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CONTINUING EDUCATION– HEPATOBILIARY TUMORS

Prognostic and Therapeutic Implications of Microvascular Invasion in Hepatocellular Carcinoma

Derek J. Erstad, MD[1] and Kenneth K. Tanabe, MD[2]

[1]Department of Surgery, Massachusetts General Hospital, Boston, MA; [2]Division of Surgical Oncology, Massachusetts General Hospital, Boston, MA

ABSTRACT Hepatocellular carcinoma (HCC) is a morbid condition for which surgical and ablative therapy are the only options for cure. Nonetheless, over half of patients treated with an R0 resection will develop recurrence. Early recurrences within 2 years after resection are thought to be due to the presence of residual microscopic disease, while late recurrences $>$ 2 years after resection are thought to be de novo metachronous HCCs arising in chronically injured liver tissue. Microvascular invasion (MVI) is defined as the presence of micrometastatic HCC emboli within the vessels of the liver, and is a critical determinant of early recurrence and survival. In this review, we summarize the pathogenesis and clinical relevance of MVI, which correlates with adverse biological features, including high grade, large tumor size, and epithelial–mesenchymal transition. Multiple classification schemas have been proposed to capture the heterogeneous features of MVI that are associated with prognosis. However, currently, MVI can only be determined based on surgical specimens, limiting its clinical applicability. Going forward, advances in axial imaging technologies, molecular characterization of biopsy tissue, and novel serum biomarkers hold promise as future methods for non-invasive MVI detection. Ultimately, MVI status may be used to help clinicians determine treatment plans, particularly with respect to surgical intervention, and to provide more accurate prognostication.

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K. K. Tanabe, MD
e-mail: ktanabe@partners.org

Hepatocellular carcinoma (HCC) is the sixth most common malignancy and the fastest rising cause of cancer-related death globally. Cirrhosis is a major risk factor for HCC, which arises from a milieu of chronically inflamed and damaged liver tissue, and both the prevalence of cirrhosis and incidence of HCC are expected to increase in the twenty-first century.[1] HCC is refractory to most therapies, and outcomes remain poor, with a 5-year survival rate of $<$ 15%.[2] Surgical resection, liver transplant, and ablation are the only established curative therapies, although 75% of patients are inoperable due to metastatic disease or local invasion at the time of diagnosis, and many of those who go on to surgery will develop recurrence either from residual microscopic disease or de novo tumorigenesis.[3,4]

The presence of vascular invasion is a critical determinant of early HCC recurrence and prognosis based on the results of multiple retrospective studies.[5–7] Vascular invasion is categorized as either microscopic or macroscopic. The latter is determined by gross tissue evaluation or radiography, whereas microvascular invasion (MVI) is determined by histologic evaluation of the tumor and surrounding hepatic tissue. MVI appears as small thrombi of malignant cells in the portal and hepatic venous systems.[8] The presence of macrovascular invasion is a contraindication to liver transplant and, in most cases, surgical resection, given the association with increased rates of recurrence, distant metastases, and poor prognosis. In contrast, the significance of MVI has historically been less appreciated, although multiple recent retrospective analyses have closely correlated MVI with adverse tumor biology and poor prognosis, and have shown that MVI is a more sensitive predictor of tumor recurrence and survival after transplant and surgical resection than standard staging criteria.

In this review, we summarize recent findings on the cellular and molecular pathogenesis of MVI, associations with outcome measures, and techniques for non-invasive

MVI prediction. Based on this information, we also discuss future directions for the management of HCC, in which application of MVI status may allow for more nuanced and effective treatment algorithms that may prevent futile interventions and improve outcomes.

PATHOGENESIS OF MICROVASCULAR INVASION (MVI)

A simple and widely accepted definition for MVI is the presence of tumor cells within a vascular lumen lined by endothelium that is visible only by microscopy.[8] MVI is observed in both portal and hepatic veins; the former is a potential source of intrahepatic metastases, while the latter is a source of distant metastatic spread, including recurrence after liver transplantation. Debate remains whether invasion of lymphatics and bile ducts or satellite disease hold similar mechanistic and clinical relevance.[9] In contrast to MVI, satellite lesions are detached micrometastatic nodules of HCC cells embedded in hepatic parenchyma, which are thought to be a result of direct tissue invasion by malignant cells, although they may also represent microvascular emboli that subsequently invaded a new region of liver. Loss of cell–cell adhesion, breakdown of surrounding extracellular matrix (ECM), use of alternative energy sources, and cellular motility are required for invasion into either luminal structures or hepatic parenchyma.[10] Among malignant cells, these capabilities are typically associated with more aggressive tumor biology, therefore MVI and satellitosis are likely to have some degree of overlapping prognostic significance and clinical relevance. Indeed, satellitosis has been independently associated with recurrence and poor outcome after liver transplantation similar to MVI.[11]

These phenotypic cellular changes associated with MVI and satellitosis are also commonly observed during epithelial–mesenchymal transition (EMT), a dedifferentiation process notable for loss of cell adhesion proteins and increased expression of genes involved in cell migration and ECM degradation.[12] Several research teams have investigated the relationship between EMT and MVI in HCC. Zhou et al. showed that tumor expression of ZEB1, a zinc-finger protein that plays a central role in EMT, correlated with vascular invasion, intrahepatic metastases, and early recurrence in 110 patients with HCC.[13] They also showed that ZEB1 silencing in vitro reduced migration of MHCC-97H cells, which was associated with reduced expression of EMT markers, including N-cadherin and vimentin. Mima et al. similarly found that low expression of E-cadherin, an epithelial marker, with concomitant increased expression of vimentin, was significantly associated with vascular invasion, extrahepatic metastases, and reduced disease-free survival (DFS) among 150 patients with HCC.[14] Finally, Wan et al. performed a meta-analysis evaluating 10 studies that included 1334 patients with data correlating expression of EMT-inducing transcription factors with clinicopathologic traits and outcome measures in HCC.[15] They found that expression of ZEB, Snail, Slug, or Twist1 was associated with multiple adverse prognostic factors, notably vascular invasion, intrahepatic metastases, and reduced overall survival (OS). In addition, recent advances in molecular characterization of HCC have revealed that a broad range of mechanisms, including transcriptional and epigenetic abnormalities, as well as aberrant expression of microRNAs and long noncoding RNAs (lncRNAs), are involved in EMT.[16] Given that both intrahepatic and distant metastatic disease are byproducts of MVI, it can be inferred from these studies that activation of EMT transcriptional programs is an important pathogenic step in the development of MVI in HCC (Fig. 1). A summary of molecular pathways involved in HCC EMT are shown in Table 1.

MVI CLASSIFICATION SCHEMA

Studies on MVI are often limited in the precision of their terminology in several ways. First, the terms *intrahepatic metastasis*, *micrometastasis*, and *microsatellite* are often used to describe both MVI and satellitosis, without distinction regarding the luminal or parenchymal location of the malignant cells. Although MVI and satellitosis are thought to have pathogenic overlap, use of these nonspecific terms precludes any mechanistic differentiation between the two processes. Second, in some instances there may be a lack of distinction between macrovascular invasion and MVI, referring only to the presence of vascular invasion. Finally, there are no agreed upon criteria or terminology to distinguish different severities of MVI burden. Regarding this latter point, there is ongoing debate about the appropriate method for classifying MVI. Factors under consideration include the number of malignant cells in the embolus, the presence or absence of vessel wall invasion, and the distance from the primary lesion, which are features thought to negatively correlate with prognosis. Accordingly, early MVI may represent only a few malignant emboli composed of small cellular clusters located within the surgical margin of a primary lesion, whereas advanced MVI may include multiple, large foci of malignant cells scattered throughout the liver, including distant lobes. The likelihood of residual malignant disease, early recurrence, and worse outcome is higher in the latter case, although there is no validated system to make this distinction.

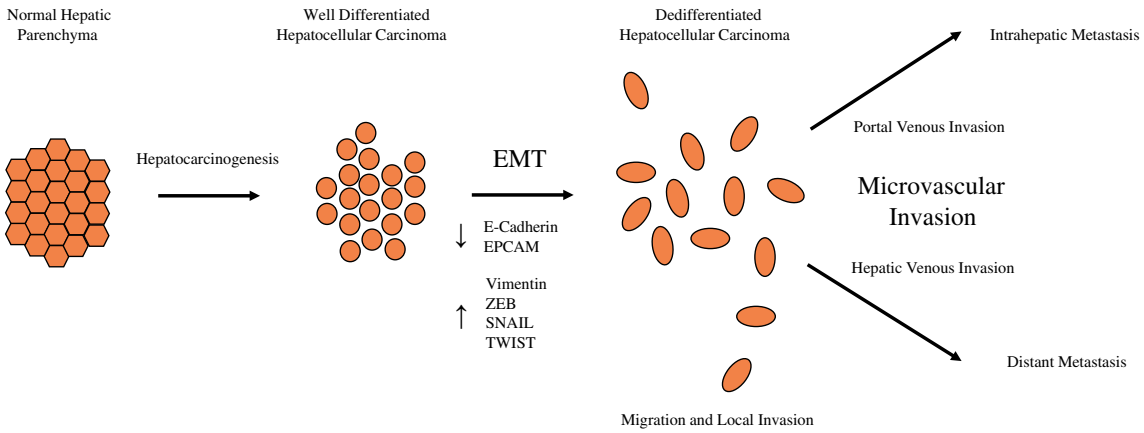

FIG. 1 Schematic of hepatocarcinogenesis, epithelial–mesenchymal transition, and microvascular invasion. *EMT* epithelial–mesenchymal transition, *EPCAM* epithelial cell adhesion molecule, *ZEB* zinc finger E-box-binding homeobox

Multiple research teams have analyzed MVI patterns that associate with prognosis in order to propose novel staging criteria. Sumie et al. evaluated 207 surgically resected HCC samples (50% MVI positive), with particular focus on the burden of vessel involvement. They stratified patients as having no MVI, mild MVI (five or fewer total blood vessels involved), or severe MVI (> 5).[17] Severe MVI tumors were associated with greater size, elevated alpha-fetoprotein (AFP) and des-gamma carboxyprothrombin (DCP) serum levels, presence of satellitosis, and worse grade. On multivariable analysis, both mild MVI [hazard ratio (HR) 1.93] and severe MVI (HR 2.87) were independent predictors of poor outcome; however, compared with mild MVI, severe MVI had shorter disease-specific survival (DSS). Iguchi et al. similarly evaluated the prognostic significance of MVI burden in 142 patients treated with living-donor liver transplant for HCC. Patients were stratified as having no MVI, low MVI (< 50 carcinoma cells observed within a vascular lumen), and high MVI (> 50 cells). High MVI status was predictive of recurrence-free survival (RFS; $p = 0.03$), and was associated with increased DCP levels and greater tumor size.[18]

In contrast, Feng et al. analyzed MVI patterns with particular emphasis on the appearance of the embolus, in which they stratified MVI into four subtypes: (1) free (non-adherent to the endothelium); (2) adhesion (adhered to the endothelium); (3) invasion (adhered and invading the endothelium); and (4) break through (penetrated the vascular wall or tumor capsule).[19] They coupled these data with the number of vessels invaded to create four MVI classes based on appearance and burden: M0 (no MVI), M1 (non-invasion type, < 5 vessels), M2 (invasion type < 5 vessels, or non-invasion type > 5 vessels), and M3 (invasion type, > 5 vessels). They did not account for distance of emboli from the primary lesion. Using this system, they substratified MVI-positive lesions to show progressive reduction in OS and RFS with advancing MVI stage. Finally, Feng et al. created a prognostic nomogram based on clinicopathologic tumor traits, serum AFP, and MVI classification for the prediction of OS that was more accurate than TNM, Chinese University Prognostic Index (CUPI), and Japan Integrated Staging (JIS) criteria in both their training ($n = 686$) and validation ($n = 225$) cohorts.

Roayaie et al. used a combined approach, evaluating both the burden and appearance of MVI in 384 surgically resected HCC specimens (34% MVI-positive), in which they identified three risk factors that correlated with outcomes: (1) invasion into a vessel with a muscular wall; (2) invasion into a vessel > 1 cm from the primary lesion; and (3) invasion into five or more vessels.[8] Based on these attributes, they created a scoring system that could accurately risk stratify MVI-positive lesions by recurrence and survival. Lesions with none or few risk factors had similar prognosis to MVI-negative HCC, whereas tumors with multiple risk factors had outcomes similar to HCC with gross macrovascular invasion.

Finally, Zhao et al. performed a similar analysis to Roayaie et al., although rather than evaluating for embolic vessel wall invasion they accounted for emboli size.[20] Thus, they stratified MVI lesions into low- and high-risk groups based on the presence of three features: (1) > 5 vessels involved; (2) > 50 carcinoma cells present in the embolus; and (3) > 1 cm from the primary lesion. Using this approach, they analyzed 295 surgically resected HCC specimens and showed that low-risk MVI patients had similar outcomes to non-MVI patients, while high-risk MVI patients did significantly worse in regard to both recurrence and survival. On subgroup analysis, anatomic liver resection was associated with improved OS and RFS compared with non-anatomic resection in high-risk MVI

TABLE 1 Molecular mechanisms associated with epithelial–mesenchymal transition

Category	Author	Year	Mediator	Associated signaling	Findings
Transcriptional	Giannelli et al.[94]	2005	Laminin-5	Snail, Slug	Laminin-5 increases Snail and Slug expression, resulting in β-catenin translocation to the nucleus and mesenchymal morphologic change
	Saxena et al.[95]	2007	Leptin	AKT, ERK1/2, STAT3	Leptin stimulates HepG2 and Huh7 cell migration via AKT, ERK1/2, and STAT3 signaling in vitro
	Fransvea et al.[96]	2008	TGF-β	SMAD-2	TGF-β inhibition with LY2109761 reduced HCC migration via SMAD-2 activation; incubation of human HCC tumor with the TGF-β inhibitor increased E-cadherin expression, a cell–cell adhesion protein
	Sun et al.[97]	2013	NANOG	SMAD-3, Snail	NANOG expression correlates with metastasis and low survival; overexpression associated with EMT; NANOG induces expression of SMAD-3 and Snail, drivers of EMT
	Ye et al.[98]	2016	HIF-1α	CCL20	HIF-1α induces expression of CCL20 in HCC, which is associated with hypoxia resistance and EMT
	Wu et al.[99]	2016	TNFα	NF-κB, Snail	TNFα induces Snail expression via NF-κB, inducing EMT, and is associated with reduced survival in humans
	Zhou et al.[100]	2015	CXCL2	CXCR5, PI3 K, AKT	CXCL2/CXCR5 axis activated PI3 K and AKT to induce EMT, and is associated with worse prognosis in humans
	Huang et al.[101]	2015	IL-8	FOXC1, PI3 K, HIF-1α	IL-8 induces FOXC1 expression via PI3 K and HIF-1α signaling, which increased metastases in a murine xenograft model
	Xiao et al.[102]	2016	ACTL6A	Notch	ACTL6A expression is associated with worse HCC prognosis; ectopic expression in vitro associated with HCC cell line migration and invasion; ACTL6A promotes HCC xenograft tumor growth in mice
Epigenetic	Niwa et al.[103]	2005	SOCS-3	STAT3, FAK	Methylation silencing of SOCS-3 was associated with increased STAT3/FAK-mediated cell migration in 5 of 10 HCC cell lines in vitro
	Lim et al.[104]	2008	Snail	HDAC1	Reactive oxygen species stress-induced Snail expression, which recruits HDAC1 to the E-cadherin promoter, inducing hypermethylation and contributing to EMT
	Ogunwobi et al.[105]	2013	c-MET	HGF	HCC circulating tumor cells undergo EMT via hypomethylation of the c-MET promoter at six different CPG sites, resulting in increased c-MET signaling and increased metastatic potential
	Xie et al.[106]	2014	SFRP	HBx	HBx expression induces DNMT1, resulting in hypermethylation of SFRPs and increased WNT-mediated signaling; decreased SFRP expression in HBV-related HCC is associated with poor differentiation
	Wang et al.[107]	2015	PTPRS	EGFR	PTPRS is downregulated in 80% of human HCCs, which correlates with worse prognosis; PTPRS interacts with and inhibits EGFR-mediated EMT; promoter methylation of PTPRS in HCC induces EMT
	Wang et al.[108]	2016	RASSF10	Bcl-2, Bcl-xl	RASSF10 hypermethylation associated with worse HCC prognosis; in vitro RASSF10 expression associated with increased apoptotic gene expression and reduced anti-apoptotic expression of Bcl-2 and Bcl-xl
	He et al.[109]	2015	FOXD3	E-Cadherin	FOXD3 promoter hypermethylation associated with EMT and worse prognosis; in vitro expression of FOXD3 associated with reduced proliferation, migration and invasion of HCC cell lines via inhibition of Snail and β-Catenin
	Li et al.[110]	2016	CLDN14	β-Catenin	CLDN14 is downregulated by EZH2-mediated promoter hypermethylation, which is associated with β-catenin activation and EMT in vitro

TABLE 1 continued

Category	Author	Year	Mediator	Associated signaling	Findings
microRNA	Li et al.[111]	2009	miR34a	c-MET, ERK1/2	miR-34a directly targets c-MET-mediated HepG2 migration via ERK1/2 in vitro
	Zheng et al.[112]	2012	miR-124	ROCK, EZH2	miR-124 downregulation in HCC is associated with worse prognosis; miR-124 binds to the 3′UTR and suppresses expression of ROCK and EZH2; this effect reduced
	Yan et al.[113]	2013	MiR-10a	EphA4, β1-Integrin	miR-10a suppressed transcription of EphA4, thereby promoting migration and invasion of HepG2 cells via suppression of the β1-integrin pathway in vitro
	Xu et al.[114]	2013	miR-148a	AKT, mTOR	miR-148a inhibits HPIP expression, thereby reducing AKT-mediated mTOR activation and EMT; HBx suppressed TP53-mediated expression of miR-148a, inducing EMT
	Brockhausen et al.[115]	2015	miR-181a	TGF-β	TGF-β induces expression of miR-181a, which is associated with EMT-like changes in vitro
	Ning et al.[116]	2014	miR-7, miR-124, miR-21	HNF4α	Reduced HNF4α expression correlates with HCC poor prognosis and EMT; exogenous HNF4α-induced expression of miR-7 and miR-124, which prevented transcription of RelA via binding of the 3′UTR
	Shih et al.[117]	2015	miR-21	MCP-1, TIMP3	MCP-1-induced expression of miR-21, resulting in increased expression of MMP9 and TIMP3, increasing cell motility and EMT in Huh7 cells
	Yang et al.[118]	2015	miR-192, miR-200b, miR-215	AP-1, ZEB1	Gα12 overexpression induces MDM2-mediated suppression of TP53 via suppression of miR-192, miR-200b, miR-215 in HCC. Downregulation of miR-192, miR-200b, miR-215 were associated with MVI in HCC samples.
	Kan et al.[119]	2015	miR-520 g	SMAD7	miR-520 g expression is associated with poor survival in HCC; miR-520 g is associated with EMT via targeting SMAD7
	Zhou et al.[120]	2015	miR-125	TGF-β, SMAD2, SMAD4	TGF-β treatment suppressed miR-125 expression; expression of miR-125 abrogated EMT and cancer stem cell marker expression, and reduced tumor mets in vivo by targeting SMAD2 and SMAD4
	Yang et al.[121]	2015	miR-99b	CLDN11	miR-99b expression associated with decreased HCC survival; miR-99b targets CLDN11, a component of tight junctions
	Wang et al.[122]	2016	miR-26b	Twist1	miR-26b inhibited EMT by suppression of Twist1-induced expression of SMAD1; miR-26b expression levels in human HCC were inversely correlated with the presence of metastases
	Sandbothe et al.[123]	2017	miR-449a/b	SOX4	miR-449a/b suppress transcription of SOX4, thereby reducing EMT in HCC
	Deng et al.[124]	2017	miR-30a	MTA1	miR-30a suppresses transcription of MTA1, which is involved in HCC EMT via ErbB2 expression

TABLE 1 continued

Category	Author	Year	Mediator	Associated signaling	Findings
Long noncoding RNA	Battistelli et al.[125]	2017	HOTAIR	Snail, EZH2	HOTAIR mediates interaction between Snail and EZH2 to induce EMT in HCC
	Yuan et al.[126]	2014	lncRNA-ATB	TGF-β	lncRNA-ATB competitively binds the miR-200 family, upregulating ZEB1/2 and STAT3 signaling, inducing EMT
	Li et al.[127]	2016	ZEB1-AS1	ZEB1	lncRNA ZEB1-AS1 is upregulated in aggressive HCC via promoter hypomethylation; ZEB1-AS1 induces ZEB1 expression and EMT
	Yan et al.[128]	2017	lncRNA-MUF	ANXA2, WNT	lncRNA-MUF upregulation is associated with HCC poor prognosis; lncRNA-MUF overexpression induced EMT via binding ANXA2 and WNT activation
	Li et al.[129]	2017	lncRNA-ROR	ZEB2	lncRNA-ROR expression is associated with poor HCC prognosis; functions by sequestering miR-145, thereby inducing ZEB2 expression and EMT
	Liu et al.[130]	2016	Lnc-FTX	WNT, MCM2	lncRNA-FTX expression is associated with improved HCC survival; functions by sequestering miR-374a, repressing WNT signaling and EMT; also binds MCM2, reducing DNA replication and inhibiting proliferation

ACTL6a actin-like 6A, *AKT* protein kinase B, *ANXA2* annexin A2, *AP-1* activator protein 1, *Bcl-2* B cell lymphoma 2, *Bcl-x* B-cell lymphoma x, *CCL20* chemokine (C–C motif) ligand 20, *CLDN* claudin, *CXCL2* chemokine (C-X-C) ligand 2, *CXCR5* chemokine (C-X-C) receptor 5, *DNMT1* DNA methyltransferase 1, *EGFR* epidermal growth factor receptor, *EMT* epithelial–mesenchymal transition, *EphA4* erythropoietin-producing hepatoma receptor 4, *Erbb2* Erb-B2 receptor tyrosine kinase 2, *ERK* extracellular signal-regulated kinase, *EZH2* enhancer of zeste 2 polycomb repressive complex 2 subunit, *FAK* focal adhesion kinase, *FOXC1* forkhead box C1, *FOXD3* forkhead box D3, *HBV* hepatitis B virus, *HCC* hepatocellular carcinoma, *HDAC1* histone deacetylase 1, *HGF* hepatocyte growth factor, *HIF-1α* hypoxia-inducible factor 1 alpha, *HNF4a* hepatocyte nuclear factor 4 alpha, *HOTAIR* HOX transcript antisense RNA, *HPIP* hematopoietic pre-B cell leukemia transcription factor interacting protein, *IL* interleukin, *lncRNA* long noncoding RNA, *MCM2* minichromosome maintenance complex component 2, *MCP-1* monocyte chemotactic protein 1, *MDM2* mouse double minute 2 homolog, *miR* microRNA, *MMP9* matrix metallopeptidase 9, *MTA1* metastasis-associated 1, *mTOR* mammalian target of rapamycin, *MVI* microvascular invasion, *NF-κB* nuclear factor kappa B, *PI3 K* phosphoinositide 3-kinase, *PTPRS* protein tyrosine phosphatase, receptor type S, *RASSF10* Ras association domain family member 10, *SFRP* secreted frizzled-related protein, *SMAD* SMA- and MAD-related protein, *SOCS-3* suppressor of cytokine signaling 3, *STAT3* signal transducer and activator of transcription 3, *TGF-β* transforming growth factor beta, *TIMP3* tissue-associated metalloproteinase 3, *TNFα* tumor necrosis factor alpha, *ZEB1* zinc finger E-box-binding homeobox 1, *3′UTR* 3 prime untranslated region

patients. Similar to other studies, a worse MVI score was associated with larger tumor size, elevated serum AFP, and high tumor grade.

In summary, multiple features of MVI carry prognostic significance. A greater burden of micrometastatic disease, large thrombi size, and presence of thrombi invasion into the vessel wall are associated with more aggressive tumor biology, increased rates of early recurrence, and reduced OS. A summary of the MVI classification schema is shown in Table 2.

CLINICAL RELEVANCE OF MVI

For operable HCC, recurrence and metastasis are the main drivers of poor prognosis. Seventy percent of patients treated with curative surgical resection will develop a recurrence within 5 years. Outcomes for liver transplant are slightly better, with a 5-year recurrence rate of 10–20%.[21,22] Early recurrences, within 2 years of tumor resection, are frequently attributed to residual intrahepatic metastases, whereas late recurrences are thought to be de novo malignancies arising from a milieu of chronically inflamed liver tissue.[23] Therefore, early recurrence depends on the biological aggressiveness of the primary tumor, particularly the likelihood for MVI and satellitosis, whereas late recurrence depends on the carcinogenic potential of cirrhotic liver tissue.[23,24]

MVI is frequently present in HCC, and highly correlates with adverse biological markers, including high grade, large tumor size, and elevated serum AFP and DCP.[6,19] Nearly two-thirds of large, high-grade HCCs may have presence of MVI on histologic evaluation.[25] Up to 25% of HCCs < 3 cm will also have MVI, suggesting there exists a subtype of HCC with inherently more aggressive biology.[26] Given the association of MVI with microscopic, residual metastatic disease after resection, MVI may actually better predict recurrence and survival than conventional grading systems. Lauwers et al. reviewed clinicopathologic data from 425 patients treated with curative resection for HCC, in which MVI was present in 51% of cases and was independently predictive of decreased survival on multivariable analysis, along with tumor grade. The authors created a prognostic nomogram based on these two malignant features that was more discerning in regard to prognosis for similar, moderately staged disease than standard Edmonson grading.[5] More recently, Lim et al. assessed outcomes in 454 patients treated with curative surgical resection, and found that the presence of MVI more accurately predicted recurrence and survival outcomes than factors included in the Milan criteria.[7] A summary of studies that evaluated the prognostic significance of MVI for both recurrence and survival are displayed in Table 3.

ACCOUNTING FOR MVI IN HEPATIC RESECTION MARGIN

Hepatic resection is the primary treatment for HCC for patients not meeting the criteria for liver transplantation, although ongoing debate remains regarding the smallest acceptable resection margin before outcomes deteriorate. Most studies on the topic have used measurement cut-offs for margin size, e.g. < 1, 1–2, and > 2 cm, or have separated patients by anatomic resection versus non-anatomic resection, with the former affording a greater margin on average. The Americas Hepato-Pancreato-Biliary Association (AHPBA) and the Society for Surgical Oncology (SSO) both recommend a resection margin of 1–2 cm.[27] There have been mixed results in the literature regarding margin size and outcome measures, although a recent systematic review by Tang et al. showed there was no discernible difference in recurrence or survival between patients who had margins < 1 cm compared with those with margins ≥ 1 cm.[28] However, most of these studies did not control for tumor biological factors such as MVI status, which may have a confounding impact. It is unlikely that all HCCs require the same margin size, but rather lesions with adverse biology, which determines the risk for intrahepatic and distant dissemination of malignant cells, should potentially be treated with a wider margin. It has been previously shown that MVI-positive HCCs experience a greater reduction in recurrence rate with a wider margin compared with MVI-negative tumors.[29]

Historically, studies on margin size have stratified HCC by tumor diameter, in part because diameter is easily measurable by radiography or pathology, and because increasing tumor size roughly correlates with tumor grade. Kojiro and Nakashima nicely demonstrated this latter concept by characterizing the histologic features of HCC with increasing size.[30] They found that small HCCs $<$ 1.5 cm are typically well-differentiated, often with fatty infiltration. However, at a diameter of approximately 1.5 cm, HCC began to display 'nodule-in-nodule' growth of high-grade cells. Further tumor growth is largely due to outgrowth of these high-grade nodules, and, by 2–3 cm in size, most HCCs were primarily high grade in appearance. However, despite this association between size and grade, tumor diameter only partially correlates with markers that predict peripheral microscopic spread, in particular MVI.[26,31,32]

TABLE 2 Proposed classification schemata for MVI

Author	Year	No. of vessels	No. of cells	Distance from primary (cm)	Appearance	Findings
Sumie et al.[17]	2014	Mild (≤ 5) or severe (> 5)	–	–	–	Severe MVI associated with ($p < 0.05$) greater size, elevated AFP and DCP serum levels, presence of satellitosis and worse grade, and decreased DSS compared with mild or no MV
Iguchi et al.[18]	2015	–	Low (< 50) or high (> 50)	–	–	High MVI associated with reduced RFS, increased serum DCP, and increased tumor size
Feng et al.[19]	2017	< 5 or > 5	–	–	1. Free	M0 = no MVI
					2. Adhered to wall	M1 = non-invasion type, < 5 vessels
					3. Invading endothelium	M2 = invasion type < 5 vessels, or non-invasion type > 5 vessels
					4. Penetrating vessel wall or tumor capsule	M3 = invasion type, > 5 vessels
						Increasing M stage was associated with worse RFS and OS. Nomogram including clinicopathologic features, AFP, and M stage more accurately predicted OS than TNM, CUPI, and JIS
Roayaie et al.[8]	2009	< 5 or > 5	–	< 1 or > 1	1. Non-invading	Scoring system based on number of risk factors. Lesions with none or few risk factors had similar prognosis to MVI-negative HCC; lesions with multiple risk factors had outcomes similar to HCC with gross macrovascular invasion
					2. Invading vessel wall	
Zhao et al.[20]	2017	< 5 or > 5	< 50 or > 50	< 1 or > 1	–	Scoring system based on number of risk factors. High-risk MVI score was associated with larger tumor size, elevated serum AFP, and high tumor grade

AFP alpha-fetoprotein, *CUPI* Chinese University Prognostic Index, *DCP* des-gamma carboxyprothrombin, *DSS* disease-specific survival, *HCC* hepatocellular carcinoma, *JIS* Japan Integrated Staging, *MWI* microvascular invasion, *OS* overall survival, *RFS* recurrence-free survival, *TNM* tumor node metastasis

TABLE 3 Studies evaluating the prognostic significance of MVI

Author	Year	No. of patients	MVI and recurrence-free survival	MVI and overall survival
Zhao et al.[131]	2017	233	HR 1.67 (95% CI 1.21–2.30), $p = 0.002$	HR 1.78 (95% CI 1.06–2.99), $p = 0.029$
Liu et al.[132]	2018	623	HR 2.02 (95% CI 1.55–2.62), $p < 0.001$	HR 1.56 (95% CI 1.18–2.06), $p = 0.002$
Park et al.[133]	2017	676	HR 1.77 (95% CI 1.39–2.24), $p < 0.001$	HR 1.55 (95% CI 1.14–2.11), $p < 0.01$
Hou et al.[134]	2016	167	Not recorded	HR 2.62 (95% CI 2.15–3.19), $p < 0.01$
Jang et al.[135]	2016	149	HR 1.91 (95% CI 1.21–3.00), $p = 0.005$	HR 2.67 (95% CI 1.49–4.74), $p = 0.001$
Du et al.[136]	2014	458	RR 1.34 (95% CI 1.24–1.51), $p = 0.012$	Not recorded
Hung et al.[137]	2013	447	Not recorded	HR 2.81 (95% CI 1.57–5.03), $p < 0.001$
Chan et al.[138]	2012	471	OR 2.29 (95% CI 1.77–2.96), $p < 0.0001$	OR 2.68 (95% CI 1.94–3.71), $p < 0.0001$
Fan et al.[139]	2011	408	OR 1.85 (95% CI 1.38–2.43), $p < 0.001$	OR 2.75 (95% CI 2.00–3.84), $p < 0.001$
Kim et al.[140]	2011	240	Not recorded	OR 2.55 (CI not recorded), $p = 0.009$
Lim et al.[7]	2009	454	Median time to recurrence (months): MVI (+) 12 (95% CI 8.9–15.8) vs. MVI (−) 42.2 (95% CI 33.9–61.8I), $p < 0.001$	HR 2.12 (95% CI 1.52–2.97), $p = 0.007$
Wang et al.[141]	2009	473	RR 1.84 (95% CI 1.40–2.40), $p < 0.05$	RR 1.93 (95% CI 1.40–2.50), $p < 0.05$
Sumie et al.[142]	2008	110	HR 2.97 (95% CI 1.69–5.24), $p = 0.002$	HR 3.51 (95% CI 1.27–9.74), $p = 0.016$

CI confidence interval, *HR* hazard ratio, *MVI* microvascular invasion, *OR* odds ratio, *RR* relative risk

Multiple research teams have evaluated the relationship between margin size, outcomes measures, and MVI status. With respect to MVI-positive HCCs < 2 cm in diameter, increased margin size may be associated with improved outcomes. Yamashita et al. evaluated 149 patients with surgically resected HCCs < 2 cm in diameter and found that the presence of MVI was associated with a significant reduction in 5-year DFS on multivariable analysis (44% vs. 72%, $p = < 0.01$); however, this effect was abrogated with a larger resection margin (≥ 0.5 mm).[33] Similarly, Roayaie et al. evaluated 132 patients with HCCs ≤ 2 cm in diameter, and found that anatomic resection significantly reduced rates of recurrence at 1 and 3 years.[31] Specifically, among MVI-positive lesions, there was a dramatic 40% reduction in 1-year recurrence with anatomic resection compared with non-anatomic resection. The findings from both studies suggest that for very early-stage HCCs with MVI, a wider margin more adequately clears peripheral intrahepatic metastases, preventing early recurrence. Importantly, the degree of baseline liver dysfunction between small and wide margin cohorts was similar in both studies, adding veracity to their conclusions. However, in reality, patients with advanced liver disease are less likely to undergo larger resections.

In contrast, there may not be a benefit associated with an increased resection margin for MVI-positive HCCs > 2 cm in diameter. Jung et al. compared anatomic resection versus non-anatomic resection in 1527 patients with HCCs 2–5 cm in diameter.[34] Tumors were also stratified based on ADV score, which is a composite of AFP and DCP levels and tumor volume that serves as a surrogate marker for adverse biology. In this analysis, among tumors with an ADV score > 4 or with the presence of MVI, anatomic resection provided no additional recurrence or survival benefit, indicating that the burden of micrometastatic disease was not addressed by a larger margin.

This assertion is also supported by two recent propensity matched analyses evaluating the extent of surgical resection for HCC. Zhao et al. examined survival outcomes in 228 HCC patients treated with either anatomic or non-anatomic resection. The average tumor diameter for both groups was 5 cm, however the margin size was greater in the anatomic group (0.6 cm vs. 0.5 cm, $p = 0.03$).[35] Anatomic resection was associated with significantly greater 5-year RFS (45.1% vs. 31.0%, $p = 0.005$), although there was no difference in OS. On subgroup analysis of MVI-positive patients only, anatomic resection was again associated with improved RFS but not OS, indicating that a modestly larger margin did not translate to improved long-term outcome. Marubashi et al. performed a similar analysis of 658 HCC patients treated with anatomic versus non-anatomic resection.[36] The average tumor size for both groups was approximately 3.5 cm, and the surgical margin was again greater with anatomic resection (0.6 cm vs. 0.5 cm, $p = 0.001$). There was no difference in RFS, early recurrence rate (< 2 years after resection), or OS between resection groups, and no outcome differences were observed on subgroup analysis of MVI-positive patients only. Taken together, these findings indicate that a modestly larger margin associated with anatomic resection does not translate to improved survival for patients with MVI-positive HCCs that is, on average, > 2 cm in diameter.

Finally, Shi et al. performed a clinical trial in which 169 patients with HCC underwent surgical resection with either a narrow (1 cm) or wide (2 cm) margin. Although the authors did not explicitly determine MVI status, they observed that a 2 cm margin was associated with a longer 5-year OS (49.1% vs. 74.9%, $p = 0.008$) only for tumors ≤ 2 cm in diameter, with no added benefit for larger lesions.[37]

Maeda et al. evaluated surgical specimens from 131 patients with solitary HCCs ≤ 2 cm in diameter, and found that all intrahepatic metastatic lesions were within 1 cm of the primary tumor edge.[38] These findings were consistent with a previous study in which 128 HCCs < 3 cm in diameter were evaluated for microinvasive disease. Only eight cases were positive for metastatic malignant cells, all found within 11 mm from the primary tumor edge.[39] Similarly, Okusaka et al. evaluated 149 surgical specimens with HCCs ≤ 3 cm, and found that 19% had evidence of microinvasive disease, with all foci located within 2 cm of the tumor edge.[40] Finally, Sasaki et al. evaluated 100 patients with HCCs < 5 cm in diameter for microsatellite disease, which they defined as either venous thrombi or intrahepatic metastases, and found a positive correlation between primary tumor size and the distance of microsatellite disease from the tumor edge.[41] In their study, among 22 patients with microsatellites found > 5 mm from the primary tumor edge (and several over 30 mm), 21 of them had tumor diameters > 2.5 cm. Finally, it should be noted that while Sasaki et al. indicate the inclusion of microvascular thrombi in their analysis, the terminology is less clear in the other studies, although it is presumed that the intrahepatic metastatic burden of disease captured both satellites and microvascular thrombi.

Taken together, these findings suggest that with respect to MVI-positive HCCs, there may be an advantage associated with a larger surgical margin for small lesions, approximately 2–3 cm in diameter, which is more often obtained with anatomic resection compared with non-anatomic resection. However, this advantage is more difficult to demonstrate for larger MVI-positive HCCs, presumably due to a greater burden of distant micrometastatic disease. Historically, the surgeon has been left to balance the competing objectives of providing an oncologically sound operation while mitigating the risk of postoperative liver

failure without any guidance in regard to individual tumor biology. However, based on the associations between MVI status, tumor size, and survival, going forward there are scenarios where preoperative MVI status could thus help guide surgical management, e.g. anatomic versus non-anatomic resection for small HCCs.

LIMITATIONS OF ABLATION FOR MVI-POSITIVE HEPATOCELLULAR CARCINOMA (HCC)

Over the last decade, advances in ablative therapy have led to improved outcomes with ablation for early-stage HCC.[42] Compared with surgical resection, percutaneous RFA (PRFA) is less invasive with a lower risk profile, which is particularly useful for patients with cirrhosis who would otherwise do poorly with hepatic resection. Accordingly, the AHPBA and SSO currently recommend PRFA as first-line therapy for HCC tumors that are < 4 cm in diameter and located away from large vascular structures in patients who are unable to undergo hepatic resection or transplant.[27] Multiple clinical trials comparing surgical resection and PRFA have been performed with mixed results, although there appears to be a trend towards improved survival and decreased rates of recurrence associated with surgical resection.[43–46] Some have criticized these trials for their inclusion criteria, citing that RFA works better for lesions < 3 cm in diameter. Therefore, inclusion of larger lesions may have obfuscated the efficacy of RFA for very early-stage HCC.[47] Multiple research teams have thus also retrospectively compared surgical resection and PRFA for HCCs < 2 cm, and again there appears to be both a recurrence and survival benefit associated with surgery.[48]

Nonetheless, as RFA continues to gain traction, there is the potential for worse outcomes among the subset of patients with MVI-positive HCCs, which includes up to 28% of very early-stage lesions, who are at increased risk for local micrometastatic disease that may not be addressed by an ablative margin.[26,49] Ueno et al. recently performed a subgroup analysis of prognostic determinants for patients with HCCs < 5 cm in diameter treated with either surgical resection or PRFA.[50] They measured three HCC markers: AFP, AFP-L3, and DCP. While they found no difference in survival between treatment modalities among all patients, for the subset of HCCs that were positive for all three markers, there was a significant survival advantage with surgical resection (5-year OS 74.9% vs. 47.6%, $p < 0.01$). The authors concluded that the presence of multiple HCC markers correlated with worse biology and increased risk for MVI and microsatellite disease. Accordingly, they predicted that the ablative margin associated with RFA provided inferior radical tumor clearance compared with surgical resection.[50] Although significant advances have been made in the execution and monitoring of ablative margins with RFA, more studies are needed to further clarify the risk of recurrence for patients with MVI or microsatellite disease, regardless of tumor size, who may benefit from a larger negative margin afforded with surgical resection.[51]

ADDITIONAL THERAPEUTIC MODALITIES FOR MANAGEMENT OF MVI-POSITIVE HCC

Neoadjuvant Antiviral Therapy

Approximately 70–90% of HCC cases are associated with hepatitis B virus (HBV) infection in East Asia.[52] It has been previously shown that active HBV infection might induce MVI through chronic inflammation, stimulation of metastasis-associated protein 1 expression, and suppression of local immune surveillance.[53–55] Concordantly, multiple studies have shown that HBV-positive HCC is associated with higher rates of MVI, and the degree of invasion correlates with HBV viral DNA load.[56,57] Given this context, Li et al. evaluated the impact of neoadjuvant HBV antiviral therapy (AVT) on the incidence of MVI among HBV-positive HCC patients treated with curative hepatic resection. In this study, 2036 patients underwent surgery alone and 326 patients were treated with at least one type of AVT for more than 90 days prior to surgery. Compared with the non-AVT group, neoadjuvant AVT was associated with a reduced incidence of MVI (38.7% vs. 48.6%, $p = 0.001$). AVT was also associated with reduced 2-year HCC recurrence (38.5% vs. 52.3%, $p < 0.001$), whereas non-AVT patients were more likely to have multiple intrahepatic recurrences involving more liver segments.[58] Based on these findings, neoadjuvant AVT for HBV-associated HCC might be of value in preventing MVI formation and worse prognosis.

Adjuvant Therapy After Curative Resection for MVI-Positive HCC

With MVI-positive HCC there remains the risk of intrahepatic microscopic metastatic disease after curative surgical resection, which is evidenced by increased rates of early local recurrence and reduced progression-free survival after hepatic resection when compared with MVI-negative HCC. Therefore, adjuvant therapy might be particularly beneficial for this subgroup of patients.

In this regard, Sun et al. evaluated the survival impact of adjuvant TACE therapy among 322 patients with MVI-positive HCC treated with curative hepatic resection. Overall, 137 patients were administered TACE

therapy, which included infusion of doxorubicin, pirarubicin or pharmorubicin, and lipiodol 4 weeks after hepatectomy. They found that TACE was associated with significantly improved 5-year RFS (35% vs. 30%, $p = 0.012$) and OS (54% vs. 43%, $p = 0.006$).[59]

Wei et al. similarly evaluated the impact of adjuvant TACE among 250 patients with MVI-positive HCC treated with curative hepatic resection. Carboplatin, mitomycin, and lipiodol were infused via the hepatic artery 4–6 weeks after hepatectomy ($n = 125$). The median DFS (17 months vs. 9 months, $p = 0.02$) and OS (44 months vs. 22 months, $p = 0.029$) were significantly longer with adjuvant TACE therapy compared with surgery alone.[60]

In another study, Ye et al. evaluated 519 patients with early- or intermediate-stage HCC treated with curative surgical resection. Overall, 260 patients were found to have MVI on postoperative histologic analysis; 158 patients (72 MVI-negative, 86 MVI-positive) were treated with adjuvant TACE at 1, 3, and 6 months after hepatectomy with infusion of lobaplatin, raltitrexed, and lipiodol. The authors found that adjuvant TACE had no impact on survival among patients with MVI-negative HCC, however, there were significant improvements in RFS and OS with adjuvant TACE therapy among patients with MVI-positive HCC (3-year OS 67.5% vs. 53.9%, $p = 0.019$).[61]

Similarly, Liu et al. evaluated 117 patients with HCC treated with curative hepatic resection, of whom 62 patients were also treated with adjuvant TACE at 1, 3, 6, and 12 months postoperatively with infusion of epirubicin, oxaliplatin, and fluorouracil. They found that adjuvant TACE therapy was associated with significantly improved 1-year tumor-free survival among MVI-positive HCC patients ($n = 50$; 12.5% vs. 42.3%, $p = 0.02$), although no difference in survival was observed among MVI-negative HCC patients.[62]

Lastly, Wang et al. evaluated the therapeutic benefit of adjuvant TACE (lipiodol, fluorouracil, and epirubicin or pharmorubicin) in patients with MVI-positive HCC treated with curative hepatectomy. They also observed significantly longer DFS and OS among patients treated with TACE, however only for patients beyond the Milan criteria.[63]

Taken together, these studies indicate that the presence of MVI, in addition to other factors such as tumor burden, might be a useful indication for adjuvant transarterial chemotherapy after hepatic resection for HCC.

TRANSPLANTATION FOR HCC: IMPLICATIONS OF MVI STATUS ON INDICATIONS AND PROGNOSIS

Liver transplantation offers the most extensive form of local disease control; however, recurrence still remains an issue in 10–20% of cases, caused by a mix of allograft recurrence, intra-abdominal lymph node metastases, and distant metastases.[64] Given the high risk and cost of this procedure, coupled with the limited supply of allografts, there is great incentive to allocate organs to patients with the best opportunity for long-term survival. Accordingly, multiple research teams have retrospectively evaluated liver transplant outcomes for HCC in order to determine biomarkers that predict recurrence and survival. Historic benchmarks for prognosis have been tumor size and number, although incorporation of biological markers such as grade, satellitosis, and MVI has greatly improved prognostic accuracy.[65] Recently, Mehta et al. developed a novel prognostic index call the Risk Estimation of Tumor Recurrence After Transplant (RETREAT), which is based on three tumor features: AFP level at transplant, MVI status, and the sum of the largest viable tumor plus the number of tumors at explant.[66] RETREAT was subsequently validated using the United Network for Organ Sharing (UNOS) database of 3276 patients with HCC who met the Milan criteria and were transplanted between the years 2012 and 2014.[67] The authors found that RETREAT score directly correlated with both survival and recurrence. Specifically, survival rates at 3 years post-transplant were 91, 80, and 58% for RETREAT scores of 0, 3, and ≥ 5, respectively. Similarly, recurrence rates at 3 years post-transplant were 1.6% for a score of 0 and 29% for a score of ≥ 5. Importantly, RETREAT was significantly more accurate at predicting recurrence after transplant compared with the Milan criteria, with the latter only accounting for tumor size and number.

Although MVI has been found to be one of the most significant predictors of tumor recurrence and death after transplant for HCC, transplant nonetheless provides the best chance for cure, particularly for high-risk patients, raising the question whether upfront transplant for HCC provides superior outcomes compared with resection followed by salvage transplant for recurrence.[68,69] Bhangui et al. performed an intention-to-treat analysis comparing both approaches for early-stage HCC.[70] Primary liver transplant ($n = 340$) was associated with superior 10-year DFS (61% vs. 21%, $p = 0.0007$) and OS (63% vs. 35%, $p < 0.0001$) compared with liver resection ($n = 130$). Of the liver resection patients, 90 recurred, although only 31 met the criteria for liver transplantation. MVI was independently predictive for both OS and DFS. Interestingly, when measured from the time of transplant, there was no

difference in long-term outcomes for recurrence and survival between primary versus salvage transplant. Taken together, these findings indicate that upfront transplant provides superior outcomes compared with liver resection, primarily due to the high dropout rate of patients who are not candidates for salvage transplant at the time of recurrence. However, for the minority of patients able to undergo salvage transplant, outcomes are similar to those for upfront transplant. Bhangui et al. concluded that in the setting of allograft scarcity, it is not possible to provide all HCC patients with a transplant. However, there is the potential for optimizing the selection of HCC patients who should be treated with upfront transplant, given that approximately 30% of patients treated with resection experience long-term cure and therefore would not require transplant, while other patients experience rapid recurrence and early exclusion from transplant criteria, and may therefore have been better treated with upfront transplant. In this regard, the authors invoke MVI as one potential selection criteria for early transplant among HCC patients.

Conversely, others have argued that MVI should be a contraindication to liver transplant as it represents a poor use of allografts. By this reasoning, although transplant for MVI-positive HCCs provides the best chance for cure among oncologic treatment options, it is nonetheless associated with inferior outcomes compared with other indications for transplant. In the current clinical landscape, a subset of patients with MVI-positive HCCs will inevitably be transplanted as first-line therapy due to the lack of an available method for preoperative MVI screening. However, in the event that a reliable non-invasive method for MVI detection becomes available, the presence of MVI may represent a contraindication to transplant by virtue of the associated inferior survival.

Finally, multiple forms of inclusion criteria beyond the Milan criteria are increasingly used for liver transplantation in the modern era. Outcomes among these patients are quite variable, with some experiencing excellent survival similar to patients who meet the Milan criteria, while others do quite poorly, such that transplant may have been futile. Sapisochin et al. recently compared survival outcomes after liver transplantation for HCC for 86 patients with HCC beyond the Milan criteria who still met the extended Toronto criteria (no number or size limit, must not have systemic symptoms, biopsy of largest tumor nodule must not show poor differentiation) with 124 patients who met the Milan criteria.[71] There was no difference in survival up to 5 years post-transplant (beyond the Milan criteria 69% vs. Milan criteria 78%, $p = 0.3$). By precluding poorly differentiated lesions with the extended Toronto criteria, there is presumably a lower likelihood of transplanting patients with MVI and intrahepatic metastases. In contrast, Mazzaferro et al. evaluated 1112 patients transplanted outside of Milan in search of prognostic markers for recurrence and survival.[6] In their study, the median largest nodule size was 4 cm and the median number of tumors was four. They found that the presence of MVI doubled all HRs irrespective of size or tumor number (33% 5-year OS), while patients who were MVI-negative and met the up-to-seven criteria (seven as the sum of the size of the largest tumor in centimeters plus the number of tumors), experienced survival outcomes similar to patients within the Milan criteria (71.2% 5-year OS). Based on these observations, it has become clear that in appropriately selected patients, transplantation for HCCs beyond the Milan criteria is a reasonable and life-saving intervention; however, transplantation for MVI-positive HCCs beyond the Milan criteria is associated with poor outcomes and should be avoided.[72]

FUTURE DIRECTIONS FOR NON-INVASIVE MVI DETECTION

Knowledge of MVI status at the time of HCC diagnosis would both help physicians make more informed management decisions and improve prognostication. However, currently, MVI can only be reliably determined by histopathologic evaluation of surgical specimens obtained from resection or liver explantation, thereby limiting its clinical utility. Core needle biopsy is hampered by the lack of sensitivity for MVI, intratumoral heterogeneity, and sampling error. There are no readily available non-invasive markers for MVI.[73] Consequently, there is a critical unmet need for a reliable non-invasive method for preoperative detection of MVI.

In this regard, one novel approach towards non-invasive MVI detection is the use of axial imaging, which has the advantages of lower risk to the patient compared with biopsy, and allows for visualization of the entire tumor and liver, providing a more complete assessment of disease burden. Segal et al. have recently shown that certain prognostic HCC gene signatures can be correlated to radiographic phenotypes using contrast-enhanced computed tomography (CECT).[74] Building on this work, Banerjee et al. proposed imaging criteria that were 76% sensitive and 94% specific for the detection of MVI by CECT in a validation cohort.[75] Radiographic features predictive of MVI included persistent arterial tumor enhancement during the venous phase, absence of a rim of hypoattenuation surrounding the tumor, and absence of a clear difference in attenuation between the tumor and surrounding hepatic parenchyma. Using this technique, they were able to accurately differentiate prognosis among similarly staged lesions [American Joint Committee on Cancer (AJCC) stage II], as well as for tumors < 3 cm.[75]

Other studies have also corroborated an infiltrative or non-smooth margin as an independent predictor of MVI on axial imaging.[76,77] Lee et al. preoperatively imaged 197 patients with HCCs $\leq$ 5 cm using magnetic resonance imaging (MRI) with the hepatobiliary contrast agent gadoxetic acid.[78] They found the following imaging findings to independently associate with MVI: arterial peritumoral enhancement, non-smooth tumor margin, and peritumoral hypointensity on the hepatobiliary phase. When at least two of three findings were present, the specificity for the presence of MVI was 92.5%, while three of three findings indicated 99.3% specificity. In addition, Taouli et al. performed a study in which they were able to demonstrate a correlation between an infiltrative HCC pattern on either CECT or MRI to genetic signatures of poor prognosis.[79]

CT quantitative imaging analysis is a relatively new technology in which pixelated variation in enhancement patterns can be measured for a region of interest and potentially correlated to biologic traits of the imaged tissue. Zheng et al. recently used this technique to measure HCC enhancement variation in 120 patients, which they correlated to MVI status.[80] They found that for HCCs $<$ 5 cm in diameter, standard clinical and radiographic markers were non-predictive for the presence of MVI. However, quantitative features based on the angle co-occurrence matrix had a positive predictive value of 63% and negative predictive value of 85%. In patients with tumors $>$ 5 cm, AFP, tumor size, and quantitative CT features were predictive of MVI. They created a multivariable model combining CT quantitative features with other predictive markers for MVI that had a positive predictive value of 72% and negative predictive value of 96%. Although this technology may not be ready for mainstream clinical use, imaging quantitative analytic techniques are early in development and may have utility going forward for non-invasively characterizing tumor biology. Lastly, multiple groups are now working to develop novel HCC-specific molecular imaging probes, which may have the potential to further improve the accuracy of MVI detection on axial imaging.[81–84]

In addition to axial imaging, others have also evaluated serum and tissue markers for their association with MVI status. Schlichtemeier et al. reviewed their series of 125 patients with surgically resected HCCs, in which 53 (42%) patients had MVI present.[85] The authors found that age $\geq$ 64 years, AFP $\geq$ 400 ng/ml, and tumor diameter $\geq$ 5.0 cm were significantly associated with MVI on univariate analysis. However, a logistic regression model created with these variables only had a sensitivity of 60% and specificity of 74% for the prediction of MVI, which is insufficient for mainstream clinical application. Hirokawa et al. evaluated 167 patients with solitary HCCs and found that protein-induced vitamin K antagonist-II (PIVKA-II) $>$ 150 mAU/mL and AFP-L3 positivity were independently associated with MVI on multivariable analysis.[86] More recently, Yu et al. evaluated 157 patients with HCCs $<$ 5 cm in diameter, and found that the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), two laboratory-based markers of systemic inflammation, were independently associated with MVI on multivariable analysis.[87] Some research teams have also looked to gene expression analyses for MVI prediction. Liu et al. evaluated serum of 108 patients with HCC, and found that presence of microRNA 125b (miR-125b) and elevated AFP, as well as increased tumor size, were independently associated with MVI on multivariable analysis.[88] In combination, these variables had a combined predictive capacity of 86% by receiver operator curve analysis, with a sensitivity of 84% and specificity of 72%. Finally, Minguez et al. performed transcriptomic analysis of HCC tissue from 214 patients, from which they developed a 35-gene signature that had a negative predictive value of 77% for MVI detection.[89]

Although many of the approaches cited above are promising, at present it appears that serum or radiographic markers alone are unlikely to have sufficient statistical accuracy for reliable MVI detection.[90] Therefore, several research teams have attempted to create multivariable nomograms with stronger predictive capacity by combining axial imaging patterns with laboratory and clinicopathologic data. Lei et al. used this approach for 1004 patients treated with surgical resection for HBV-related HCC. On multivariable analysis, they found seven variables highly correlated with MVI: tumor diameter, multiple nodules, poorly formed capsule, AFP $>$ 20 ng/mL, platelet count $<$ 100,000 per microliter, HBV DNA load $>$ 1000 IU/mL, and presence of a typical dynamic enhancement pattern of MRI. Combining these features, they constructed a nomogram with a negative predictive value of 83.2%, allowing for identification of low-risk MVI patients.[57]

Cucchetti et al. developed a weighted algorithm with output-based feedback called an artificial neural network (ANN) to predict HCC tumor grade and MVI status based on serum AFP and axial imaging findings, including tumor volume, number of nodules, and diameter of the largest nodule. They analyzed 250 HCC specimens and showed that their ANN had an accuracy of 93% in identifying tumor grade, and 91% accuracy in detecting MVI.[91] This study represents one of the first examples of applying computer learning to clinical evaluation of HCCs.

A simpler but nonetheless useful approach is to set risk thresholds. Kobayashi et al. utilized preoperative positron emission tomography/CT (PET/CT) coupled with serum AFP-L3 to predict MVI in 60 patients with HCCs $<$ 3 cm.

They found that a standardized uptake value (SUV) > 3.2 and an AFP-L3 level ≥ 19% had a combined sensitivity and specificity of 88.9% and 82.4%, respectively, for the prediction of MVI.[92] Shirabe et al. employed a similar approach, using a combinatorial prediction schema of tumor size > 3.6 cm, serum DCP level > 101 mAU/mL, and SUV on PET/CT > 4.2, which had a sensitivity and specificity for the prediction of MVI of 100% and 90.9%, respectively, in a cohort of 34 patients.[93]

Most of these studies were validated using small sample sizes and will need to be investigated in larger trials before wider application. As imaging technologies continue to improve, implementation of axial imaging criteria based on CT, MRI, and PET/CT seem to hold the most immediate promise of providing a means to preoperatively discern MVI. In addition, advances in artificial intelligence and the search for novel serum biomarkers also provide hopeful avenues for improving preoperative, non-invasive MVI detection.

CONCLUSIONS

MVI is defined by the presence of micrometastatic HCC emboli within the vessels of the liver. Multiple retrospective trials have shown that the presence of MVI is a critical determinant of recurrence after surgical resection and liver transplant. MVI highly associates with adverse biological features, and has been shown to be a more accurate predictor of long-term survival than conventional staging criteria. Unfortunately, MVI can only be determined based on postoperative surgical specimens, limiting its potential value in guiding personalized treatment. Molecular and imaging technologies hold promise as novel methods for reliable, non-invasive detection of MVI, which could have a paradigm-shifting, beneficial impact on the management of HCC.

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