Review

Hepatocellular Carcinoma

Definition:

Hepatocellular carcinoma (HCC) is the most often primary cancer of the liver, accounting from 85% till 90% of all primary liver cancers (El-Serag HB & Rudolph KL, 2007).

Epidemiology:

Hepatocellular Carcinoma (HCC) is the 6th most common cancer in the world, but the second most common cause of cancer death (Theise ND et al, 2014). There has been a marked increase in HCC related annual death rates during the past two decades (Lozano R et al, 2012).

Geographical distribution of HCC varies throughout the world. The burden of HCC has been increasing in Egypt with a doubling in the incidence rate in the past 10 years (Gomaa AI et al, 2008).

The highest age-adjusted incidence rates ([20/100,000) are recorded in East Asia (North and South Korea, China, and Vietnam) and sub-Saharan Africa (Yang JD& Roberts L R, 2010). Approximately 75% of liver cancers occur in Asia, with China accounting for more than 50% of the world’s burden (Lai CL et al, 2003).

HCC represents an important public health problem in Egypt. In many Egyptian regional registries, liver cancer is the first most common cancer in men and the second in women (Baghdady I et al, 2014).

In Egypt, liver cancer forms 11.75% of the malignancies of all digestive organs and 1.68% of the total malignancies. HCC constitutes 70.48% of all liver tumors among Egyptians (Mokhtar N et al, 2007).
HCC represents the main complication of cirrhosis, and shows a growing incidence in Egypt, which may be the result of a shift in the relative importance of hepatitis B virus (HBV) and HCV as primary risk factors (Gomaa AI et al, 2014), and improvements in screening programs and diagnostic tools (El-Serag HB, 2001).

In HCC, male predominance is more obvious in population at high risk with male to female ratio 3.7:1 (Mohamed NH, 2000). Besides the higher ratio of males in HCV infection, the male predominance in HCC may be explained by greater exposure of males to other environmental carcinogens, a role for sex hormones, and higher DNA synthetic activities in male cirrhotic patients compared to females (P. Tangkijvanich et al, 2004).

Risk Factors:

Worldwide, approximately 80% of HCC cases are caused by hepatitis B virus (HBV) and/or hepatitis C virus (HCV) infection, especially in the setting of established cirrhosis or advanced fibrosis (Yang JD& Roberts LR, 2010).

HCC is multifactorial in etiology and varies by region. Nonetheless, several major and minor causal associations with the tumor have been identified (Table 1).

Table (1). Risk Factors of Hepatocellular Carcinoma.
1) Environmental risk factors

- **Cirrhosis:**

  It has been recognized that the most important clinical risk factor for the development of HCC is cirrhosis. Approximately 80% of HCCs develop in cirrhotic livers (*Llovet JM et al, 2003*).

  The high rate of co-existing cirrhosis in HCC patients and the emergence of HCC in prospectively followed cirrhosis patients have led to the assumption that pre-existing cirrhosis is an important prerequisite for hepatocarcinogenesis, although some HCCs do arise in the absence of cirrhosis (*Gao J et al, 2012*).

- **Hepatotropic Viruses:**

  **a. Chronic HBV infection:**

  HBV is an enveloped DNA virus containing a partially double stranded, circular DNA genome, classified into the Hepadnaviridae family. The 3.2 kb HBV genome consists of four major open reading frames, P, S, C and X, encoding the reverse transcriptase essential for viral replication, the viral envelope proteins, the nucleocapsid (core)
protein and the X protein, respectively. There are at least 300 million HBV carriers in the world and 8–20% of the populations in endemic areas, including China, are carriers (SHAPIRO CN, 1993).

HBV transmission in adults occurs primarily by blood exchange and sexual contact, while children often acquire infection from their infected mothers at the time of birth (ZIMMERMAN RK et al, 1997).

Moreover, HBV was one of the first viruses to be implicated as a cause of a human cancer, (Blumberg BS et al, 1975) and evidence for its hepatocarcinogenicity is now beyond doubt (Kew MC, 2010).

Approximately 240 million people are chronic HBV surface antigen (HBsAg) carriers, with a large regional variation of HBsAg positive patients between low (2%) and high (8%) endemicity levels (Schweitzer A et al, 2015).

The incidence of HCC ranges from 340 to 804 per 100,000 HBV-positive males and 120 to 178 per 100,000 positive females per year, with odds ratios of 20.4 and 15.6, respectively, having been calculated (Parkin DM et al, 2001).

Chronic HBV infection and HCC have closely parallel geographical distributions, and chronic infection with the virus is implicated in the genesis of as many as 85% of the HCCs that occur with such high frequency in ethnic Chinese and Black African populations (Kew MC, 2010).

In individuals chronically infected with HBV, host factors influence the risk of malignant transformation. Sex and age are important in this regard. Chronically infected males are at greater risk of developing HCC than infected females, the ratio being 2:1 to 3:1 in most populations (Blumberg BS et al, 1975).
HBV is the dominant risk factor for HCC, causing up to 80% of HCCs worldwide. HBV acts as a pro-oncogenic agent indirectly and directly (CHISARI F V et al, 2000). The indirect mechanism involves the ability of HBV infection to induce hepatocellular necrosis, chronic inflammation and regeneration of the liver, eventually leading to cirrhosis and predisposition to HCC (MURAKAMI T et al, 1998).

The direct carcinogenic mechanisms have been related to the integration of HBV DNA into the host genome. As HBV DNA insertions are frequently associated with deletions, amplifications or chromosomal translocations, HBV DNA integration may result in chromosomal instability (HILDT E et al, 2002).

When the expressions of tumour suppressor genes and proto-oncogenes responsible for regulating cell growth, differentiation and apoptosis are altered, selective growth advantage of infected cells may trigger the onset of tumour and cancer progression (PATERLINI–BRECHOT P et al, 2003).

One such molecule is the HBx protein, which is a multifunctional regulator for a number of host processes by interacting with the virus and host factors (MURAKAMI S, 2001). For example, the HBx protein plays a role in modulating gene expression by interacting with promoters of oncogenes, cytokines and growth factors (MURAKAMI S, 1999).

HBx also interacts with transcription activators such as the leucine zipper family, p53, and Smad4. The abnormal activation of the signal transduction in the hepatocytes may result in oncogenic alterations and outgrowth of genetically damaged cells (MURAKAMI S, 1999).
b. **Chronic HCV infection:**

HCV is an enveloped single stranded ribonucleic acid virus with a 9.6 kb genome, classified into the Flaviviridae family. There are six major genotypes with several subtypes based on the genomic sequence heterogeneity (Simmonds P, 2004).

The prevalence of chronic HCV infection ranges from 0.3% in Switzerland to more than 20% in Egypt (Hutin Y et al, 2003). The ages of the patients influence progression of the infection: 5% of patients less than 40 years of age and 20% of those over 40 years of age progress to cirrhosis in less than 20 years (Kew M et al, 2004).

A strong correlation between HCV infection and intravenous treatment for schistosomiasis was frequently reported (Halim AB et al, 1999). Schistosomiasis, trematode blood flukes, is endemic in tropical areas of Africa, South America, Asia and the Caribbean. Only S. japonicum which is not present in Egypt has been classified as possibly carcinogenic in humans (Chou YH et al, 2003).

Approximately one-quarter of those with overt hepatitis will ultimately develop cirrhosis, and a significant proportion, usually but not invariably those with cirrhosis, progress to HCC (Kew MC, 1998).

Chronic HCV infection is the dominant cause of HCC in resource-rich countries. In Japan, Italy, and Spain, resource-rich countries with an intermediate incidence of HCC, HCV accounts for as much as 83% of HCCs (Kew M et al, 2004).

Average time to develop HCC after initial exposure to HCV is about 28 years and usually about 8-10 years after the development of liver cirrhosis (Vukotic R et al, 2007).
Patients with chronic HCV infection are at a greater risk of malignant transformation than those with persistent HBV infection (*Hutin Y et al, 2003*). Patients with HCV-induced HCC are generally older than those with HBV-induced tumors: the difference in age in most countries is about 10 years, although it is 20 years in sub-Saharan Black Africans (*Zampino R et al, 2013*).

The risk is highest among cases with cirrhosis where HCC develops at rate of 1–4% per year, though rates up to 8% have been reported in Japan (*Fattovich G et al, 1997*).

Most infected adults (up to 80 %) develop chronic HCV infection is consistently associated with substantially increased HCC risk in prospective and retrospective studies Fig. (1) (*Yoshizawa H, 2002*).

![Figure (1). Estimated progression rates to cirrhosis and hepatocellular carcinoma in hepatitis C infection.](image)

Unlike the HBV infection, the HCV genome does not integrate into the host genome (*BRANDA M& WANDS JR, 2006*), and almost all HCC patients infected by HCV suffer from cirrhosis, suggesting that cirrhosis is the major risk factor of HCC development in individuals with HCV (*HU KQ&TONG MJ, 1999*).
The polymerase enzyme of ribonucleic acid viruses such as HCV lacks efficient ‘proofreading’ ability, resulting in constant mutation and escape from the host immune response. Although the underlying mechanism of HCV-induced HCC development remains unclear, the core protein of HCV has been reported to have a significant role in HCC development in chronic hepatitis C (MORIYA, K, et al, 1998).

c. HBV/HCV coinfection:

Three meta-analyses have confirmed that patients with dual HBV/HCV infection have an increased risk of HCC (Shi J et al, 2005). Different mechanisms have been hypothesized as being associated with development of HBV- or HCV related HCC (Donato F et al, 1998). Both viruses could play an active role at different steps of the carcinogenic process when they are present together in hepatocytes, and may be synergistic in causing HCC (Cho LY et al, 2011).

Aflatoxins B1 (AFB1):

Aflatoxins are naturally occurring metabolites of the fungi Aspergillus flavus and Aspergillus parasiticus. They are structurally related difuranocoumarin derivatives, some of which are mutagenic and carcinogenic. These fungi are widely distributed in nature and are frequent contaminants of a number of staple foods, but particularly maize, ground nuts, rice, and sorghum. Aflatoxins pose serious health hazards to humans as a result of their toxic, teratogenic, mutagenic, and highly carcinogenic properties (Williams JH et al, 2004).

Aflatoxins are the most common known noninfectious food-borne risk factors for HCC. It is estimated that 4.5 to 5.5 billion people worldwide are at risk of exposure to these toxins (Wild CP & Turner PC, 2002).
Aflatoxin B1 is a major hepatocarcinogen, which acts in part by causing mutations of codon 249, a mutational hotspot of the p53 tumor suppressor gene. Aflatoxin B1 exposure, however, is more common in areas where HBV is the dominant virus, including sub-Saharan Africa, Southeast Asia, and China. Within these areas, higher levels are found among rural than urban populations (Wild CP & Hall AJ, 2000), among males than females (Plymoth A et al, 2009), and among persons chronically infected with HBV (Sun CA et al, 2002).

AFB1 is the most potent known hepatocarcinogen (Brown RL et al, 1999). It is metabolized in the liver by p450 enzymes into AFB1–8,9-exo-epoxide, which is highly reactive and forms derivatives with DNA, RNA, and proteins, and which can react with the p53 tumor suppressor gene (Hsu IC et al, 1991).

In turn, AFB1 binds to DNA to form the predominant promutagenic 8,9 dihydro-8-(N7-guanyl)-9-hydroxy AFB1 adduct. The latter can be converted into a more stable AFB1-formaminopyrimidine adduct, which causes guanine to thiamine transversion mutations. AFB1-formaminopyrimidine, incorporated into double-stranded DNA, is mutagenic, whereas the dominant species in single-stranded DNA blocks DNA replication (Kirk GD et al, 2000).

Aflatoxin B1 is metabolized by CYP2E1, which is induced by alcohol. Thus, alcohol may have an incremental genotoxic effect on aflatoxin B1 (Bulatao-Jayme J et al, 1982).

There is a synergistic association between aflatoxin B1 and HBV in increasing the risk of HCC. Compared with persons with neither risk factor, the risk of HCC is reported to be fourfold greater among persons with elevated levels of aflatoxin B1, sevenfold greater among chronic HBV carriers, and 60-fold greater among persons with both factors (Qian
Evidence suggests that there is also a synergistic effect between aflatoxin B1 and HCV infection (Kuang SY et al, 2005).

Alcohol abuse:

Abuse of alcohol is common in the Americas and Western Europe, and is increasing in Asia. More than 18 million adults in the USA (7% of the USA adult population) abuse alcohol (Grant B et al, 1994).

Chronic alcohol abuse (consumption in excess of 80 g/day) is complicated by the development of HCC. Such abuse for more than 10 years increases the chance of HCC development approximately fivefold (Morgan TR et al, 2004).

Alcohol acts synergistically with preexisting chronic liver disease, such as HCV, HBV, and fatty liver disease, as well as lifestyle choices, such as smoking and obesity, to further increase the risk of HCC in these disease states (Berman K et al, 2011).

Pesticides:

Occupational exposure pesticides may have a contributory role in the etiology or progression of HCC. A major segment of the Egyptian population (i.e, around 26%) is employed in agriculture and uses pesticides routinely to control insects, weeds, rodents, and fungal infections of crops and livestock. Studies suggested that exposures to organophosphorus and carbamate pesticides, as a result of increasing discharge of untreated industrial wastes and agricultural irrigation waste water, are additive risk factors to current HCV and HBV infection among rural males (Ezzat S et al, 2005).

Future investigation should address the possible hepatocarcinogenicity of pesticides using biomarkers of exposure and
other techniques to better estimate dose-response relationships (Anwar WA et al, 2008).

**Tobacco smoking:**

The effect of tobacco in the development of HCC is biologically plausible, due to the carcinogenic potential of several of the ingredients in tobacco that are metabolized in the liver (Marrero JA et al, 2005).

A Korean study has found a 50% increase in the risk of primary liver cancer for current male smokers compared to never smokers (Yun YH et al, 2005).

However, a population based case-control study from the United States did not observe a significantly increased risk of primary liver cancer among current male smokers (Zhu K et al, 2007).

A prospective study of 12,008 men observed that smoking significantly increased the risk of HCC only in anti-HCV-positive patients but not in those who were anti-HCV-negative when compared to anti-HCV-negative nonsmoking individuals (Sun CA et al, 2003).

In Egypt, a preliminary case-control study showed significantly higher percentage of HCC patients used to smoke for more than 20 years, more than 20 cigarettes/day (Abdou Moustafa EF et al, 2009).

**Oral contraceptive steroids:**

Oral contraceptives (OCs) appear to be associated with the development of benign liver tumors such as hepatic hemangioma, hepatocellular adenoma or focal nodular hyperplasia (Tajada M et al, 2001).
Malignant transformation can occur within the context of hepatic adenomas after 11 years mean duration of OCs use (Ito M et al, 2003). The frequency of HCC among hepatic adenomas appears to vary from 5% to 18% (Micchelli ST et al, 2008). In Egypt, 10.8% of married women aged 15-49 years were relying on OCs (Awadalla HI, 2012).

2) Host-related risk factors

- **Host genetics:**

  HCC develops in only a small percentage of those infected with HCV or HBV. Host genetic makeup may be an important factor that influences progression to HCC (Qin H et al, 2010). Two meta-analyses identified variants of tumor necrosis factor (TNF) associated with a higher risk of HCC (Guo YM et al, 2010). They showed that TNFa-308 AA and AG variants (versus GG) were associated with a significantly increased risk of HCC. A recent meta-analysis concluded that null genotypes of glutathione S-transferase (GST) genes (GSTM1 or GSTT1) were associated with an increased risk of HCC (White DL et al, 2008).

- **Metabolic syndrome:**

  In recent years, the incidence of obesity has increased alarmingly in many resource-rich countries, none more so than in the USA. In the great majority of these obese individuals, the obesity is attributed to the metabolic syndrome (Bianchini F et al, 2002).

  Paralleling this increase in obesity has been an increase in the incidence of HCC, and it has become increasingly evident that the burgeoning incidence of obesity in the inhabitants of resource-rich countries has been the dominant reason for the striking increase in the incidence of the tumor in these countries (Calle EE et al, 2002).
For example, the occurrence of HCC in the USA has tripled during the past 2 decades (El-Serag HB, 2001), and the tumor, paralleling the epidemic of obesity, is now the most rapidly increasing cause of cancer deaths in that country (Altekruse SF et al, 2009).

This syndrome is defined as a constellation of metabolic and other abnormalities indicated by the presence of central obesity (BMI in excess of 30 kg/m² or increased waist circumference), plus at least two of the following clinical or biochemical components: fatty infiltration of the liver in the form of NAFLD or NASH; type 2 insulin resistance; hyperinsulinemia or overt type 2 diabetes mellitus; cirrhosis; hyperlipidemia; and systemic arterial hypertension (Marchesini G et al, 1999).

The incidence of the metabolic syndrome in different parts of the world ranges from 9% to 34%, (Takamatsu S et al, 2008) and it is estimated that as many as 90% of obese adults will develop the syndrome (Ford ES et al, 2004). NAFLD is currently the most common liver disease in resource-rich countries (Lazo M & Clark JM, 2008). Ten to twenty-six percent of patients with NAFLD progress to NASH, and 8.6% of the latter become cirrhotic, although the latter progression may take many years (Nobili V et al, 2014).

Obesity is present in 33% to 100% of patients with NAFLD, and the risk of steatosis is appreciably higher in obese than in non-obese individuals (Borena W et al, 2012). NAFLD is commonly associated with insulin resistance and hyperinsulinemia (Nobili V et al, 2014). Patients with steatosis are at risk for developing cirrhosis and HCC (Lazo M and Clark JM, 2008).

Cohorts with NASH and cirrhosis have a risk of developing HCC (Ascha MS et al, 2010), which is as high as 12.8% over a 3.2-year
median follow-up period (Schlesinger S et al, 2013). Although NAFLD is currently the most common liver disease in resource-rich countries, the incidence of HCC complicating NAFLD is lower than that of HCC complicating NASH (4%–27%) (Nobili V et al, 2014).

- Diabetes mellitus (DM):

  DM is an independent risk factor for HCC development in patients with chronic HCV, but also in patients without viral hepatitis (van der Meer AJ et al, 2012). The mechanism for the association between DM and HCC is not fully discerned, but these diseases share common risk factors, such as obesity and steatosis (Marrero JA et al, 2005).

  Direct effects of hyperinsulinemia and insulin signaling have also been suggested as contributing factor (Chettouh H et al, 2015). The relationship between HCV, insulin resistance, cirrhosis, and HCC is complex. Insulin resistance is higher among patients with chronic HCV than in matched controls (Hui JM et al, 2003). Liver cirrhosis causes insulin resistance and leads to hyperinsulinemia, and this seems to contribute to the progression of liver disease (Garcia-Compean D et al, 2009).

  Some possible mechanisms explained this association. Most non-insulin dependent diabetics show hyperinsulinemia. Thus, insulin or its precursors may interact with liver cells to stimulate mitogenesis or carcinogenesis (Moore MA et al, 1998).

  Another possible pathway is that a p53 mutation (an apoptotic factor) was noted frequently in HCC patients with diabetes rather than non-diabetic, this might provide an evidence for a molecular mechanism involving this common association (Hsu HC et al, 1994).
Hereditary hemochromatosis:

Hereditary hemochromatosis, a rare autosomal recessive genetic disorder characterized by excess iron absorption, is caused by mutations in the HFE gene and/or other mutations in the iron metabolism machinery (Powell LW et al, 2000).

The altered iron metabolism seen in hereditary hemochromatosis leads to excess iron storage in the liver and the subsequent development of liver cell damage. Several studies have shown that the diagnosis of hereditary hemochromatosis confers a consistent and markedly elevated risk for the development of HCC (Elmberg M et al, 2003).

An Egyptian study revealed that the frequencies of HD and DD genotype of H63D mutation were significantly increased among HCC patients compared to control group and to cirrhosis group (Gharib AF et al, 2011).

In fact, patients with excess total body iron secondary to other etiologies such as β thalassemia or iron overload in people of African descent have been shown to have a higher risk of HCC in the absence of genetic hemochromatosis (Borgna-Pignatti C et al, 2004). Regardless of etiology, surveillance for HCC should be undertaken in case of iron overload (Moyo VM et al, 1998).

Membranous obstruction of the inferior vena cava:

Membranous obstruction of the inferior vena cava (MOIVC) is an occlusive lesion of the inferior vena cava, usually complete, but occasionally with a small central opening, which is located close to the entrance of the inferior vena cava into the right atrium or just below the level of the diaphragm. Although usually in the form of a membrane of variable thickness, it may take the form of a fibrotic occlusion of variable
length. The condition occurs more often in men than in women, and most patients are in their 30s, 40s, or 50s (Kew MC & Hodkinson HJ, 2006).

MOIVC is rare in most countries, but occurs more frequently in Southern Africa, India, Japan, Nepal, the People’s Republic of China, and Korea. For example, it occurs in 4% of sub-Saharan Black Africans. It is uncertain whether the lesion is a congenital vascular anomaly or the result of organization of a thrombus in the hepatic portion of the inferior vena cava. The lesion impedes hepatic venous drainage, causing chronic hepatic venous congestion and centrilobular fibrosis of the liver (Kew MC & Hodkinson HJ, 2006).

The reason for the malignant transformation is unknown, although it is not thought to be a direct consequence of the occlusive lesion. Rather, the centrilobular necrosis and regeneration resulting from the hepatic venous hypertension and congestion, by acting as a tumor promoter, predisposes to one or more environmental carcinogens prevalent in those countries where HCC often complicates MOIVC. This belief is supported by the geographical distribution of the complicating HCC in Southern Africa (Simson IW, 1982).

**Autoimmune hepatitis (AIH):**

AIH is a disease of unknown etiology affecting females mainly (Meza-Junco J et al, 2007). It is an inflammation of the liver that occurs when immune cells mistake the liver’s normal cells for harmful invaders and attack them. The risk of HCC among AIH patients with cirrhosis is 1.9% per year. This is comparable to HCC risk among patients with cirrhosis secondary to HBV, HCV or alcohol-related liver disease (Dawn BM et al, 2007).
In Egypt, an epidemiological study over last 15 years including 1,759 HCC patients found that 0.5% of patients had suffered from AIH (Saleh SM et al, 2012).

HCC incidence of about 1% has been reported from different geographic areas among chronic AIH dependent liver cirrhosis (Geramizadeh B et al, 2013).

**Others:**

- **Alpha1 antitrypsin deficiency (A1ATD):**

  Epidemiology studies revealed that severe alphal antitrypsin deficiency (A1ATD) is a significant risk factor for cirrhosis and HCC unrelated to HBV or HCV infections. However, predisposition to HCC in moderate A1ATD is rare, and probably occurs in combination with HBV and/or HCV infections or other unknown risk factors (Topic A et al, 2012). It is proposed that accumulation of polymers of A1ATD variants in endoplasmic reticulum of hepatocytes leads to damage of hepatocytes by gain-of-function mechanism (Carlson JA et al, 1989).

  The increased frequency of mutant A1AT deficiency alleles together with the existence of HFE mutant alleles among HCV liver cirrhosis Egyptian patients may warrant us to do further studies assessing their relevance for risk stratification for disease progression (Settin A et al, 2006).

- **Hereditary Tyrosinemia**

  Hereditary Tyrosinemia is an autosomal recessive inborn error of tyrosine metabolism caused by a deficiency of fumaryl acetoacetate hydrolase (FAH). Hepatomegaly with focal hepatic lesions is the commonest presentation. It is increasingly recognized among Egyptian children; this may be explained by the high rate of consanguinity among Egyptians (El-Karaksy H et al, 2011).
Tyrosinemia might be complicated by the development of HCC (Montalto G et al, 2002). Thus, dietary or pharmacological management of hereditary tyrosinemia might offer a strategy for prevention of HCC in these cases (Ashorn M et al, 2006).

- **Wilson’s disease (WD):**

  WD is a rare autosomal recessive metabolic disorder with an incidence of 1:40,000 (Frydman M et al, 1990). HCC is well described despite adequate copper chelating therapy, although the true incidence is difficult to establish (Ryder et al, 2003).

  The mechanism of tumorigenesis in hepatic WD is complicated and may be multifactorial. Copper overload plays a critical role in the initial liver injury that eventually leads to chronic inflammation and cirrhosis (Xu R et al, 2007).

- **Primary biliary cirrhosis:**

  The incidence of HCC in stage 4 primary biliary cirrhosis is about the same as in cirrhosis due to hepatitis (Bruix & Sherman, 2005), however, available data suggest that women, even with established cirrhosis, have a low risk but males have a similar risk to patients with alcohol related cirrhosis (Ryder et al, 2003).

### Aetiology and Risk Factors of HCC in Egypt:

Egypt is responsible for most of cases of HCC in Africa (Ezzat et al, 2005). Meanwhile the latest decade, a considerable increase was observed among Egyptian patients with chronic liver disease related HCC (from 4.0% to 7.2%) (National Cancer Registry of Egypt, 2010) (Abdel Hamid et al, 2011). Hepatitis B and hepatitis C are the main causes of liver disease (Zekri et al, 2012).

Egypt is one of the highest HCV prevalence worldwide (Egyptian Ministry of Health, 2007) (Khattab et al, 2010).
Hassan et al. (2001) found anti-HCV antibodies to be 75.8% in the HCC patients and in 42.9% of the controls while Hepatitis B surface antigen (HBsAg) was present in 15.2% of the patients and in 2.9% of the controls. Also, (Qabil, 2002) found nearly the same results, 70% of the patients were positive for HCV and 8% demonstrated HBV antibodies.

Pathogenesis of HCC

As with most types of cancer, hepatocarcinogenesis is a multistep process typically requiring inflammation and cirrhosis and also involving different genetic alterations that ultimately lead to malignant transformation of the hepatocyte, although HCC can occur in the setting of mild liver disease as well (Moradpour D& Blum HE, 2005).

The molecular contribution of the multiple factors and their interactions in hepatocarcinogenesis are still poorly understood suggesting that HCC is genetically a very heterogeneous tumor. As shown in (Figure 2), malignant transformation of hepatocytes might occur regardless of the etiologic agent through a final common pathway of increased liver cell turnover, induced by chronic liver injury and regeneration in a context of inflammation and oxidative DNA damage (Moradpour D& Blum HE, 2005).
HCC is characteristically silent and slow growing, with most patients having few symptoms until very late in the disease; often these symptoms are related to decompensated liver disease rather than directly due to the tumor growth or metastatic disease (*Bialecki ES & DiBisceglie AM, 2005*).

*Figure (2).* Pathogenesis of hepatocellular carcinoma. AFB1, aflatoxin B1. Reprinted with permission from Moradpour and Blum (*Moradpour D & Blum HE, 2005*).

As with other malignancies, the natural history of HCC relates to patterns of growth, the severity of the underlying cirrhosis if cirrhosis is present, and the effect of the tumor on liver function including blood flow in those patients with marginal reserve as well as the propensity to invade vasculature and spread to surrounding and distant organs. Features of HCC have been shown to indicate more aggressive behavior, including poorly differentiated histology, lack of fibrous capsule, large size (>5 cm in diameter), vascular invasion, early metastases, and elevated serum levels of alpha-fetoprotein (AFP). HCC is the most common form of liver
cancer and might be unifocal, diffuse, or multifocal at presentation (Sheu JC et al, 1985).

Tumor growth rates have a wide range of variability even among patients from the same region and regardless of disease stage. The development of liver cell dysplasia is a well-recognized premalignant finding in patients with cirrhosis of any etiology (Bialecki ES & DiBisceglie AM, 2005).

The term dysplasia as well as the role of small and large cell dysplasia as a precursor condition of HCC are still being defined as imaging modalities improve, and surgical specimens including explant tissue are being examined both on a microscopic tissue level and with the use of special stains and genomics (Lee JS & Thorgeirsson SS, 2005).

Maturation of HCC requires adequate vasculature, which is derived primarily by the hepatic arterial network. Characteristic satellite lesions might subsequently develop and eventually progress to form multinodular disease and invasion of large vascular structures (Bialecki ES & DiBisceglie AM, 2005).

Pathology of HCC:

Gross pathology:

Grossly, the cut surface of an HCC may be yellow in color from fatty infiltration. The tumor tissue tends to be soft and friable (Abeloff et al, 2000).

The right lobe is involved more frequently than the left, although bilobar involvement is seen in as many as 80% of cases of multicentric primary. In advanced cases, the large veins (portal or hepatic) tend to be involved (Molmenti et al, 1999).
Hepatocellular carcinoma occurring in the cirrhotic liver may appear as solitary mass, multifocal masses or as a diffusely infiltrating mass, satellite nodules may be present. A capsule is present in 30% to 67% of cases. When HCC occurs in a normal liver; it is typically a solitary well defined mass. Hemorrhage is common within HCCs in both cirrhotic and non-cirrhotic liver (Molmenti et al, 1999).

**Microscopic picture:**

Hepatocellular carcinoma is classified histologically into well differentiated, moderately differentiated or undifferentiated (pleomorphic) forms (Kew, 2002).

**Classification:**

The WHO has classified types of HCC on the basis of structural organization of tumor cells. The WHO has reviewed and accepted the following histological classification of HCC (Aguayo & Patt, 2001):

1. **Trabecular (sinusoidal):** tumor cells are arranged in cords of variable
cell thickness separated by sinusoids, fibrosis is absent or minimal.

   **Pseudoglandular (acinar):** tumor cells form glandular like structures, canaliculi with or without bile are common.

2. **Compact:** Cells form a solid mass, sinusoids are inconspicuous.

   **Scirrhouos:** Significant fibrous stroma separate cords of tumor cells.
Diagnosis of HCC

Clinical Features:

1. Non-specific signs and symptoms:

   Few patients may have weight loss, sense of poor appetite or early fullness of the stomach, or a visible mass in the upper abdominal part. These symptoms often signify the presence of an advanced hepatic lesion (Sugano S et al, 1994).

2. Clinical aspects of cirrhosis:

   Clinical signs and symptoms of hepatic cirrhosis that is often present in patients with HCC, usually mask the presence of an underlying early hepatocellular carcinoma (Trevisani F et al, 1995).

   Patients who develop hepatocellular carcinoma (HCC) usually have no symptoms other than those related to their chronic liver disease. Suspicion for HCC should be heightened in patients with previously compensated cirrhosis who develop decompensation such as ascites, encephalopathy, jaundice, or variceal bleeding. These complications are often associated with extension of the tumor into the hepatic or portal veins or arteriovenous shunting induced by the tumor (Sugano S et al, 1994).

2.1. Hepatomegaly:

   Hepatomegaly can be an expression of the tumor mass. In case of HCC, the palpable edge of the liver is more often irregular, hard, with nodular consistency. Hepatomegaly is more often present in patients without advanced cirrhosis (Lam et al, 2004). In case of large tumors, the mass can cause asymmetry of the abdomen (Sanders C F et al, 1968).
2.2. Vascular bruit:

Through auscultation of the abdomen, an arterial bruit can be heard in patients affected by HCC. This clinical sign is thought to be caused by the presence of an arteriovenous fistula in the context of the tumor (Sherman HI et al, 1979), suggesting the presence of a highly vascularized HCC (Okuda KO et al, 1999).

3. Abdominal pain, portal vein thrombosis and rupture of HCC:

Frequent manifestation of onset of HCC is abdominal pain. The pain is usually mild, located in right hypochondrium and it can radiate to the right shoulder. Abdominal pain is more frequent in non-cirrhotic patients, and in case of portal thrombosis (Trevisani F et al, 1995).

Portal vein thrombosis has been found in (14-44%) of autopsies of patients with HCC. Patients with both cirrhosis and hepatic carcinoma have the highest risk to develop portal vein thrombosis. Portal vein thrombosis is reported to be diagnosed during investigation for acute abdominal pain in 18% of cases in cirrhotic patients (Ogren M et al, 2006).

Splenomegaly has been reported to be present in 75-100% of patients with portal vein thrombosis (Sobhonslidsuk A & Reddy KR, 2002). Bleeding from esophageal varices or from portal hypertensive gastropathy is the most common presenting symptom of portal vein thrombosis in cirrhotic patients (Amitrano L et al, 2004).

In 43% of cases of Portal vein thrombosis in cirrhotic patients, diagnosis is done during a routine echo-Doppler examination (Amitrano L et al, 2004).
Rare presenting features include acute abdominal catastrophe from rupture of HCC with intra-abdominal bleeding or extra hepatic manifestations (eg, hypercalcemia, hypoglycemia, thyrotoxicosis (Choi BG et al, 2001).

HCC rupture is also a rare complication of therapeutic procedures on HCC. For example after transcatheter arterial chemoembolization (TACE), HCC rupture occurs in less than 1% of patients (Battula N et al, 2007).

3.1. Gastrointestinal bleeding:

A particular manifestation of HCC can be a bleeding from esophageal varices. This presentation is not frequent, occurring as first clinical sign only in 1%-8% of cases of HCC (Lang BH et al, 2004) (table 2). Variceal bleeding is caused by higher pressure in portal district which in turn can be caused by tumor invasion of this venous system and portal hypertension (Chen CH et al, 1998).

Additionally, 50 % of causes of gastrointestinal hemorrhages are represented by hypertensive gastropathy, peptic ulcer and direct tumoral invasion of digestive tract (Yeo W et al, 1995).

3.2. Jaundice:

Jaundice is a frequent sign of presentation of HCC. Some studies indicated that it is present at the diagnosis of HCC in 28% of African patients, but less frequent in Chinese, Japanese or European countries (table 2). Different pathologic conditions linked to HCC can explain the onset of jaundice. Jaundice can be expression of hepatic failure, due to extensive tumor infiltration of a cirrhotic liver or by worsening of the underlying hepatitis that can occur in presence of HCC (Lau W et al, 1997).
The neoplastic obstruction can occur due to intraluminal biliary obstruction, extraluminal neoplastic compression or clot formation secondary to hemobilia caused by tumor invasion of biliary tree (Lau WY et al, 2000).

4. Fever

Fever of Unknown Origin can be a way of presentation of HCC (Hayashi T et al, 1995). Fever occurs more frequently in patients with massive HCC and in non-cirrhotic individuals (Trevisani F et al, 1995).

Table (2). Clinical signs and symptoms of hepatocellular carcinoma in different geographic regions (Lang BH et al, 2004).

<table>
<thead>
<tr>
<th>Clinical signs and symptoms in different geographic regions (%)</th>
<th>Black African</th>
<th>Japan</th>
<th>China</th>
<th>Europe (Italy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>-</td>
<td>-</td>
<td>29.9</td>
<td>38</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>95</td>
<td>46</td>
<td>51</td>
<td>38</td>
</tr>
<tr>
<td>Ascites</td>
<td>51</td>
<td>27</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>Palpable mass</td>
<td>92</td>
<td>23</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>-</td>
<td>-</td>
<td>54</td>
<td>90</td>
</tr>
<tr>
<td>Ankle Edema</td>
<td>-</td>
<td>17</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>Jaundice</td>
<td>28</td>
<td>17</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Fever</td>
<td>35</td>
<td>17</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Hemoperitoneum</td>
<td>-</td>
<td>7</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Variceal Bleeding</td>
<td>2</td>
<td>8</td>
<td>-</td>
<td>4</td>
</tr>
</tbody>
</table>

5. Age differences in presentation:

Patient’s age can influence the clinical presentation of HCC. Signs and symptoms at presentation of HCC described in patients affected by hepatitis B are significantly different in patients younger and older than 40 years. Younger patients present more often with pain, hepatomegaly and ruptured HCC. Older patient present more often with ankle oedema.
and ascites. This is explained by the fact that in patients affected by viral hepatitis, advanced cirrhosis is more frequent in the older ones (Lam CM, 2004).

6. Extrahepatic metastases:

Metastases from HCC, spread through lymphatic or hematic system, are more frequently placed in abdominal and thoracic lymph nodes, lung, bones, and adrenal glands. Less frequent sites of metastases are brain, spleen and breast (Uchino K et al, 2011). Rarely metastases can also be detected in digestive tube, pancreas, seminal vesicle and bladder (Katyal S, 2000).

When HCC is diagnosed, extrahepatic metastases are present in more or less 40% of cases, and several signs and symptoms can be caused by this condition. Regional lymph nodes are affected in up to 60% of metastatic HCC, while distant lymphatic stations are involved only in 12% of these cases (Katyal S, 2000).

Pain and pathologic fractures can be caused by osteolytic metastases. Severe pain is present in 90 % of patients with bone metastases (Natsuizaka M et al, 2005).

6. Paraneoplastic syndromes:

Paraneoplastic syndromes occur in 19-44% of patients affected by HCC. The presence of paraneoplastic syndromes is described to be related with younger patients, with larger size tumor (>10 cm) and with presence of portal vein thrombosis (Luo JC et al, 1999).

Among patients that have paraneoplastic syndromes during the clinical course of HCC, most of them have a single paraneoplastic manifestation. Hypercholesterolemia, erythrocytosis, hypoglycemia and
hypercalcemia are some of the most common paraneoplastic manifestations of HCC. Only 7% of patients have 2 of these syndromes, and rarely can be present all of them (<1% of cases) (Table 3) (*Luo JC, 1999*).

Various cutaneous manifestations have been reported as paraneoplastic syndromes caused by HCC. In rare cases, dermatomyositis has been described in patients affected by HCC, either associated or not to viral hepatitis (*Apostolidis L, 2009*).

Arterial hypertension has been described as a paraneoplastic manifestation of HCC. Some cases of severe arterial blood pressure associated with high plasma level of angiotensin-I, accompanied with hypokalemia have been reported (*Arai H, 1999*).


**Table (3).** Some of the reported paraneoplastic syndromes (*Luo JC, 1999*).

<table>
<thead>
<tr>
<th>Paraneoplastic Syndromes</th>
<th>Usual Manifestations</th>
<th>Reported Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoglycemia</td>
<td>Hypercalcemia</td>
<td>Arterial Hypertension</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>Erythrocytosis</td>
<td>Dermatomyositis/Polymyositis</td>
</tr>
<tr>
<td>Thrombocytosis</td>
<td>Pityriasis Rotunda</td>
<td>Porphyria cutanea tarda</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pemphigus</td>
</tr>
<tr>
<td>Sign of Leser-Trelat</td>
<td>Diarrhea</td>
<td>Neurological manifestations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Membranous glomerulonephritis</td>
</tr>
<tr>
<td>Dy sfibrinogenemia</td>
<td>Cryofibrinogenemia</td>
<td>Carcinoid syndrome</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myasthenia grave</td>
</tr>
</tbody>
</table>
Imaging Modalities:

❖ Ultrasonography (US):

Imaging of the lesion usually begins with US (if not already performed), which provides information on the shape, echogenicity, growth pattern and vascular involvement of the tumour (CHU YU et al, 1996).

US can detect large tumours with high sensitivity and specificity, but has limitations in identifying smaller lesions, which is an essential requirement for improved outcomes in this disease. Expertise of the operator and use of dedicated equipment seem important to optimize results; where these are available, US detects 85% of tumour <3 cm, 85–95% of tumours 3–5 cm in diameter and can achieve 60–80% sensitivity in the detection of tumours measuring 1 cm (COLOMBO M et al, 1991).

The combination of AFP and US improves detection rates. Screening with US has been suggested at six monthly intervals on the basis of tumour doubling times (MIMA S et al, 1994).

Contrast-enhanced US has a higher accuracy of biopsy, which lowers the false negative rate of malignant lesions. Disadvantages of US include association with obesity and the requirement for necessary operator experience. Moreover, US has difficulties in differentiating between benign and malignant lesions in the context of nodular cirrhosis (Bellissimo F et al, 2015).

❖ Computed tomography (CT):

CT is used for detection and evaluation of the extent of HCC (DALLA PALMA et al, 1992). The most reliable diagnostic tests are
triple-phase helical CT and triple-phase dynamic contrast enhanced magnetic resonance imaging (MRI) (Arguedas MR et al, 2003).

Hepatic angiography has fallen out of favor in most practice settings. HCC derives its blood supply predominantly from the hepatic artery, whereas the remainder of the non-tumorous liver receives both arterial and portal blood. The hallmark of HCC during CT scan or MRI is the presence of arterial enhancement followed by delayed hypointensity of the tumor in the portal venous and delayed phases, ie, washout. The presence of arterial enhancement followed by washout has a sensitivity and specificity of 90% and 95%, respectively (Marrero JA et al, 2005). CT showed better sensitivity than US but poorer sensitivity than MRI (Chou R et al, 2015).

- Magnetic resonance imaging (MRI):

  MRI has been recently proposed to be better for defining tumor morphology than CT scanning, although this remains controversial and needs to take place at centers with a special interest and expertise in this area (Sherman M, 2004).

  4 studies that have compared the accuracy of CT and MRI for HCC diagnosis, using the explanted liver as the gold standard (Burrel M et al, 2003) (Rode A et al, 2001). These show that MRI is slightly better in the characterization and diagnosis of HCC when compared with CT scan. The performance of CT and MRI is affected by the size of the lesions. For example, in tumors larger than 2 cm, MRI is reported to have an accuracy 90%; however, in tumors smaller than 2 cm, this level is reduced to 33% (Ebara M et al, 1986).
Nuclear image

18Fluorodeoxyglucose (FDG) PET is generally accepted to have low sensitivity (50–68%) for intrahepatic HCC (Talbot JN et al, 2006)( Park JW et al, 2008) and is therefore not considered to be useful for diagnosis of HCC, except perhaps in cases of poorly differentiated HCC where it may show better results (Yamamoto Y et al, 2008).

The role of 18-FDG is limited to evaluation of extra hepatic disease (Yoon KT et al, 2007), with sensitivity of 13–84%, depending on the size of the lesions (M. Sugiyama et al, 2004).

Dual tracer imaging with the addition of 11C-acetate improves sensitivity for intrahepatic disease from 37–49% for 18-FDG and 11-C alone to 90% when combined (Yoon KT et al, 2007).

Newer tracers such as 18F-choline (Talbot JN et al, 2009) and 18F-thymidine have shown slightly better results, but further experience is needed. At present, PET plays a small role in imaging assessment of HCC, but tumour-specific tracers may be the key to its use in future (F. Eckel et al, 2009).

Laboratory Studies

Biomarkers for hepatocellular carcinoma

The usefulness of tumor markers is determined by their sensitivity and specificity, which vary depending on the diagnostic level used as the gold standard in a disease, and also depending on the cutoff level of tumor markers (Taketa K & Ikeda S, 2001).

Advances in genomics and proteomics platforms and biomarker assay techniques over the last decade have resulted in the identification of
numerous novel biomarkers and have improved the diagnosis of HCC (Figure 3) (Nobuhiro Tsuchiya et al, 2015)

Figure (3). Hepatocellular carcinoma tumor markers.

A. Embryonic antigen

1. Alpha-fetoprotein:

   However, serum levels of AFP do not correlate always with other clinical features of HCC such as size, stage, or prognosis, and AFP is the most common used marker for HCC. Because of AFP is normally produced during gestation by the fetal liver and yolk sac, the serum concentration of AFP can be increased during pregnancy with tumors of gonadal origin (both germ cell and non-germ cell) and in a variety of other malignancies (El-Bahrawy M, 2010).

   Elevated serum AFP can also be seen in patients with chronic liver disease without HCC such as acute or chronic viral hepatitis, particularly in hepatitis C (Collier J & Sherman M, 1998).
It is generally accepted that serum levels greater than 500 mcg/L (normal in most laboratories is between 10 and 20 mcg/L) in a high-risk patient are diagnostic of HCC (Wu JT, 1990). On the other hand, HCC is often diagnosed at a lower AFP level in patients undergoing screening (El-Bahrawy M, 2010). Not all tumors secrete AFP, and serum concentrations are normal in up to 40% of small HCCs (Chen DS et al, 1984).

Persistently elevated AFP values in a patient with cirrhosis have an increased risk of developing HCC compared with those who have fluctuating or normal levels (Colombo M et al, 1991).

The sensitivity and specificity of AFP for HCC are highly dependent on the cut-off value above which AFP is considered positive. Values ranging from 10 to 500 ng/mL have been used in the literature as diagnostic cut-off to detect HCC. While the threshold abnormal level for serum AFP is often taken as 20ng/mL, the cut point for the suggestion or diagnosis of HCC varied among different studies especially the earlier ones, e.g., 10.5 μg/L, 25μg/L, 50μg/L, 100μg/L, 200μg/L, 400μg/L and 500μg/L as sensitivity decreases with increasing cut-off values, the specificity increases (lopez JB, 2005).

The sensitivity, specificity, and predictive value for the serum AFP in the diagnosis of HCC is still controversial issue. There is no strict cut-off value. Commonly accepted value is >20 mcg/L and a review which have five studies showed that sensitivity was 41–65% and specificity was 80–94% based upon a cutoff value of >20 mcg/L (Gupta S et al, 2003).

An increase in AFP is associated with increased tumor size and stage, extrahepatic metastasis, portal vein thrombosis, and decreased survey (Ioannou GN et al, 2008). Patients with AFP levels >1000 mcg/L
have an extremely high risk of recurrent disease following transplantation, irrespective of the tumor size (Pomfret EA et al, 2010).

Although ASLD and EASL guidelines recommend using US alone to achieve this goal given concerns about the suboptimal sensitivity and specificity of AFP (Bruix J& Sherman M, 2011), and when AFP used in combination with US, its sensitivity reaches up to 63% for early-stage HCC (Mehta A& Singal AG, 2015).

2. Lens culinaris agglutinin-reactive fraction of AFP (AFP-L3):

Development and applications of biological chemistry have determined that total AFP can be divided into three different glycoforms (AFP-L1, AFP-L2 and AFP-L3 according to their binding ability to the lectin lens agglutinin (LCA). High percentage of AFP-L3 has been demonstrated to be associated with pathologically malignant characteristics, worse liver function, larger tumor mass and poor survival in HCC patient (Khien V et al, 2001).

Lens culinaris agglutinin-reactive AFP (AFP-L3) is a newly developed assay, highly sensitive fraction of AFP (hs-AFP-L3) that has been used as a diagnostic and prognostic marker of HCC. In patients with AFP < 20 ng/mL, measurements of AFP-L3% by the highly sensitive method before treatment was more useful for diagnosis and prognosis of HCC than by the conventional method (Toyoda H et al, 2011).

3. Glypican-3 (GPC3):

GPC3 is oncofetal protein being one of members of heparin sulfate proteoglycans anchored to plasma membrane through glycosyl-phosphatidyinositol. It is involved in regulation of cell proliferation and survival during embryonic development and functions as a tumor suppressor (Sung YK et al, 2003).
GPC3 is absent in hepatocytes of healthy subjects and patients with nonmalignant hepatopathy, and can be detected in about 50% of HCC patients and 33% of HCC patients seronegative for AFP (Nakatsura T et al, 2003).

A number of studies showed that a very high specificity (90–100%) associated with serum GPC-3 in patients with HCC, but the sensitivity of serum GPC-3 remained relatively low; however, if GPC-3 measured combined with AFP, sensitivity appears to improve (Hui Liu et al, 2010).

B. Proteantigen

1. Heat shock protein (HSP):

Heat shock protein (HSP) is a highly conserved stress response protein that is expressed under stress conditions, including carcinogenesis and inflammation (Chai Y ET AL, 1999). HSP is also a group of proteins that play a major role in the folding of cellular proteins (Kiang JG & Tsokos GC, 1998). HSPs promote carcinogenesis through inhibiting the pathways of tumor suppression (Melcher A ET AL, 1998).

Luk J et al. (2006) have demonstrated overexpression of HSP70 and HSP27 in HCC tissues and in the microenvironment of HCC that can increase tumor growth and metastasis.

Sakamoto et al. (2008) reported the significant overexpression of HSP70 in early HCC compared with precancerous lesions, and in advanced HCC compared with early HCC. HSP70 has been shown to be negative in other benign nodular lesions, hepatocellular adenoma and focal nodular hyperplasia. Hence, HSP70 might work as a molecular marker to differentiate between benign and malignant liver nodules.
2. Squamous cell carcinoma antigen (SCCA):

SCCA is a member of the family of serine protease inhibitors named serpins and has been reported to be overexpressed in HCC tissues (Giannelli G et al, 2005).

Guido M et al. (2008) showed that the expression of SCCA increased in the early stages of HCC. The sensitivity and specificity of SCCA in detecting HCC were found to be 84% and 46% respectively.

Zhou L et al. (2006) have reported that SCCA is an important diagnostic marker’s supplement for the diagnosis of HCC because SCCA, unlike AFP, has high sensitivity and low specificity.

3. Golgi protein 73 (GP73):

GP73 (also known as Golph2 and GOLM1) is a type II Golgi-specific membrane protein that is coded by the GOLM1 gene on chromosome 9q21.33 (Kladney RD et al, 2000).

It is significantly elevated in various types of cancer, such as lung adenocarcinoma (Zhang F et al, 2010), seminomas (Fritzsche FR et al, 2010) and renal cell cancer (Fritzsche FR et al, 2008).

Results of recent studies have shown that the serum GP73 is significantly elevated in primary hepatic carcinoma (PHC) (Shi Y et al, 2011). In liver cirrhosis it is not only elevated, but also higher than in HCC, whether infection is caused by HBV or hepatitis C virus (HCV) (Tian L et al, 2011).

By contrast, in normal liver, GP73 is expressed by biliary epithelial cells, but minimally by hepatocytes. Thus, GP73 is a significant factor in many hepatic diseases. (Riener MO et al, 2009). The sensitivity of diagnosis of HCC (76.9%) was markedly elevated compared with AFP.
(48.6%), suggesting GP73 is a novel and effective serum biomarker for the diagnosis of HCC (Zhou Y et al, 2012).

Additional investigations identified fucosylated GP73 (FC-GP73). Compared with total GP73, FC-GP73 improves the sensitivity and specificity of diagnosis of HCC from 65-90 to 90-100%, respectively. For GP73-negative or low levels, detection of FC-GP73 is a viable option (Drake RR et al, 2006).

In general, it is important that correlation between GP73 and tumor size, stage, recurrence and prognosis of HCC remains to be clarified. Thus, role of GP73 in the diagnosis remains to be extensively investigated (Zhang Y et al, 2012).

4. Tumor-associated glycoprotein 72 (TAG-72):

TAG-72 is a glycoprotein complex that is found on the surface of cancer cells and overexpressed in the majority of human adenocarcinoma including gastric, colon and pancreatic cancer whereas it is lightly expressed in normal tissue (Sheer DG et al, 1988).

Recent studies found that the expression of TAG-72 is significantly elevated in HCC tissues compared with normal liver tissues. Its increased expression may promote tumor invasion and metastasis. Furthermore, overexpression of TAG-72 is closely correlated with poor survival in patients with HCC (Zhang Y et al, 2012).

Thus, TAG-72 is a potential prognostic marker for HCC, which has important clinical implications. Moreover, anti-TAG-72 monoclonal antibody has been used for the clinical detection of tumors (Milenic DE et al, 2010).

5. Zinc-a2-glycoprotein (ZAG):
ZAG is a member of the class I major histocompatibility complex (MHC-I) family and may have a role in the induction of the immune response (Hassan MI et al, 2008). Due to its high homology of the amino acid sequence with the lipid mobilizing factor (LMF), we considered it as a novel adipokine. ZAG is down regulated in human obesity (Mracek T et al, 2011).

Wang F et al. (2012) have demonstrated that ZAG is a soluble glycoprotein and up regulated in several types of cancer such as breast, lung and prostate cancers and HCC and it can be considered as a novel candidate biomarker for these cancer types; But it can be said, the function of the ZAG under physiologic and cancerous conditions, especially HCC, needs to be investigated more.

C. Enzymes and Isozymes

1. Des-gamma-carboxyprothrombin (DCP):

Des-γ-carboxy prothrombin (DCP) is a prothrombin precursor produced in hepatocellular carcinoma (HCC). DCP lacks the activity of interacting with other coagulation factors (Inagaki Y et al, 2011).

DCP has been considered as a specific marker for diagnosis of HCC (Zhou L et al, 2006). Elevated serum DCP levels have been found in 44–81% of HCC patients (Yuen MF et al, 2005). High level of DCP has been considered to be associated with large tumor and recurrence of HCC. Recent studies have shown that DCP might play important roles in the development of HCC. A significant association between an elevated DCP level and worse tumor behavior has been established (Gotoh M et al, 2003).

Elevated serum DCP is significantly related to portal vein invasion and/or intrahepatic metastasis. It recommends simultaneous
measurements of serum alpha-fetoprotein and protein induced by vitamin K absence for detecting hepatocellular carcinoma (Bertino G et al, 2010).

DCP can be elevated in other conditions besides HCC. Conditions such as obstructive jaundice, intrahepatic cholestasis causing chronic decrease in vitamin K, and ingestion of drugs such as warfarin or wide-spectrum antibiotics can result in high concentrations of DCP. Simultaneous determinations of AFP and DCP are useful for monitoring recurrence in patients with HCC after treatment, but the decrease to normal levels of a single marker does not always indicate the absence of tumor recurrence (Aoyagi Y et al, 1996).

2. γ-glutamyl transferase (GGT):

GGT, a plasma membrane-bound enzyme, is synthesized in the microsomes of human cells. It is highest in embryo livers and decreases rapidly to the lowest levels after birth (Yao DF et al, 2007).

The activity of serum GGT is extremely low in healthy adults, but stimulations such as cholestasis and inflammation can improve the level of serum GGT to varying degrees. GGT mRNA is widely distributed in the liver tissues of healthy adults, patients with liver disease, benign liver tumor and HCC (Singhal A et al, 2012).

However, the sensitivity of GGT to detect small HCC is only 43.8% (Cui R et al, 2003). This enzyme is therefore only used to aid in the diagnosis of HCC. In addition, it has been previously shown that simultaneous determination of GGT, DCP and AFP can significantly improve the rate of diagnosis of HCC (Wang CS et al, 2005).

3. α-l-fucosidase (AFU):
AFU is a lysosomal enzyme widely present in human cells, blood and body fluid. It can be detected in the serum of healthy adults, although its activity increases in the serum of HCC patients. Additionally, it does not correlate with tumor size or the level of AFP (Tangkijvanich P et al, 1999).

It has been reported that the sensitivity, specificity, and diagnostic accuracy of AFU at the cut-off value of 2.3005 μmol/l/min were 90, 97.5 and 94.9%, respectively. The combined detection of AFP and AFU can improve the sensitivity, specificity, and diagnostic accuracy of AFP from 70, 85 and 79.7%, respectively, to 95, 100 and 99.1%, respectively (Fawzy Montaser M et al, 2012).

D. Cytokines

1. Transforming growth factor-β1 (TGF-β1):

TGF-β1 is a pluripotent growth factor belonging to a superfamily of polypeptide signaling molecules involved in the regulation of cell proliferation, differentiation, embryo formation, angiogenesis, and immune functions (Worster AA et al, 2000).

It is significantly produced in tumor cells, inhibits the proliferation of tumor specific cytotoxic T lymphocytes (CTL) and NK cells and stimulates the growth of tumor cells (Giannelli G et al, 2002).

TGF-β1 and TGF-β1 mRNA may be used as sensitive indicators to diagnose HCC which is induced by HBV, with the sensitivity and specificity being 89.5 and 94.0%, respectively when TGF-β1 is >1.2 μg/l (Dong ZZ et al, 2008).

2. Vascular endothelial growth factor (VEGF):
VEGF is a signal protein expressed by cells that stimulates vasculogenesis and angiogenesis. In addition vascular endothelial growth factor (VEGF) is has a special role in angiogenesis by promoting new vessel formation an inducing tumor invasion and metastasis. Also overexpression of VEGF has been shown in various malignancy condition (Duffy AM et al, 2004).

Xiang ZL et al. (2011) have reported that expression of VEGF is strongly high in HCC patients versus healthy people. Furthermore, expression of VEGF is correlated with tumor recurrence and poor prognosis and survival. Thus, the overexpression of VEGF can serve as a biologic tumor marker in predicting poor prognosis and survival in HCC patients.

E. Growth Factors and Their Receptors

1. Tumor-Specific Growth Factor (TSGF):

Tumor specific growth factor (TSGF) is a factor that helps in inducing the growth of tumor blood vessels similar to VEGF. Liang YR, et al. have demonstrated that TSGF significantly correlates with hyperplasia of tumor tissue and microenvironment capillary vessels whereas similar correlation has not been shown between TSGF and hyperplasia of non-tumor blood vessels (Liang Y et al, 2002).

Researches extensively indicated the high sensitivity of TSGF for detection of neoplasm condition. TSGF secretes into peripheral blood by malignant tumors during their growing period (Yu B et al, 2009).
Lawicki S et al. (2009) have reported that serum levels of TSGF can indicate the existence of tumor; therefore, TSGF can be used as a diagnostic marker in diagnosis of various cancer types such as HCC.

2. Epidermal Growth Factor Receptor Family (EGFR):

EGFR is the cell-surface receptor tyrosine kinases, a members of the epidermal growth factor family (EGF family), that has a major role in regulating cell proliferation and consists of four related transmembrane tyrosine kinase receptors: EGFR (erbB-1), c-erb-2 (Her-2/neu), c-erb-3 (HER-3), and c-erb-4 (HER-4) (Wieduwilt M & Moasser M, 2008).

Osada S et al. (2007) have indicated that high levels of EGFR expression are closely correlated with early recurrence and poor prognosis following resection of hepatocellular carcinoma.

3. Fibroblast Growth Factor (FGF):

Fibroblast growth factors (FGFs) and their receptors play essential roles in regulate many function of cell such as regulating cellular proliferation, survival, migration and differentiation. Poon RT-P et al. have demonstrated that serum levels above the median of >10.8 pg/mL can decrease disease-free survival in HCC patients (Poon RT-P et al, 2001).

F. Molecular Biomarkers:

1. AFP mRNA:

AFP mRNA is considered the most valuable marker for circulating HCC cells and is only present in active HCC cells. If other interferences such as genital tumors and peripheral blood are excluded, AFP mRNA could be used as a significant marker for spreading of HCC in blood. However, use of this marker is controversial, possibly due to the blood-
borne dispersion of normal liver cells and tumor cells and the mis-
transcription of mRNA encoding AFP by peripheral mononuclear cells
(Singhal A et al, 2012).

The positive rate of AFP mRNA in the recrudescent patients was
82.4%, significantly higher compared with the group without recurrence.
Thus, AFP mRNA effectively predicts tumor recurrence and metastasis
following surgery (Zhi H et al, 2007).

2. MicroRNAs (miRNAs):

Approximately 500 miRNA genes have been identified and found to
be important components of complex functional pathways controlling
important cellular processes, such as proliferation, differentiation, and
apoptosis. In the development of human cancer, miRNAs have been
determined to function both as oncogenes and as tumor suppressor genes

MicroRNAs (miRNAs) associated with HCC development have
been investigated as biomarkers to diagnose the disease. Some of these
miRNAs have been shown to accurately predict poor prognosis in HCC
(Han ZB et al, 2012).

A panel of seven miRNAs (miR-122, miR-192, miR-21, miR-223,
miR-26a, miR-27a and miR-801) has been shown to have high diagnostic

MicroRNAs (miR-21) was the only one that was up regulated in the
HCC cells. A sensitivity of 87.3% and specificity of 92% were shown for
miR-21 (Zhao YJ et al, 2013).

3. Insulin-Like Growth Factor II (IGF-II) mRNA:
Expression of IGF-II mRNA can be a valuable tumor marker for diagnosis, metastasis, and following postoperative recurrence in HCC. Himoto T et al. have reported that the assay of serum insulin-like growth factor- II (IGF-II) (at the cut-off value of 4.1 mg/g, prealbumin) has sensitivity of 63%, specificity of 90%, and accuracy of 70% in the detection of HCC patients. The simultaneous assay of IGF-II m-RNA and AFP (at the cutoff value of 50 ng/mL) can improve the sensitivity to 80% and accuracy to 88% (Himoto T et al, 2005).

4. Albumin mRNA:

Albumin is a family of globular proteins; Albumin is a soluble protein and is commonly found in blood and is synthesized by the liver. The albumin’s mRNA is in human blood and could be a clinically potential biomarker for liver pathologies. Cheung ST et al. have demonstrated that high serum albumin’s mRNA level predicted the 2-year recurrence rate with sensitivity and specificity of 73% and 70%, respectively (Cheung ST et al, 2008).

5. Gamma - Glutamyl Transferase mRNA (GGT mRNA):

GGT mRNA can be found in the serum and liver tissues of healthy adults, patients with liver disease, benign liver tumor, HCC, and secondary tumors of the liver. Presence of type B GGT mRNA in cancerous tissue was significantly associated with high serum level of AFP and poor survival in HCC (Zhou L et al, 2006).

Other serum markers for liver cancer

❖ Osteopontin(OPN):

Osteopontin is a glycoprotein produced by several different cell types, particularly bone and epithelial cells, and highly expressed in
various cancers, including HCC. For differentiating early HCC from cirrhosis, the plasma osteopontin at an optimal cutoff of 91 ng/mL was found to be superior to AFP at a cutoff of 20 ng/mL, with an AUROC (95% CI) of 0.73 (0.62–0.85) versus 0.68 (0.54–0.82), a sensitivity of 75% versus 46%, and a specificity of 62% versus 93%, respectively (Shang S et al, 2012).

The combination of osteopontin and AFP at these cutoffs had better performance than either test alone, with an AUROC (95% CI) of 0.81 (0.70–0.91), a sensitivity of 83%, and a specificity of 63% (Shang S et al, 2012).

Cytokeratin 19:

Cytokeratin 19 (CK19) is a novel HCC biomarker that has been consistently linked to a poor clinical prognosis in patients. The simultaneous detection of CK19 and GPC3 expression in HCC patients was shown to be a predictive indicator of higher risks of cancer invasion and metastasis, as well as worse treatment outcome (Feng J et al, 2016).

Furthermore, the combination of CK19 and GPC3 demonstrated better diagnostic sensitivity (90.6%) compared with the use of GP3 alone (54.2%) when applied for the detection of HCC in a study cohort of 518 patients (Yu JP et al, 2015).

Consistently, several other studies also confirmed a correlation between increased CK19 expression and a lower survival rate and/or a shorter remission period in HCC patients (Sun DW et al, 2015).
Annexin A2:

Annexin A2 is a calcium-dependent, phospholipid-binding protein commonly found in the cell surface (Lokman NA et al, 2011).

Many biological functions of Annexin A2 are related to cell mobility and protein interaction with the actin cytoskeleton, as well as endocytosis. Due to these roles, Annexin A2 has also been implicated in the development and metastasis of HCC. Not surprisingly, the overexpression of Annexin A2 was revealed to be an indicator of the general degree of HCC tumor malignancy in patients and showed an inverse correlation with their survival rates (Zhang H et al, 2015).

Annexin A2 also demonstrated higher sensitivity and specificity than AFP (83.2% and 67.5% for Annexin A2, compared to 54.7% and 81.3% for AFP) for the detection of early-stage HCC (Sun Y et al, 2013).

Midkine (MDK):

Midkine (MDK) is a heparin-binding growth factor that has been associated with tumor migration and proliferation (Muramatsu T, 2010).

Not surprisingly, MDK is often overexpressed in various human tumors, making it an attractive target in tumor detection and treatment. A clinical study on a cohort of 388 HCC patients and 545 hospital enrollees diagnosed with other diseases identified MDK as a discriminating tissue and serum biomarker with better sensitivity (86.9%, serum MDK) than AFP (51.9%) (Zhu WW et al, 2013).

The distinguishing power of MDK remained evident even for very early-stage HCC. These results were echoed in another study that confirmed that the MDK-based predictive model was dramatically more sensitive than its AFP counterpart (90% versus 40%) in differentiating
between patients with early-stage HCC and those with cirrhosis (Shaheen KY et al, 2015).

- **Soluble urokinase plasminogen activator receptor:**

  Soluble urokinase plasminogen activator receptor (suPAR) is the circulating form of the glycosylphosphatidylinositol-linked membrane protein, urokinase-type plasminogen activator receptor (uPAR). Soluble urokinase plasminogen activator receptor (suPAR) was recently established as a biomarker for the level of activation of the immune system and cancer metastasis. Serum levels of suPAR are elevated in patients with ovarian cancer, colon cancer, and HCC (Chounta A et al, 2015).

  The study revealed that within the subgroup of the high-risk European Association for the Study of Liver (EASL), a suPAR concentration of > 9.56 ng/mL yielded sensitivity of 76.0%, specificity of 90.4%, and positive and negative predictive values of 54.3% and 96.2%, respectively, for the eventual development of HCC. Based on these results, suPAR has potential as an early predictor to evaluate the risk of the development of HCC (Chounta A et al, 2015).

- **Thioredoxins (TRXs):**

  Thioredoxins (TRXs) are thioloxidoreductases that are ubiquitously expressed and involved in several biological processes such as, regulation of protein states, cellular apoptosis and proliferation, and protection against oxidative stress (Nordberg J & Arnér ES, 2001).

  The expression of TRXs is increased in many neoplasms, and has been shown to correlate with prognosis, specifically in lung and colorectal carcinoma (Raffel J et al, 2003).
Li et al. (2015) reported on the potential availability of a TRX for the detection of early stage HCC (well-differentiated, < 2 cm HCC). In this study, the sensitivity and specificity of TRX (74.9% and 87.5%, respectively) were higher than for AFP (68.6% and 75.2%, respectively).

Liver Biopsy

Even targeted liver biopsy does not have perfect diagnostic accuracy. The overall sensitivity is approximately 90% for the diagnosis of HCC, depending on the size and location of the lesion (Durand F et al, 2001).

Percutaneous, ultrasound-guided liver biopsy (the Menghini method) has become a worldwide standard. The method is simple, rapid, inexpensive, and quite safe, although controlled prospective data about it are scarce. Significant complications arise in about 1% of biopsies, with less than 0.1% mortality (Rockey DC et al, 2009).

The main complications are post-interventional hemorrhage and bile leakage; injuries to other organs (gall bladder, lung and kidney) and bacteremia are rare (Ewe K, 1981). The risk of hemorrhage depends on the type of hepatic disease present and on the presence or absence of portal hypertension (Caldwell S & Northup PG, 2010).

Laparoscopic liver biopsy yields more information than percutaneous liver biopsy, as it enables macroscopic inspection of the hepatic surface. Performing a biopsy under laparoscopic vision also ensures that the tissue cylinders will be large enough for further processing in the pathology department (Lohse AW, 2011).

Immunohistochemistry:

Immunostains can be used to study hepatocellular carcinoma and to distinguish hepatocellular carcinoma from other focal lesion or other
malignancies, especially intrahepatic cholangiocarcinoma and metastatic adenocarcinoma (Varma V, 2004).

A large variety of immunophenotypical markers of HCC has been described, including highly specific markers (HepPar1, albumin, fibrinogen, α1-anti-trypsin and Alfa-Fetoprotein) (Ishak KG, 2001).

In cases of poorly differentiated tumors, such markers display insufficient performance and additional markers are useful. Among them, Glypican-3 (GPC-3), an oncofetal protein, seems to be the more efficient with more than 80 % of HCC immunopositive (Shafizadeh N et al, 2008).

Interestingly, immunophenotyping, based on a panel of antibodies, has shown its performance in the differential diagnosis of early HCC and dysplastic nodules (Di Tommaso L et al, 2007).

Furthermore, genomic studies provided molecular classifications of HCC based on gene expression (Villanueva A et al, 2007). Such analysis allowed the identification of subgroups of patients according to etiological factors, stage of the disease, recurrence and survival (Villanueva A et al, 2008).
Clinical Practice Guidelines for Hepatocellular Carcinoma

Surveillance and Diagnostic Algorithms

Although HCC was once thought to be a special type of cancer prevalent only in Southeast Asia and Africa, it has rapidly become more common in other regions, particularly Europe and the United States, which has led to greater interest in the diagnosis and treatment of HCC worldwide (Bruix J ET AL, 2011).

Consequently, the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver and the European Organisation for Research and Treatment of Cancer (EASL-EORTC) have published clinical practice guidelines for liver cancer (Bruix J et al, 2011).


1. American Association for the Study of Liver Diseases (AASLD) practice guidelines:

Essentially, for the AASLD and EASL-EORTC surveillance and diagnostic algorithms, only cirrhotic patients are considered candidates for surveillance, and surveillance is performed with ultrasound every 6 months. If a nodule ≤1 cm is found, ultrasound examinations are performed every 3 months, and dynamic computed tomography (CT) or magnetic resonance imaging (MRI) scans are not performed unless the nodule exceeds 1 cm (figure 4) (Bruix J, Sherman M,2011).
If arterial enhancement and venous equilibrium phase washout are observed with either modality, HCC can be diagnosed noninvasively. If a diagnosis cannot be made by CT or MRI, HCC can be diagnosed if this imaging hallmark is observed in another dynamic study. Liver biopsy is recommended only when these imaging features are not observed (Bruix J & Sherman M, 2011).

**Figure (4).** AASLD practice guidelines for HCC surveillance and diagnosis. MDCT=multidetector CT; US=ultrasound. Modified with permission from (Bruix J et al, 2011).

**2-EASL-EORTC practice guidelines:**

The algorithm in the EASL-EORTC guidelines differs only in some minor respects from the AASLD algorithm. When a tumor ≤1 cm is detected, both algorithms propose that CT and MRI examinations should not be performed, but the AASLD algorithm recommends an ultrasound examination every 3 months, whereas the EASL algorithm recommends an ultrasound examination every 4 months. In addition, although the EASL guidelines present different diagnostic algorithms for 1- to 2-cm
nODULES and nodules >2 cm, this distinction is essentially meaningless, and the EASL algorithm is largely the same as the AASLD algorithm (figure 5) (*Bruix J & Sherman M, 2011*).

The EASL and AASLD algorithms have identical recommendations for cirrhotic patients: they both recommend only an ultrasound examination every 6 months and do not recommend the use of tumor markers (*Bruix J & Sherman M, 2011*).

![Figure 5](image)

**Figure 5.** EASL-EORTC practice guidelines for HCC surveillance and diagnosis (*Bruix J, Sherman M, 2011*).

**3-Japanese evidence-based clinical practice guidelines:**

The Japanese evidence-based clinical practice guidelines for HCC (fig. 6) deliberately divide patients into an extremely high-risk group (hepatitis B or C cirrhosis) and a high-risk group (patients with chronic hepatitis B, chronic hepatitis C, or non-viral cirrhosis) and recommend an ultrasound examination every 3–4 months along with measurement of three tumor markers [AFP, proteins induced by vitamin K absence
(PIVKA-II), and AFP-L3] every 3–4 months for the extremely high-risk group *(Song P et al, 2013)*.

They also recommend CT and MRI examinations every 6–12 months as an optional screening method. For the high-risk group, they recommend an ultrasound examination every 6 months and measurement of three tumor markers every 6 months *(Kudo M et al, 2013)*.

The Japanese diagnostic algorithm differs from the European and American algorithms in that it recommends gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid-enhanced MRI (EOB-MRI), contrast-enhanced ultrasound (CEUS), or CT angiography as an optional imaging test when a nodule shows early contrast enhancement without late washout and is ≥1 cm; if a diagnosis is not confirmed, then liver tumor biopsy is recommended *(Japan Society of Hepatology, 2010)*.

The Japanese algorithm takes a more rigorous approach to noninvasive diagnosis. If there is no early contrast enhancement and the tumor diameter is >1.5 cm, the same optional tests can be performed; however, if it is ≤1.5 cm, the patient should be followed every 3 months and another dynamic CT or MRI performed if the tumor enlarges or tumor marker levels increase. If the tumor size is unchanged or regresses, the algorithm recommends the regular surveillance schedule be resumed *(Kokudo N et al, 2015)*.
Figure 6. Japanese evidence-based clinical practice guidelines for HCC surveillance and diagnostic algorithm (SongP et al, 2013). AFP=alpha-fetoprotein; DCP=des-gamma carboxyprothrombin (also known as PIVKA-II). Optional testing=EOB-MRI, CEUS, CT angiography or tumor biopsy. aCT/MRI are used for some patients even if the nodule(s) are not visualized using ultrasound because of poor visualization capability. Contrast-enhanced ultrasound may be considered for patients with renal impairment and/or allergies to contrast media of CT/MRI. Modified with permission from Kokudo Net al. (SongP et al, 2015).

4- Japan Society of Hepatology (JSH) Consensus-Based Clinical Practice Guidelines:

As in the Japanese evidence-based guidelines, the consensus-based clinical practice guidelines for liver cancer (fig.7) use identical definitions for the extremely high-risk group and high-risk group. They are also
similar to the evidence-based guidelines in that they recommend surveillance with dynamic CT or MRI every 6–12 months for cirrhotic patients because nodules may not be detected on ultrasound alone. However, the consensus-based algorithm differs in that it recommends EOB-MRI, which has higher detection sensitivity than CT, as the first-line modality for surveillance every 6–12 months \textit{(Kudo M et al, 2014)}.

This algorithm classifies nodules as having one of three patterns: arterial hypervascularity and late washout, hypervascularity but no late washout, or arterial hypovascularity. Hypervascular nodules with washout can be diagnosed as HCC by imaging alone. Hypervascular nodules without washout can be diagnosed as HCC if they are hypointense in the hepatobiliary phase of EOB-MRI. Biopsy is recommended if the nodules are isointense or hyperintense in the hepatobiliary phase \textit{(Ichikawa T et al, 2014)}.

Nodules that are hypovascular on EOB-MRI have strong malignant potential if they are hypointense in the hepatobiliary phase and therefore, in these cases, Sonazoid CEUS is performed. If the nodule is hypervascular on Sonazoid CEUS or there is a defect in the Kupffer phase, it is definitively diagnosed as HCC, although biopsy can be performed as an optional test. This is because such cases may include not only well-differentiated HCC but also moderately and poorly differentiated HCC, which could affect the treatment options neither hypovascular on Sonazoid CEUS nor shows a defect in the Kupffer phase, biopsy is recommended if the nodule is 1–1.5 cm or larger in order to differentiate between early HCC and a dysplastic nodule \textit{(Ichikawa T et al, 2014)}. 
Even small nodules 1–1.5 cm or smaller have a strong potential to become hyper vascular if they are hypointense in the hepatobiliary phase of EOB-MRI, so intensive follow up with EOB-MRI is recommended every 3–6 months. A fundamental principle of these consensus-based guidelines is that for institutions that cannot perform EOB-MRI so frequently, it is acceptable to substitute dynamic CT for both surveillance and follow up (Kudo M et al, 2014).

Figure (7). Consensus-based surveillance and diagnostic algorithm for HCC. Gd-EOB-DTPA=gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid-enhanced; DN=dysplastic nodule. SPIO=superparamagnetic iron oxide; CTAP=CT during arterial portography; CTHA=CT during hepatic arteriography. Optional examinations such as SPIO MRI, CTAP, CTHA and highly sensitive tumor marker measurement are recommended in difficult to diagnose cases. aCavernous hemangioma may show hypointensity on equilibrium (transitional) phase of dynamic Gd-EOB-DTPA MRI (pseudo-washout). Cavernous hemangioma should be excluded by other sequences of MRI and/or other imaging modalities. bCavernous hemangioma usually shows hypointensity on hepatobiliary phase of Gd-EOB-DTPA MRI. Cavernous hemangioma should be excluded by other sequences of MRI and/or other imaging modalities. cBiopsy may be considered for confirmation. Modified with permission from (Kudo M et al, 2014).
STAGING OF HEPATOCELLULAR CARCINOMA

After making the diagnosis of HCC, the next step in the management of the patient is staging. The goal of cancer staging is to separate patients into different groups based on their predicted survival to help determine the most appropriate treatment modality (Marrero JA et al, 2010).

Staging systems for HCC should include 4 related aspects: tumor stage, degree of liver function impairment, patient’s general condition, and treatment efficacy. Prognostic systems assessing just 1 of these aspects (CTP, TNM, performance status) have a marginal usefulness. The Okuda staging is unable to distinguish between early and advanced HCC and serves mostly to identify end stage individuals (Bruix J et al, 2001).

The Child-Turcotte-Pugh (CTP) (Table 4) model is primarily an assessment of liver function and is intended to predict prognosis and stratify disease severity to facilitate transplant allocation (Christensen E et al, 1984). While still used as a complementary tool to help with treatment decisions or evaluate progression and/or regression of disease, the CTP model has largely been replaced by the Model for End-stage Liver Disease (MELD) score (Ravaioli M et al, 2006).
Table (4). Child-Turcotte-Pugh Score for Assessment of Liver Function.

<table>
<thead>
<tr>
<th>Clinical or Biochemical Parameter</th>
<th>One Point</th>
<th>Two Points</th>
<th>Three Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>&lt;2</td>
<td>2–3</td>
<td>&gt;3</td>
</tr>
<tr>
<td>Serum albumin (g/dL)</td>
<td>&gt;3.5</td>
<td>2.8–3.5</td>
<td>&lt;2.8</td>
</tr>
<tr>
<td>International normalized ratio</td>
<td>&lt;1.7</td>
<td>1.7–2.3</td>
<td>&gt;2.3</td>
</tr>
<tr>
<td>Ascites</td>
<td>None</td>
<td>Mild</td>
<td>Moderate to severe</td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>None</td>
<td>Grade I–II</td>
<td>Grade III–IV</td>
</tr>
</tbody>
</table>

Source.—Reprinted, with permission, from reference 25.
Note.—Severity of liver disease is graded as CTP A–C, on the basis of a patient’s total score: A = 5–6, B = 7–9, and C = 10–15.

It is well known that the functional impairment of the underlying liver disease has a significant impact on prognosis, irrespective of the tumour stage (Bruix J & Sherman M, 2005). For this reason, systems that include the anatomical characteristics of the tumor only, such as the American Joint Committee on Cancer (AJCC)/International Union Against Cancer (UICC) staging system that stratifies patients using a Tumor-Node-Metastasis (TNM) classification, do not have per se a good predictive capability (Edge SB et al, 2010).

Several staging or scoring systems for HCC have been proposed (Figure 8), such as the Okuda staging system (Okuda K et al, 1985), GRETCH (Groupe d’Etude et de Traitement du Carcinome Hépatocellulaire) scoring system (Chevret S et al, 1999), CUPI (Chinese University Prognostic Index) staging system (Leung TW et al, 2002), CLIP (Cancer of the Liver Italian Program) scoring (The Cancer of the liver Italian Program (CLIP) Investigators, 2000), BCLC (Barcelona Clinic Liver Cancer) staging system (Llovet JM et al, 1999), JIS (Japan Integrated Staging) (Kitai S et al, 2008), Tokyo scoring system (Tateishi

Figure (8). Timeline of hepatocellular carcinoma staging system. AJCC: American Joint Committee on Cancer; UICC: International Union Against Cancer; CLIP: Cancer of the Liver Italian Program; GRETCH: Groupe d’Etude et de Traitement du Carcinome Hépatocellulaire; BCLC: Barcelona Clinic Liver Cancer; CUPI: Chinese University Prognostic Index; JIS: Japan Integrated Staging Score; TNM: Tumor Node Metastasis.

The majority of these classifications, however, have been constructed with patients at advanced tumor stage, and this explains why even patients at supposed early stages achieved poor outcomes (Chevret S et al, 1999). In (Table 5) are represents the parameters included in these staging systems, some of these classifications have been externally validated in separated groups.

Table (5). Factors included in each staging system for HCC.

<table>
<thead>
<tr>
<th>Staging system</th>
<th>Size</th>
<th>Nodules</th>
<th>Met</th>
<th>PVT</th>
<th>AFP</th>
<th>CH</th>
<th>Alb</th>
<th>Bil</th>
<th>ALP</th>
<th>Ascites</th>
<th>PS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNM</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Okuda</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>CLIP</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>FRENCH</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>BCLC</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>JIS</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>CUPI</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

Met: Metastasis; PVT: Portal vein thrombosis; AFP: Alafetoproteina; Alb: Albumin; Bil: Bilirubin; ALP: Alkaline phosphatise; PS: Performance status; CLIP: Cancer of the Liver Italian
Program; **BCLC**: Barcelona clinic liver cancer classification; **CUPI**: Chinese University Prognosis Index; **JIS**: The Japan Integrated Staging; **TNM**: Classification of malignant tumours; **FRENCH**: French classification of hepatocellular carcinomas; **CH**: Child-Pugh.

While comparative studies have demonstrated that CLIP scores are more accurate than the Okuda model for determining prognosis, the BCLC staging system has the greatest predictive power for survival rates *(Grieco A et al, 2005)*. Indeed, the BCLC staging system has emerged as the most accurate and comprehensive cancer model to show consistent prognostic determination *(Cillo U et al, 2006)*.

**Pathologic tumor-node-metastasis (pTNM):**

TNM classification (Table 6) considers the size and number of nodules, vascular invasion, and bilobar involvement. Hepatic function is not considered as a factor in staging, although it is an important factor for the prognosis of these patients *(Greene F et al, 2002)*. It therefore fails to accurately predict survival in patients undergoing hepatic resection for HCC *(Izumi R et al, 1994)*.
Table (6). TNM classification of hepatocellular carcinoma (Greene F et al, 2002).

<table>
<thead>
<tr>
<th>Classification</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Solitary tumor ≤2 cm in the widest diameter without vascular invasion.</td>
</tr>
<tr>
<td>T2</td>
<td>Solitary tumor ≤2 cm in the widest diameter with vascular invasion; or multiple tumors limited to one lobe, none of them &gt;2 cm in the widest diameter, without vascular invasion; or solitary tumor &gt;2 cm in the widest diameter, without vascular invasion.</td>
</tr>
<tr>
<td>T3</td>
<td>Solitary tumor &gt;2 cm in the widest diameter with vascular invasion; or multiple tumors limited to one lobe, none of them &gt;2 cm in the widest diameter, with vascular invasion; or multiple tumors limited to one lobe, some of them &gt;2 cm in the widest diameter, with or without vascular invasion.</td>
</tr>
<tr>
<td>T4</td>
<td>Multiple tumors in more than one lobe; or tumor(s) invading a large portal branch or one or more suprahepatic veins. Solitary tumor &gt;2 cm in the widest diameter with vascular invasion.</td>
</tr>
</tbody>
</table>

Stage of TNM classification

<table>
<thead>
<tr>
<th>Stage</th>
<th>Tumor</th>
<th>Nodes</th>
<th>Metastasis</th>
<th>Regional Lymph Nodes</th>
<th>Distant Metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>T1 / N0 / M0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>T2 / N0 / M0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III A</td>
<td>T3 / N0 / M0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III B</td>
<td>T1 / N1 / M0</td>
<td>T2 / N1 / M0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV A</td>
<td>T4 / any N / M0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV B</td>
<td>any T / any N / M1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

T = tumor; N = nodes; M = metastasis; N0 = without regional lymph nodes; N1 = with regional lymph nodes; M0 = without distant metastasis; M1 = with distant metastasis.

The Okuda system:

The Okuda system was the first staging system to be widely used that included parameters that reflect the biology of the tumor and the underlying liver disease (Table 7) (Okuda K et al, 1985).

This system is highly effective at identifying a subgroup of patients, Okuda stage III, who have a very poor prognosis and probably should be treated with supportive care only (Slmonetti RG et al, 1997).

This leaves only 2 remaining stages, which may limit its ability to separate patients into clinically relevant groups. Okuda stages I and II are heterogeneous and include patients with good prognosis especially with liver transplantation and those with poor prognosis whatever treatment is given (Fannati F et al, 2000).
Table (7). Okuda Staging System *(Okuda K et al, 1985)*

<table>
<thead>
<tr>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size &lt;50% of liver</td>
<td>&gt;50% of liver</td>
</tr>
<tr>
<td>Ascites Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Serum albumin &gt;3 g/dl</td>
<td>&lt;3 g/dl</td>
</tr>
<tr>
<td>Bilirubin &lt;3 mg/dl</td>
<td>&gt;3 mg/dl</td>
</tr>
</tbody>
</table>

Okuda I: No positive factor; Okuda II: 1 or 2 positive factors; Okuda III: 3 or 4 positive factors.

The Cancer of the Liver Italian Program score (CLIP):

The CLIP proposed a scoring system (CLIP score; table 8) in 1998 that accounts for both liver function and tumor characteristics relevant to prognostic assessment for patients with HCC *(Bruix J & Sherman M, 2005)*. The CLIP score was derived from a retrospective evaluation of 435 Italian patients with HCC diagnosed from 1990 to 1992 *(CLIP investigators, 1998)*.

Table (8). Cancer of the Liver Italian Program (CLIP) classification of hepatocellular carcinoma *(Bruix J & Sherman M, 2005)*.
At multivariate analysis, independent predictive factors of survival were Child-Pugh stage, tumor morphology, AFP, and portal vein thrombosis. A simple scoring system was thus produced, assigning linear scores (0/1/2) to the covariates (*CLIP investigators, 2000*).

**Barcelona staging classification (BCLC):**

The BCLC classification has emerged as the standard classification for clinical management of HCC (*Llovet JM et al, 2003*). This classification has been externally validated (*Marrero JA et al, 2005*) and is endorsed by AASLD and EASL (*Bruix J & Sherman M, 2005*).

The BCLC staging system (table 9) has come to be widely accepted in clinical practice and is also being used for many clinical trials of new drugs to treat HCC (*Forner A et al, 2010*).

**Table (9). BCLC staging system**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very early (stage 0)</td>
<td>PS 0, Child-Pugh A, single HCC &lt; 2 cm</td>
</tr>
<tr>
<td>Early (stage A)</td>
<td>PS 0, Child-Pugh A-B, single HCC or 3 nodules &lt; 3 cm</td>
</tr>
<tr>
<td>Intermediate (stage B)</td>
<td>PS 0, Child-Pugh A-B, multinodular HCC</td>
</tr>
<tr>
<td>Advanced (stage C)</td>
<td>PS 1-2, Child-Pugh A-B, portal vein invasion, nodal metastases, distant metastases</td>
</tr>
<tr>
<td>Terminal (stage D)</td>
<td>PS &gt; 2, Child-Pugh C</td>
</tr>
</tbody>
</table>

Abbreviation: PS, ECOG performance status.
This classification takes into consideration hepatic function, portal hypertension, bilirubins, symptoms related to the tumor, tumor morphology, presence of distant metastases and vascular invasion. This is the only classification that correlates prognostic data with therapeutic possibilities (Llovet JM et al, 1999).

**Modified International Union for Cancer Control (mUICC) staging system:**

The Korean Liver Cancer Study Group (KLCSG) and the National Cancer Center (NCC) have adopted the fifth version of the modified International Union for Cancer Control (mUICC) staging system as a primary staging system for HCC (Table 10) (Ueno S ET AL, 2002).

**Table (10). Modified Union for International Cancer Control Staging System (Ueno S ET AL, 2002).**

<table>
<thead>
<tr>
<th>Stage</th>
<th>T</th>
<th>N</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>T1 (all 3 criteria*)</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>II</td>
<td>T2 (2 of 3 criteria*)</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>III</td>
<td>T3 (1 of 3 criteria*)</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>IVA</td>
<td>T4 (none of 3 criteria*)</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>IVB</td>
<td>T1-4</td>
<td>N1</td>
<td>M0</td>
</tr>
</tbody>
</table>

*Criteria: (1) Number of tumors: solitary; (2) Diameter of the largest tumor: ≤ 2 cm; (3) No vascular or bile duct invasion: Vp0, Vp1, No, B0. Adapted from 10.

The mUICC staging system appears more advantageous for assessing the prognosis of small HCC because it sets the size cut-off to 2 cm unlike the American Joint Committee on Cancer (AJCC)/UICC, which used a cut-off of 5 cm (Korean Liver Cancer Study G, 2015).
**Albumin-bilirubin score (ALBI):**

Contributing factors related to tumor characteristics and liver function status is sometimes difficult to separate clinically. *Johnson and associates* developed the ALBI system to solve the lack of validation of Child-Pugh score in patients with advanced HCC (Table 11) (*Johnson PJ, 2015*). According to Johnson and associates, the ALBI score is calculated as follows: ALBI score = (log [bilirubin in μmol/L] × 0.66) - (albumin in g/L × 0.085).

The advantages of the ALBI score are its simplicity and its validity in large, contemporary, and internationally diverse HCC populations (*Knox JJ, 2015*). Therefore, it is logical and practical to replace the Child-Pugh with the ALBI model during planning of future trials (*Chan AW et al, 2016*).

**Table (11).** Albumin-Bilirubin Grading Score (adapted from Johnson and associates) (*Johnson PJ et al, 2015*).

<table>
<thead>
<tr>
<th>ALBI Score</th>
<th>ALBI Grade</th>
<th>Median Survival, mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ -2.6</td>
<td>A1</td>
<td>85.6</td>
</tr>
<tr>
<td>&gt; -2.6 and ≤ -1.39</td>
<td>A2</td>
<td>46.5</td>
</tr>
<tr>
<td>&gt; -1.39</td>
<td>A3</td>
<td>15.5</td>
</tr>
</tbody>
</table>

*Abbreviations:* ALBI, albumin-bilirubin

**Hong Kong Liver Cancer staging system:**

A large study was conducted at Queen Mary Hospital with prospectively collected data of 3856 patients with HCC (predominantly patients with hepatitis B virus-related disease) treated from 1995 to 2008 (Table 12) (*Yau T et al, 2014*).

Data on patient performance status, Child–Pugh grade, tumor status (size, number of nodules, and presence of intrahepatic vascular invasion),
and presence of extrahepatic vascular invasion or metastasis were included, and randomly separated into training and test sets for analysis (*Yau T et al, 2014*).

**Table (12).** Hong Kong Liver Cancer Scoring System (adapted from Yau and associates) (*Liu PH et al, 2016*).

<table>
<thead>
<tr>
<th>Liver Tumor Status</th>
<th>Size</th>
<th>Number of Nodules</th>
<th>Intrahepatic Venous Invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>≤ 5 cm</td>
<td>≤ 3</td>
<td>No</td>
</tr>
<tr>
<td>Intermediate</td>
<td>≤ 5 cm</td>
<td>≤ 3</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>≤ 5 cm</td>
<td>&gt; 3</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>&gt; 5 cm</td>
<td>≤ 3</td>
<td>No</td>
</tr>
<tr>
<td>Locally advanced</td>
<td>≤ 5 cm</td>
<td>&gt; 3</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>&gt; 5 cm</td>
<td>≤ 3</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>&gt; 5 cm</td>
<td>&gt; 3</td>
<td>Any</td>
</tr>
<tr>
<td>Diffuse</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
</tr>
</tbody>
</table>

Tumors in the liver are classified into early, intermediate, and advanced based on 0, 1, or ≥ 2 adverse prognostic factors.

**The American Association for the Study of Liver Disease (AASLD):**

For the best assessment of prognosis, the American Association for the Study of Liver Disease recommended that the staging system should take into account tumour extent, liver function and physical status. The impact of treatment should also be considered when estimating life expectancy. Currently, the BCLC system is the only system that takes into consideration the treatment options available for patients (*BRUIX J & SHERMAN M, 2005*).
Management

The therapeutic approach in patients with HCC depends on several factors such as liver function, size and number of nodules, tumour extension, age and comorbidities existence (Lencioni R & Llovet JM, 2010).

Currently, available treatments can be divided into surgical treatments (resection or transplantation), percutaneous ablation (Chemistry: Acid ethanol acetic or thermal: Microwave, laser, radiofrequency and cryoablation), chemoembolization, radioembolization and systemic treatment. The goal of curative treatments should be to obtain a complete response, according to modified RECIST radiological criteria (Reig M et al, 2014).

The recommendation of selection for different treatment strategies are based on evidence-based data and local experience and capacities (Lencioni R & Llovet JM, 2010).

The treatment algorithms in the AASLD and EASL-EORTC guidelines are identical (fig. 9). Basically, they use the Barcelona-Clinic Liver Cancer (BCLC) staging system and define very early stage cancer (<2 cm, single nodule, Child-Pugh A) as stage 0 and cases involving single nodules or ≤3 nodules of ≤3 cm as early stage (stage A) HCC. They define multinodular HCC as intermediate stage (B), cases involving vascular invasion or extrahepatic spread as advanced stage (C), and cases where the patient has Child-Pugh C cirrhosis or a performance status ≥2 as terminal stage (D), with a different treatment option recommended for each stage (Kudo M, 2014).
They recommend resection (Mise Y et al, 2013) and ablation (Lin SM, 2013) or transplantation (Chan SC, 2013) for very early stage and early stage HCC, transcatheter arterial chemoembolization (TACE) (Raoul JL et al, 2014) for intermediate-stage HCC, sorafenib for advanced-stage HCC and best supportive care for terminal-stage HCC (Kudo M, 2014).

![Figure (9). AASLD/EASL-EORTC treatment algorithm for HCC.]

PST=performance status; CLT=cadaveric liver transplantation; LDLT=living donor liver transplantation; RF/PEI=radiofrequency ablation/percutaneous ethanol injection; OS=overall survival; mo=months; Target 20% means expected target population is 20%. The numbers in parentheses after the overall survival in months indicate the OS range of reported series. Modified with permission from Bruix J et al. and EASL-EORTC (Bruix J & Sherman M, 2011).

In contrast, the Japanese evidence-based clinical practice guidelines (fig. 10) recommend resection as the first-line treatment in patients with Child-Pugh A or B liver function with a single tumor, with ablation as an alternative when the tumor is ≤3 cm. Resection and ablation are both options for patients with 2–3 tumors that are ≤3 cm, and resection is
considered the first-line treatment and embolization the second-line treatment for patients with 2–3 tumors ≥3 cm \((Kudo M, 2012)\).

Embolization is considered the first-line treatment and hepatic arterial infusion chemotherapy (HAIC) the second-line treatment for patients with ≥4 tumors \((Kudo M, 2012)\).

It should be noted that chemotherapy includes oral drugs (sorafenib) and HAIC. Liver transplantation is recommended for patients with Child-Pugh C cirrhosis who meet the Milan criteria, but the maximum age is generally 65 years. Palliative care is recommended for patients who do not meet the Milan criteria \((Kudo M, 2012)\).

**Figure (10).** Japanese evidence-based clinical practice guidelines for hepatocellular carcinoma. Treatment algorithm. aThe Child-Pugh classification may also be used when non-surgical treatment is considered. bCan be selected for tumors with a diameter of ≤3 cm. cOral administration and/or hepatic arterial infusion are available. dA single tumor ≤5 cm or 2–3 tumors ≤3 cm in diameter. ePatients aged ≤65 years. Modified with permission from Kokudo N et al \((Kokudo N et al, 2015)\).
1. **SURGERY**

  The only proven curative therapy for HCC remains surgical, being either hepatic resection or orthotopic liver transplantation (OLT). Therefore, patients with single, small (<5 cm) HCC, or with no more than three lesions, each <3 cm in diameter, should be referred for surgical assessment *(PRIMROSE JN, 2002)*.

**A) Tumor Resection:**

  First option for patients who have optimal profile according to BCLC staging system should be resection *(Bruix J & Shereman M, 2012)*. Surgical resection is the choice of treatment for patients with single nodules, no underlying cirrhosis and good liver function *(Raza A & Sood GK, 2014)*.

  Resection also can be performed for multifocal HCC inside Milan criteria or in the case of mild portal hypertension when patients are not suitable for OLT, although whether such patients could benefit from other locoregional therapies, avoiding the risk of surgery and liver decompensation after surgery, has been debated *(Pawlik TM et al, 2005)*.

  Less than 25% of HCC patients are suitable for surgical resection due to several factors, including the presence of cirrhosis, anatomically unresectable disease, extrahepatic spread and vascular spread. The results of surgical treatment of HCC are inferior to the results of treating hepatic metastases from colorectal cancer, although the same general surgical principles apply in both conditions *(RYDER SD, 2003)*.

  Complications associated with surgical resection include haemorrhage, bile leakage, stress ulceration complicated with bleeding, transient haemobilia, atelectasis and inflammatory changes in the right lung *(SNARSKA, J et al, 1997)*.
Hospital mortality rates for resection alone vary from 5 to 24% (BAIN I et al, 1997). Mortality was mainly due to hepatorenal or cardiorespiratory failure and also occasionally to myocardial infarction or disseminated intravascular coagulation (SNARSKA J et al, 1997).

- Non-cirrhotic liver:

  The indications for resection are lesions limited to one lobe, mild hepatic dysfunction and absence of extra hepatic spread of the cancer. The 1 year survival rate post-resection varies between 50 and 80%, with a 5 year maximum survival of 50% (ZIBARI GB et al, 1998). In non-cirrhotic HCC patients, partial hepatectomy is associated with a 5 year survival of 30–68% (NAGASUE N et al, 1986).

- Cirrhotic liver:

  In the presence of cirrhosis, the risks from surgery are increased. Cirrhosis affects post-operative survival in several debilitating ways and remains the major determinant of post-operative survival for the following reasons:

  - Liver regeneration cannot occur in the cirrhotic remnant.
  - Recurrent HCC may develop in the hepatic remnant (BUSUTTIL R.W et al, 2005).
  - Pre-operative clotting is abnormal in cirrhosis.
  - Hepatic reserve is poor (KAWARADA Y et al, 1994).

  With cirrhosis, operative mortality is higher, and of those who survive the surgery, a 5 year survival of 25–30% has been recorded (FARMER et al, 1994).

  Wu et al. (1996) suggested that the size of the tumour is also a significant determining factor in survival. Of 2051 cases, ‘small’ (<5 cm
in diameter) and ‘very small’ (<3 cm in diameter) tumours had post-resection 5 year survivals of 79.8% and 85.3%, respectively.

Incidence of HCC recurrence, however, has been reported to vary between 20 and 70%, with almost all relapses occurring within two years of surgery (NAGASUE N et al, 1986).

B) Liver Transplantation:

Among patients with unresectable disease, the most viable surgical option is often liver transplantation, frequently in conjunction with adjuvant therapy such as TACE or percutaneous ablation (Llovet JM et al, 2005).

However, liver transplantation is not appropriate for all patients, and thorough evaluation is necessary to prudently allocate the scarce resources available (Wong R & Frenette C, 2011).

In 1996, Mazzaferro and colleagues, published a landmark prospective study involving less than 50 patients who were transplanted for HCC under predefined criteria (single HCC ≤5 cm or 3 HCC ≤3 cm each), known as the Milan criteria, and showed a 4-year survival of 75%.

Subsequent experiences of OLT for HCC inside the Milan criteria confirmed a survival rate exceeding 70% at 5 years, with recurrence in less than 15% (Kudo M et al, 2011). These outcomes are also similar to expected survival rates for patients undergoing transplantation for cirrhosis without HCC (Figueras J et al, 1997).

Recent interest has focused on the use of a down staging approach in which patients with HCC exceeding transplantation criteria are treated with locoregional therapy (ie, TACE and/or ablation therapy) to decrease
the tumor burden to the point of meeting transplantation criteria (*Yao FY et al, 2008*).

Some experts suggest offering OLT to patients who achieve effective down staging, while others favor OLT as a rescue treatment in patients who do not achieve an effective response (*De Carlis L et al, 2012*).

*Yao FY et al. (2008)* published a down staging protocol using TACE and/or RFA and have shown survival rates of 96.2% at 1 year and 92.1% at 4 years among patients who received transplants.

2. Nonsurgical Therapies for Localized Hepatocellular Carcinoma:

A) Percutaneous Local Ablation: Radiofrequency Ablation (RFA) and Percutaneous Ethanol Injection (PEI):

Percutaneous local ablation, which includes RFA and PEI, is the standard of care for BCLC stage 0-A not suitable for surgery. RFA relies on a needle electrode to deliver a high-frequency alternating current, resulting in frictional heating of the tissue and subsequent necrosis (*Mc Gahan JP et al, 1992*).

Injection of 95% ethanol directly into a tumor through a needle can induce local coagulation necrosis and a fibrous reaction, as well as thrombosis of tumor microvasculature and tissue ischemia (*Livraghi T et al, 1992*).

In tumors 3 cm or larger, both RFA and PEI achieve complete necrosis in 80% to 90% making percutaneous local ablation competitive with resection (*Chen MS et al, 2006*).
Before the advent of RFA, PEI was the most widely accepted, minimally invasive method for treating such patients. However, RFA continues to demonstrate the most predictable efficacy in both small and large tumors, and studies suggest that patients treated with RFA have superior survival and local recurrence–free rates compared with those of PEI (Cho YK et al, 2009).

Although there is no absolute tumor size beyond which RFA should not be considered, the best outcomes are in patients with a single tumor that is less than 4 cm in diameter or in patients who have no more than 3 HCC nodules (Tanabe KK et al, 2004).

RFA is also used for treating recurrent HCC in the liver following partial hepatectomy (Choi D et al, 2004). Local recurrence rates for RFA and PEI are variable, ranging from 2% to 50% up to 3 years after treatment (Hori T et al, 2003). Some studies demonstrate 5-year survival rates of 70% among patients with tumors less than 2 cm (Wong R et al, 2011).

**B) Transarterial Chemoembolization (TACE):**

Among patients with large multifocal HCC or those whose tumor characteristics are not appropriate for surgical or ablative therapy, TACE is recommended as a first-line, non-curative treatment for BCLC stage B multinodular asymptomatic tumors without vascular invasion or extrahepatic spread (Mancuso A, 2013).

TACE involves the injection of a chemotherapeutic agent that is mixed with embolic material and administered selectively into the feeding arteries of the tumor to potentially obtain higher intratumoral drug concentrations compared with intravenous therapy, with occlusion
of the blood vessel causing infarction and necrosis (Lammer J et al, 2010).

The improvement in survival in treated patients may range from 20% to 60% at 2 years (Llovet JM & Bruix J, 2003). Nevertheless, it is clear that the relevance of the improvement compared with the outcome if untreated is largely dependent on the patient’s baseline characteristics regarding tumor stage, liver function and general health status (Bruix J & Sherman M, 2005).

Absolute contraindications to TACE include main portal vein thrombosis, severe encephalopathy, biliary obstruction, and Child-Pugh C cirrhosis. TACE causes some degree of ischemic hepatic damage, which has the potential to lead to hepatic decompensation, with a rate of up to 20% in one series (Chan AO et al, 2002).

Side effects of chemoembolization are those of the chemotherapeutic agent used (usually doxorubicin), in addition to the complications of arterial embolization, namely pain, fever, hepatic decompensation and, rarely, infarction of organs other than the liver (Kasugai H et al, 1989).

Serious complications occur in 3–5% of treated patients. In addition, the use of TACE is associated with a ‘postembolization syndrome’ of fever, pain and vomiting in over 60% of patients. These complications are thought to be secondary to stretching of the liver capsule, pancreatitis, gall bladder infarction, peptic ulceration and necrosis (Belli L et al, 1997).

This is a transient side effect and in most cases can be controlled by non-steroidal anti-inflammatory drugs or hydrocortisone. Less common complications include hepatic failure, liver abscess, arteritis, and ruptured HCC (Liu CL et al, 1998).
C) Yttrium-90–Labeled Microspheres Radioembolization:

An alternative means of delivering focal radiotherapy uses radioactive isotope Y90-labeled microspheres and selectively delivers them to the tumor via the hepatic artery (Salem R et al, 2010).

This technique has the major advantage of being indicated in the case of portal vein neoplastic thrombosis, which is one of the major contraindications for TACE, and its toxicities have proven to be well tolerated (Kulik LM et al, 2008).

However, Y90 is contraindicated in patients with significant hepatopulmonary shunting because it could result in very high levels of pulmonary radiation exposure (El-Serag HB, 2004).

Tumor necrosis and survival depend on the tumor risk and Child-Pugh scoring systems, but response rates are similar to those obtained with TACE (Kooby DA et al, 2010).

D) Systemic Therapy:

Systemic therapies examined in the past, including both cytotoxic and hormonal agents, have provided limited or no benefit for patients with HCC. In 2007, the tyrosine kinase inhibitor (TKI) sorafenib was approved for use in advanced HCC based on an improvement in survival compared with placebo (Cheng AL et al, 2009).

Despite initial responses to sorafenib, most patients with HCC experience a loss of efficacy, which may be due to “resistance” via escape/compensatory mechanisms (Frenette C & Gish R, 2012).

In addition, 20% to 38% of patients discontinue its use due to adverse effects. As with other TKIs, sorafenib also has had class adverse effects, including skin-related toxicities, hypertension, proteinuria,
diarrhea, and cytopenias as well as life threatening complications, such as thromboembolism, bleeding, and bowel perforation. Liver failure also has been reported more frequently in patients whose liver disease is Child-Pugh stage B/C (Roodhart JM et al, 2008).

The mainstay of palliative therapy for advanced HCC is sorafenib, which is indicated strictly in advanced HCC (BCLC stage C) or HCC progressing after surgical or loco regional therapies in patients with well-preserved liver function and good performance status (Mancuso A, 2013). Studies are ongoing to determine the role of sorafenib as adjuvant therapy with surgical or loco regional therapy. Determining efficacy and safety in the substantial portion of patients with advanced HCC remains a challenge (Frenette C & Gish R, 2012).

Other TKIs are in development to treat HCC, both in the first-line setting and for use following sorafenib failure. Agents with anti-angiogenic properties in phase 2 and 3 development for the treatment of patients with HCC include bevacizumab (Avastin, Genentech), ramucirumab, ABT-869, everolimus, and ARQ 197 (Frenette C & Gish R, 2012).

Prognosis

There are 4 main groups of prognostic factors: tumor related, factors related to underlying liver status, factors related to the general health status of the patient, and factors related to the efficacy of treatment (Liu PH et al, 2016).

Independent negative predictive factors of HCC outcome include α-fetoprotein levels of $\geq 20$ ng/mL, serum albumin of $< 3.5$ g/dL, creatinine of $\geq 1$ mg/dL, bilirubin of $\geq 1$ mg/dL, alkaline phosphatase of $\geq 200$ IU/L, presence of ascites, maximal tumor size $>5$ cm, multiple tumor nodules,
presence of vascular invasion, presence of extrahepatic metastasis and poor performance status (Liu PH et al, 2016).

Other factors that predict survival include tumor histology, living in high- versus low-incidence regions of hepatitis B and C, having antiviral therapy for hepatitis B virus-related HCC, variant estrogen receptors, diabetes mellitus, vascular endothelial growth factor and insulin like growth factor 1 (Villa E et al, 2003).

Pathological prognostic factors are useful not only in predicting survival but also to stratify patients into risk groups for relapse. Tumour grade, size of tumour, microvascular invasion, portal vein tumour thrombus and the presence of tumour microsatellite lesions have all been found to predict survival (Zhou L et al, 2006).

With advances in understanding of tumour biology, molecular biomarkers of carcinogenesis have been investigated with regard to both their prognostic significance and their potential as therapeutic targets (Mann CD et al, 2007).

HCCs are largely resistant to radio- and chemotherapy. Long-term survival is likely only in patients with small, asymptomatic HCC that can be treated by surgical resection, including liver transplantation, or non-surgical methods, including percutaneous ethanol or acetic acid injection and percutaneous radiofrequency thermal ablation (Sugo H et al, 1999).

The high mortality / incidence ratio reported by the cancer registries indicates that the majority of cases do not survive more than 1 years, this is confirmed by most clinical series particularly in developing countries (Bosch, 2002).
Patients at terminal stages bear a poor prognosis, with less than a 6-month life expectancy and no survival benefit from treatment. Old series characterized these patients as Okuda stage III, or as Performance Status 3–4. These patients deserve symptomatic treatment (Llovet JM ET AL, 1999).

A rare fibrolamellar variant of HCC occurs in young patients without underlying liver disease and has a favorable prognosis because it often can be successfully resected (Josephs D & Ross P, 2010).
Alpha-Fetoprotein-L3

History

Historically alpha fetoprotein’s presence was observed in both normal fetuses as well as in abnormal conditions. In 1956 Bergstrand and Czar found a fetal component not commonly found in adults which was first detected as a post albumin migrating protein in fetal serum using paper electrophoretic techniques. However, it was only in 1963 that AFP gained interest after discovery of AFP in adults during carcinogenesis (Koteish A & Thuluvath PJ, 2002).

Later, in 1970, this antigen was named “alpha-fetoprotein” (AFP) and has been long recognized as the first onco-developmental biomarker. Currently, AFP is considered as a “golden standard” among tumor-specific molecular biomarkers (Debruyne EN & Delange JR, 2008).

AFP is synthesized by the yolk sac during early fetal life and later on by the fetal liver. Synthesis reaches its maximum at a concentration of 3 g/L at 12–16 weeks of gestation. Subsequently, after birth, serum AFP concentration drops rapidly below 10μg/L within the first 18 months. In adults, under normal conditions, serum AFP concentration is 5 to 10 μg/L (Evi et al, 2008).

A. Structure of AFP

AFP is a 70-kD glycoprotein consisting of 591 amino acids and 4% carbohydrate residues, encoded by a gene on chromosome 4q11-q13 (Johnson PJ et al, 2002).

AFP is synthesized as a precursor that undergoes post-translational processing with cleavage of a signal peptide. The amino acid sequence of immature AFP polypeptide chain was deduced from its mRNA nucleotide
sequence and the translation product was shown to contain 609 residues (figure 11) (*Morinaga T et al, 1983*).

Initial studies showed that the signal peptide contains 19 residues, and the mature protein is composed of 590 residues. However, later the N-terminal amino acid residue of mature AFP molecule isolated both from cultured human hepatoma HepG2 cells and embryonic tissues was shown to be arginine instead of threonine and, therefore, the mature human AFP molecule was demonstrated to contain 591 residues (figure 12) (*Dudich I et al, 1999*).

![Molecular configurations of human AFP and human albumin](image)

**Figure** (11) Molecular configurations of human AFP (Left) and human albumin (Right) based on the predicted secondary structures. The amino acid residues participating in the formation of alphahelices, beta-sheets, beta-turns, and random coils are indicated by respectively. The loops formed by disulfide bonding are filled in black. Stars indicate extra turns introduced in human AFP at amino acid residues 195-198 and 504-507 where the probabilities of beta turn occurrence were higher. Black squares represent the carbohydrate residues attached to asparagine-232 in AFP. The first loop at the amino terminus in AFP is formed assuming cysteines 18 and 67 participate in a disulfide linkage (*Morinaga T et al, 1983*).
**Figure (12).** Model of tree-dimensional structure of human AFP generated on the basis of homology with serum albumin. Domains I, II and III are indicated. Secondary structure elements are represented by alpha-helices and irregular structures.

**B. AFP Gene:**

The AFP gene is mapped on chromosome 4 (4q11–q13). This gene belongs to albumin gene family along with serum albumin, a group-specific component, also known as vitamin D-binding protein and alpha-albumin (protein found in human central nervous system) genes (Belanger et al., 1994).

Similar to their genes, these proteins are highly homologous in primary structure (Belanger et al., 1994). All of them are synthesized in liver and secreted into blood, providing delivery of their bound ligands to different tissues. All the albumin family genes are located on the same chromosome. The AFP, serum albumin, and alpha-albumin genes are positioned near each other and have a common direction of transcription (Figure 13) (Guan et al., 1996).
Figure (13): The human albumin gene cluster and AFP gene 5'-regulatory region structure (P, promoter; S, silencer; E1-EII, minimal enhancers of AFP gene; ALB, alpha-albumin gene) (Lazaravich et al, 2000).

All the albumin proteins consist of three homologous domains. This suggests that the coding genes originate from common origin, which, in turn, arose as a result of triplication of a primary gene corresponding to one domain of the protein (Gibbs et al, 1998).

The data about the clustered localization and about the evolution of the albumin genes, the dynamics of their synthesis, as well as the structure of their regulatory regions, suggest that the expression of these genes is interconnected and has common principles of regulation (Gibbs et al, 1998).

The main product of AFP gene transcription in fetal liver is a 2.1 kilobase (kb) mRNA. Besides, 1.7, 1.4, and 1.0 kb mRNAs are detected in fetal and regenerating liver and in carcinogenesis, respectively (Wan et al, 1989). The shorter forms of mRNA, 1.4 and 1.0 kb dominate in adult liver (Lemire et al, 1991).

Apparently, expression levels of the multiple mRNA forms are controlled by different mechanisms and can be changed independently. All the products of AFP gene transcription can be translated. The 2.1 kb mRNA corresponds to polypeptides weighing 68 and 70 kD. Different forms of AFP are now under studies for their functions (Lemire et al, 1991).
**C. AFP Gene Regulation:**

Transcription is the determining level for AFP gene regulation. This is confirmed by correlation of AFP mRNA amount and the protein synthesis level in various systems. However, it is impossible to exclude completely the participation of post transcriptional mechanisms in the regulation of AFP level (*Lienard et al, 2006*).

**1) AFP Gene Promoter:**

A sequence of base pairs (bp) of the AFP gene are characterized by tissue-specific promoter activity and contain multiple overlapping binding sites for ubiquitous and tissue-specific transcription factors. In vivo in the absence of the enhancer, the AFP promoter is inactive. A considerable similarity of AFP and other albumin gene promoter organization can be noted, in particular, the presence and localization of CCAAT-box and hepatocyte necrosis factor -1(HNF1) binding sites within (*Lazarevich, 2000*).

**2) AFP Gene Enhancers:**

Within the human AFP gene regulatory region two enhancer elements have been revealed: .4.0/.3.7 and.3.7/.3.3 kb. In the proximal enhancer the HNF1 binding site is localized (*Lazarevich , 2000*). All of these elements enhancers are able to stimulate AFP promoters. Like AFP promoter, the enhancers are tissue-specific and are not active in non-hepatic cells (*Lienard et al, 2006*).

Each of the enhancers is able to stimulate serum albumin as well as AFP promoter. Probably, at some stages of development and in some hepatoma lines, in which the upstream serum albumin enhancer is non-active, the intergenic enhancers control the expression of the two genes independently, and the corresponding promoters do not compete with
each other due to their interaction with the different enhancers sites (Lienard et al, 2006).

3) **AFP Gene Silencer Elements:**

These silencer are able to suppress AFP promoter. At least two silencers, .1822/.951 and .402/.169 bp, have been revealed in the human AFP gene regulatory region. The distal silencer is more powerful; it inhibits the activity of homologous and heterologous enhancers according to their localization (Lazaravich et al, 2000).

**D. Glycoforms of Alpha –Fetoprotein:**

Since AFP is a glycoprotein, research has been carried out on its glycosylation and the possible diagnostic use of its variation in glycosylation. Yoshima et al, unraveled in 1980 the carbohydrate moiety of AFP and detected three oligosaccharide fractions: a neutral fraction and acidic fractions 1 and 2. The two acidic fractions differ from the neutral fraction only in the presence of, respectively, 1 and 2 extra sialic acid components. These three fractions were later identified as asialylated AFP (neutral fraction), monosialylated AFP (acidic fraction 1) and disialylated AFP (acidic fraction 2) (Johnson et al, 1997).

Accordingly the IEF technique identifies three main bands based on terminal sialylation: band +I (disialylated), +II (monosialylated) and +III (asialylated). These three bands differ by their isoelectric point. Monosialylated AFP (msAFP) was considered specific for HCC (Johnson et al, 2000).

Lectin-electrophoretic techniques separate total AFP into several glycoforms based on difference in affinity towards lectins, such as Concanavalin A (Con A), Lens culinaris agglutinin (LCA) and erythroagglutinating phyto-hemagglutinin (E-PHA) (Evi et al, 2008).
Con A is a mannose binding lectin with highest affinity to high-mannose type biantennary sugars *(Krusius et al, 1982)*. LCA shows high affinity towards fucosylation at the innermost N-acetylglucosamine residue of the biantennary sugar chain of AFP *(Aoyagi et al, 1988)*.

E-PHA is most reactive to the AFP-glycoform with one exposed and one sialylated galactose residue (Figure 14) *(Taketa et al, 1993)*. According to their binding capacity towards LCA lectin, three different glycoforms of AFP were identified, namely AFP-L1 (LCA nonreactive), AFP-L2 (intermediate reactive versus LCA) and AFP-L3 *(Breborowicz et al, 1988)*.

AFP-L3 is the LCA-affinitive AFP glycoform, with an additional α1-6 fucose residue attached at the reducing terminus of N-acetylglucosamine. AFP-L3 appears to be produced only by cancer cells *(Du et al, 1991)*.

**Figure (14).** Representative pattern of AFP glycoforms obtained by lectin affinity electrophoresis, with different lectins and combined antibody-affinity blotting, for benign and malignant diseases *(Taketa et al, 1990)*.
E. AFP Receptors:

Four cell surface receptors for AFP have been described that might bind various forms of AFP in addition to the native protein. These receptors have been described as both endothelial components and epithelial cell surface membrane receptors (Torres et al, 1992).

Eighteen, 31, and 60-kD cell surface receptors have been found in the vascular endothelium of many tissues (heart, lung, epididymus, etc.). The 18 and 31-kD binding proteins represent scavenger receptors that bind chemically modified or denatured albumin and AFP, while the 60-kD form is an endothelial cell surface sialoglycoprotein found only on continuously lined (not sinusoidal) endothelium. However, a canonical AFP cell surface receptor has been localized on monocytic, reproductive, immunologic, and tumor cells, notably, hepatomas and MCF-7 breast cancer cells (a type of breast cancer cell lines) (Alava et al, 1999).

This AFP receptor is a 62 to 67-kD protein first detected on human breast cancer cells and later purified from monocyte cell membrane preparations. High AFP receptor concentration is correlating with the tumor cell growth (Tsuboi, 2006).

F. AFP Synthesis:

During embryogenesis AFP can be detected in visceral endoderm of the yolk sac (Lazarevich, 2000). At this stage, AFP is a dominant serum protein. Later, the maximum level of its expression is observed in fetal liver and, at significantly lower levels, in embryonic gut and in some other organs (Tyner et al, 1990).

At the end of the embryonic period of development, at the same time as the morphological restructuring of the liver, a drastic decrease in AFP blood level and reduction of AFP-producing cell number take place.
Simultaneously albumin blood level increases and the main adult serum protein substitute the embryonic one. This switch is carried out on the transcriptional level (Lazarevich, 2000).

Shortly after birth AFP concentration in blood decreases 104- fold, in parallel with the increase in serum albumin level and induction of alpha-albumin (Cooke et al, 1991).

Coordinated regulation of expression levels has been described for many other systems, e.g., for the globin gene cluster and liver-specific apolipoprotein genes. AFP gene expression is repressed reversibly in adult liver. It can be restored during the course of liver regeneration induced by partial hepatectomy, when up to 2/3 of the organ is removed surgically, or by acute intoxication that causes necrosis of the hepatocytes bordering central veins. Simultaneously with AFP induction, serum albumin synthesis decreases (Lazarevich, 2000).

An elevation of AFP serum level is observed in the case of acute viral hepatitis and, to a lesser extent, in liver cirrhosis. AFP blood level elevation is observed in primary liver tumors, teratocarcinomas, and gut tumors (Abelev et al, 1999).

In the case of embryonic carcinomas, teratocarcinomas, yolk sac tumors, and hepatoblastomas, an increase in AFP level is observed in 80-90% of cases and appears to be an important diagnostic marker. Many teratocarcinomas and hepatomas are characterized by a decrease in albumin synthesis along with an increase in AFP. However, in a number of cell cultures, e.g., human hepatoma Hep G2, AFP and albumin are produced simultaneously. As in normal tissues, in most of the teratocarcinomas and hepatomas, a dependence of albumin and AFP synthesis on the differentiation state is observed (Abelev et al, 1999).
G. Clinical Utility of AFP-L3:

1) AFP-L3 and Down Syndrome:

A small portion of this substance eventually passes into the mother’s bloodstream. Fetal plasma concentration increases to a maximum (approximately 3.0-4.0 g/L) between 13-14 weeks of gestation. Maternal serum levels peak at about 30 weeks (about 250 mg/L). After birth, maternal and infant AFP rapidly decline (Kabili G et al, 2004).

In 1984 Merkatz and Co-workers, reported that maternal serum AFP (MSAFP) in the second trimester from pregnancies affected with fetal trisomy 21 was lower (AFP < 0.7 MoM) than in normal pregnancies (Merkatz IR et al, 1984).

Masaki Azuma et al. (2001) examined the utility of analyzing AFP microheterogeneity assessed by lectin affinity in Down’s syndrome (DS) screening. Maternal sera and amniotic fluids were collected from women who were carrying DS fetuses and unaffected pregnancies around 16 weeks of gestation. The percentages of AFP which reacted with LCA (AFP-L2, 3) were determined by lectin affinity electrophoresis. AFP-L2, 3 levels were significantly increased in both maternal serum and amniotic fluid from DS-affected versus unaffected pregnancies. These data suggest that the measurement of AFP-L3 in maternal serum is a potential biochemical marker for Down syndrome.

Masaki Azuma et al. (2001) stated that combination of AFP-L3 and the triple test which is the conventional method gives better diagnostic performance. They found that knowing the percentage of AFP-L3 in maternal serum was effective for discriminating between Down's syndrome-affected pregnancies and unaffected pregnancies. The
percentage of AFP-L3 in maternal serum identified 55% of Down's syndrome cases with a 5% false-positive rate.

Elevated levels of AFP can be found in certain conditions such as: spina bifida, anencephaly (failure of brain and skull development), fetal death, abdominal wall defects, twin gestation, or inaccurate dating of pregnancy (Duric K et al, 2003).

2) AFP-L3 and Testicular Tumors:

In non-seminomatous germ cell tumors (NSGCT) the role of AFP, HCG and LDH as tumor markers is well established in diagnosis, staging, monitoring therapy, follow up and prognosis (Lange PH et al, 1977).

AFP is produced in approximately two third of the patients with GCT containing yolk sac elements (embryonal carcinomas, teratocarcinomas, teratomas and endodermal sinus tumors). Ninety percent of patients with NSGCT at any stage of the disease have elevated serum levels for AFP, HCG or both before treatment. AFP levels above 10,000 ug/ml are known to be a poor prognostic factor with a five-year survival of 48% (Scardino PT et al, 1977).

Kamoto et al. (2002) reported the usefulness of the LCA-reactive AFP using a liquid-phase binding assay (LBA) for the detection of residual and recurrent tumor, and also for discrimination of false positive elevation in non-seminomatous germ cell tumors (Kamoto et al, 2002).

During therapy, AFP and HCG should decline to normal according to their half-life of three to six days and 18 to 36 hours respectively, indicating an effective treatment (Vogelzang NJ et al, 1982).

3) AFP-L3 and Teratoma:
Kinoshita et al. (2006) studied 5 cases of teratoma from 2003 to 2006, all of which were neo infantile and treated in their department. The total AFP level and the L3 fraction (%) were measured to assess the usefulness of the L3 fraction as a diagnostic marker. In all, both the total AFP and the L3 fraction were high in the presence of malignant tumors. The L3 to AFP fraction was 10% and decreased after surgical treatment. These results indicated that the level of the L3 fraction accurately confirmed the existence, or the malignant potential of germ cell tumor.

4) AFP-L3 and HCC:

Alpha-fetoprotein L3 (AFP-L3) is generated from malignant liver cells; its measurement helps to differentiate HCC from benign hepatic diseases (Leerapun et al, 2007).

AFP glycoforms in combination with other proteins have been also proposed to be biomarkers for early detection of HCC. In particular, levels of lens culinaris agglutinin-reactive AFP (AFP-L3) and des-gamma carboxyprothrombin (DCP) were significantly higher in patients with HCC than in those without HCC (Durazo FA et al, 2008).

AFP glycoform measuring is more important than measuring of AFP. There are three types of glycoforms, the most important one in diagnosis of HCC is lens Culinaris aglutinin reactive 3 alpha fetoprotein (AFPL3) (Li D et al, 2001).

By contrast, AFP-L3, as an LCA-bound heterogeneity, exists only in the serum of patients with HCC at a cut-off value of 15%, with a sensitivity and specificity of 96.9 and 92%, respectively, in detecting HCC (Singhal A et al, 2012).

The sensitivity and specificity of AFP-L3 are both relatively satisfactory as compared with AFP. Moreover, AFP-L3 does not correlate with AFP, thus the former can be used as an independent and significant
factor for the early diagnosis of HCC. Specifically, for HCC patients with a total AFP of 10-200 ng/ml, when the cut-off value of AFP-L3 is 35%, the diagnostic specificity for HCC reaches 100%, thereby improving the early diagnostic rate (Leerapun A et al, 2007).

A regular surveillance program for patients at risk for development of Hepatocellular Carcinoma (HCC) is recommended by clinical practice guidelines worldwide. The following surveillance algorithm is currently in place at the University of California San Diego (UCSD) (Figure15). Dr. Yuko Kono, an Associate Clinical Professor at the UCSD Health System, performs regular surveillance on her patients at risk for HCC development. Serum biomarkers lectin-reactive alpha-fetoprotein (AFP-L3), alpha-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) are performed routinely in combination with ultrasound at a frequency of every 6 months. If ultrasound is negative, but one or more of the HCC biomarkers are positive, the patients are escalated to enhanced imaging for further evaluation (National Comprehensive Cancer Network (NCCN), 2017).

Studies have shown that although each biomarker can be clinically useful on its own for risk assessment of HCC, due to the heterogeneity of HCC tumors, using the biomarkers in combination can yield better clinical performance (Hann HW et al, 2014). As such, the HCC biomarkers in combination (AFP-L3, AFP and DCP) are increasingly becoming part of surveillance protocols in U.S. clinics (Gish R, 2014).
Methods of Assay of AFP-L3:

1) Enzyme Linked Immunosorbent Assay (ELISA):

Enzyme Linked Immunosorbent Assay (ELISA) technique is an immunoassay for the in-vitro quantitation of human AFP-L3 protein in human sera. The AFP-L3 kit is a solid phase phase sandwich ELISA. Samples, including standards of known AFP-L3 concentrations and unknowns are pipetted into wells coated by AFP-L3 specific antibody. During the first incubation, the AFP-L3 and a biotinylated monoclonal antibody specific for AFP-L3 are simultaneously incubated (Toyoda H et al, 2006).

After washing, the enzyme (streptavidin-peroxidase) is added. After incubation and washing to remove the entire unbound enzyme, a substrate solution which is acted on by the bound enzyme is added to induce a colored reaction product. The intensity of this coloured product is directly
proportional to the concentration of AFP-L3 present in the samples. The lower detection limit of ELISA method for AFP L3 is 0.1ng/mL. The CV% within-plate, inter-plate is less than 10%. There is no significant cross reactivity or interference the linearity is from 0.6 to 40ng/mL (ToyodaH et al, 2006).

2) Lectin Affinity Electrophoresis and Western Blotting:

AFP-L3 studies were done by lectin-affinity electrophoresis coupled with antibody-affinity blotting. Sera with AFP > 200 ng/mL are diluted to approximately 100 ng/mL. Each prepared sample is applied to wells on the lectin agarose gel. Before electrophoresis, the electrophoretic chamber and electrode buffer are cooled to below 15°C. Electrophoresis is carried out at a constant voltage of 200 V for 50 minutes (Hau-Tsai Cheng et al, 2007).

After electrophoresis, separated AFP bands are blotted on nitrocellulose membranes pre-coated with affinity-purified antibodies to human AFP for 30 minutes at 37°C with 4 mL of rabbit AFP antibody solution. Then, the membrane is exposed to affinity-purified goat rabbit immunoglobulin G antibodies labeled with horse-radish peroxidase for 30 minutes at 37°C. For coloring reaction, 10 mL of the enzyme-substrate solution is added. The system is kept at room temperature for 30 minutes and then gently washed and dried after color is developed. AFP bands are then quantified through densitosmetric analysis (Hau-Tsai Cheng et al, 2007).

Measuring of these bands of tested serum and those of standard is done simultaneously. An example of bands appearing after electrophoresis is shown in (Figure 16). The results of AFP-L3 are expressed as percentage of AFP-L3 to total AFP. Both sensitivity and specificity of AFP-L3 for prediction of HCC are 71% at a cutoff value of
15%. The sensitivity decreases to 66.5% while the specificity increases to 82% if the cutoff ratio is raised to 17.5% (Hau-Tsai Cheng et al, 2007).

Figure (16). Lectin-affinity electrophoresis for AFP. A strong AFP-L3 (AFP-L3 to total AFP ratio, 70%; white arrow).

3) Liquid Phase Binding Assay:

Laboratory assay for AFP-L3 used to be based on lectin affinity electrophoresis and Western blotting (Shimizu et al, 1996).

These assays were tedious with long turnaround times. Recently, an automated assay for measuring the AFP-L3 has been developed and introduced for clinical use. The new automated method for measurement of AFP-L3 is an immunoassay based on the liquid phase binding of L3 subspecies of AFP with two specific monoclonal antibodies labeled with peroxidase and polysulfide tyrosine peptide, respectively. The bound and free AFP isoforms are separated by affinity liquid chromatography (Yamagata et al, 1998).

The concentration of bound AFP-L3 is then determined fluorometrically after a simultaneous assay of total AFP, the ratio of concentration of AFP-L3 and total AFP is calculated. Results are reported as percentage ratio of AFP-L3 to total AFP (Yamagata et al, 1998).
The cut-off value for a positive test is currently set at 10%, yielding a sensitivity of 56% and specificity of 95% which was derived from receiver operating characteristic curve analysis (Takeda et al, 1993).

Inter-assay CV for AFP-L3 ranges from 2.8% to 5.8%. Recovery varies from 92.6% to 105.6%. The automated assay shows good correlation with lectin-affinity electrophoresis. Endogenous substances do not interfere with the assay (Davi et al, 2001).