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Chapter

Hepatocellular Carcinoma: Diagnosis and Surveillance

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Abstract

Hepatocellular carcinoma arises commonly on the background of liver cirrhosis. Patients presenting with clinical symptoms have advanced stage and often are unsuitable for curative therapies. Diagnosis of hepatocellular carcinoma is commonly performed by multiphase computed tomography (CT) and / or magnetic resonance imaging scans (MRI). Contrast enhanced ultrasound and MRI with hepatobiliary contrast agents are better in characterizing small lesions. Tumor markers play an adjunct role in diagnosis. For HCC in cirrhotic liver biopsy is seldom required and diagnosis is based on typical imaging features of non-rim arterial phase hyperenhancement and washout on delayed phase and pseudocapsule appearance. This is due to differential blood supply of liver parenchyma, regenerative nodules and tumor. Biopsy is only required in noncirrhotic liver, vascular liver diseases, atypical imaging features. Surveillance programs involving high risk groups can help in early detection of lesions which are amenable for curative therapies. Biannual ultrasound with or without alfa fetoprotein are commonly used surveillance tests. Multidisciplinary teams provide platform for care coordination, reassessments of clinical course, and fine changes in treatment plans required for management of this complex group of patients.

Keywords: hepatocellular carcinoma, surveillance, tumor markers, multiphase computed tomography, multiphase magnetic resonance imaging, LI-RADS, multidisciplinary team

1. Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of liver. It is sixth most commonly diagnosed cancer and fourth leading cause of cancer related mortality worldwide [1]. Most cases are diagnosed late in course of disease so that curative treatments could not be offered to such patients and hence incidence to mortality ratio for HCC approaches 1 [2]. Incidence of HCC is likely to increase due to increase in population and aging, as well as changing distribution of risk factors like obesity, hepatitis B and C virus infection and alcohol consumption [3]. Diagnosis at early stages and implementing surveillance programs in high risk population may reduce mortality [3]. This chapter focuses on diagnosis and surveillance for HCC.

2. Diagnosis of hepatocellular carcinoma

Diagnosis of hepatocellular carcinoma is primarily based on imaging with multiphase computed tomography (CT) scan and/or multiphase magnetic resonance

imaging (MRI) scan. Like in other cancers, biopsy is required in selective cases where there is diagnostic dilemma. HCC usually becomes symptomatic only in advanced stages of the disease hence clinical features are seldom useful for the diagnosis of disease. Tumor markers are useful blood test in supporting diagnosis and prognostication of most of HCC however, they have their own limitations in early diagnosis of HCC. This section throws light on clinical features, imaging investigations and tumor markers and there role in diagnosis of hepatocellular carcinoma.

2.1 Clinical features of HCC

Early HCC are asymptomatic and are usually picked up during surveillance imaging. Classic clinical triad of right upper quadrant abdominal pain, palpable lump and weight loss is noted in 90% of the symptomatic patients [4]. New onset of abdominal pain and abdominal distension due to ascites are common in patients with underlying liver cirrhosis [5, 6]. Rapid worsening of portal hypertension indicates invasion of portal vein by tumor leading to tumor thrombosis [6]. Generalized weakness, anorexia and weight loss are common symptoms noted in 90%, 74% and 55% patients respectively [7]. Catastrophic presentation in the form of tumor rupture, hemoperitoneum and shock occurs in 3–15% of cases [8]. Hepatomegaly with irregular or nodular surface is common finding in nearly 84% cases [7]. Arterial bruit is present in minority of cases (2.6%) [9]. Ascites in HCC is most commonly due to underlying decompensated cirrhosis or due to tumor invasion of hepatic veins, portal vein or peritoneum and is often hemorrhagic [9]. Paraneoplastic manifestations of HCC include type B hypoglycemia due to increased production of insulin like growth factors by tumor, hypercalcemia, hypertension, carcinoid syndrome, clubbing, polycythemia, porphyria, thyrotoxicosis, migratory thrombophlebitis, watery diarrhea, sexual changes like feminization, gynecomastia [9].

2.2 Imaging diagnosis of hepatocellular carcinoma

Almost 90% of HCC develop on the background cirrhotic liver [10]. Regenerative nodules form in cirrhotic livers obtain majority of blood supply from portal vein, like the normal liver parenchyma. As the nodule progresses from regenerative to dysplastic and then into HCC, there is shift in blood supply from portal vein to hepatic artery [10]. Hence HCC obtains majority of the blood supply from hepatic artery. This forms the basis of diagnosis of HCC by non-invasive methods using multiphase computed tomography scan (CT) and magnetic resonance imaging (MRI). Radiology forms the cornerstone in diagnosis of HCC in cirrhotic liver. Non-invasive methods are applied to nodule ≥1 cm in cirrhotic liver due to high pretest probability [10].

Technical details related to machine, required images and additional images to be taken while evaluating liver nodule are mentioned in **Table 1**.

2.2.1 Typical appearance of hepatocellular carcinoma in cirrhotic liver on multiphase CT or MRI scans include

1. Non-rim arterial phase hyperenhancement AND

2. Non-rim washout in portal venous phase

3. Enhancing capsule appearance in portal venous phase or delayed venous phase

Technical details	Multiphase CT scan	MRI scan
Machine specifications	• Multidetector CT with more than 8 detector rows • Slice thickness 3 mm • Injection rate 4 milliliter/second	• 1.5 T or 3 T • Torso phased array coil
Required images	• Non-contrast • Arterial phase at 30 seconds with bolus tracking • Venous phase at 65 seconds • Delayed phase at 240 seconds	• Unenhanced T1 weighted in phased and opposed phase imaging • T2 weighted imaging (fat suppression per institutional preference) • All contrast agents in T1 weighted imaging. • Precontrast imaging • Arterial phase • Portal venous phase • Delayed venous phase
Additional images	• Multiplanar reformations • Precontrast in patients with locore-gional treatment	• Diffusion weighted images • Subtraction imaging • Multiplanar acquisition

Table 1.
Technical details, required images and additional images to be obtained while evaluating liver space occupying lesion [11].

4. Increase in size of mass > 50% in <6 months measured in same phase sequence and plane (if possible). To measure the size of lesion largest outer edge to outer edge dimensions should be taken.

5. Ancillary features for diagnosis of HCC include hyperintensity on T2-weighted MRI, hyperintensity on diffusion-weighted MRI, intra-lesional fat, lesional iron sparing, corona enhancement, presence of capsule, mosaic architecture, nodule-in-nodule architecture, intralesional hemorrhage however, these features do not have specificity of 100% and do not allow conclusive diagnosis of HCC.

2.2.2 Comparison of multiphase CT and MRI with extracellular contrast agents performance in detecting HCC

Table 2 shows comparative performance of multiphase CT and MRI in HCC with various sizes [12].

For all sizes and tumors with <1 cm MRI with extracellular contrast agents appears to be more sensitive than CT scan with comparable specificity and diagnostic odds. Hence for small lesions MRI with extracellular contrast agents may be preferred modality over CT scan. Having said this availability, cost, longer scan times, more technical complexities, expertise, several patient factors like ascites, difficulty in breath holding, claustrophobia may limit its use as the first investigation for evaluation of liver lesion in cirrhotic patients. CT scan on the other had is technically relatively simple, less number and short duration of sequences, widely available and less costly than MRI. However, radiation exposure is the disadvantage of the CT scan. Hence multiple factors like availability, cost, patient related factors, tumor size, radiation are necessary to be considered to choose between CT scan and MRI as first investigation for evaluation of liver lesion [12].

Tumor size	Sensitivity (CT vs. MRI)	Specificity (CT vs. MRI)	Diagnostic odds CT vs. MRI
All sizes	0.69 vs. 0.84 (p = 0.0003)	0.92 vs. 0.94 (p = 0.83)	22 vs. 43 (p = 0.24)
< 1 cm	0.48 vs. 0.69 (p = 0.049)	0.46 vs. 0.69 (p = 0.08)	2.05 vs. 2.3 (p = 0.8)
1–2 cm	0.64 vs. 0.7 (p = 0.15)	0.87 vs. 0.88 (p = 0.78)	13 vs. 17 (p = 0.78)
2 cm	0.79 vs. 0.88 (p = 0.09)	0.9 vs. 0.87 (p = 0.71)	25.79 vs. 64.66 (p = 0.47)

Table 2.
Comparative performance of multiphase CT and MRI in HCC with various sizes.

2.2.3 Role of MRI with hepatocyte specific contrast agents in diagnosis of HCC

This technique uses Gadoxetic acid as a contrast agent. Approximately 50% of the administered dose is taken up by hepatocyte and excreted into the bile ducts and remaining half was excreted by kidneys [13]. Images are taken in two phases: Transitional phase taken at 2–5 minutes after contrast agent and hepatobiliary phase taken after 20 minutes of contrast injection [13]. Lesions with functional hepatocytes take up the contrast in hepatobiliary phase and appear hyperintense. Those without functional hepatocytes like high grade dysplastic nodules or HCC do not take the contrast in hepatobiliary phase and appear hypointense compared to background liver parenchyma [13]. These early HCC or high grade dysplastic nodules may not show typical arterial hyperenhancement resulting in missing some of the early HCC lesions. Addition of hepatobiliary phase to conventional dynamic MRI sequences increases likelihood of identifying malignant nodules and reduces the risk of overlooking malignant lesions [13–15]. Signal intensity of lesion on hepatobiliary phase is also a prognostic factor with hypointense lesions on hepatobiliary phase which are non-hypervascular, non-HCC have a higher risk of progression to typical HCC as compared to those lesions which are iso- or hyper-intense [16, 17].

2.2.4 Role of contrast enhanced ultrasound in diagnosis of HCC

It is performed with intravenous injection of a microbubble contrast agent. Real-time imaging is performed continuously for the 1st minute to capture the arterial phase. This is followed by intermittent scanning every 30–60 seconds for up to about 5 minutes to evaluate washout [11]. Typical appearance of HCC on Contrast enhanced ultrasound (CEUS) shows non rim arterial phase hyperenhancement and washout in delayed phase >60 seconds to differentiate it from mass forming cholangiocarcinoma which show early washout. It requires expertise and cannot scan entire liver at a time like CT or MRI [11]. CEUS has low sensitivity for detection of lesion as compared to CT and MRI but has higher specificity as compared to CT and MRI especially for small nodules (< 20 mm) 92.9% vs. 76.8% vs. 83.2% [18]. CEUS as second imaging modality has highest specificity 76.8% (after MRI) and 70.7% (after CT) for diagnosis of HCC [19].

2.2.5 Liver imaging reporting and data system (Li-RADS)

Liver imaging reporting and data system (Li-RADS) provides standardization for hepatocellular carcinoma (HCC) imaging. Li-RADS defines eight unique

diagnostic categories LR 1 to 5, LR-M for malignant but not specific for HCC, LR-TIV for tumor in vein, LR-TR for treated lesion, based on imaging appearance that reflect the probability of HCC or malignancy with or without tumor in vein. Term LR-NC (non-categorizable observation) is used when observation that cannot be meaningfully categorized due to lack of one or more major criteria. LI-RADS criteria are to be applied for liver nodules in cirrhotic livers and lesion >1 cm. **Table 3** describes the each Li-RADS category and risk of HCC and non-HCC malignancy [11].

LI-RADS is not applicable for liver lesions in noncirrhotic liver, vascular liver diseases, sinusoidal obstruction syndrome, chronic inflow obstruction and hereditary hemorrhagic telangiectasia.

2.2.6 Role of Fluorodeoxyglucose positron emission tomography (FDG-PET) in diagnosis of HCC

FDG uptake is seen only in 40% of patients with HCC, so FDG-PET scan is not useful for diagnosis of HCC [20]. Uptake on 18F-FDG-PET has some potential prognostic significance and is associated with poor prognosis, increased serum alpha-fetoprotein and vascular invasion. Therefore, it may facilitate the selection of patients for surgical resection or liver transplantation [21].

2.2.7 Diagnosis of portal vein thrombosis- tumoral vs. non-tumoral (bland thrombus)

Cirrhosis without HCC is associated with portal vein thrombosis with prevalence ranging from 1% in compensated cirrhosis to as high as 25% in patients with advanced liver disease requiring liver transplantation [22]. Macrovascular invasion of the portal vein is a major prognostic factor frequently seen in HCC. Portal vein thrombosis may create diagnostic dilemma in patients with cirrhosis and HCC. Presence of arterial phase hyperenhancement, diffusion weighted MRI with high b values, venous expansion with diameter > 23 mm, thrombus in continuity with parenchymal HCC are the findings which point towards the diagnosis of tumoral portal vein thrombosis [23, 24].

2.3 Pathological diagnosis of hepatocellular carcinoma

HCC diagnosis in cirrhotic liver is based on imaging criteria mentioned above. However biopsy is required in patients with vascular liver diseases, non-cirrhotic livers, inconclusive radiological investigations, elevation of CA 19.9 or carcinoembryonic antigen (CEA) and liver lesion without HCC risk factors [24]. Samples for histological diagnosis of HCC can be obtained by image guided (ultrasound / CT scan) biopsy sometimes by diagnostic laparoscopy. Resected specimens and explants after liver transplants need evaluation for resection margin and histological assessment [24].

2.3.1 Gross appearance

HCC takes three forms nodular, massive or diffusely infiltrating type. Nodular form is often associated with liver cirrhosis. Massive form is associated with satellite nodules and has potential to rupture. Diffuse infiltrating type causes involvement of large part of liver and its vascular structures mainly portal vein, and is associated with poor prognosis [25].

Li-RADS category	Description	Interpretation	Risk of overall malignancy	Risk of HCC
LR-NC	Observation that cannot be categorized into specific category due to inability to assess one or more major criteria.	Noncategoriz-able observation	—	—
LR-1	Benign observation with 100% certainty	Benign	0%	0%
LR-2	High probability of being benign observation. No major features, LR-M features, ancillary features favoring malignancy	Probably benign	13%	14%
LR-3	Nonmalignant and malignant entities each have moderate probability. Nonrim APHE without any other major features OR Arterial phase iso/ hypoenhancement with size <20 mm and ≤ 1 additional major feature or > 20 mm and no major feature.	Moderate probability of being malignant or nonmalignant	38%	40%
LR-4	High probability of HCC but not 100% certainty. Non rim APHE and < 10 mm and ≥ 1 additional major feature 10–19 mm with capsule >20 mm with ≥1 additional major feature OR <20 mm with 2 additional major features	Probably HCC	74%	80%
LR-5	100% certainty of being HCC Nonrim APHE and 10–19 mm with non-peripheral washout OR 10–19 mm with ≥50% size increase in <6 months >20 mm with ≥1 additional feature	Definitely HCC	94%	97%
LR-TIV	Presence of soft tissue in vein regardless of mass in parenchyma	Malignancy with tumor in vein	—	—
LR-M	Targetoid mass with: Rim APHE Peripheral washout Delayed central enhancement Targetoid diffusion restriction Nontargetoid mass not meeting LR-5 criteria and without TIV with >1 of following Infiltrative appearance Marked diffusion restriction Necrosis or ischemia	Probably or definitely malignant but not specific for HCC	36%	93%

Table 3.
LI-RADS criteria with description of terminologies, risk of overall malignancy and risk of HCC.
[APHE – Arterial phase hyperenhancement, TIV- tumor in vein].

2.3.2 Microscopic appearance

Microscopically HCC can be well differentiated, moderately differentiated, undif-ferentiated and progenitor cell. Most common variety is well differentiated type. It can

be of trabecular type or acinar type (pseudoglandular type). Malignant hepatocytes are polygonal with large hyperchromatic nuclei. Bile production is present. Moderately differentiated HCC can be of solid, scirrhous, sarcomatoid and clear cell varieties. Solid type tumor shows small hepatocytes with areas of necrosis, inconspicuous fibrous tissue and absent bile production. In scirrhous variety abundant connective tissue stroma is noted separating hepatocytes. Clear cell variety has cells having high glycogen content. Undifferentiated HCC has pleomorphic cells with variable sized nuclei. Progenitor cell HCC have their origin from stem cells of liver. These tumors may appear similar to HCC or mixed cholangiohepatocellular carcinoma [25]. On biopsy specimens differentiation of small HCC from high grade dysplastic nodules is challenging. Diagnosis of HCC needs to be supplemented with three marker panel as recommended by International Consensus Group of Hepatocellular Neoplasia and the World Health Organization. This is because features of interstitial and vascular invasion can be missed on biopsy specimens. Combination of HSP70 (HSPA7), glypican 3 (GPC3), and glutamine synthetase (GS) has sensitivity and specificity of 72% and 100%, respectively in surgically resected specimens and its specificity is validated in biopsy specimens [26, 27]. Several immunohistochemical markers useful in diagnosis of hepatocellular carcinoma include Arginase-1 which is most sensitive and specific marker for hepatocellular differentiation. Hepatocyte paraffin-1 (Hep Par-1) has both sensitivity and specificity greater than 80% for HCC. Polyclonal carcinoembryonic antigen (pCEA) shows typical canalicular pattern and has sensitivity of 92% and 88% for well differentiated and moderately differentiated HCC [28].

HCC is heterogenous tumor in pathogenesis, behavior, phenotype and has different genetic signatures as described by recent studies. As mentioned above several different subtypes are described. 5th edition of world health organization classification of digestive system tumors integrates histopathologic features and molecular signatures of these tumors. **Table 4** shows morphological features, molecular signatures of different HCC subtypes as per 5th Edition of WHO Classification of Digestive system tumors [29, 30].

2.3.3 Risks associated with biopsy of the lesion

Biopsy is associated with risk of bleeding in 3–4% cases and severe bleeding requiring transfusion in 0.5% cases [31]. Risk of needle track seeding of tumor cells is about 2.7% [32]. Sampling errors can occur for small lesions <2 cm [33].

2.4 Role of tumor markers in diagnosis of HCC

Tumor markers are the substances which can be measured in cells, tissues, body fluids, indicate presence of cancer and help in prognostication. Ideal tumor marker should be highly sensitive and specific so as to diagnose lesions early HCC. Alfa fetoprotein (AFP) is used since long time for surveillance and diagnosis of hepatocellular carcinoma [34]. Now with identification of new molecular signatures, our understanding of pathological processes involved in HCC is improved leading development of newer biomarkers. This section will through light on old and new tumor markers and their utility in diagnosis of HCC [34].

2.4.1 Alfa fetoprotein (AFP)

AFP is a glycoprotein produced by fetal liver. After birth levels of AFP fall and its synthesis is repressed in adult life. It is expressed under some pathological conditions like chronic liver disease, cirrhosis, HCC, germ cell tumors and cholangiocarcinoma [35]. It is the most extensively studied biomarker for surveillance and diagnosis of

Variant	Histopathology	Molecular signature	Comments
Steatohepatic	Features of steatohepatitis in tumor.	IL-6/JAK/STAT activation	Less often vascular invasion or satellite nodules. Prognosis similar to conventional HCC
Clear cell	>80% cells demonstrates clear cytoplasm due to glycogen.	Not known	Slightly better prognosis compared to conventional HCC. Needs differentiation from clear cell type of renal cell carcinoma.
Macrotrabecular	Prominent thick trabeculae.	TP53 mutation FGF9 amplification.	Associated with HBV infection, vascular invasion, poor differentiation, high alfa-fetoprotein.
Scirrhous	Tumor cells mixed with dense fibrous stroma.	TSC1/TSC2 mutations, transforming growth factor beta activation.	Large tumors, vascular invasion, infiltrative growth.
Chromophobe	Cells have clear cytoplasm, focal areas of nuclear atypia.	Alternate lengthening of telomere phenotype.	Prognosis similar to conventional HCC.
Neutrophil rich	Diffuse tumoral infiltration by neutrophils.	Granulocyte monocyte colony stimulating factor production.	Elevated leucocyte count, interleukin-6. Poor prognosis.
Lymphocyte rich	Lymphocytic infiltration of tumor.	Not known.	Favorable outcome to conventional HCC.

Table 4.
Shows morphological features, molecular signatures of different HCC subtypes as per 5th edition of WHO classification of digestive system tumors.

HCC. AFP is elevated in nearly 70% patients with HCC. When cut-off value of 20 ng/ml is used AFP has sensitivity of 59.9% and specificity of 93% while at the cut-off value of 200 ng/ml sensitivity drops to 22% and specificity of 100% [35, 36]. AFP can be falsely elevated in patients with viral infections like hepatitis B and C. Positive predictive value of AFP in diagnosing HCC in patients with viral etiologies and non-viral etiologies was 70% vs. 94% in one study using cut-off of 20 ng/ml [34]. AFP also has prognostic significance with values ≥400 ng/ml have higher tumor burden, bilobar involvement, tumoral portal vein thrombosis and diffuse and massive variety of tumors [35]. Limitations of AFP measurement include false negative in small HCC and 30% of large tumors do not have elevated levels [35]. False positive in chronic liver disease, cirrhosis, HCC, germ cell tumors and cholangiocarcinoma [34, 35]. AFP-L3 glycoform of AFP is detected in approximately 35% of <3 m size HCC. Cut-off level of 15% has sensitivities ranging from 75%–96.9% and specificities of 90–92% [35]. Higher levels of AFP-L3 are associated with worse liver function, poor histology and large tumor mass and portal vein invasion [35].

2.4.2 Glypican-3

It is proteoglycan in plasma membrane. It produced by tumor cells but not elevated in non-HCC liver diseases. It can be detected in 40–53% of HCC patients and 33% of HCC patients with negative for both AFP and PIVKA-II. Addition of Glypican-3 measurements to AFP improves sensitivity from 50–72% [34, 35].

2.4.3 Des-gamma-carboxyprothrombin or protein induced by vitamin K absence or antagonist II (PIVKA-II)

It is abnormal product from liver carboxylation disturbance during the formation of thrombogen [34, 35]. It is overproduced in HCC patients. Sensitivity and specificity of PIVKA-II at the cut-off level 40 mAU/ml is 51.7% and 86.7% while at the cut-off value of 125 mAU/mL in discriminating HCC from nonmalignant hepatopathy sensitivities and specificities were 89% and 86.7% [37, 38]. In combination AFP-L3, AFP and DCP achieved 60.6% sensitivity and 100% specificity while DCP combined with AFP alone increased sensitivity from 65–87%, but specificity dropped from 84–69% [39, 40]. Japanese clinical guidelines recommend the combined use of PIVKA-II and AFP for the diagnosis of HCC, management of high-risk population, and prognosis of anticancer treatment [41].

2.4.4 Long noncoding RNAs (Inc RNAs)

Recent evidences have shown that long noncoding RNAs (lncRNAs) are involved in cancer diagnosis and prognosis. Pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and AUC for lncRNAs in the diagnosis of HCC were 0.83, 0.80, 4.2, 0.21, 20, and 0.88, respectively [42].

Table 5 summarizes newer biomarkers under evaluation for diagnosis of HCC.

2.5 Fibrolamellar hepatocellular carcinoma (FL-HCC)

Rare variant of HCC accounting for only 1% cases. In contrast to conventional HCC, FL-HCC is common in young patients aged <40 years, occurs in normal liver and has normal AFP levels [53]. FL-HCC is chromosomally stable tumor and displays genomic homogeneity in contrast to conventional HCC. Mutations in AFP, TP53 beta-catenin and surviving are not seen in FL-HCC, however increased expression of anterior gradient-2, CD133, CD44 and nuclear factor-kB pathway are seen in FL-HCC. Chromosomal imbalances involving chromosomes 1, 7 and 8 are noted in aggressive FL-HCC [54–56]. FL-HCC is typically large tan colored well-circumscribed firm mass without underlying chronic liver disease or cirrhosis. Central stellate scar is seen in 75% cases. Microscopically it is composed of cluster or sheets of large polygonal or spindle shaped cells with eosinophilic cytoplasm and prominent nuclei. Fibrous stroma is seen around the tumor cells. It has capsule and central scar [57, 58]. On immunohistochemistry it shows hepatocyte paraffin 1, CK7, CD133, CD44, α-1-antitrypsin, fibrinogen, C-reactive protein, carcinoembryonic antigen and copper [55, 59]. Patient presents with abdominal pain, malaise, weight loss and abdominal lump [57]. On ultrasound FL-HCC has no specific features [60]. On computed tomography scan tumors are well defined with lobulated outline. It has hypodense large >2 cm central scar and radiating fibrotic bands are more common. Central scar may show calcification. On contrast enhancement in arterial phase it shows heterogenous hyperattenuation. On the portal venous phase and delayed phase, approximately 50% of fibrolamellar HCCs become isoattenuating to liver. However, they may also be hyperattenuating (36%) or hypoattenuating (16%). Central scar may show delayed enhancement in 25–65% cases. Venous and biliary obstruction is rare [60]. On MRI, FL-HCC is hypointense on T1 imaging and hyperintense on T2 images. Central scar is hypointense on T1 and T2 weighted images. On contrast injection, it shows heterogenous enhancement which becomes iso or hypointense in delayed phase [60]. Nodal metastasis occur in 50–65% of FL-HCC and commonly occur in hepatoduodenal ligament and hepatic hilum. Cornerstone for treatment is surgical resection with

Serial Number	Tumor marker	Comments
1	Serum Gamma-glutamyl transferase II isoenzyme [43]	74% for all HCC and 34% for small HCC. In combination with AFP, PIVKA-II sensitivity may be improved.
2	Alpha-I-fucosidase [44]	Activity increases in HCC patients. Sensitivity and specificity at 870 nmol/ml per hour is 81.7% and 70.7% respectively
3	Alfa-fetoprotein mRNA [45, 46]	Serum AFP mRNA detected by reverse-transcription polymerase chain reaction (RTPCR) is correlated with portal vein thrombosis, number of nodules of tumor, tumor diameter, stage and post-operative recurrence.
4	Human telomerase reverse transcriptase mRNA (hTERT) [47, 48]	It has sensitivity and specificity of 88.2% and 70% and levels correlate with AFP concentration, tumor size, tumor differentiation.
5	Vascular endothelial growth factor(VEGF) [49]	Serum VEGF levels per platelet count are increased >1.4 picogram/10^6 in patients with HCC and correlate with stage, portal vein thrombosis, response to therapy and survival.
6	Interleukin-8 [50]	It is chemokine having direct effect on tumor cells, angiogenesis, tumor migration. Serum levels are significantly elevated in HCC patients compared to healthy adults and correlate with tumor size, venous invasion, advanced stage, absence of capsule and poor prognosis
7	Transforming growth factor-beta 1 [51]	Serum levels elevated in HCC. At cut-off 800 pg./ml sensitivity and specificity is 68% and 95%.
8	Tumor-specific growth factor (TSGF) [52]	Serum TSGF reflects the existence of tumor. It has been indicated that TSGF can be used as a diagnostic marker in detecting HCC, and its sensitivity can reach 82% at the cut-off value of 62 U/mL. With other markers like AFP, ferritin sensitivity and specificity can reach up to >90%

Table 5.
Summary of new tumor markers in HCC.

adequate lymph node dissection. The 5 year overall survival rate after partial hepatectomy was 70%. Radioembolization using ^{90}Y is helpful in unresectable FL-HCC. Liver transplantation is therapeutic option in selected patients [60, 61].

2.6 Diagnosis of HCC in noncirrhotic liver

About 10% HCC can occur in noncirrhotic liver. Risk factors include alcohol (21%), chronic hepatitis B(30.60%), chronic hepatitis C infection (14.36%), diabetes (40%), family history (13.85%) and cryptogenic (39%). Other risk factors include aflatoxin B, metabolic liver diseases, chemical and industrial carcinogens like vinyl chloride. HCC in noncirrhotic liver present as advanced disease, larger in size [62]. Male to female ratio is 2:1. Hepatomegaly, abdominal pain, malaise, weight loss and anorexia are common presenting features [62, 63]. On ultrasound, lesion can be hypoechoic, hyperechoic due to intralesional fat or mixed echogenicity due to necrosis. On unenhanced CT, lesions appear as hypodense circumscribed masses. Few of them show calcifications, hemorrhagic areas and necrosis. On contrast injection, it does show arterial phase hyperenhancement and washout in delayed phase but specificity is lower as other lesions like hepatocellular adenoma and hypervascular metastasis. On MRI these tumors have variable T1 and T2 weighted images depending on degree of fat, necrosis and fibrosis. On contrast injection, features are similar to CT scan. Liver biopsy is often required for diagnosis [62, 63].

3. Surveillance for hepatocellular carcinoma

Surveillance is defined as periodic application of diagnostic test to individuals who have specific risk factors for disease. Surveillance depends on the incidence of the surveyed disease in the target population, the availability of efficient diagnostic test(s) at bearable costs and acceptability for the target population, and the availability of treatments and their effectiveness if disease is diagnosed early in course of disease. Primary objective of surveillance program is early diagnosis of disease so that curative treatments can be offered to the patients [64].

3.1 Target population for surveillance

While deciding the appropriate population it is necessary to consider incidence of HCC in the population, probability that curative therapies can be offered to the patients who are diagnosed as having the disease and cost effectiveness of surveillance. In case of HCC, application of curative therapies not only depend on extent of tumor but also on underlying liver function. Hence appropriate patients should be enrolled in the surveillance program [3, 24].

3.1.1 Cirrhotic patients

Nearly 90% HCC develop on the background of cirrhosis of liver. The annual incidence of HCC is 2.0–6.6% in patients with cirrhosis [24]. Cost-effectiveness studies in western patients have shown that surveillance for HCC would be beneficial if the incidence is 1.5%/year or greater, irrespective of etiology of cirrhosis [65]. However, advanced cirrhosis with Child score C or Child score B with gross ascites, hepatorenal syndrome, clinical jaundice do not qualify for curative therapies for HCC and do not warrant surveillance unless they are considered for liver transplantation. Child A cirrhotic patients or those decompensated cirrhotic patients who are listed for liver transplant warrant surveillance as diagnosis of HCC modifies the priority and decision to transplant [66–68].

3.1.2 Noncirrhotic patients

HCC can develop in noncirrhotic liver in patients infected with hepatitis B virus. The risk varies with geographical distribution and is higher in Asia and Africa than Western countries. Higher levels of HBV replication, age and gender (males higher than females) are the risk factors for development of HCC which is lower than cirrhotic but definitely higher than general population [69, 70]. In a cohort study of males belonging to multiple race and age-groups, risk of HCC was highest among Asian Pacific Islanders, followed by whites and African Americans. Also, regardless of race, annual incidence of HCC was more than 0.2% for all patients older than 40 years with high levels of alanine aminotransferase [71]. A similar HCC incidence rate of 0.2 per 100 person-years has been observed in inactive carriers with chronic HBV infection from East Asian countries. Asian females >50 years of age and patients with family history of HCC are also at increased risk of HCC. Hence, surveillance should be offered in the above subset of patients as these patients are noncirrhotic with preserved liver function and fit for curative resection for HCC [66, 67]. Patients with chronic hepatitis B on therapy with advanced fibrosis or cirrhosis at baseline should also be enrolled under surveillance program [72, 73]. Various scoring systems are available which can help in stratifying the patients based on risk of HCC and those with significant risk should be offered surveillance [74]. Examples of such scoring systems include GAG-HCC score, LSM-HCC score, PAGE-B score, REACH-B score. REVEAL risk model [74].

Patients with chronic hepatitis C infection with bridging fibrosis are at increased risk of development of HCC. Transition from advanced fibrosis to cirrhosis cannot be accurately determined [75]. Several studies show that liver stiffness assessment performed by transient elastography correlates with risk of development of HCC [76, 77]. Hence these patients warrant surveillance for HCC. Patients with chronic HCV infection previously treated, who have achieved sustained virological response but had advanced fibrosis or cirrhosis need HCC surveillance [74].

Prevalence of nonalcoholic fatty liver disease (NAFLD) is increasing in all part of the world. Nonalcoholic steatohepatitis (NASH) is associated with morbidity and mortality due to cirrhosis and its complications and development of HCC [3]. Similar to cirrhotic patients with other etiologies, patients with NASH cirrhosis should be included in surveillance program. A systematic review and metanalysis of studies on HCC in noncirrhotic NASH subjects showed that these subjects were at greater odds of developing HCC than non-cirrhotic subjects of other etiologies (OR 2.61, 95% CI 1.27–5.35, P = 0.009) [78]. The incidence of HCC in patients with non-advanced fibrosis is expected to be insufficiently high to deserve universal surveillance, given the large prevalence of NAFLD in the general population [79]. American society of gastroenterology clinical practice update on screening and surveillance of HCC in NAFLD suggest to use two noninvasive tests to assess level of fibrosis [79]. Those patients with significant fibrosis on both tests to be enrolled in the screening program. Genetic studies have shown the presence of the PNPLA3 risk allele is increased in those NAFLD with HCC. However limited availability of the test restricts its use in clinical practice [79].

Patients with Wilson's disease, autoimmune liver disease and alpha 1- antitrypsin deficiency have lower risk of developing HCC unless cirrhosis is developed. Hence routine surveillance is not recommended [24].

3.2 Surveillance tests

Surveillance tests should be sensitive, easily available to large population, less costly, safe, acceptable to the people and permits early diagnosis of disease. Surveillance tests used for HCC surveillance can be classified as radiological, serological or combination of both. Section 2.2 and 2.4 describe imaging and tumor markers, their sensitivity, specificity and accuracy.

3.2.1 Radiologic surveillance tests

Ultrasonography (USG) of liver is the most commonly used method for surveillance. It is non-invasive, relatively inexpensive, easily available and without any associated risk of radiation. It has the sensitivity of 84% for any stage HCC and 63% for early-stage HCC [80]. In patients with cirrhosis, USG may have a suboptimal performance due to the presence of fibrous septa and regenerative nodules, which appear as a coarse pattern on ultrasound and may mask the presence of a small tumor. In a meta-analysis, the sensitivity and specificity of USG for detection of HCC at any stage were 84% (95% CI, 76–92%) and 91% (95% CI, 86–94%), respectively, but, the pooled sensitivity of ultrasound was only 47% (95% CI, 33–61%) for detection of early-stage HCC [81]. Hence, it is recommended that USG of liver for HCC surveillance should be done by an expert radiologist. Compared to ultrasonography, computed tomography and MRI had better sensitivity and specificity for diagnosis of early HCC (Refer to Section 2.2 for details). However use of radiation, complex imaging techniques, availability, cost of imaging are the important limiting factors. While comparing 6-monthly USG and yearly triphasic CT for HCC surveillance, it was found that biannual ultrasound was more sensitive (71.4%) when compared to CT (66.7%) with lower overall cost [82].

3.2.2 Serological tests

Serological test for early diagnosis of HCC include AFP, PIVKA II, AFP-L3, alpha fucosidase and glypican. (Refer to Section 2.4). Out of all AFP is most widely studied. In a study evaluating the biomarkers AFP had the best area under the receiver operating characteristic curve (0.80, 95% confidence interval [CI]: 0.77–0.84), followed by des-gamma carboxy-prothrombin (DCP) (0.72, 95% CI: 0.68–0.77) and lectin-bound AFP (AFP-L3%) (0.66, 95% CI: 0.62–0.70) for early-stage HCC and the sensitivity of AFP was 66% [83]. As a serological test alone for surveillance AFP has suboptimal performance however, it may used if ultrasound is not easily available [84, 85]. One problem with use of AFP as surveillance test is that only in 10–20% of early HCC have elevated AFP and on the other hand AFP can be falsely elevated in chronic hepatitis B and C infections [24]. Instead of single biomarker for surveillance combination of multiple biomarkers are being increasingly studied. GALAD, which includes gender, age, lectin-bound AFP % (AFPL3%), AFP, and des-gamma carboxy prothrombin (DCP) studied in a multinational phase II study involving 6,834 patients (2,430 HCC and 4,404 chronic liver disease), achieved sensitivities ranging from 60–80% for early HCC detection. Another panel including AFP, fucosylated kininogen, age, gender, alkaline phosphatase, and alanine aminotransferase demonstrated a c-statistic of 0.97 (95% CI 0.95–0.99) for early HCC detection. A methylated DNA marker panel had a c-statistic of 0.96 (95% CI 0.93–0.99), with a sensitivity exceeding 90%, for early HCC detection in a phase II study. Although these studies appear promising further research is needed in this field [3].

3.2.3 Combination of both

Meta-analysis had shown that combination of AFP and USG to be superior to only USG or AFP alone. Ultrasound with vs. without AFP detected early-stage HCC with 63% sensitivity (95% CI, 48–75%) and 45% sensitivity (95% CI, 30–62%), respectively (P = .002) [86]. The benefit of AFP in addition to ultrasound was consistent across subgroups, including prospective studies, studies conducted in the United States, and studies conducted after the year 2000 [86]. Counter argument to this approach is that, although addition of AFP to USG helps in detection of 6–8% additional tumors does not balance the increase in false positive results resulting due to active inflammation causing raise in AFP levels in absence of HCC, adding to cost of screening without significant benefit [24].

3.3 Surveillance interval

It depends on rate of tumor growth and incidence of cancer in the population [24]. Median doubling time of an HCC lesion is 6.5 months +/− 5.7 months [87]. Analysis of prospectively maintained multi-center Italian database showed a better overall median survival of 40.3 months in the 6-monthly surveillance group, compared to 30 months in the 12-monthly surveillance group (P = 0.03) [88]. Subsequently a French study evaluated impact of shortening of surveillance to 3 months. It showed that 3-months surveillance group had higher incidence of non-malignant lesions, similar number of patients in both 3-months and 6-months group were detected with HCC at an early stage (79% vs. 71%; P = 0.40) and similar proportions received curative therapies (62% vs. 58%; P = 0.88) [89]. Hence it appears that 6 months interval is optimal.

3.4 Benefits of surveillance

Cancer surveillance programs are aimed to detect tumors early so that curative treatments can be provided to patients. Evidence in favor of surveillance programs

	EASL	AASLD	APASL	JSH	INASL
Target population	• Cirrhotic Child A and B • Child C listed for transplant • Noncirrhotic • HBV high risk for HCC • Noncirrhotic F3 fibrosis as per risk	• All cirrhotic patients • HCV cirrhotic post antivirals SVR achieved.	• Cirrhosis any etiology. • Chronic HBV and HCV infection with high risk.	• **Extremely high risk**: Cirrhosis related HBV and HCV • **High risk:** Cirrhosis nonviral, Chronic hepatitis B and C	• Child A and B cirrhotics • Child C cirrhotics on transplant list • High risk noncirrhotic chronic HBV and HCV
Ultrasound	✓	✓	✓	✓	✓
CT/MRI	X	X	X	✓ **In extremely high risk group 6–12 monthly.**	X
AFP	X	✓+/−	✓ Cut-off 200 ng/ml	✓	✓
Other markers	X	X	X	PVIKA II AFP-L3	X
Surveillance Interval	6 months	6 months	6 months	**Extremely high risk-** 3–4 monthly. **High risk** – 6 months	6 months

Table 6.
Recommendations, screening tests, screening interval by various societies across the world. (SVR-sustained virological response).

in HCC has remained controversial. One randomized controlled trial support-ing HCC surveillance with 6-monthly abdominal ultrasound was performed in more than 18,000 Chinese patients and showed a 37% reduction in mortality risk in screened patients [90]. Other studies are retrospective, observational and has suffered some biases. Lead time which means the given proportion of survival benefit is due to early diagnosis due to surveillance and length time bias arises due to detection of slow growing tumors during surveillance programs where as fast growing tumors become symptomatic early in their course [3]. Surveillance programs can create a state of anxiety in mind of patients. Additional tests and financial burden if screening tests are indeterminate. There is also possibility of overtreatment of tumor which might never become symptomatic [3]. Considering dismal prognosis of HCC, all societies recommend screening of at risk patients for HCC [24, 33, 91–93].

3.5 Summary of recommendations by various societies

Table 6 summarizes recommendations, screening tests, screening interval by various societies across the world [24, 33, 91–93].

4. Role of multidisciplinary team in surveillance and diagnosis of HCC

Optimal care of patients with HCC involves specialists from multiple disci-plines like gastroenterology/hepatology, surgical oncologist, liver transplant team, medical oncologist, radiologist, interventional radiologist, primary care physician, radiation oncologist, pathologists, palliative care specialist, nursing staff and dieticians. Multidisciplinary teams (MDTs) have evolved to facilitate care coordina-tion, reassessments of clinical course, and fine changes in treatment plans required for these complex group of patients. MDTs provide platform to facilitate prompt diagnosis of HCC by reviewing patients imaging, tumor markers and also assessing the need for biopsy which is associated with complications like bleeding and needle track seeding. As mentioned in previous sections, diagnosis of HCC is primarily based on imaging and there are restricted indications for biopsy of lesion. Experts in MDTs can also play a role in suggesting next investigation if one of the diagnostic investigation is inconclusive [94].

5. Conclusion

To conclude, small HCC rarely become symptomatic. HCC can be a cause for new onset decompensation. Diagnosis of HCC requires multiphase computed tomography or MRI scan. In cirrhotic liver, diagnosis of HCC is based on typical imaging features and rarely needs biopsy. In noncirrhotic liver and vascular liver diseases biopsy may be required to confirm diagnosis. Contrast enhanced ultra-sound and MRI with hepatobiliary contrast agents are promising modalities for evaluation of small and indeterminate nodules. Tumor markers play adjunct role in diagnosis but has prognostic significance. Pathologically HCC is heterogenous tumor with multiple subtypes with distinct molecular signatures. HCC surveil-lance in high risk groups with biannual ultrasound with or without alfa-fetoprotein helps in early detection of lesions which are amenable to curative treatment. Multidisciplinary teams provide platform for care coordination, reassessments of clinical course, and fine changes in treatment plans required for this complex group of patients.

Conflict of interest

The author declare no conflict of interest.

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