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REVIEW

Changing role of histopathology in the diagnosis and management of hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is one of the most common and fatal cancer in the world. HCC frequently presents with advanced disease, has a high recurrence rate and limited treatment options, which leads to very poor prognosis. This warrants urgent improvement in the diagnosis and treatment. Liver biopsy plays very important role in the diagnosis and prognosis of HCC, but with technical advancements and progression in the field of imaging, clinical guidelines have restricted the role of biopsy to very limited situations. Biopsy also has its own problems of needle tract seeding of tumor, small risk of complications, technical and sampling errors along with interpretative errors. Despite this, tissue analysis is often required because imaging is not always specific, limited expertise and lack of advanced imaging in many centers and limitations of imaging in the diagnosis of small, mixed and other variant forms of HCC. In addition, biopsy confirmation is often required for clinical trials of new drugs and targeted therapies. Tissue biomarkers along with certain morphological features, phenotypes and immune-phenotypes that serve as important prognostic and outcome predictors and as decisive factors for therapy decisions, add to the continuing role of histopathology. Advancements in cancer biology and development of molecular classification of HCC with clinic pathological correlation, lead to discovery of HCC phenotypic surrogates of prognostic and therapeutically significant molecular signatures. Thus tissue characteristics and morphology based correlates of molecular subtypes provide invaluable information for management and prognosis. This review thus focuses on the importance of histopathology and resurgence of role of biopsy in the diagnosis, management and prognostication of HCC.

Key words: Hepatocellular carcinoma; Biomarker; Biopsy; Histopathology; Immunohistochemistry; Targeted therapy; Molecular; Diagnosis; Prognosis

Core tip: Liver biopsy plays important roles in the diagnosis and prognosis of hepatocellular carcinoma. However biopsy related complications and limitations

along with advancements in imaging have restricted its role to very limited situations. In recent time, studies on tissue biomarkers, molecular classifications and targeted therapies for hepatocellular carcinoma (HCC) with their clinic-pathologic correlations have highlighted that morphologic variants and subtypes can serve as importance surrogates of molecular signatures, thus renewing the interest in tissue analysis. Tumor biopsy thus is increasingly being recognized as an invaluable tool for the diagnosis, management and prognostication of HCC.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer in the world[1-4], with an increasing incidence each year. It is also one of the most lethal human malignant tumor with > 600000 deaths per year worldwide[1,4] making it the third leading cause of cancer related death[5,6]. Dismal prognosis of HCC is attributable to advanced disease at presentation, high rates of metastases and recurrence along with the limited and unsuccessful treatment options available[7,8]. Also, Amongst the primary liver cancers, HCC is the most common, accounting for 70%-85% of all the histological types[9,10]. Major risk factors of HCC are infection with hepatitis B and hepatitis C, excess alcohol intake, obesity, diabetes and metabolic diseases[7,11]. These risk factors cause repeated episodes or sustained state of inflammation, resulting in progressive fibrosis and cirrhosis, along with development of preneoplastic lesions with stem cells acting as a nidus for HCC[12,13]. Literature indicates that 70%-97% of patients with HCC have underlying cirrhosis of the liver at the time of diagnosis[14]. Poor clinical outcome makes it imperative to advance our understanding of HCC at the cellular level and improve methods for the early diagnosis and treatment particularly targeted therapies. HCC is diagnosed by the non-invasive methods of imaging and tumor markers and by the invasive techniques of biopsy and aspiration. Lesion biopsy in HCC, like other solid organs provide valuable information about the diagnosis, prognosis and in certain circumstances, guide about treatment decisions, however biopsy in the HCC and cirrhotic milieu is controversial and is superseded by imaging[6,15]. Certain biopsy limitations especially needle tract seeding, sampling errors and small risk of morbidity along with the technical advancements in imaging, undermined the importance of tissue analysis. This led most of the international guidelines on HCC to restrict the role of liver biopsy to characterize the lesions in non-cirrhotic liver or those with equivocal imaging. However, imaging technologies also have certain caveats, cautioning against abandon of histopathology assessment for HCC. Tumor histopathology, besides being an important diagnostic tool, plays numerous other important roles such as distinguishing from metastasis and other primary benign or preneoplastic lesions, in prognostication and influencing treatment decisions, which cannot be substituted by imaging techniques or tumor markers. With increasingly accumulating data on prognostic and therapeutic importance of specific phenotypes and distinct molecular-morphologic correlates, there is resurgence of interest in the role of tissue evaluation.

ROLE OF IMAGING IN THE DIAGNOSIS OF HCC

The recent diagnostic approach for HCC is based on imaging studies, restricting the role of histopathology to only certain situations. Clinical guidelines of American Association for the Study of Liver disease, European Association for the Study of the Liver (EASL) and Asian-Pacific Association for the Study of Liver have changed the diagnostic criteria for HCC[2,16-18], recommending radiology in view of the remarkable advances in techniques that have led to very high sensitivity and specificity for the diagnosis of HCC[19]. A recent systematic review and meta-analysis for studies comparing CT with extracellular contrast-enhanced MRI or gadoxetate-enhanced MRI in adults with cirrhosis and suspected HCC, found that all them performed better for HCC $\geqslant$ 2 cm in comparison to lesions < 1 cm. For all tumor sizes, studies showed significantly higher sensitivity for MRI over CT, with no difference in the specificity between techniques[20]. Typical imaging findings of intense uptake of contrast during the arterial phase followed by decreased enhancement and washout during the portal phases, based on the neo-arterial supply feeding the HCC, by even a single contrast enhanced imaging study is considered sufficiently specific for the diagnosis of HCC[2,16-18,21]. Thus, biopsy is not advocated for the diagnosis of HCC, if typical features are present on dynamic imaging technique[16-18,22].

IMAGING LIMITATIONS

Imaging techniques falter in certain situations and histopathology assessment becomes mandatory for the diagnosis of all equivocal lesions, irrespective of the size[6], reported in 10%-15% of patients[23]. Lesion in a cirrhotic patient that lacks typical imaging characteristics, histopathological evaluation is the recommended diagnostic tool. Imaging alone has been found to be insufficient to diagnose well-differentiated HCC.

Biopsy has advantage over imaging as comparison with non-lesional liver tissue provides vital information, particularly for the diagnosis of well-differentiated HCC. Horigome *et al*[24] reported that digital subtraction

angiography and magnetic resonance imaging in the absence of biopsy, could diagnose HCC in only 58% of well-differentiated HCC. Dynamic imaging has been shown to have a sensitivity of 35%-71% in different series for tumors of less than 2 cm size[25-29], attributable to hypovascularity of small HCC. Another limitation of imaging is the requirement of pathologic diagnosis for all nodules developing in the non-cirrhotic background[6]. Also, false positives, as high as 33%[30,31], have been reported with the radiology techniques for diagnosing HCC. A study reported that many nodules detected by ultrasound were not found on computed tomography[32,33]. Similarly, 41% and 30.8% of the imaging based cases turned out to be non-HCC on biopsy or follow-up[30]. Without biopsy confirmation, these cases might get subjected to unnecessary transplantation, resection and other therapies. Strength of tissue analysis to determine the malignant potential and histogenesis of liver lesions is another major constraint with imaging. Combined hepato-cholangiocarcinoma (cHCC-CC) is difficult to diagnose and characterize by the imaging alone[34]. Lack of skill and absence of advance imaging technologies in many centers, are other constraints, requiring histopathology assessment for confirmation. Such limitations of imaging compel tissue confirmation in a significant number of cases.

Role of serum α-feto protein (AFP) in the diagnosis of HCC although controversial, is still recommended by certain guidelines[35]. In situations, where imaging is atypical, AFP levels are useful for diagnosing HCC when biopsy needs to be avoided in view of the risk of tumour seeding[36-38]. Studies have shown that increasing AFP levels before liver transplantation are associated with an increased risk of tumor recurrence and decreased survival following transplantation[39,40]. Serum AFP levels are not influenced by technical factors, skill, observer variability thus still has an important role in surveillance, diagnosis, prediction of outcome and monitoring treatment response[41].

BIOPSY SHORTCOMINGS

The declining interest for biopsy is due to several issues. There are risks associated with the procedure, that include morbidity due to the most frequent complication, *i.e.*, pain[42,43] and bleeding especially in patients with cirrhosis who are at a higher risk of this complication. Incidence of such minor complications is 5.9%[44]. Significant hemorrhage occurs in 0.5%[45]. There is slight risk of mortality[46,47], with incidence of 0.11% reported in experienced centers[48]. Evaluation of sixty-four series reporting 7649 TJLBs revealed minor and major complication rates to be 6.5% and 0.56%, respectively with mortality in adults to be 0.09%[49]. However, a large series of 16648 guided biopsies and 3035 therapeutic procedures performed in 13222 patients, overall mortality was reported in 0.06%[50].

Needle track seeding of malignant cells is another important concern, especially in patients who might otherwise benefit from liver transplantation. The reported incidence of tumor seeding following a liver biopsy ranges between 1.6%-5.1%[51-55], however one of the largest series has reported this complication in only 0.76% in their experience[19,56] In a systematic review and meta-analysis of eight observational studies, it was shown that the incidence of needle tract tumour seeding following biopsy of a HCC is 2.7% overall, or 0.9% per year[57]. Other studies have emphasized not to preclude the biopsy if management can be altered based on the biopsy interpretation, supporting this is the fact that 2.5% unnecessary surgery are conducted if the patient is not biopsied[58].

Technical challenges are another section which limits the utility of biopsy. Small lesions which are < 2 cm are often difficult to target, leading to high false-negatives[19]. Distinction of well-differentiated HCC from preneoplastic and regenerative focus is also a problem area. Characteristic histomorphologic profile in combination with reticulin stain and immunohistochemistry for HSP70 and Glutamine synthetase, can differentiate hepatocellular adenoma from HCC, however well-differentiated HCC is often difficult to distinguish[59-61]. Tumor heterogeneity, necrosis and inadequacy or failed sampling of the suspected lesion, all add to the inferior results of biopsy with the risk of mismanagement after diagnostic errors. Negative predictive value is very low in such setting (14%)[51,62,63].

HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY FOR THE DIAGNOSIS AND PROGNOSTICATION OF HCC

Tumor histopathology, besides being an important diagnostic tool, plays numerous other important roles such as distinguishing from other lesions, prognostication and influencing treatment decisions, which cannot be substituted by imaging techniques or tumour markers. Assessment of histological parameters in tumour resection specimens has been shown to predict recurrence and metastatic potential and thus indicate the need for salvage transplantation[64-67]. Biopsy tissue and archived blocks are important source for teaching, knowledge sharing, correlation with translational research and biomarker development. Several histopathology parameters had been extensively studied and shown to be significant predictors of prognosis, highlighting the role of tissue analysis in HCC. The most studied parameters which are also linked to prognosis are tumor number, size, cell differentiation and grade, presence of satellite nodules, pTNM stage[68]. (Figure 1)

IMPORTANCE OF GROSS PATHOLOGY DESCRIPTION

HCC is a heterogeneous tumor with varied gross and

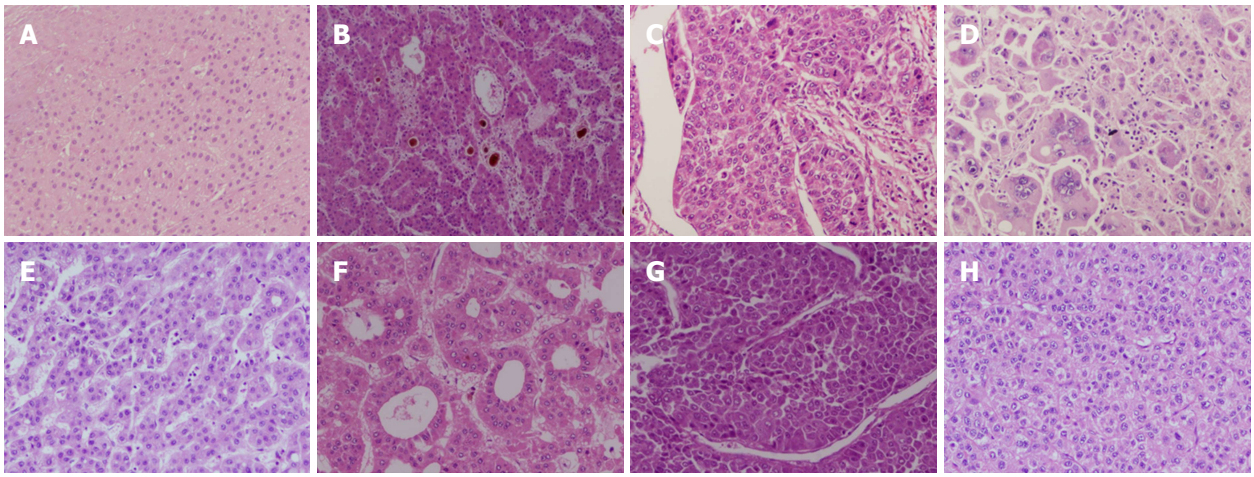

Figure 1 Hepatocellular carcinoma Edmondson and Steiner grading. Grade 1 (A); grade 2 (B); grade 3 (C); and grade 4 (D). Most common patterns in histopathology of hepatocellular carcinoma: Microtrabecular (E); pseudoglandular (F); macrotrabecular (G); and compact (H). (HE stain).

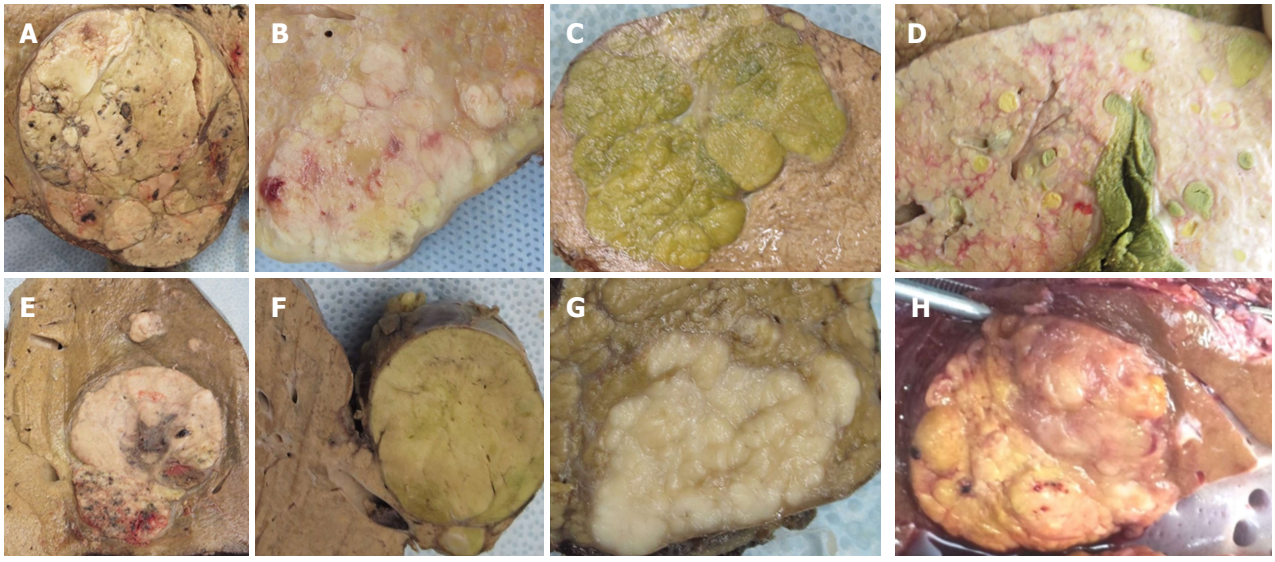

Figure 2 Gross morphology of hepatocellular carcinoma. Single expanding nodular hepatocellular carcinoma (A); vaguely nodular with perinodular extension (B); Multinodular (C); multicentric with cirrhotomimetic appearance (D); nodular with satellite nodules (E); pedunculated (F); infiltrative (G); and hepatocellular carcinoma in non-cirrhotic background (H).

microscopic appearances. A study from Seoul had shown that gross features are also independent predictor of overall and disease-free survival regardless of tumor size. They classified 242 HCC resection specimens based on the gross appearance into vaguely nodular, expanding nodular, multinodular confluent, nodular with perinodular extension and infiltrative types[5]. Infiltrative type had the worse prognosis whereas vaguely nodular and expanding nodular had more favorable prognosis. Similar results in small HCC and in HCC > 10 cm[69,70] and in patients treated with RFA[5,71], verify that gross morphology is an important predictor of prognosis. (Figure 2)

ROLE OF MORPHOLOGICAL PARAMETERS AND HISTOLOGICAL SUBTYPES

Four major growth patterns in HCC are trabecular (70%), solid (20%), pseudo glandular (10%) and macrotrabecular (1%)[72]. Of these, macrotrabecular pattern has recently been shown to have significant clinical relevance[73,74]. Morphology based segregation into histological subtypes[72,75] such as fibro lamellar carcinoma[76,77], lymphoepithelioma like carcinoma[78], steatohepatitis HCC[79], combined hepatocholangio-carcinoma[75], and histological subtype with stem cell markers[80,81] have independent prognostic importance. Others such as Clear cell HCC has been demonstrated to be smaller, better differentiated with lower rates of vascular invasion[82,83], whereas the sarcomatoid HCC is a poorly differentiated subtype[72]. Several recent studies have also highlighted distinctive clinical, biological and molecular characteristics associated with these phenotypes and subtypes, with creditable prognostic implications[84,85] (Figure 3).

In a recent study by Ziol *et al*[74] Macrotrabecular-massive is a newly described subtype of HCC, found in

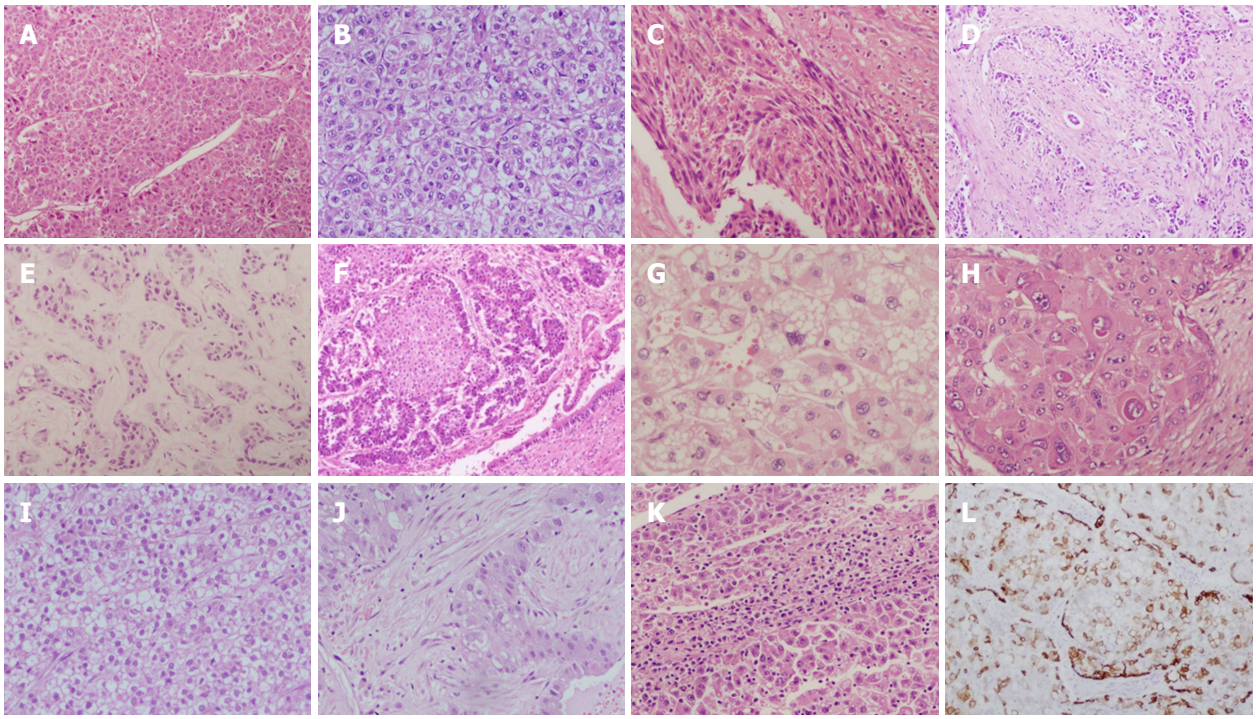

Figure 3 Hepatocellular carcinoma variants, subtypes and histological features. Macrotrabecular (A); steatohepatitic (B); sarcomatoid (C); cholangiocellular (D); sclerosing (E); combined HCC-CC (F); HCC with foam cells (G); HCC with giant cells and hyaline bodies (H); clear cell (I); fibrolamellar (J); HCC with immune cells (K); CK19 positive stem cells (L). HCC: Hepatocellular carcinoma.

12% of the cohort of 237 HCC surgical samples and 284 HCC liver biopsies. Authors have defined this entity by the presence of a predominant (> 50%) macrotrabecular architecture (more than 6 cells thick). This phenotype was associated with poor prognostic factors like tumor size, α-Feto protein (AFP) level, satellite nodules, and vascular invasion and was found to be an independent predictor of early and overall recurrence[74]. Lauwers *et al*[86] noted macrotrabecular -predominant architecture in 26.6% of their 425 cases of resected HCC. In comparison to the compact architecture they had worse overall survival. Prognostic information derived from phenotypes reemphasizes the potential benefits of biopsy add to reviving its importance[74].

Combined hepatocellular-cholangiocarcinoma (cHCC-CC) representing 0.4%-14.2% of primary liver cancers is an aggressive tumor associated with poor outcome[34,87-90]. Histopathological evaluation is crucial for diagnosis, as this entity lacks the typical imaging characteristics thus often misdiagnosed by radiology. cHCC-CC was first described by Allen and Lisa in 1949[91] was updated by Goodman in 1985[92]. In 2010, WHO classified it into classical subtype and subtypes with stem cell features[92]. In a study of sixty-two patients of cHCC-CC, stem cell subtypes (WHO criteria), were found in various amount and combinations in all of their patients with typical subtype in 16%, intermediate cell type in 83.9% and cholangiolocellular type (CLC) in 71%[93]. cHCC-CC has been shown in a study, to have 1-year and 3-year survival rates of 81.9% and 47% respectively, which suggest a better prognosis than CC but worse compared with HCC[88]. Similarly, another study has found 1, 3 and 5 year overall survival rates of 53%, 26% and 12% respectively, supporting their biological behavior intermediate between HCC and CC[90,94]. Knowledge of mixed tumor by biopsy evaluation prior to surgery, can guide the type of resection including the lymph node dissection. Recently, consensus terminology for primary liver carcinomas with both hepatocytic and cholangiocytic differentiation has been published[8] which emphasizes that stem cell phenotypes and features can coexist within combined HCC-CC and should be reported in a descriptive report. Sub classifying stem cells is not necessary. Presence of two other types of primary liver cancers - CLC and intermediate cell carcinoma were described, which may coexist with HCC, intrahepatic cholangiocarcinoma or cHCC-CC. Although the minimum cut-off of HCC and CC to qualify for the diagnosis of cHCC-CC is uncertain, the accepted criteria to qualify as CLC is > 80% of the tumor comprising CLC[8]. This phenotype is very important, with prognostic implications[93]. CLC exhibits increased expression of ABC transporters and is linked with a worse prognosis, chemo resistance, and an aggressive behavior[95]. Meta-analyses of twelve articles involving 1344 patients showed that the presence of cancer stem cells (CSCs) was significantly associated with a poor histological grade[96]. HCC expressing stem cells marker keratin 19 (K19), also known as HCC with "stem cell features" or "progenitor features" display immunohistochemical expression of K19 in > 5% of tumor cells[97,98]. This particular tumor subtype gene expression profile with oval cells and fetal

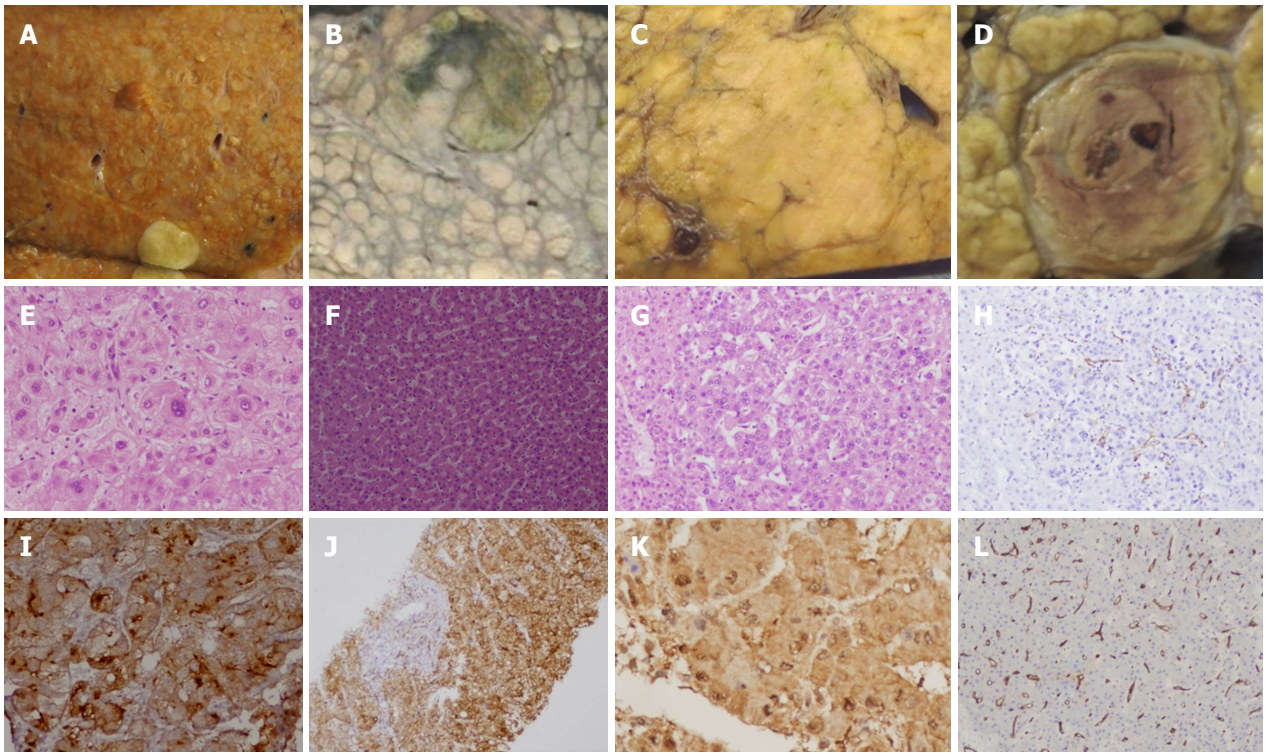

Figure 4 Dysplastic lesions and early hepatocellular carcinoma. Gross morphology of small distinctly nodular HCC (A); small vaguely nodular HCC (B, C); HCC with nodule in nodule appearance (D). Microphotographs of large cell change (E) and small cell change (F) in dysplastic nodules. Nodule in nodule with low grade dysplasia surrounding central high grade dysplastic nodule (G) on HE stain and focal CD34 positive (H) on immunohistochemistry. Glypican-3 (I), glutamine synthetase (J), HSP-70 (K) and diffuse CD34 (L) immunostaining in well-differentiated HCC. HCC: Hepatocellular carcinoma.

hepatoblasts[97-99]. It has adverse clinical outcome with worse postoperative survival rate.

ROLE OF TISSUE ANALYSIS FOR SMALL HCC

Small suspicious nodules are difficult to detect and characterize by the imaging modalities. Vascular characteristics and capsule formation are not completely developed hence such lesions lack the typical imaging characteristics. Histopathology evaluation is required in around 60% of such cases, for early and correct diagnosis[24]. Hepatocellular nodules were classified by the International working party in 1995[100] and further characterized by the International consensus group for hepatocellular neoplasia in 2009[101], which classified small HCC (< 2 cm in size) as early HCC and progressed HCC. (Figure 4)

Pathology based differentiation of HCC from other nodular lesion found in chronic liver disease such as large regenerative nodule, low grade dysplastic nodule (LGDN) and high grade dysplastic nodule (HGDN) has become one of the most important accomplishments and indications of biopsy, gaining importance in view of surveillance programs and early detection of HCC. Biopsy is often recommended for nodules 1.0 cm or larger to make a differential diagnosis between early HCC and a DN[102]. Immunohistochemsitry provides crucial support in such diagnostic dilemmas. Glypican-3, heat shock protein 70 and glutamine synthetase are used as single panel for delineation of HCC from other suspicious nodules[72]. LGDNs and large regenerative nodules (LRNs) are negative for the panel. In a study, all negative phenotype was noted in 100% of LRNs, 100% of LGDNs, 72.7% of HGDNs and 3.1% of early HCC[103-105]. Studies have shown that if two of these three stains are positive, the sensitivity and specificity for the detection of HCC is 60%-70% and 100% respectively[10]. Tommaso *et al*[104], has reported that addition of clathrin heavy chain to this panel, improved diagnostic accuracy to 84.3% for small HCCs. Hepatocyte paraffin 1, arginase 1, polyclonal CEA, CD10 and several other tissue markers that are frequently performed for confirmation of diagnosis, differentiation of HCC from metastatic carcinoma, identifying HCC in poorly differentiated and necrotic tumors as well as a protocol tissue based diagnosis confirmation before enrolment in phase Ⅲ clinical trials of novel drugs[68,106,107].

BIOPSY FOR PROGNOSTICATION

Grading of HCC cellular differentiation, pathological tumor node metastasis stage and vessel invasion are reported as the most important histoprognostic features[22,72,108].

Edmondson and Steiner system of HCC (ES) grading, published in Cancer in 1954[109], is the most widely adopted classification[86]. It divides HCC into four grades based on histological differentiation with grade 1 being

the best differentiated. Current three-tier classification of HCC into well-, moderately- and poorly differentiated HCC is also based on this system, combining architectural and nuclear features[110,111], pTNM staging based on 8th edition of AJCC staging, which is based on number, size and vessel invasion, provides crucial prognostic information, guides about therapeutic decisions and is an essential component of research and clinical studies. 8th ed. of pTNM lays emphasizes on separation of small HCC and on vessel invasion by modifying pT1 and pT4 respectively[112].

Macro vascular and Micro vascular (MVI) invasion is the major predictor of prognosis of HCC and are associated with more advanced tumor stage, disease progression, local invasion and distant metastasis[86,113]. Identification of MVI is feasible only on histopathological examination of resected surgical specimens[114]. Incidence of MVI after surgical resections and liver transplantation has been shown to be between 15% and 57%[114-116]. Pawlik *et al*[115], reported MVI to occur at the rates of 25%, 40%, 55% and 63% in HCC < 3, 3-5, 5-6.5, and > 6.5 cm. Similarly, Yamashita *et al*[117] has also shown 28.9% of the small HCC has MVI, with 1-year recurrence rates of 7.5% and 23.3% for patients without and with MVI. A systematic review of 20 observation studies investigating prognostic role of MVI in patients who had undergone liver transplantation or resection, highlight the adverse impact of MVI on disease free and overall survival[118]. Different studies have graded MVI based on the number of vessels invaded[119] as well as have sub typed them into adhesion, invasion and breakthrough types and found association with long-term survival[120]. Distance of embolized vessel from the main tumor has prognostic significance with 1 cm cut-off shown in studies to predict very poor outcome[121]. MVI detection helps to identify patients at risk of development of distant metastasis post-resection and guides for the need of adjuvant therapy[114]. There is an urgent need for prediction of MVI in biopsies to guide therapeutic strategy. However, currently neither the detection of MVI in biopsy is possible nor any validated surrogate markers of MVI are available[122]. A recent pilot study assessed the performance of IHC panel of three biomarkers of MVI (H4K16ac, H4K20me2, PIVKA-Ⅱ) in a test set of 64 HCC surgical specimens and 42 core needle biopsies of HCC. In this study combination of PIVKA-Ⅱ with H4K20me2 showed the best accuracy for prediction of mVI, with very high specificity and PPV in HCC core needle biopsies[123].

Several studies have highlighted the prognostic relevance of histological grade, stage and MVI[124-126]. Analysis of eighteen registries comprising 570 transplanted patients revealed MVI in 16% and poor differentiation of tumor in 12%. These features were significant risk factors for dismal cancer specific survival[127]. Similar, prognostic significance of MVI and grade 3, in predicting overall survival, disease free survival and recurrence, had been found in 151 patients transplanted for HCC, by Donat *et al*[124] and by Lauwers *et al*[86] in their 425 HCC resections. In a study of 116 patients of large HCC resection, MVI and ES grade were reported as predictors of early recurrence[128]. Poor differentiation in a single HCC or in the largest HCC in a pre-transplant biopsy indicates aggressive tumour biology associated with poor prognosis. Certain centers exclude transplantation and assign patients to other treatment modalities, based on such prognosis and management related effects of biopsy[129,130].

ROLE OF IMMUNOHISTOCHEMISTRY IN PREDICTING PROGNOSIS

Several biomarkers have been investigated in HCC for prognostication and treatment decisions. K19 expressing HCCs show aggressive behavior, poor differentiation, high proliferative index and high recurrence rate[131-133]. Similarly, expression of stem cell marker CD133 is associated with higher tumor grade, advanced disease stage, higher recurrence rates and shorter overall survival[134]. EpCAM expression also demonstrates poor prognosis[5,134]. In a recent study, progressed HCC with > 5% tumor fraction expressing stem/progenitor cell markers CK19, EpCAM and CD133, displayed more aggressive behavior with increased likelihood of recurrence, chemo-resistance and metastasis[135].

Tumor tissue expression of high Wnt-1 shows correlation with nuclear B-catenin accumulation and increased rate of tumor recurrence. In a study of 142 patients of HCC, correlation of 3 groups: Biliary/stem cell marker positive group, Wnt/B-catenin signaling related marker positive, and both negative group was done with other prognostic features. Biliary/stem cell marker positive group demonstrated poor tumor differentiation, high frequency of portal vein invasion, intrahepatic metastasis and high proliferative activity[132].

HCC are hyper vascular and tumor angiogenesis is a known important prognostic factor. Immunohistochemical staining for endothelium specific markers CD31, CD34 or vWF allow semi quantitative assessment of micro vessel density, which is a significant prognostic indicator[136,68]. Li *et al*[137] performed meta-analysis of 12 articles comprising a total 1138 HCC patients. Survival outcomes showed positive correlation between poor prognosis and high micro vessel density levels.

Molecular markers with prognostic significance are analyzed by tissue markers and abundant literature is available on studies of phenotypic correlates. DNA ploidy, cell proliferation markers, Cell surface proteins Glypican-3, cytoskeleton proteins Fascin, enzymes Histone deacetylase, transcription factors BATF2, tumor suppressor genes TP53, adhesion molecule E-cadherin, cell cycle regulators, oncogenes, tumor angiogenesis related markers and several others related to Notch, Hippo, Hedgehog and other signaling pathways, have been analyzed in tissues and shown to be of prognostic significance[103,138].

IHC markers are also increasingly being used for the decisions of molecular targeted therapy and as a

predictor of therapeutic response[22,139]. Six areas of genomic alterations which have targetable potential for HCC are TERT promoter mutation, p53 pathway, oxidative stress pathway, Wnt-B catenin pathway, epigenetic, AKT/mTOR and MAP kinase pathway[22]. Evaluation of c-MET at the tissue level had been related to response to Tivantinib[106,140,141] with several such phase Ⅱ and phase Ⅲ ongoing trials are linked to molecular analysis of tissue samples.

Immune biomarkers such as immune checkpoint-inhibiting antibodies anti-PD-1, anti-PD-L1 and anti-CTLA-4 are useful for decisions regarding adjuvant therapy[142-144]. PD-L1 can predict response to anti-PD-1 antibody. A recent study assessed 294 HCC samples for the expression of PD-1, PD-L1 and CTLA-4. High PD-L1 staining was associated with poor disease-free survival and simultaneous increased expression of PD-L1 and CD68+ TIL was reported to be an important prognostic factor related to immune checkpoint pathway in HCC[145,146]. Advances related to determination and response assessment to the targeted therapies are dependent on archived tissues in institutions and biobanks. This further highlights the revival of importance of tissue procurement and analysis. Phase Ⅲ studies of novel targeted therapies require biopsy confirmation of diagnosis, and assessment of treatment response as in a study of 30 patients of HCC treated by Radiofrequency ablation (RFA) and check point inhibitor[147].

MORPHOMOLECULAR CLASSIFICATIONS: REINCARNATION OF TISSUE ANALYSIS IN HCC

Histopathological features can predict prognosis and lately, linkage of prognosis to distinct biological phenotypes has been demonstrated[84,85]. HCC phenotype may be associated with activation of specific oncogenic pathways thus histopathological parameters and molecular markers acting in concert can be very useful prognostic predictors[85]. HCC phenotypes are associated with distinct molecular pathways and such association is recently being the focus point due to the tremendous scope for advancement in HCC therapy. Transcriptomic classifications by Tan *et al*[84] and Calderaro *et al*[85] deserve special mention in this context.

In the study by Calderaro *et al*[85], CTNNB1 and TP53 defined two distinct tumor phenotypes in a large series of 343 cases of surgically resected HCC. Using pathology, immunohistochemistry, gene expression profiling and sequencing; patho-molecular correlates were classified into G1-G6 based on transcriptomics. Tumors associated with CTNNB1 mutations were large, well-differentiated, cholestatic, with microtrabecular/pseudo glandular pattern whereas TP53 mutated tumors were poorly differentiated, with multinucleated, pleomorphic cells arranged in compact pattern and displayed frequent vascular invasion. G1-G3 subclasses demonstrated correlation with histological features of poor differentiation, frequent macro vascular invasion, foci of clear cells, sarcomatous change, compact and macrotrabecular pattern, and foci of pleomorphic and multinucleated cells. Whereas G4-G6 subclasses, revealed low cell proliferation, association with small tumor size, lack of satellite nodules or micro vascular invasion, and tumors were well-differentiated.

In the study by Tan *et al*[84], 96 tumor tissues were used for the development of clinic pathological indices predictive of HCC molecular subclass. HCC transcriptome had been characterized into 3 subtypes S1-S3[148], determined by genome-wide transcriptome profiling, with potential therapeutic targets. S1 reflected aberrant activation of the WNT signaling pathway, S2 was characterized by proliferation as well as MYC and AKT activation, and S3 was associated with hepatocyte differentiation[46,148]. Predictive indices in the study by Tan *et al*[84] were validated in 99 HCC tumors. Phenotypic molecular correlation with S1-S3 based on transcriptomic analysis, revealed steatohepatitic HCC and immune cell infiltrates represented S1, macrotrabecular or compact pattern, lack of pseudo glands belonged to S2 and microtrabecular low histological grade and lack of steatohepatitis and clear cell patterns constituted S3 subclass. Macrotrabecular pattern/S2 showed activation of therapeutically targetable oncogene YAP and stemness markers EPCAM, keratin 19[84].

Similar histomorphology correlates of molecular characteristics were reported in scirrhous subtype and TSC1/TSC2 mutations and steatohepatitis subtype with IL-6/JAK/ STAT activation[85].

SUMMARY: RENEWED INTEREST IN BIOPSY WITH ROLE REDEFINED

Histopathological evaluation of HCC tissue has time tested applications in the diagnosis and prognosis (Table 1). Pathology combined with immunohistochemistry is essential for the differentiation of HCC from preneoplastic lesions, from metastatic diseases and other primary liver tumors. Certain biopsy limitations especially needle tract seeding, sampling errors and small risk of morbidity along with the technical advancements in imaging, undermined the importance of tissue analysis. This led most of the international guidelines on HCC to restrict the role of liver biopsy to characterize the lesions in non-cirrhotic liver or those with equivocal imaging. Other advantages of biopsy including the role in prognostication, therapy decision, research and clinical trials along with teaching and archiving in biobank, all suffered indirectly due to lesser availability of tissue specimens. With increasingly accumulating data on prognostic and therapeutic importance of specific phenotypes and distinct molecular-morphologic correlates, there is resurgence of interest in the role of tissue evaluation. Analysis of tumor biopsies allows clinicians to fine tune therapies. Molecular subclasses with phenotypic surrogates will be valuable for predicting response to specific targeted therapies

Table 1 Role of tumour tissue analysis in hepatocellular carcinoma

Diagnostic	Prognostic/theragnostic
Distinguishing HCC from metastasis	Prognostic histomorpholic parameters-tumour grade, vessel invasion, pTNM stage
Distinguishing benign/preneoplastic lesions from HCC	Identification of Histological variants of prognostic importance
Diagnosis confirmation of small HCC	Tissue biomarkers-for prognostication
Diagnosis of liver nodules in non-cirrhotic background	Tissue biomarkers-for assessing presence of therapeutic targets and drug development
Diagnosis of atypical variants of HCC, which have atypical Imaging findings	Histologic surrogates of clinically relevant molecular signatures-for predicting prognosis
Combined HCC-CC	Histologic surrogates of clinically relevant molecular signatures-as predictors of potential responders for targeted therapies
Diagnosis confirmation of HCC in phase Ⅲ trials of new drugs	

HCC: Hepatocellular carcinoma.

Table 2 Based on the evidence from published literature, algorithm based on the role of tissue diagnosis is inserted

Mandatory	Potentially necessary/helpful	Unwarranted
Lesion in a cirrhotic patient that lacks typical imaging characteristics	Well-differentiated HCC	HCC, if typical features are present on dynamic imaging technique
All nodules developing in the non-cirrhotic background	< 2 cm sized HCC, due to hypovascularity in small HCC and lack of typical imaging findings	AFP levels very high in the absence of other known causative tumours
Combined hepato-cholangiocarcinoma	Predictors of prognosis	Curative resection possible due to risk of needle tract seeding, if biopsied
Atypical variants of HCC	Teaching and biobanking	
Identifying stem cell phenotypes	Distinguishing HCC from regenerative nodule, dysplastic nodule, hepatic adenoma, FNH	
Phase Ⅲ trials of novel drugs	Morphologic surrogates of molecular signatures	
Tissue biomarker development and studies	Surrogate biomarkers of MVI in liver biopsy	

HCC: Hepatocellular carcinoma; MVI: Micro vascular; AFP: α-fetoprotein.

for HCC. HCC morphologic correlates of prognostically important molecular signatures need to be explored further to alleviate the HCC complications of recurrence, intra-hepatic and distant metastasis, responsible for dismal prognosis of HCC and to discover useful biomarkers (Table 2).

CONCLUSION

Histopathological analysis of HCC plays very important role in the diagnosis, prognosis and management decisions. Despite the shortcomings of biopsy and advancements in imaging and molecular characterization of HCC, value of biopsy is unshaken, with several recent facets further empowering tissue analysis.

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