We believe that there will be continued modifications in the MELD system to reach this goal.

Continued

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Liver Transplantation for Hepatocellular Carcinoma

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The preferred therapy for hepatocellular carcinoma (HCC) apparently confined to the liver is surgical removal of the tumor. When tumor characteristics and hepatic reserve are such that resection cannot be safely performed, liver transplantation becomes a consideration. Transplantation is appealing in this setting, since removing the entire liver eliminates cirrhosis and occult intrahepatic metastases, and prevents future de novo HCC's. Applicability of transplantation, however, is limited by the shortage of donor organs. Much work has thus focused on identifying those patients with HCC who without transplant have a poor outlook, but with transplantation have outcomes similar to those achievable in transplant candidates without HCC. Tumor characteristics including large diameter, multiple nodules, vascular invasion, and poor differentiation are associated with increased risk of tumor recurrence after transplant despite the absence of detectable extrahepatic disease. Mazzaferro et al in 1996 published in the New England Journal of Medicine a series of patients with either a single HCC ≤ 5cm, or 2-3 nodules all ≤ 3cm, and no detectable vascular invasion, who were transplanted and achieved 4-year survival of 75%. Current clinical practice in much of the world has adopted these criteria as the basis for organ allocation in patients with HCC, restricting access to transplantation either by outright denial of candidacy or by failing to grant priority sufficient to receive a donor organ to patients with tumors beyond these limits. In the U.S., despite the priority granted to patients with qualifying tumors, the waiting time for a liver may be quite long. Prevention of tumor progression while waiting has thus become an important aspect of pretransplant HCC management. Chemoembolization, ethanol injection, and radiofrequency ablation are the most commonly employed modalities in this setting. An important aspect of the overall problem facing this patient population in the U.S. is the fact that, in most cases, hepatitis C is the underlying liver disease; as the

result of recurrent hepatitis C, by 5 years after transplant 25% of patients once again have cirrhosis, and overall survival is significantly diminished independent of tumor recurrence. Current areas of controversy concerning transplantation for HCC include whether to expand eligibility/priority criteria, how to equitably prioritize those who qualify, and whether patients with HCC that initially exceeded criteria but that as a result of treatment has been downstaged to the acceptable limits should be accorded priority. Ongoing developments in imaging that permit more precise staging, and in molecular characterization that predict biological behavior, will allow for continual refinement in the selection of HCC patients for transplantation so that the maximum number who may be helped will be, while those destined to recur after transplant will be spared that unnecessary and very costly ordeal.

Living Donor Liver Transplantation for Hepatocellular Carcinoma

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The incidence of hepatocellular carcinoma (HCC) continues to increase at an alarming rate. The role of liver transplantation in patients with HCC has continued to evolve over the last 15 years as the medical community and governmental agencies have struggled with the issue of equity versus utility of transplantation in this patient population. Recent studies showed that carefully selected patients with HCC could be successfully transplanted with a survival rate equivalent to patients transplanted without HCC (75% survival at 4 yrs) and less than a 10% risk of recurrence. In 1996, the Mazzaferro (Milan) criteria (a single lesion <5 cm or 3 lesions <3 cm, without gross vascular invasion) became adopted by the United Network of Organ Sharing (UNOS) and the majority of transplantation centers as the benchmark for which patients with HCC were deemed eligible candidates for OLT. The decision to provide Medicare coverage to patients with unresectable HCC who were within Milan criteria later followed and was implemented on September 1, 2001.

The first successful adult-to-adult LDLT was performed in 1998. The advantage of LDLT for patients with HCC is the lack of the lengthy waiting period that traditionally accompanies deceased donor transplantation and subsequently, a decline in the dropout rate due to disease progression. In the "pre-MELD era" there have been two decision analysis studies examining the utility and cost effectiveness of LDLT in patients with HCC. They concluded that LDLT conferred a substantial survival advantage in patients with early stage HCC (3.5 cm) who would otherwise have waited over 7 months for a deceased donor liver transplant (DDLT). LDLT has also become a chance for cure in patients who fall outside the Milan criteria and therefore are not considered candidates for DDLT.

The Model for End- Stage Liver Disease (MELD) adopted on February 27, 2002 further changed the allocation of organs, with a significant advantage given to patients with T1 (MELD 24) and T2 (MELD 29) lesions, who otherwise would not have been transplanted on the basis of synthetic dysfunction alone. This had a profound effect on the number of transplants performed for the indication of HCC and also made LDLT less warranted in patients with a small HCC. In February 2003, the allocation system was changed from 24 and 29 MELD points to 20 and 24 MELD points awarded to patients with T1 and T2 lesions, respectively. More recently, it was decided to do away with the MELD advantage for patients with T1 lesions. The downgrading of patients with HCC may make LDLT a more justifiable procedure in the future, especially as the number of patients with HCC steadily rises. Also there is increasing data to support the superiority of transplant over resection in patients with small tumors.

Currently there is no consensus on the use of LDLT for HCC. The data regarding LDLT in patients with HCC is limited to a few reports from single centers. One such recent report was quite promising. Despite over half of the patients having tumors outside the Milan criteria, no significant differences in survival or recurrence rate have been seen when compared with deceased donor liver transplant (DDLT). While longer follow up periods are required in a larger cohort of patients, this data does supports the use of LDLT in patients with HCC. The National Institutes of Health having recognized the need to accrue outcomes of a large, diverse population of donors and recipients undergoing LDLT, created the "Adult-to-Adult Living Donor Transplant Cohort Study" (A2ALL). The goal is the development of an adequately powered study to generate meaningful guidelines for the use of LDLT, including HCC as an indication for LDLT.

At Northwestern Memorial Hospital, we have noted an increase in recurrence when evaluated stage for stage in patients who have undergone "fast tracking" to transplant, defined as LDLT, partial liver transplants (splits), domino liver transplant, or MELD upgrade for HCC (unpublished data). We hypothesis that a subset of "fast tracked" patients with a more biologically aggressive tumor, who normally would drop off the list due to tumor progression, may not be allowed adequate time for the tumor to declare itself prior to LDLT. Due to the enormous implications this may have on LDLT for HCC, this warrants further study.

The role of LDLT in patients with HCC will continue to evolve as our experience in large trials, such as A2ALL, provides a sizeable cohort of patients to be studied. Clinical trials are needed to gain a better knowledge of the biological behavior of HCC on an individual basis. It is evident that the current exclusion of patients from transplant based on size and number of tumors alone is inadequate. The use of neoadjuvant therapy in patients scheduled for LDLT and its effect, if any, on the biological nature of tumors is also an area to be explored. LDLT for HCC greatly increases the access to transplant amongst patients who may be ineligible (based on size and/or number of tumors) for DDLT and/or become so while awaiting transplant. However, the utility of this approach becomes crucial due to legitimate concerns for the safety and well being of the donor. Reliable and reproducible predictors of survival and recurrence are essential to appropriately balance these two entities.

Hepatocellular Carcinoma in the Woodchuck Model of Hepatitis B Virus Infection

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Experimental Woodchuck Hepatitis Virus Infection. The woodchuck hepatitis virus (WHV) was described originally by Summers and colleagues in a colony of woodchucks (*Marmota monax)* maintained at the Philadelphia Zoological Garden that had experienced high rates of chronic hepatitis and HCC.[1] It was concluded that WHV belonged to the hepatitis B virus (HBV) family of viruses and the woodchuck hepatitis virus (WHV) is now classified with HBV as a member of the family Hepadnaviridae, genus *Orthohepadnavirus*.[2] To exploit the woodchuck as an experimental animal model, a breeding colony of woodchucks was established at Cornell University which serves as a source of experimental woodchucks for studies of the pathogenesis WHV infection, for preclinical antiviral drug development, and for studies of hepatocarcinogenesis.

Woodchucks born in the colony are inoculated at birth with diluted serum from standardized infectious pools obtained from chronic, WHV-carrier woodchucks. The rate of chronic WHV infection following neonatal inoculation is 60% or higher. Kaplan-Meier survival analyses have been performed comparing chronic WHV carriers, woodchucks in which neonatal WHV infection was resolved (WH viremia cleared and anti-WHs antibody detected), and control woodchucks born and raised under similar laboratory conditions but not infected with WHV. All WHV carriers were dead by 56 months of age, and the lifetime risk of HCC was 100%. In contrast, 42% of the woodchucks with resolved WHV infection and 62% of uninfected controls were alive after 56 months. Although the rate of HCC was significantly higher in chronic WHV carriers, HCC developed in 17% of woodchucks in which neonatal WHV infection resolved. HCC was not observed in the uninfected, laboratory-reared, control woodchucks of this study and HCC is rare in woodchucks unrelated to WHV infection. The rate of HCC in woodchucks with experimentally

induced, chronic WHV infection was similar to that observed in woodchucks with naturally acquired chronic WHV infection, and the presence of preneoplastic foci of altered hepatocytes with progressive aneuploid change also was similar. These results provide direct experimental evidence for the carcinogenicity of WHV and, by analogy, for other hepadnaviruses (HBV, California ground squirrel hepatitis virus [GSHV], and arctic ground squirrel hepatitis virus [AGSHV]) in which naturally acquired infection has been associated with HCC.[2]

Histogenesis of Experimental HCC. HCC generally is recognized as a multistage process. In rodent models of chemically induced HCC, microscopic foci of phenotypically altered hepatocytes (FAH) precede development of adenoma and/or HCC. Such FAH also are characteristic of chronic WHV infection. Dysplastic changes in hepatocytes have been described in humans with chronic HBV infection and in some cases have been considered to be precancerous in nature. More recently, FAH have been described in the livers of patients undergoing liver transplantation for chronic, end stage viral hepatitis or HCC and are essentially identical to those caused by chemical hepatocarcinogens in rats and mice and that are characteristic of chronic WHV infection. The earliest detection of FAH in chronic WHV carriers was at six months of age. By 9-10 months, 50 % had such lesions and thereafter, almost all the livers of chronic WHV carriers contained FAH.

Integrated hepadnaviral nucleic acid sequences have been demonstrated in the cellular DNA of most hepatic tumors from individuals infected with HBV and in woodchucks chronically infected with WHV suggesting a direct molecular role of hepadnaviruses in hepatocarcingenesis. Integration of hepadnaviral nucleic acid sequences is considered to be a critical mutagenic event that results in alteration of the expression of cellular regulatory genes (protooncogenes, tumor suppressor genes) and ultimately in the neoplastic transformation of hepatocytes. Buendia and her colleagues demonstrated that N-myc mRNA was over expressed in 60% of woodchuck HCCs they examined, and this transcript was not detectable in normal woodchuck liver. Woodchucks were found to have two N-myc loci. One N-myc locus was homologous to other mammalian N-myc genes. The other was an

intronless gene with the characteristic structure of a retrotransposon and was called N-myc2. N-myc2 has been mapped to the X chromosome. Expression is highly restricted, and the brain is the only normal woodchuck tissue in which N-myc2 RNA has been detected.

We have examined 55 hepatocellular neoplasms and matched non-tumorous hepatic tissue from 13 chronic WHV-carriers and the frequency of WHV DNA integrations and of N-myc rearrangements compared in tumors of different size and histologic grade. Fifty-one of the tumors were classified histologically as HCC. Seven grade 1 HCCs contained WHV DNA integrations in 43% but none had N-myc rearrangements. Twenty grade 2 HCCs had WHV DNA integrations in 80% and in 38% N-myc rearrangements were present. In 24 grade 3 HCCs, integrations of WHV DNA were detected in 79% and N-myc rearrangements in 74%. In two other grade 3 HCCs, rearrangements of N-myc were recognized in the absence of detectable WHV DNA integrations. The 12 largest tumors in the series all were grade 2 or 3 HCCs, and in 83%, both WHV DNA integrations and N-myc rearrangements were demonstrated. The proliferative stimulus and/or other growth advantage apparently provided by the molecular alterations suggested their direct etiologic role in viral hepatocarcinogenesis.

Chemoprevention of Experimental HCC. Three long-term, chemoprevention studies have been performed in chronic WHV carriers with antiviral nucleosides. The first was a life time study initiated when the woodchucks were 8 months of age. Twenty carriers were treated with lamivudine (5 mg then 15 mg/kg/ day) and 20 carrier controls were treated with placebo. Serum WHV DNA decreased by 4 to 5 logs in lamivudine treated woodchucks and the antiviral effect was sustained for approximately 1 year. Thereafter, recrudescence of viral replication was detected that was associated with mutations of the WHV polymerase B domain gene. There was a significant delay in the development of HCC in lamivudine treated woodchucks and a corresponding increase in survival. The median time to death in placebo treated controls was 32 months and in lamivudine treated woodchucks 44 months (p=0.01).

In a second study reported by Colonno, et al,[4] entecavir, a guanosine nucleoside analogue with potent antiviral activity against WHV and HBV, was used to assess the influence of long term suppression of viral replication on hepatocarcinogenesis in woodchucks. Beginning at 8 months of age, WHV carriers were given entecavir orally (0.5 mg/kg/day) for 8 weeks then weekly for 12 months. Drug then was withdrawn from 6 woodchucks, 3 of which had a sustained antiviral response and developed no evidence of HCC during the next 2 years. Weekly treatment was continued in 5 of the woodchucks for an additional two years. At 4 years of age, there was no evidence of HCC in 4 of the 5 (80% HCC free survival). In historical WHV carrier controls, the HCC free survival rate at 4 years of age was 4%. Hepatic expression of viral antigens and covalently closed circular WHV DNA levels were significantly reduced in entecavir treated woodchucks. Entecavir significantly delayed development of HCC and prolonged the life of treated woodchucks compared to historical controls ($p<0.001$).

In the third study reported by Menne, et al,[5] 11 one year-old woodchucks were treated for 32 weeks with the highly potent nucleoside, clevudine (L-FMAU, 1-(2-fluoro-5-methyl-beta-L-arabinofuranosyl)-uracil, 10 mg/kg day) and 10 received placebo. Half of the L-FMAU treated woodchucks and half of the placebo recipients then received 4 doses of alum adsorbed, WHV surface antigen vaccine during the next 16 weeks. Vaccination alone elicited low-level antibody to WHsAg in most carriers but did not affect serum WHV DNA, serum WHsAg or liver enzyme responses. Carriers treated first with clevudine to reduce serum WHV DNA and WHsAg and then vaccinated developed a robust anti-WHs response and normalized liver enzymes. Following vaccination, WHsAg-specific cell-mediated immunity (CMI) was demonstrated in both vaccinated groups, but was significantly enhanced in carriers treated initially with clevudine, and was broadened to include WHV core antigen (WHcAg) and selected peptide epitopes of WHcAg and WHsAg. It was concluded that vaccination with WHsAg following

treatment with clevudine disrupted virus-specific humoral and cell-mediated immune tolerance in chronic WHV infection and enhanced the immune response profiles beyond those seen with either drug or vaccine monotherapies. Clevudine-vaccine combination therapy resulted in immune response profiles that resembled those observed during resolution of acute, experimental infection. Clevudine treatment caused sustained reductions in viral load for a period of more than 18 months following drug withdrawal. HCC development was delayed and survival was increased in both L-FMAU treatment groups compared to controls.

Summary and Conclusions. Woodchucks have been valuable in the studies of the pathogenesis of hepadnaviral infection, for the preclinical development of antiviral drugs, and for investigation of viral hepatocarcinogenesis. The results of the three chemoprevention studies in the woodchuck model demonstrated that prolonged suppression of WHV replication inhibits viral hepatocarcinogenesis and enhances survival. Taken together and by analogy they suggest such therapy should be of value in the clinical management of patients with HBV infection. New types of HCC chemoprevention and therapy[5,6] can be evaluated in this model under controlled, experimental conditions, within a reasonable time frame, and the effects on tumor growth and survival determined. The woodchuck seems to be well suited for experimental studies of combination therapy with the new, highly potent, second and third generation antiviral nucleosides and of nucleoside treatment followed by immunotherapy.

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Prevention of Hepatocellular Carcinoma in Chronic Hepatitis C

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The association of hepatocellular carcinoma, (HCC) with chronic viral hepatitis B or C, has long been recognized, particularly in countries where the prevalence of these chronic viral infections is high. In 1999 a study of three databases indicated that between 1976 and 1995 the incidence of HCC had risen in the USA. This study showed that the incidence of HCC to be highest in black men (6.1/100,000 from 1991-1995) followed by that for white men (2.8/100,000) over the same time period[1]. There was a 41% increase in the mortality rate from HCC and the incidence had increased significantly in the 40-60 year old age group over this time period compared to earlier periods. Although this study did not include information regarding viral hepatitis it is highly likely that these data indicating an increase in the incidence of HCC in the USA is related to a surge in high risk behavior during the 1960's and 70's and thus a question of viral hepatitis when the majority of the study population were aged between 12-49 years of age.

Risk Factors for HCC

Primary prevention strategies are most effective when a specific single etiology is known. However, hepatocarcinogenesis is likely the result of a long-term multi-stage process with the involvement of multiple risk factors. Numerous studies, mostly retrospective, have examined a variety of potential risk factors for HCC in chronic Hepatitis C. For the most part, these risk factors appear to be similar across all populations with a few geographic variations. There are risk factors that cannot be altered such as gender. Worldwide chronic viral hepatitis is a more severe disease in men than women, both in terms of liver disease progression and HCC risk. Similarly in all studies, the risk of HCC increases with age over 50 years and with increasing duration of infection.

Co-infection with Hepatitis B is a risk factor which clearly could be prevented by vaccination. Other potentially preventable or at least reducible risk factors for HCC in patients with chronic Hepatitis C include excessive alcohol consumption, iron overload and diabetes. Two other factors which are potentially preventable through early intervention with antiviral therapy include persistent elevation in serum amino transferase levels and the presence of cirrhosis.

Effect of Antiviral Therapy on HCC Risk

Much debate surrounds the issue of whether or not antiviral therapy affects the rate of HCC in individuals chronically infected with Hepatitis C. Antiviral therapy has been licensed since the early 1990's, hence there are no long-term randomized controlled trials to examine this issue. The next best way to analyze the effect of antiviral therapy on HCC risk is to examine the data which compares the rate of HCC in individuals given antiviral therapy with those individuals, who for whatever reason did not receive antiviral therapy. Whereas early studies from Europe[2,3,4] suggested that standard Interferon monotherapy did not reduce rates of HCC in treated patients with cirrhosis and Hepatitis C, neither did this treatment effect viral clearance. The majority of long-term follow-up studies from Japan have involved many thousands of infected patients and they do indicate a reduction in the incidence of HCC in individuals with significant fibrosis given antiviral therapy but only in those who achieved either a sustained virologic or biochemical response[5,6]. No benefit is reported in non-responders. However, for reasons that are not clear, HCC occurs with much greater frequency, in individuals with chronic Hepatitis C in Japan than in individuals from the Western world. Two meta analyses have been performed to examine the efficacy of antiviral therapy in reducing the incidence of HCC, they have somewhat conflicting results. One suggests there is minimal benefit[7], and the other suggests that a benefit is seen in those who achieve a sustained virological response[8].

As HCC in the West is almost entirely limited to those individuals who have already developed cirrhosis, it would be logical to assume that eradication of Hepatitis C prior to the development of cirrhosis would prevent HCC, But few patients chronically infected with Hepatitis C progress to cirrhosis (only 20-30%) and only 1-4% of those with cirrhosis develop HCC per year, thus most individuals without cirrhosis given antiviral therapy would never have likely developed HCC! Nevertheless, there are multiple benefits of successful antiviral therapy in terms of both reducing co-morbidities and very likely overall mortality at least from subsequent liver failure. Thus, antiviral therapy in individuals with progressive liver disease due to Hepatitis C may be considered part of the preventive strategy for subsequent HCC.

There are also a few reports which suggest that Interferon is effective as secondary prevention in individuals who have undergone resection for HCC.

Other Preventive Strategies

Unfortunately, current antiviral therapies only give rise to achieve a sustained virologic response in about half of those individuals who are able to undergo therapy. Those individuals most at risk for HCC, i.e. older men with cirrhosis, are frequently the very patients who either cannot be given current antiviral therapies because of significant contraindications or who are likely to have a very low sustained virologic response because of their age and severity of underlying liver disease. Thus, it is urgent that other preventative strategies be sought, particularly for this patient population.

Reports on the effects of other biological response modifiers, (other than Interferon) include as primary prevention TJ-9 and Glycyrrhizin. These drugs have been studied both prospectively and retrospectively and they suggest these agents may limit the

development of HCC in patients with cirrhosis, particularly those infected with Hepatitis C[9,10]. These two agents have not been assessed outside Japan. Chemo prevention strategies employing the acyclic retinoid, polyprenoic acid[11] in a prospective randomized control trial suggested that this agent was beneficial in the prevention of a second primary hepatocellular carcinoma following earlier surgical resection of HCC. The advantage of TJ-9 and polyprenoic acid is that they are orally administered whereas Glycyrrhizin requires intravenous infusion.

The Future

There remain many individuals with chronic Hepatitis C at high risk for HCC who have not responded or cannot tolerate current antiviral therapies who are either quite unaware that they are at high risk for HCC or who are aware of the risk, but medical data is insufficient to provide advice as to how to minimize that risk. Once all other modifiable risk factors have been optimized, it is appropriate to consider evaluation, either of another biological response modifier or a chemo preventive agent in the context of multi-center randomized controlled trials.

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Prevention of HCC in Chronic Hepatitis B

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There is strong evidence for an etiological association between chronic HBV infection and HCC. The most effective means of preventing HBV-related HCC is prevention of HBV infection via vaccination. HBV vaccine is the first vaccine that has been demonstrated to prevent cancer. A study of liver cancer among Taiwanese children found that the average annual incidence of HCC in children 6 to 14 years of age declined from 0.70 per 100,000 between 1981 and 1986 to 0.57 between 1986 and 1990, and to 0.36 between 1990 and 1994 ($P<0.01$)[1]. The decrease in incidence of HCC coincided with the implementation of a nationwide hepatitis B vaccination program in Taiwan in July 1984 and an ensuing decline in prevalence of HBsAg from 9.8% in 1984 to 0.7% in 1999 among persons younger than 15 years of age.

For the 300 million persons with chronic HBV infection worldwide, HBV vaccination would not be effective in viral clearance or in preventing HCC. Several factors have been reported to increase the risk of HCC among HBV carriers: male gender, older age (or longer duration of infection), Asian or African race, cirrhosis, family history of HCC, exposure to aflatoxin, alcohol and tobacco, coinfection with HCV and HDV, and more recently viral factors including HBV genotype, core promoter variants, and presence of HBeAg. Apart from environmental agents, most of these risk factors are not reversible. Thus, prevention of HCC in persons with chronic HBV infection is a major challenge.

The mechanisms by which HBV infection causes HCC are unclear. Two pathways have been proposed. One involves chronic inflammation, generation of reactive oxygen species, chromosomal mutations, hepatocyte regeneration, and clonal selection of transformed hepatocytes. The other pathway evokes direct oncogenic potential of HBV

through chromosomal integration or trans-activation of cellular genes. It is likely that both pathways contribute to HBV-related hepatocarcinogenesis. Accordingly, persistence of the virus, a high replication rate, and chronic necroinflammation increase the risk of HCC. Thus, treatment that is effective in eradicating HBV or in sustained suppression of HBV replication and the accompanying necroinflammation may prevent HCC among persons who are infected with HBV.

Using sensitive amplification assays, many studies have demonstrated that HBV DNA persists for decades even among persons who have serological recovery from transient HBV infection. Nevertheless, the incidence of HCC is markedly reduced in persons who have recovered from HBV infection. In the landmark study by Beasley et al., in which 22,707 Taiwanese men were followed for a mean of 8.9 years, the incidence of HCC was significantly lower in immuned persons compared to carriers, 5 versus 495 per 100,000 per year. Another study from Taiwan reported that HCC was not detected in any of the 163 patients with chronic HBV (but no HCV or HDV) infection who had spontaneous HBsAg clearance. Thus, treatment that can result in HBsAg clearance may reduce the incidence of HCC among persons with chronic HBV infection. Long-term follow-up of patients who responded to interferon or lamivudine treatment found that subsequence clearance of HBsAg occurred in 20-70% of Caucasian patients but in less than 5% of Asian patients. Because very few chronic hepatitis B patients who received antiviral therapy clear HBsAg, prevention of HCC using antiviral therapy is effective only if the risk of HCC is also reduced in patients who fail to clear HBsAg but have sustained HBeAg seroconversion or in the case of patients with HBeAg-negative chronic hepatitis, sustained suppression of HBV replication and normalization of aminotransferase levels.

Based on the postulated mechanisms of HBV-related hepatic carcinogenesis, it would seem logical that the risk of HCC is lower in patients with lower levels of HBV replication. However, many studies found that most HBsAg positive patients with HCC are HBeAg negative with undetectable serum HBV DNA using hybridization assays. In these studies, HBeAg and HBV DNA were tested at the time of diagnosis of

HCC, and may not reflect HBV replication levels during the years or decades prior to the detection of HCC. The only study that prospectively evaluated the effect of HBV replication on the risk of HCC included 11,893 Taiwanese men followed for a mean of 8.5 years. HBeAg test result at the time of enrollment was used as a marker of HBV replication. The incidence rate of HCC per 100,000 person-years was 1169 among men who were HBsAg positive and HBeAg positive, 324 for those who were HBsAg positive only, and 39 for those who were HBsAg negative[2]. This study confirmed that among persons with chronic HBV infection, the risk of HCC was increased in those who had higher levels of HBV replication.

Longitudinal follow-up studies of patients with chronic HBV infection showed that patients who had spontaneous HBeAg seroconversion had reduced risk of cirrhosis and liver-related mortality, but these studies failed to show a significant reduction in incidence of HCC. The negative result may in part be related to small sample size and short duration of follow-up, and the low overall rate of HCC.

Two reports of long-term follow-up of chronic hepatitis B patients, who received interferon therapy, reported a decrease in incidence of HCC. In one study, 67 interferon-treated and 34 untreated HBeAg positive Taiwanese men, who participated in a randomized trial of interferon, were followed for 1-12 years. HCC was detected in 1 (1.5%) of the 67 treated patients and in 4 (12%) untreated patients ($p=0.04$)[3]. In another study, 209 interferon-treated and 195 untreated HBeAg negative Greek patients were followed for 1-14 years; the incidence of HCC was similar in the 2 groups but treated patients with sustained biochemical response had lower incidence of HCC. Because of the slow rate of HCC development, individual studies are unlikely to demonstrate an effect of antiviral treatment or of treatment response on the risk of HCC. However, a meta-analysis of 12 studies with 1187 patients who received interferon and 665 untreated patients followed for a mean of 5 years reported a point estimate of HCC among treated patients to be 1.9% (95% CI, 0.8%-3.0%) and for untreated patients 3.2% (95% CI, 1.8%-4.5%)[4].

The ultimate proof that antiviral therapy can prevent HCC in patients with chronic HBV infection relies on data from prospective randomized controlled clinical trials. Such trials will need to enroll a large number of patients with high risk of HCC followed for an adequate duration. To date, only 1 such trial has been conducted. Preliminary results of this trial were recently presented. In this trial, 651 Asian patients with compensated chronic HBV infection, who were positive for HBeAg and serum HBV DNA (using bDNA assay), with Ishak fibrosis score ≥4 were randomized to receive lamivudine or placebo in a 2:1 ratio[5]. After a median follow-up of 32 months (0-42), HCC was diagnosed in 17 (3.9%) lamivudine-treated patients and 16 (7.4%) placebo controls, hazard ratio 0.49 (95% CI 0.25-0.99) (p=0.047). When the 5 HCC cases in year 1 were excluded, the hazard ratio for the treated group was 0.47 (p=0.052). This elegant trial demonstrated that antiviral therapy can reduce the incidence of HCC in patients with chronic HBV infection. The encouraging results of this trial should stimulate studies using other antiviral agents that are safe for long-term use but with lower risk of drug resistance.

In summary, prevention of HBV-related HCC is best accomplished by preventing HBV infection via vaccination. Concerted efforts among the scientific community, public health officials, and philanthropists are needed to ensure universal childhood HBV vaccination globally. For persons who are already chronically infected, development of more effective antiviral therapies that are affordable and have long-term safety and efficacy with low risk of drug resistance will reduce the incidence of HCC by sustained suppression of HBV replication and reduction of necroinflammatory liver damage. Additional studies are needed to define the optimal timing to initiate therapy, the end-points of treatment, and the subset of patients who are most likely to benefit when the goal of treatment is prevention of HCC rather than short-term virological response.

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Chemoprevention Strategies for HCC

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Unlike many human cancers, the etiology of liver cancer is well understood. Infection with hepatitis viruses coupled with dietary exposure to the fungal toxin, aflatoxin, results in multiplicative increases in risk, and accounts for much of the disease. While primary prevention entailing vaccination against hepatitis viruses and avoidance of aflatoxin exposure is appealing, these strategies will require considerable time and resources to be successful. In the developing world, where the burden of liver cancer is highest, immediate, practical, and economical approaches are mandatory. Thus, targeted chemoprevention may be most appropriate for the current generation of individuals at risk.

Chemoprevention involves the use of natural or synthetic agents to block, retard, or even reverse the carcinogenic process. Two approaches have been evaluated for reducing the body burden of aflatoxins— interceptor molecules and inducers of carcinogen detoxication pathways. Chlorophylls and their water soluble salts (chlorophyllins) are constituents of the human diet and have been found to be effective anti-carcinogens in several animal models. Chlorophyllin is a mixture of sodium-copper salts of chlorophyll that is marketed as an over-the-counter drug for controlling odor and an accelerant in wound healing, and is extensively used as a food additive for coloration. Chlorophyllin can act as an 'interceptor molecule' through the formation of tight molecular complexes with carcinogens such as aflatoxin, thereby diminishing bioavailability by impeding their absorption. In a clinical trial performed in Qidong, China, chlorophyllin consumption at each meal led to an overall 55% reduction in median urinary levels of excreted aflatoxin-DNA adducts compared to placebo[1]. However, supplementation of diets with foods rich in chlorophylls might represent a more practical means of administration.

Activities of enzymes that are involved in the metabolic detoxication of aflatoxin are influenced by nutritional status, age, hormones and exposure to drugs or other xenobiotics. Oltipraz, a drug originally developed for the chemotherapy of schistosomiasis, is an effective inducer of enzymes that detoxify carcinogens (*e.g.*, glutathione S-transferases (GSTs) and UDP-glucuronosyltransferases) and is a potent anticarcinogen in animals. Administration of oltipraz in a placebo-controlled, randomized, double-blind clinical trial also conducted in Qidong resulted in a 2.6-fold increase in the excretion of aflatoxin-mercapturic acid, a detoxication product of the reactive, DNA-damaging intermediate of aflatoxin[2]. However, pharmaceutical-based interventions for populations at highest risk for liver cancer, such as those in Southeast Asia, China and sub-Saharan Africa, might not be a very practical approach to chemoprevention. Drugs like oltipraz are typically expensive and therefore beyond the reach of those who would benefit the most.

Natural products are not *de facto* safer than synthetic agents, although patterns of long-term ingestion of certain food types provide guides for identifying promising compounds or foods themselves. A decrease in risk of HCC is associated with increased yellow-green vegetable consumption, which contain a range of biologically active phytochemicals. Particularly encouraging are the findings that edible plants belonging to the family Cruciferae and genus Brassica (*e.g.*, broccoli, cauliflower, Brussels' sprouts) contain substantial quantities of isothiocyanates-mostly in the form of their glucosinolate precursors. The major isothiocyanate in 3-day old broccoli sprouts, sulforaphane, is an exceedingly potent inducer of protective enzymes and inhibitor of carcinogenesis in rats[3]. Studies of such plant materials in high-risk cohorts for HCC are in progress. In support of this approach, several controlled feeding clinical studies have indicated that botanically defined (but of unknown glucosinolate composition and content) vegetable diets elevate production of detoxication enzymes, such as GSTs.

Another material under investigation is green tea, in particular green tea-derived polyphenols. Green tea polyphenols are highly effective chemopreventive agents in a variety of animal models for different organ sites, including liver. Inverse associations have been observed in humans with green tea consumption and risk of development and time of onset of cancer. A study is underway in Guangxi, China, to evaluate the modulating effects of green tea polyphenols on aflatoxin and oxidative stress biomarkers. Reductions in levels of oxidative stress biomarkers have been observed in smokers consuming green tea. As results from food and beverage-based trials are compiled, it may be possible to combine foodstuffs to accomplish multiple modes of anticarcinogenic actions.

Chronic hepatitis or cirrhosis commonly occur in the pre-neoplastic stages of HCC, and are seen in almost 80% of all cases worldwide. Cirrhosis results from constant inflammation, cell proliferation and fibrosis secondary to many viral and chemical insults. Because cirrhosis is a common precursor to HCC, surveillance programs aimed at early detection of HCC in cirrhotic patients have been undertaken. While the effects on overall survival with such screening are unresolved, such individuals might benefit from chemoprevention. Recent preclinical studies have also shown that oltipraz regenerates cirrhotic liver[4]. If cirrhosis could be reversed in humans, the benefit could be enormous. It has also been observed that liver cancer recurs in 50% or more of patients who have undergone liver resection or other treatment of the initial tumor. Such a population was targeted in a Japanese chemoprevention trial of polyprenoic acid, a acyclic retinoid[5]. Polyprenoic acid has demonstrated chemopreventive efficacy in experimental models of HCC, and has been shown to suppress cell growth and induce differentiation in human liver cancer cell lines. A significant decrease in the development of second primary hepatomas was observed in individuals receiving the polyprenoic acid compared to placebo.

Although cancer prevention, in its ideal form, entails permanent reduction or elimination of tumor development, short of successful universal vaccination and eradication of carcinogenic exposures, such a goal is not realistic. Chemoprevention provides opportunities to create molecular detours, but not necessarily roadblocks, to impede the carcinogenic process. Numerous animal studies have demonstrated that chemopreventive interventions act to not only reduce cancer incidence, but to extend tumor latency as well. These latter effects alone presage strong public health benefit.

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Poster Abstracts

POSTER 1

Inpatient Costs of Hepatocellular Carcinoma in the United States: A Retrospective Claims Data Analysis, 1993-2001

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Background: Hepatocellular carcinoma (HCC) is the 8th leading cause of cancer deaths in the United States, accounting for over 10,000 deaths annually. Although precise data are not available, it is presumed the majority of HCC cases result from the chronic sequelae of hepatitis B virus (HBV) or hepatitis C virus (HCV) infection. There are few studies on the costs of hospitalization and care for patients with HCC in the United States.

Objective: To determine inpatient costs of HCC in the United States using retrospective insurance claims data.

Methods: Hospital inpatient admission records in an employment-based health insurance claims database (MarketScan® Databse), which includes 3.5-5.0 million enrollees annually, were analyzed for 1993-2001. All patients >=18 years old admitted to the hospital with a primary diagnosis of HCC (ICD-9-CM code 155.0) were included in the analysis. For each patient identified, all admissions in each year were included except those related to liver transplantation or unlikely to be due to HCC based on review of primary and secondary diagnoses for that admission. Cost estimates were calculated from actual paid claims, adjusted for inflation using the medical care component of consumer price index, and are reported in 2002 US$.

Results: A total of 272 HCC patients with 408 inpatient admissions were identified during the 9-year period: 67% were male and the median age was 55 years (range: 18-73 years). The average annual number of admissions per patient was 1.5 (95% confidence interval [CI]: 1.3-1.6) (range: 1.2-1.7) and the average length of hospital stay was 10.7 days (95% CI: 9.0-12.4) (range: 8.2-15.3 days). The average annual cost of inpatient care per patient was $32,996 (95% CI: $26,658-$39,333) (range: $20,500-$44,355). Of the average

annual cost per patient, 84% (95% CI: 82%-87%) was for hospitalization and 7% (95% CI: 6%-8%) for physician costs. Among the patients, 27% (95% CI: 19%-35%) incurred annual costs < $10,000, 35% (95% CI: 28%-41%) $10,000-$25,000, 23% (95% CI: 16%-30%) $25,000-$50,000, 10% (95% CI: 6%-14%) $50,000-$100,000, and 5% (95% CI: 3%-7%) >$100,000.

Conclusions: The annual cost of inpatient care for HCC in the United States is >$30,000 per patient hospitalized, more than twice the average annual cost for all hospital admissions (~$12,000), and nearly eight times the per capita annual medical care expenses (~$4,176). With >10,000 annual deaths, HCC causes substantial economic burden to society, which underscores the need to support HBV and HCV prevention activities including immunization (for HBV), counseling and testing, and appropriate medical evaluation and treatment.

POSTER 2

Laparoscopic Evaluation of Masses in End Stage Liver Disease Patients

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The Model of End-Stage Liver Disease (MELD) scoring system is the current standard for determining recipient priority for liver transplantation. Under this system, a patient diagnosed with hepatocellular carcinoma (HCC) receives a considerable increase in their priority score. As a result, many transplant programs have adopted an aggressive cancer surveillance program, which frequently utilizes surgical exploration, to confirm the presence of and provide treatment for tumors.

Methods: All potential liver transplant candidates who were explored for a radiographic mass were identified. Patient demographics, tumor characteristics, and patient survival were examined. All patients with tumors identified by intra-operative laparoscopic ultrasound underwent radiofrequency ablation (RFA).

Results: Sixty-four patients underwent surgical exploration for radiographic evidence of a liver mass(es). Three (7%) patients were managed via an open incision; one laparoscopic patient required conversion to improve access to safely ablate a dome lesion. Fourteen (21%) patients underwent laparoscopy without ablation: absence of diagnostic lesions (n=7), metastatic disease (n=5), or benign tumor biopsy (n=2). Mean hospital stay for the series was 36 hours (range 20 to 50 hrs). Complications included new onset of ascites (n=5), worsening of ascites (n=23), and wound infection (n=4). No treatment-related mortality was incurred. Of the 46 patients who received RFA, 16 (34%) were excluded from transplantation: newly diagnosed pulmonary hypertension (n=2), unabated alcohol usage (n=2), poor physiologic age (n=2), lack of social support (n=5), morbid obesity (n=2), metastatic disease (n=1), and death [variceal bleed, recurrent HCC] (n=2). Twenty-seven (42%) explored patients were transplanted, while 9 (14%) patients were excluded from transplantation based

upon findings identified during the procedure. With the diagnosis of HCC, 24 patients in need of therapeutic transplantation had their MELD scores increased from 14 to 22 points. Six patients are currently awaiting either completion of their evaluations or transplantation. Time from RFA to transplant ranged from 2 weeks to 1 year. Examination of explants identified one (2%) liver in which HCC was missed. Of the 24 patients who underwent RFA and subsequent transplantation, all had evidence of focal necrosis at the RFA site, which varied from 30 to 99%. While those with RFA-to-transplant intervals greater than 3 months had the highest percentages of necrosis, those with shorter intervals, though demonstrating a lower degree of necrosis, displayed extensive apoptotic tumors.

Conclusion: Laparoscopic evaluation and RFA of hepatic tumors in cirrhotic transplant candidates is both safe and efficacious. With continued changes in the MELD scoring system, laparoscopy can provide both accurate staging and interventional therapy for candidates awaiting transplantation. In this series, laparoscopic findings saved 14% of patients from an unnecessary open exploration at the time of organ availability. The findings of this series also suggest there is a linear rate of necrosis found in cirrhotic livers following ablation.

POSTER 3

Prognostic Factors Analysis of 753 Hepatocellular Carcinoma: The Impact of Hepatitis C Virus on the Outcome of Hispanic Patients

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Background and Purpose: Over the past 2 decades, a significant increase in Hepatocellular carcinoma (HCC) incidence was reported in the United States. The risk of HCC development is highly related to chronic infection with hepatitis B virus (HBV), hepatitis C virus (HCV), heavy alcohol consumption and diabetes mellitus. Nevertheless, the impact of these risk factors as predictors of HCC patients' survival has not been entirely elucidated after taking into consideration the effect of clinical characteristics of HCC patients.

Methods: We have developed a database that includes all HCC patients diagnosed at University of Texas M. D. Anderson Cancer Center. It includes demographic information, HCC risk factors, pathological and radiological evidence of cirrhosis with Child–Pugh classification, tumor-node-metastases (TNM) disease stage, baseline values of liver enzymes, a feto-protein (AFP), and treatment data.

Patients: Between January 1992 and November 2003 we recorded 753 (males, 69.3%; females, 30.7%) pathologically confirmed HCC patients. The overall mean age was 61.7 ± 12.8. The race distribution of all patients (White, 65.1%; Black, 8.1%; Hispanic 16.1%; Asian, 9.3%; other, 1.5%) was comparable to M D Anderson general referral pattern.

Statistical Analyses: Survival times were calculated from the date of pathology diagnosis and were censored for patients who were alive at the last follow up. Median survival was calculated using Kaplan Meier product-limit method and survival rates were compared using the log rank test. Cox proportional hazards model was used for univariate and multivariate analysis of all prognostic factors. The relative importance of prognostic factors was measured by x^2 value based on the

Wald test of the coefficient associated with each factor in the Cox model. In addition, we measured the multivariate Hazard Risk Ratios (HRR) and 95% Confidence Interval (CI) for these factors.

Results: The overall median survival of HCC patients was 11.4 months (95% CI, 9.7-13.1 months). We found no significant impact of heavy alcohol consumption, family history of liver cancer, diabetes, and cigarette smoking on HCC outcome. The ever exposure to treatment was the most significant predictor of long survival in these patients. The next most significant prognostic factors were cirrhosis (Child-Pugh class B and C) followed by high AFP level, stage IV disease, Hispanic race, and male gender (Table 1). The median survival times were 16.1 months (95% CI, 13.4–18.8) and 5.1 months (95% CI, 3.9–6.3) for treatment and non-treatment groups respectively, Log Rank p value <0.0001. Treated patients were further categorized into 5 groups 1) chemotherapy only (n=153); 2) chemotherapy with other non-surgical interventions (n=188); 3) chemoembolization only (n=18); 4) surgical resection only (n=5); and 5) surgical resection with any other treatment (n=100). The respective median survival times (months) were 9.5 (95% CI, 7.1-11.8); 15.2 (95% CI, 11.7-18.7); 14.0 (95% CI, 5.1-22.9); 23.3 (95% CI, 15.5-31.1); and 25.6 (95% CI, 18.1-32.5) respectively. However, including these treatment categories in the Cox model with other prognostic factors, both HRR and x^2 (Wald Statistics), indicated that group 5 treatments was the best predictor for long survival while group 3 treatment was the poorest. Since Hispanic HCC patients experienced poor outcome as compared to other races, restricted multivariate Cox regression in Hispanics was performed. It showed that patients with chronic HCV infection were at 4 times higher risk for poor HCC survival outcome than those with no virus infection, HRR=3.9 (95% CI 1.2–13.3). This risk was highly modified by the presence of diabetes mellitus; the estimated HRR was 18.6 (95% CI, 1.8–187.9).

Conclusion: This is the largest study to assess the main prognostic factors for HCC outcome. We are the first to report the impact of HCV as a prognostic factor for HCC outcome among Hispanic patients with chronic HCV infection and which can be significantly modified by diabetes mellitus. Given the high prevalence of HCV and diabetes mellitus in Hispanic population, further large-scale clinical epidemiology studies are warranted among these high-risk populations to investigate the underlying mechanisms of hepatocarcinogenecity, susceptibility to treatment response, and disease outcome.

Table 1: Cox Regression Analysis for 753 Patients with confirmed diagnosis of HCC

Variable	N (%)	DF	x^2 (Wald)	HRR	95% CI	P value
Ever exposed to treatment	504 (66.9)	1	76.304	0.4	0.3–0.5	<0.0001
Cirrhosis (Child-Pugh B&C)	171 (22.7)	1	34.114	1.8	1.5–2.3	<0.0001
AFP (>100 ng/mL)*	417 (55.4)	1	9.360	1.3	1.1–1.6	0.002
TNM stage IV-A/IV-B	514 (68.3)	1	7.832	1.3	1.1–1.6	0.005
Hispanic race	121 (16.1)	1	7.671	1.6	1.1–2.2	0.006
Sex (Male)	525 (69.7)	1	3.824	1.2	1.0–1.5	0.05
Virus (HCV or HBV)	306 (40.6)	1	2.256	1.0	0.8–1.5	0.09
Age (year unit)	-	1	0.257	0.8	0. –1.1	0.6

*** Cutoff point:** median level among HCC patients with stage 1-II, non-cirrhotic, HCV-, HBV-

POSTER 4

Demographics from the ETHECC© Trial: A Randomized Comparison Between THYMITAQ® and Doxorubicin for the Treatment of Unresectable Hepatocellular Carcinoma (HCC) in Terms of Survival

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Introduction: ETHECC© is currently the largest and most advanced (in terms of study enrollment) prospective controlled randomized Phase III study in unresectable HCC and is a pivotal trial for the |FDA approval of THYMITAQ® in HCC. The randomization of this trial is stratified by CLIP score and Performance Score (PS) and involves the comparison of nolatrexed (THYMITAQ®) to doxorubicin (ADRIAMYCIN®) in patients with unresectable HCC. Nolatrexed is a direct and potent Folate Analogue inhibitor of Thymidylate Synthase (TS) that binds directly to the Folate site of TS. The ETHECC© trial is being conducted in North America (US and Canada), Europe and South Africa. Currently, more than 75% of the 446 subjects that need to be enrolled into the trial have been accrued.

Materials and Methods: The present communication relates to the overall demographics of the HCC population studied in the ETHECC© trial. Based on a requirement by the Data Safety Monitoring Board (DSMB), the statistics provided are not separated by treatment group. The data provided is based on the database employed for the tables and listings that were generated for the DSMB meeting that took place 23 January 2004. A total of 294 subjects are associated with the data provided in the tables generated for this abstract.

Results: The median age of the population was 62.0 years of age; the median weight and height was 72.7 Kg and 170.0 cm, respectively. The male-to-female ratio was 5:1. The distribution by ethnicity was: Caucasian, 71.8%; Black, 15.0%; Asian, 8.2%; and Other, 5.1%. The distribution by histology was: HCC, 87.4%; Fibrolamellar, 1.4%; and Presumptive HCC, 11.3%. The distribution by risk was: HBV, 16.6%; HCV, 27.0%; Alcoholism, 18.6%; and Cirrhosis (no diagnosis), 37.7%. The distribution by extension of disease: localized to liver, 55.1%; and metastastic, 44.9% (Location: Lung, 29.6%; Bone, 14.4%; Lymph

nodes, 31.1%; and Other, 25.0%). The distribution by Child-Pugh was: A, 72.9%; B, 26.4%; and C, 0.7%. The distribution by CLIP score was: 0, 6.8%; 1, 26.4%; 2, 37.0%; 3, 28.8%; and 4, 1.0%. The PS (Karnofsky) distribution was: 60, 0.7%; 70, 15.2%; 80, 20.7%; 90, 41.7%; and 100, 22.0%.

Conclusions: To our knowledge, this is the largest Phase III study to date where the demographic data already shows the importance of HCV in the etiology of HCC in North America and European countries. The data from this trial will either confirm or refute the activity of doxorubicin and that of THYMITAQ® in this disease without a standard of care and will provide valid information regarding outcomes in subgroup populations by PS and CLIP scores. Patient enrollment is expected to be completed by the end of 2004.

References

DSMB Demographics Tables and Listings-January 23, 2004.

POSTER 5

Clinical Study of Carbon Ion Radiotherapy for Hepatocellular Carcinoma

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Background: With the development of the linear accelerator, reports on clinical research into Hepatocellular Carcinoma (HCC) treatment with a high-energy X ray beam have been published. In recent years, radiation therapy using a proton beam has been credited with marked achievements mainly because of its excellent dose distribution resulting from well-localized energy deposition at the end of the beam path, called the Bragg peak. Since carbon ion radiation is known to possess the Bragg peak and biologically unique characteristics resulting in a higher cytocidal effect than that of proton beams, it is expected to produce higher therapeutic effects on cancers.

Patients and Methods: To investigate whether these unique properties are clinically useful, we performed clinical studies with carbon ion radiotherapy for HCC. We first performed a phase I/II study to evaluate the toxicity and anti-tumor effect of the therapy with 15-fraction/5-week irradiation for 24 patients with 24 lesions. We then conducted the second clinical study of a phase I/II study using a short-course therapy regimen with 12-fraction/3-week, 8-fraction/2-week or 4-fraction/1-week irradiation for 82 patients with 86 lesions. Based on the results of these clinical trials, we carried out the third clinical study of a phase II study using the fixed 4-fraction/1-week regimen to confirm its clinical efficacy for 44 patients with 47 lesions. Fifty-eight % of the patients had previously undergone other treatments. A further phase I/II study using a 2-fraction/2-day irradiation regimen started from April 2003.

Results: No severe adverse effects and no treatment-related deaths have occurred up to now. In the first study, local control rates were 92% and 82% at 1 year and 2 to 5 years, respectively. In the second study, they were 93% and 87% at 1 year and 2 to 5 years, respectively. In the third study, they were 97% and 89% at 1 and 2 years, respectively. There were no differences in local control rate among the different fractionation regimens and between the patients with a tumor diameter equal to 5 cm or smaller and the ones with a tumor larger than 5 cm in diameter. Overall survival rates in the 55 patients without previous treatments and other lesions were 93% and 60% at 1 and 3 years, respectively.

Conclusion: Carbon ion radiotherapy seemed to be safe and effective for patients with HCC. However, further long-term observation will be needed to confirm its therapeutic efficacy.

POSTER 6

Tumor Characteristics and Risk for HCC with HBV, HCV, and Aflatoxin-associated P53 Mutations

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HCC is the most common cancer in The Gambia resulting from endemic HBV infection and dietary aflatoxin exposure. In aflatoxin-exposed areas, a "hotspot" mutation in HCC occurs in the p53 gene at codon 249. We examined markers of HBV and HCV infection and of aflatoxin-associated 249 ^{ser} TP53 mutations in plasma DNA among 408 controls and 216 HCC cases. Participants were recruited from three tertiary hospital sites in The Gambia. HCC was confirmed by either pathology (25%) or ultrasound lesions with AFP>100ng/ml. HCC cases had a median age of 48 years and a 4:1 male:female ratio. Over 90% of HCC cases presented with abdominal pain, anorexia, and hepatomegaly while multifocal disease (63%) and ultrasonographic cirrhosis (80%) was common. HBV carriage, HCV antibody, and 249 ^{ser} TP53 mutations were detected in 61%, 19%, and 40% of HCC cases and in 16%, 3%, and 4% of controls, respectively. Adjusted HCC risk was significantly increased with chronic HBV infection (OR 20, 95%CI 10-39), HCV infection (23, 8-64) and plasma 249ser p53 mutations (21, 8-51). Only minor differences in clinical, ultrasonographic or biochemical factors were observed by the differing etiologic factors. In summary, HCC presents at very advanced stage in The Gambia. HBV is the predominant causal virus but HCV is more important among older cases. Coinfection with HBV and HCV appears additive while our findings suggest a multiplicative effect on HCC risk resulting from chronic HBV infection and the mutational effect of aflatoxin on codon 249 of the p53 gene.

POSTER 7

The Long Term Therapeutic Efficacy of Percutaneous Holmium Injection for the Treatment of Small Hepatocellular Carcinoma

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Local ablation therapy induces local tumor necrosis by selective injection or thermoablation. However, incomplete tumor ablation due to inhomogeneous penetration may lead to local recurrence and intra-hepatic spread. Holmium-166 (Ho166) is a radioactive isotope derived from natural Holmium-165. We used Holmium-Chitosan complex (Milican®, Dong Wha Pham. Seoul, Korea) for holding radioactive material at the injection site. The aim of this study was to evaluate the long-term therapeutic efficacy of percutaneous Holmium injection (PHI) therapy for the treatment of small HCC.

Forty patients (male:female 27:13, mean age 57.4 years, mean follow-up duration 41 months) with HCC less than 3 cm (mean 2.3 cm) in maximal diameter were enrolled. They were not suitable for surgery or refused surgery. HCC was diagnosed by pathological confirmation (16 cases) or typical clinical findings. The mean amount of Ho166 injected was 47.4 mCi (range 30-60). Two months following a single session of PHI, complete necrosis was achieved in 31 of 40 patients (77.5%) with HCC less than 3cm, and in 11 of 12 patients (91.7%) smaller than 2 cm. Among completely necrotic nodules, the cumulative local recurrence rates at 1, 2, and 4-year were 6.67%, 10.26%, and 23.19%. The tumor recurred in 34 patients and out of which 22 patients recurred at another intra-hepatic site without local recurrence. The 1, 2, and 4-year cumulative local recurrence rates were 20.75%, 23.58% and 34.08%. The 1, 2, and 4-year tumor recurrence rates were 56.08%, 66.42% and 90.4%. The survival rates at 1, 2, and 4-year were 89.74%, 71.79% and 66.67%, respectively. There was no serious complication.

Conclusions: PHI was effective procedure as a new local ablation therapy for the treatment of small HCC, especially smaller than 2cm. Randomized controlled trial will be needed.

POSTER 8

Patients with Carcinomatosis of the Explant Liver Form a Distinct Clinicopathological Subgroup with a Poor Outcome

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Correct donor liver allocation to cirrhotic patients with hepatocellular carcinoma (HCC) is only possible when all factors that determine HCC-recurrence and survival are well known. This issue is gaining importance because of the shortage of donor livers and increase of time on the waiting list.

Current UNOS-criteria give priority to patients with a limited number of small HCCs (stage T1/2). These criteria are based on studies in which the correlation between number, size and pathological features of HCCs in the explant liver and outcome was studied. In these reports, explants were sliced at intervals of at least 1 cm and sometimes intervals were variable (reviewed in Am J Surg 183 :309).

However, routine slicing of the liver with 1- to 2-cm thick intervals does not suffice, because a considerable proportion of focal lesions are missed when this rather broad sectioning is applied (Eur Radiol 11: 1631). In recent pathological-radiological correlation studies by our and other groups (Liver Transpl 8: 749, Hepatology 38: 1034), the "golden standard" was used, which means slicing of explant livers at 5-mm intervals and classification of all focal lesions according to the IWP-criteria.

Thus, some patients might have been understaged in the previous follow-up studies, leading to a possible distortion of the correlation between staging and outcome, especially when the number of patients in the study is rather small.

Continued

To gain insight in this issue, we evaluated the clinicopathological and imaging data from patients that were transplanted between 2000 and 2004 and had at least 1 HCC in the explant liver. Pretransplant UNOS-staging by imaging was T2 or less. The "golden standard" was applied and the data were then compared with those of previous studies.

In total, there were 31 patients included in the study and two subgroups could be discerned based on the numbers of HCC nodules. The large majority (28 pts; 90%) had 1 to 5 HCCs (mean: 2.5). The diameter of the largest nodule showed a wide range from 2 to 70 mm (mean: 24 mm). 20 of these patients (71%) fell within the UNOS-criteria, while 2 and 6 patients were UNOS T3 and T4a, respectively. The total tumor diameter in these 8 patients was maximally 99 mm, indicating that underestimation of tumor burden by imaging was rather limited. The follow-up data of these 28 patients are being evaluated.

The second group consisted of 3 patients (10%) with HCV and with 10 or more HCCs, i.e. 10, 20 and 30 nodules, which is referred to as "carcinomatosis". These patients had 1 or 2 "main" nodules with a diameter of 20 to 45 mm, while the other nodules were much smaller (2-12 mm). In all main nodules, a considerable proportion of tumor cells was of the clear cell type. The small HCCs resembled each other and consisted of poorly differentiated tumor cells. Microvascular invasion was prominent. These findings strongly suggests that carcinomatosis arises from a single HCC that metastasizes within the liver early in its evolution, while the first group of patients represents the "classical" progression of HCC with more gradual growth of one or more HCCs and slower development of intrahepatic metastasis. The tumor burden in the latter patients was assessed rather accurately on imaging, so patients who manifestly exceed UNOS-criteria are not transplanted, which explains why there were no patients in our study with 6-9 HCCs. In carcinomatosis, only the main nodules representing "the tip of the iceberg" were detectable by imaging, leading to a stage of maximally T2 and transplantation of these 3 patients. One of 3 carcinomatosis patients was transplanted 4 months ago and is alive without recurrence. The other 2 died of recurrence 6 months after transplantation.

Interestingly, Krinsky et al. recently described 9 patients with innumerable small HCCs in their explant and 4 of them died rather rapidly due to recurrence (Liver Transpl 2002: 1156). In contrast, an entity resembling our carcinomatosis cases is not described in any of the follow-up studies in which the "golden standard" was not used. We speculate that reports of T2-patients with a rapid recurrence (e.g. Hepatology 33: 1394) may actually have been unrecognised cases of carcinomatosis.

In conclusion, liver carcinomatosis emerges from our data as a distinct entity with a poor outcome. We propose that all future studies on posttransplantation outcome of HCC-patients use the "golden standard", which means a detailed investigation of the explant with slicing at 5-mm intervals.

POSTER 9

The Long-term Efficacy and Safety of Proton Irradiation for Hepatocellular Carcinoma: Clinical Analysis of 162 Patients in a Late Phase II Study

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Aims and Backgrounds: Proton beams can deliver a high dose to hepatocellular carcinoma (HCC) without proportionally increasing the non-cancerous liver tissue dose. Proton beams has Bragg peak, which limits distribution of the beam. This specific characteristic results in both a marked reduction in the amount radiation that reaches no targeted areas and an increase in the amount of radiation hitting the targeted lesion. The aim of the present study is to elucidate the long-term effects, safety and indication of proton radiotherapy in patients with HCC.

Materials and Methods: From November 1985 to July 1998, 162 patients having 192 HCCs were treated with proton radiotherapy with or without lipiodol-TAE, TACE and/or PEI. Patients in the present series included those not suited for surgery for various reasons such as liver dysfunction, multiple tumors and recurrence following surgical resection or concomitant illnesses. A median total dose was 72 Gy; a median fraction dose was 4.5 Gy.

Results: The five-year local control rate for evaluable 137 patients having 166 HCCs was 88.4%. No significant difference in local control rate at five years was observed between combination proton therapy group (89.7%) and proton monotherapy group (86.8%). And no correlation was found among local control, and maximal tumor diameter. Ten of 27 patients in stage IIIA had tumor involving a major branch of the portal vein. Thrombosis shirked markedly without inducing hepatic insufficiency after irradiation. The five-year survival rate of stage IIIA was 28.3%. The overall survival rates after the completion of irradiation were 43.2% and 24.0% at 3 and 5 years. And The five-year survival rate of proton irradiated virgin cases with HCC was 37.2%, that was significantly higher than that of non-virgin cases.

According to the Cox regression analysis, the severity of the coexisted LC and the tumor number were ranked as factors affecting survival of the treated patients. Patients with good liver functions and single tumor irrespective of size were selected; five-year survival rates were 51.1% in the present study. Major advantage is the minor adverse effects and the treatment didn't cause any pains or other symptoms, providing with good PS even for stage IIIA and Child-Pugh C class cases.

Conclusions: In conclusion, proton irradiation for HCC was shown to be effective, safe, and well tolerable in this phase II study. It might be used as a one of the therapeutic option with curative intent as surgery especially for single HCC virgin cases with Child-Pugh A and furthermore some of HCC could be indicated irrespective of size, location, vascularity, portal thrombosis and even with severe complications.

POSTER 10

Practice Guideline for Diagnosis and Treatment of Hepatocellular Carcinoma of Korean Liver Cancer Study Group and National Cancer Center

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Background: Hepatocellular carcinoma (HCC) is the 3rd most common cancer and annual incidence is over 10,000 cases in Korea. While hepatitis B virus is major cause of Korean HCC, the impact of alcoholic liver disease is on a rising trend. The 5-year survival rate of HCC is only 9.6%, mainly due late diagnosis, tumor biology and underlying chronic liver diseases. Because almost eighty percent of HCC is diagnosed in late, not early stage, we launched last year a nationwide surveillance program to screen high risk groups (HBV or HCV carriers or liver cirrhosis, over 40 years old) and formulated this practice guideline, with special emphasis on advanced stage of HCC.

Methods: Forty-five experts from KLCSG and National Cancer Center participated in a special committee for practice guideline of HCC. Based on the quality of scientific evidence, the consensus was made for diagnosis and treatment strategy after considering the medico-social situations in Korea.

Results: Required and optional tests and clinical (non-invasive) diagnosis criteria for HCC are formulated. The first decision based on both Child-Pugh score and modified UICC tumor staging is for operability. The second decision for respectability is based on localization of tumor and residual liver function. Chemoembolization or local ablation therapy is allowed for resectable tumor in certain conditions such as at borderline risk or non-invasively diagnosed. Unresectable tumors are classified into either a group with inadequate residual liver functions or the other with extensive or macrovascular invasion or distant metastases. Indications of liver transplantation, chemoembolization, local ablation, radiation therapy and chemotherapy for unresectable HCC are presented.

Conclusion: This guideline is expected to be useful for clinical management and research for HCC patients.

POSTER 11

Hepatocellular Carcinoma and Cirrhosis: Global Estimates of Fractions Attributable to Viral Hepatitis Infection

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Introduction: Estimates of the global burden of disease (GBD) associated with hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are needed to help guide resource allocation and prevention policies. To support these efforts, we estimated the fractions of hepatocellular carcinoma (HCC) and cirrhosis that can be attributed to chronic HBV or HCV infections worldwide.

Methods: Fractions of HCC and cirrhosis attributable to viral hepatitis infections were estimated for the regions defined in the World Health Organization GBD 2000 project. Data were abstracted from published studies that examined the prevalence of both hepatitis B surface antigen and HCV antibody (and/or HCV RNA) among patients diagnosed with HCC or cirrhosis. Attributable fractions were derived by averaging the observed prevalences, with adjustment to account for coinfection.

Results: The fraction of HCC attributable to HBV infection ranged from 16-69%, with the highest fractions occurring in the Western Pacific B region (69%), which includes China, and the Southeast Asia B region (50%), which includes Indonesia and Thailand. The fraction of HCC attributable to HCV infection ranged from 13-74%, and was highest in the Western Pacific A region, which includes Japan. The attributable fractions of cirrhosis due to HBV and HCV ranged from 4-61% and 13-76%, respectively; the highest fraction due to HBV occurred in the Western Pacific B region while the highest fraction due to HCV occurred in the Western Pacific A region. In nearly all regions, HBV and HCV contributed to over half of HCC and cirrhosis cases. When these fractions were applied to the GBD 2000 HCC and cirrhosis mortality estimates, HBV and HCV infections together accounted for approximately 960,000 deaths worldwide.

Conclusions: HBV and HCV infections together account for the majority of HCC and cirrhosis worldwide. These findings highlight the need for comprehensive approaches aimed at reducing transmission and the long-term sequelae associated with chronic infection with these viruses.

POSTER 12

The Impact of the MELD Scoring System upon Liver Transplantation for Patients with Hepatic Malignancies

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The emphasis on cancer found in the MELD system has changed the priority for organ allocation in liver transplantation. The intent was to offer patients with malignancies a chance for liver replacement before lesions either metastasized or became too large such that transplantation was not feasible.

Purpose: To evaluate the impact of MELD on liver transplantation in our center and to assess any changes in outcomes, practice strategy or patient survival.

Methods: Histologic, morbidity, and mortality data on all patients with known or incidental tumors transplanted at our center were reviewed.

Results: A total of 67 (13.8%) patients with primary liver malignancy were transplanted out of 487 liver transplants performed between 1988 and 2003. In the pre-MELD era (1988-2002), 38 (9.4%) of the 403 transplants performed were for primary liver malignancy. However, in the MELD era (2002-present), a significantly higher number of the 84 transplant recipients, 29 (34.5%), had liver malignancies ($p<0.05$).

Histology	Pre-MELD (n=38)	MELD (n=29)	P Value
Hepatocellular	84.2% (n=32)	89.7% (n=26)	NS
Cholangiocarcinoma	10.5% (n=4)	3.5% (n=1)	NS
Sarcoma	5.3% (n=2)	0	NS
Carcinoid/ hemangioendothelioma	0	6.9% (n=2)	NS
% Tumor patients transplanted	9.4% (n=38)	34.5% (n=29)	P < 0.05

	Pre-MELD (n=38)	MELD (n=29)	P Value
Incidental diagnosis	44.7% (n=17)	37.9% (n=11)	NS
Poor histologic grade	5.3% (n=2)	10.3% (n=3)	NS
Recurrences	13.2% (n=5)	6.9% (n=2)	NS
1 year survival	92.1% (n=35)	100% (n=29)	NS

If patients with incidental cancers are excluded, 5.2% of patients undergoing OLT had malignancies in the pre-MELD era, vs 21.4% during the MELD era. Incidentally discovered cancers comprised 4.2% of explants in the pre-MELD era (17/403), whereas in the MELD era, 13.1% of explants were noted to contain incidental lesions ($p<0.02$). Wait time to achieve transplantation was significantly different between the 2 groups. It took an average of 34 days for newly-listed cancer OLT candidates in the MELD era to receive a transplant, vs 256 days for like candidates before the advent of MELD.

Conclusions: The number of patients being transplanted for primary liver malignancy has increased significantly since the MELD system was instituted. This may in part be due to increased vigilance during evaluation. There were no statistically significant differences between the two eras in regard to poor histologic grade, recurrence, or transplant outcomes. The increased percentage of incidentally-found cancers in the MELD group is of interest and deserves further study. As expected, the time to transplant was profoundly shorter for patients listed with primary liver malignancy in the MELD era compared to pre-MELD.

POSTER 13

Retrospective Chart Review to Determine Dropout Rates of Patients with Hepatocellular Carcinoma (HCC) Listed for Liver Transplantation

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In the USA, current United Network of Organ Sharing (UNOS) organ allocation policies are based on Model for End Stage Liver Disease (MELD) scores to assign a priority for cadaveric orthotopic liver transplantation (OLT). However, many HCC patients do not have a calculated MELD score that provides them with an urgent priority for OLT. Patients with HCC are now assigned arbitrary MELD scores to provide timely access to OLT. An equitable policy would equate HCC progression beyond acceptable transplantation criteria with death on the waiting list. However, limited information is available regarding HCC progression over time, especially in patients receiving pre-OLT therapy for the tumor such as chemoembolization.

Thus, our aim was to analyze dropout rates on the waiting list due to disease progression for patients with HCC treated with chemoembolization.

Methods: Between January 1994 and August 2001, 54 patients with HCC were listed for OLT. All patients met the current European Association for the Study of Liver Disease (EASL) consensus criteria (J Hep 35:421-430,2001) for the diagnosis of HCC and Milan/UNOS indications for transplantation. Patients underwent chemoembolization prior to OLT, and were assessed every three months for disease progression until OLT. This evaluation included laboratory evaluation with an alpha-fetoprotein and a CT scan of the abdomen and chest. Subsequent chemoembolization were performed for viable tumors and/or a rising alpha-fetoprotein.

Results: Forty (74%) patients had unicentric lesions while 14 (26%) had multicentric disease. A total of 8 (15%) patients were removed from the OLT list due to either tumor progression or death. For HCC patients who were transplanted, the median time on the waiting list was 211 days (range 28-1099 days). There were no significant differences in age, gender, tumor characteristics (size, multicentricity) and serum alpha-fetoprotein levels in those who underwent OLT vs. those who dropped out.

In conclusion, in our patient population, neoadjuvant chemoembolization for patients with HCC has a cumulative dropout rate of 15% over 6 months.

POSTER 14

Hepatitis B, Alcohol but not Ethnicity Affect Survival in Hepatocellular Carcinoma

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Background/Aims: In the US, Hawaii has the highest incidence of hepatocellular carcinoma (HCC) and has an ethnically diverse population. It is an ideal location to study HCC in the context of various ethnicities and risk factors.

Methods: 262 HCC patients were referred to our tertiary medical center from August 1992 to August 2003. Demographics, ethnicity, birthplace, hepatitis B (HBV), Hepatitis C (HCV), significant alcohol use (ETOH), diabetes, smoking and risk factors for viral hepatitis including intravenous drug abuse (IVDA), transfusions, tattoos and vertical transmission were noted. Tumor stage, Child's classifications, CLIP (Cancer of the Liver, Italian Program) score, treatment and survival were recorded.

Results: Gender, age, HBV, HCV, ETOH, IVDA, and diabetes differed significantly between Asians, Non-Asians and Pacific Islanders. There were also specific differences in type of viral hepatitis within Asian subgroups. AFP level, smoking, transfusions, stage and resectability did not differ between groups. Asians were more likely to have HBV infection, while Non-Asians were more likely to have HCV infection. Factors that decreased survival included: presence of HBV, ETOH, AFP >20 ng/ml, CLIP score >2 and advanced Childs class. There was no difference in survival for the following: HCV, IVDA, transfusions, smoking, vertical transmission, diabetes or birthplace. There was no difference in survival by ethnicity, but when Asians were combined with Pacific Islanders, median survival (1.52 yrs vs 3.54 yrs) and survival at 1 and 3-year was significantly worse than for Non-Asians. ($p<0.05$) And finally after COX regression analysis for HBV infection and ETOH was performed, there was no difference in survival by ethnicity.

Conclusions: In this diverse population, various ethnicities have different risk factors for HCC. The presence of HBV and ETOH use had a negative impact on survival. Regardless of birthplace, patients who were Asian or Pacific Islander had a decreased survival when compared to Non-Asians but this may be accounted for by their high prevalence of HBV infection. Hepatitis B infection, ETOH, and AFP level are more important factors in survival than ethnicity.

POSTER 15

Tissue Specific Ligands for Targeted Drug Delivery

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A challenge in healthcare in this century will be the successful development of an intrabody, intracellular delivery of gene-specific and/or enzyme-specific drugs to a targeted organ. In particular, one disease that would greatly benefit from this tissue-specific drug delivery would be hepatocellular carcinoma (HCC), where an effective therapy remains elusive. For HCC, current chemotherapeutic treatments involve either one or more drug delivery approaches: first, the non-specific approach, which includes most cytotoxic drugs administered by transdermal, i.v. and oral routes. These regimens have been shown to be only minimal effective with a large systemic toxicity to the patient. The second approach has been site-specific delivery, one example is the invasive procedures requiring surgery to implant intra-hepatic artery pumps and devices. This approach has been mildly effective. A third approach is the tissue-specific drug delivery, which so far has no clinical demonstration. For this approach a ligand-directed drug or prodrug is administered to the patient that would target a specific enzyme within a cell found in a single organ/tissue. The benefits to the patient are an increase in efficacy, coupled with a reduction of systemic toxicity, as exemplified by the anticancer drug, the side effect of these drugs to healthy tissue is a serious concern. In addition, tissue-specific drug delivery can revitalize an off-patent drug, to regain patent protection. This tissue-specific delivery technology could resurrect drugs previously found to be too toxic. FDA approvals for these revitalized drugs are more likely and more rapid, since FDA and the medical community would already have clinical experience with the drug itself without attachment to a harmless delivery system. It is reasonable to expect that after the approval of

the first tissue-specific drug as an example, future drugs developed by this concept would be approved more readily as well. To realize the above benefits, we plan to develop tissue-specific drug delivery system with high efficacy against HCC as a demonstration of the concept in this approach.

Cell Works has chemically synthesized a structurally defined and homogeneous neoglycopeptide, named LIV-1. This potential anti-cancer drug consists of a tri-antennary, N-acetylgalactosamine-bearing glycopeptide ligand, YEE(ahGaINAc)$_3$, covalently connected to a payload of FUDR through simple linker. This synthetic hepatic ligand YEE(ahGaINAc)$_3$ was first designed and synthesized by Dr. Y. C. Lee and his team at JHU. Our collaboration led to the development of LIV-1, now at the late pre-clinical stage. LIV-1 was constructed by rational design with the A-L-P molecular framework. To date, our research has demonstrated that tissue specific ligand-directed receptor-mediated drug delivery is less toxic to asialoglycoprotein (ASGP) receptor-negative cell types (those of non-hepatic origin) and more cytotoxic towards targeted ASGP-R positive cells, particularly rapidly dividing cells.

As a potential anti-cancer drug, LIV-1 can deliver the active metabolite of FUDR (FdUMP) to inhibit thymidylate synthase, (an essential enzyme for DNA synthesis) selectively to hepatoma cells regardless of their location. This selectivity may provide a therapeutic advantage to LIV-1 with an enhanced efficacy over traditional FUDR treatment. *In vitro* studies were undertaken to characterize the cellular uptake and subsequent impact on cellular proliferation and toxicity. With each of these studies, the biological impact of LIV-1 with that of the unconjugated FUDR was compared. These experiments demonstrated that receptor-mediated endocytosis of FUDR was increased in human hepatoma cells through ligand-directed delivery. This increased uptake was specific and led to enhancements of the *in vitro* inhibition of cellular proliferation and subsequently toxicity of human hepatoma cells. The effect of LIV-1 was about 4-6 fold higher than the effect of FUDR on cancer cell growth inhibition, and this growth inhibition on hepatoma cells was directly related to the applied LIV-1 concentration. The toxicity of LIV-1 (reduction of viable cells in culture) increases

with time down to 60% killing after 96 hours for human hepatoma cells, while for the same time period the toxicity of FUDR did not change with time and had only ~20% toxic effect. FDA required animal toxicity testing with rat and beagle dog were completed with no toxicity found at 80 mg/kg level for the rat and at 8 mg/kg level for the dog, 2 times a week for a 4 week cycle.

Animal studies illustrated direct and specific hepatic delivery of LIV-1 to both the athymic mouse liver and xenografted human hepatomas. This specific delivery resulted in the inhibition or, in some cases, eradication of xenografted human hepatoma growth in athymic mice with only mild systemic toxicity. Data from supplemental *in vitro* and *in vivo* experiments did show the Ligand-Linker portions of LIV-1 (A-L) was not toxic.

In summary, LIV-1 will soon be tested in clinics as the first anti-tumor drug using liver targeted delivery technology. Biological *in vitro* and *in vivo* studies have demonstrated that LIV-1 can indeed inhibit thymidylate synthase and not harm normal, non-dividing human liver cells in culture. Benefits of LIV-1 are demonstrated in the effectiveness of floxuridine without the toxicity of the parent drug. The organ-targeted delivery of LIV-1 is similar to hepatic artery infusion delivery of floxuridine, however with greater specificity and efficacy and without the need of a pump, which is expensive, inconvenient, and often ineffective. Finally, with the rapid approval of Xeloda (a prodrug form of FUDR approved in 10 months as an example), one could expect FDA approval, based on an expedited clinical trial for LIV-1.

POSTER 16

Characteristics and Outcomes of African American Patients with Primary Hepatocellular Carcinoma in the USA: The Nationwide Inpatient Sample

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Background: The incidence and mortality rate of primary hepatocellular carcinoma (HCC) in Black Americans (BA) is 2-3 times greater than in White Americans (WA). The higher prevalence of hepatitis B (HBV), hepatitis C (HCV), and alcoholic liver disease (ALD) among BA may partially explain this difference. Whether racial differences in socioeconomic status, i.e., poverty, and co-morbid medical conditions such as obesity, diabetes, HIV/AIDS contribute to the higher incidence is unknown. It is also unclear whether utilization of therapeutic procedure for HCC differs in the two racial groups.

Objective: The aim of the current study was to compare demographic, clinical characteristics, and inpatient outcomes of BA and WA patients with HCC using a nationally representative sample.

Methods: We analyzed all BA and WA inpatient cases diagnosed with HCC from the year 2000 Nationwide Inpatient Sample (NIS) from the Healthcare Utilization Project. Demographic variables included age, sex, and 4 levels of median household income by zip code. Clinical variables included HBV, HCV, ALD, non-ALD, HIV, diabetes, and obesity. Inpatient outcomes included in-hospital death and HCC procedure utilizations, i.e., liver transplantation, partial hepatectomy, and chemotherapy. Chi-Square and Fisher's exact test were used to compare the proportion of each variable in BA and WA cases.

Results: We identified 162 BA and 774 WA HCC cases, using ICD-9-CM Code 155.0. BA cases were significantly younger (mean age of 53.2 vs. 59.2 years, $p<0.0001$). Fifty two percent of WA cases were 61-80 year; 54% of BA cases were 41-60 year ($p<0.001$). BA had a lower median household income. Twenty seven percent of BA cases had income less than $24,999 comparing to only 3.6% of WA cases ($p<0.0001$). BA cases were more likely to have HBV (22.2% vs. 3.1%,

p<0.001), HCV (34% vs. 21%, p=0.004), HBV-HCV co-infection (8.1% vs. 1.6%), obesity (2.5% vs. 0.26%, p=0.001), and HIV infection (3% vs. 0.7%, p =0.006). ALD (15% vs. 9.2%, p=0.05) and diabetes (21% vs. 15%, p=0.07) were slightly more common among WA cases. Yet, diabetes plus either HBV or HCV was more common in BA cases. There was no difference in in-hospital death rate between the two races (16% in BA vs. 14.9% in WA, p=0.07). Also, utilization of liver transplantation, partial resection, chemotherapy, and other therapeutic procedures (i.e., chemo-embolization, percutaneous alcohol injection) were not significantly different between BA and WA cases.

Conclusions: BA HCC cases were significantly younger and had lower income than WA cases. The higher incidence of HCC among BA might be due to a higher prevalence of HBV, HCV, HBV-HCV co-infection, obesity, and viral hepatitis associated with diabetes.

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Speaker List

Hepatocellular Carcinoma: *Screening, Diagnosis, and Management*

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