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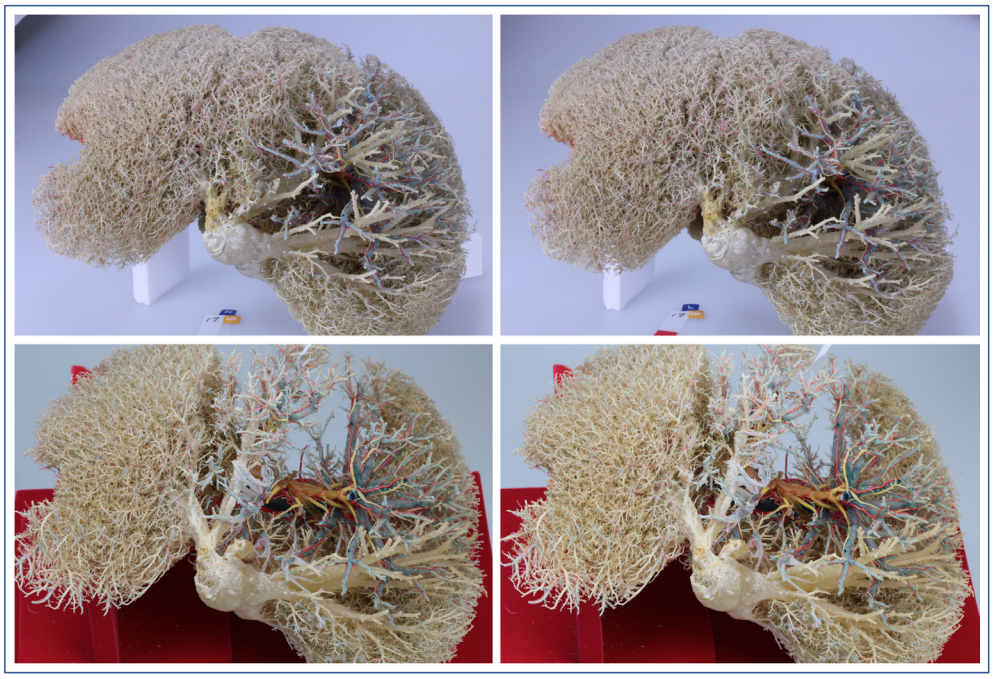

Cranial view of the liver cast and the bifurcation of the portal vein after complete removal of the caudate branches. PAGE 328-336

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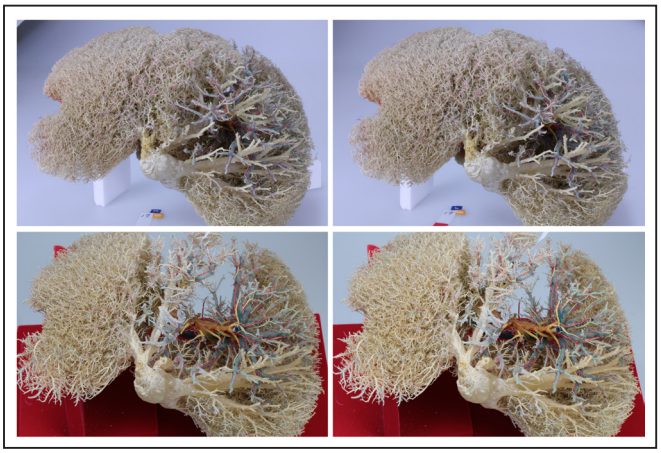

Cranial view of the accomplished liver cast and view of the bifurcation of the portal vein after complete removal of the caudate branches. (PAGE 328-336)

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EDITORIAL
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What liver surgeons have achieved in the recent decade for patients with hepatocellular carcinoma?

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Abstract: In the past decade, there has been remarkable progress in surgical treatment for hepatocellular carcinoma (HCC) based on evidence created by epoch-making prospective trials or national registry big data analysis. A head-to-head randomized controlled trial comparing liver resection and local ablation for small oligo HCCs (SURF trial) demonstrated comparable recurrence-free survival provided both modalities are feasible. Survival benefit of liver resection for HCC with vascular invasion was demonstrated by two propensity scored matched analyses based on Japanese national data. Furthermore, expanded HCC criteria for living donor liver transplantation were developed based on Japanese national data, and this "5-5-500 rule" was accepted by the social insurance system in Japan. The recent remarkable progress in promising new anti-HCC agents may open the door for effective neoadjuvant or adjuvant treatment in combination with surgery.

***Keywords*:** hepatocellular carcinoma, liver resection, vascular invasion, living donor transplantation, molecular targeted agents, immune-check point inhibitors

In the past decade, there has been remarkable progress in surgical treatment of hepatocellular carcinoma (HCC) based on evidence created by epoch-making prospective trials or national registry big data analysis. Here we focus on three recent major developments from Japan: *i*) a head-to-head randomized controlled trial comparing liver resection and local ablation, *ii*) survival benefit of liver resection for HCCs with vascular invasion, and *iii*) expanded HCC criteria for living donor liver transplantation. Future outlook of combining surgery with promising new anti-HCC agents are also discussed.

Role of liver resection for small oligo HCCs

Although both liver resection and local ablation (radiofrequency ablation: RFA) are considered potentially curative treatments for small oligo HCCs, retrospective studies have suggested better local tumor control by liver resection (*1,2*). There have been at least 5 randomized controlled trials (RCTs) conducted to compare liver resection and RFA (*3-7*, Table 1). Most of the previous studies were reports from mainland China, Hong Kong, or Taiwan where hepatitis B is a major etiology. Three of the studies failed to show the benefits of liver resection over RFA for patients with small oligo HCCs on the long-term outcome in terms of recurrence free survival (RFS) nor overall survival (OS), and only one reported a significantly better outcome for surgery (*5*). Since inclusion criteria for the latter study was within Milan criteria, RFA for tumors over 3 cm may have inferior local control which may have affected the outcome of the RFA arm. In general, patient numbers for the previous studies were relatively small and could be under-powered.

Since 2008, a similar head-to-head multicenter study called SURF trial (Comparison between SUrgery and RFA) has been conducted in Japan. Inclusion criteria were primary HCC ≤ 3 cm in diameter with ≤ 3 nodules. Liver function should be ≤ Child-Pugh 7. Before randomization, patient condition and tumor location were reviewed by both surgeons and hepatologists to check the feasibility of liver resection and RFA. Once informed consent was obtained, patients were randomized with stratification by trial site, age, HCV infection, tumor number, and size. Primary co-endpoints were RFS and OS. Although the targeted patient number (n = 600) was not achieved, 308 cases were registered, which is larger than any of the previous trials (Table 1). Surgical resection and RFA were both safe therapeutic approaches and both of them provided similar RFS after a 3-year follow-up period (*7*). It would be safe to conclude curability for small oligo HCCs is similar between liver resection and RFA, however, technical feasibility of RFA in terms of proximity to major vessels should be carefully evaluated before selecting the optimal treatment option for each patient.

Table 1. Randomized clinical trials comparing resection and RFA for small oligo HCCs

Author (*Ref.*)	Year	Sites	Size	Tumor No.	Child-Pugh	Patient No.	Conclusion
Huang, *et al*. (*3*)	2005	Taiwan	≤ 3 cm	≤ 2	A,B	76	N.S.
Chen, *et al*. (*4*)	2006	Hong Kong, Guangzhou	≤ 5 cm	1	A	180	N.S.
Huang, *et al*. (*5*)	2010	Chengdu	≤ 3 cm (Milan criteria)	≤ 3	A,B	230	Favor SUR
Feng, *et al*. (*6*)	2012	Chongqing, Ji'nan	≤ 4 cm	≤ 2	A,B	168	N.S.
Izumi, *et al*. (*7*)	2019	Japan 118 sites	≤ 3 cm	≤ 3	A,B	308	N.S.

N.S.: not significant, SUR: surgery.

Surgery for vascular resection

HCCs with vascular invasion are considered as very advanced stage and liver resection is not recommended in treatment guidelines in Western countries (*8,9*). In Asian countries, liver resection has been attempted for selected cases and is recommended in APSL (*10*) and Japanese guidelines (*11*) as long as it's technically feasible. However, there have been no randomized controlled trials or even large-scale registry data analysis to address this issue. Recently, propensity score analyses using Japanese national registry data were conducted to investigate the survival benefit of liver resection for HCC patients with vascular invasion in portal vein (PVTT) or hepatic vein (HVTT) (*12,13*).

Data for 6,474 HCC patients with PVTT registered between 2000 and 2007 were analyzed. Of these patients, 2,093 who underwent liver resection (LR) and 4,381 who received other treatments were compared. The median survival time (MST) of the LR group was 1.93 years longer than that of the non-LR group (2.74 years *vs*. 0.81 years; $p < 0.001$) and 1.03 years longer than the non-LR group (2.41 years *vs*. 1.38 years; $p < 0.001$) in a propensity score-matched cohort (*12*). Similarly, data for 1,021 Child-Pugh A HCC patients with HVTT without inferior vena cava invasion were analyzed. The median survival time of the LR group ($n = 540$) was 2.89 years longer than that of the non-LR group ($n = 481$, 4.47 *vs*. 1.58 years, $p < 0.001$) and 1.61 years longer than the non-LR group (3.42 *vs*. 1.81 years, $p = 0.023$) in a propensity score-matched cohort (*13*). These studies provide a second best level of evidence for this clinical question. The randomized controlled study may not be feasible for the patient population due to heterogeneity of the disease and technical difficulty.

Expanded criteria

Since 1994, Milan criteria have been the gold standard for selecting HCC patients for successful liver transplantation (*14*), however, these criteria are too strict and expansion of indication criteria has long been debated. Due to a very severe scarcity of deceased donors in Japan, living donor liver transplantation (LDLT) has been a mainstay in this setting. Table 2 shows a list of expanded criteria proposed by Japanese centers. Exclusion of HCC with vascular invasion and extrahepatic disease was consistent among all of the expanded criteria. The University of Tokyo proposed a so-called 5-5 criteria (≤ 5 nodules, ≤ 5 cm in diameter) (*15*), and Kyoto University group proposed ≤ 10 nodules, ≤ 5 cm in diameter, and des-gamma-carboxy prothrombin (DCP) ≤ 400 mAU/mL (*16*). Kyushu University group also proposed their own expanded criteria: no limitation in tumor number, ≤ 5 cm in diameter, or DCP ≤ 300 mAU/mL (*17*). They reported non-inferior long-term outcome for patients fulfilling their expanded criteria compared to that for Milan criteria.

Table 2. Expanded LDLT Criteria for HCC in Japan*

Institution (*Ref.*)	Number	Size (cm)	Tumor marker
Tokyo Univ. (*15*)	≤ 5	≤ 5	Any
Kyoto Univ. (*16*)	≤ 10	≤ 5	DCP ≤ 400
Kyushu Univ. after 2007 (*17*)	Any	≤ 5	or DCP ≤ 300
Kyoto Univ. before 2006 (*16*)	Any	Any	Any
All-Japan (*18*)	≤ 5	≤ 5	AFP ≤ 500

*Exclusion of HCC with vascular invasion and extrahepatic disease is consistent among all of the expanded criteria. AFP: alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin.

Recently, new expanded criteria were proposed by the Japanese Liver Transplantation Society based on a retrospective data analysis of the Japanese nationwide survey. A total of 965 HCC patients undergoing LDLT were included, and 301 (31%) were beyond the Milan criteria. The Greenwood formula was applied to investigate new criteria, which enabled a maximal enrollment of candidates while securing a 5-year recurrence rate below 10%, by examining various combinations of tumor numbers and serum alpha-fetoprotein values, and maintaining the maximal nodule diameter at 5 cm. After thorough statistical scrutiny, new expanded criteria for LDLT candidates with HCC, the "5-5-500 rule" (nodule size ≤ 5 cm in diameter, nodule number ≤ 5, and alfa-fetoprotein value ≤ 500 ng/mL), were established as a new condition with a 95% confidence interval of a 5-year recurrence rate of 7.3%. These criteria expanded the eligible patient pool by 19% (*18*). In 2019, the "5-5-500 rule" was applied as inclusion criteria for listing HCC patients by the Japanese Organ Sharing System. This rule was also accepted for Japanese Social Insurance Coverage for LDLT in 2020.

Table 3. Randomized controlled trials on adjuvant treatment after curative treatment for HCC

Intervention (*Ref.*)	Title of the Study	Registry code	Recruitment	Result
Uracil-Tegafur (*21*)	–	UMIN C000000445	Aug. 1997-2002	N.S.
Sorafenib (*22*)	STORM	NCT00692770	Aug. 2008-Nov. 2014	N.S.
Peretinoin (*23*)	NIK-333 phase 2/3	JapicCTI-060250	Feb. 2005-Dec.2009	N.S.
Nivolumab	ONO-4538-70	NCT03383458	Dec. 2017-	Ongoing
Duravalumab ± Bevacizumab	EMERALD-2	NCT03847428	April 2019-	Ongoing
Atezolizumab + Bavacizumab (*24*)	IMbrave 050	NCT04102098	Dec. 2019-	Ongoing

N.S.: not significant.

Future surgery and new drugs

Since the introduction of sorafenib in 2007 (*19*), there has been tremendous progress in molecular targeted drug or immuno-checkpoint inhibitors for advanced HCC. A combination of liver resection and advanced drug therapy may work in two ways: adjuvant therapy after curative resection and neoadjuvant or conversion therapy for initially unresectable HCC. The 5-year recurrence rate is known to be as high as 70-80% even after curative resection (*20*), and there has been a number of adjuvant treatments including Uracil-Tegafur (*21*), sorafenib (*22*), and peretinoin (*23*) to reduce tumor recurrence, but without success (Table 3). Following introduction of immuno-checkpoint inhibitors, there have been at least 3 randomized trials, which are still ongoing, to test adjuvant therapy using these agents (*24*, Table 3). Results of these trials are expected to be available within a few years.

Initial response rate (RR) of the first molecular targeted drug, sorafenib, was only 3% and strategy of conversion surgery was not feasible with such a low RR (*19*). RR of the second approved 1st line agent Lenvatinib jumped up to 24% (*25*), and more recent combination therapies demonstrated RR at around 30-40%. Currently, a few prospective studies for neoajuvant therapy are ongoing with results expected soon.

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Clinical implications of WNT/β-catenin signaling for hepatocellular carcinoma

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Abstract: Immune checkpoint inhibitors have entered clinical practice for the treatment of hepatocellular carcinoma (HCC). Several previous studies for other cancers have revealed that tumor mutation burden, tumor PD-L1 expression and cytotoxic T-cell infiltration are predictive of treatment response. The genetic analysis of HCC has shown that β-catenin mutation might be a biomarker predicting the poor response against immune checkpoint inhibitors. β-catenin is a transcription factor downstream of WNT signaling and somatic mutations of this gene are the third most common in HCC. WNT signaling is an important signal for organogenesis and is also involved in the maintenance of stem cells in several organs. Recently, clinical and basic studies have shown the specific roles of WNT/β-catenin signaling in many aspects of hepatic function and carcinogenesis including metabolic zonation and inflammation, and sub-classification and radiologic features of HCC. Base on the review on the recent advances of research investigating WNT/β-catenin signaling associated with hepatocytes, we speculate the clinical role of this signal on the immunotherapy for HCC, which suggests that an era of genetic mutation profiles may be coming to add to the HCC treatment algorithm.

***Keywords*:** hepatocellular carcinoma (HCC), immune checkpoint inhibitor, WNT, β-catenin

Immune checkpoint inhibitors have entered clinical practice for the treatment of hepatocellular carcinoma (HCC): first-line trials of nivolumab (*1*) and second-line trials of pembrolizumab (*2*) initially showed promise of efficacy, but neither of these trials failed to demonstrate statistical efficacy. However, in 2020, the combination of the PD-L1 antibody atezolizumab with the angiogenesis inhibitor VEGF antibody, bevacizumab, showed a significant overall survival benefit in a trial comparing it to sorafenib, which has long played an important role in the treatment of HCC as a first-line treatment (*3*).

Treatment of other cancers with immune checkpoint inhibitors is controversial because they are very effective in 20-30% of cases and can reduce progression of disease over time, but have little or no effect in some cases. Because of the high cost of the drug, it is required to discover the predictive factor to narrow down the list of patients who might benefit from treatment. The best known predictor of response is the tumor mutation burden (*4*). Mutation-produced neo-antigens are thought to induce active tumor immunity. Pembrolizumab has been used in patients with MSI-high mutations due to deterioration of mismatch repair enzymes, regardless of the type of cancer. In addition to tumor mutation burden, tumor PD-L1 expression and cytotoxic T-cell infiltration into the tumor tissue may also be predictive of treatment response (*5*).

The recent genetic analysis of HCC has shown that β-catenin mutation might be a biomarker predicting the poor response against immune checkpoint inhibitors. As WNT/β-catenin signaling has been studied as an important signal for hepatic organogenesis and carcinogenesis, we review recent basic and clinical findings on this signal as a factor that may be relevant to immunotherapy, which is coming soon against advanced HCC.

β-catenin mutation in HCC

Harding *et al*. performed a clinical sequence of 127 patients with HCC treated with molecularly targeted therapy using NGS. Of the 31 patients treated with immune checkpoint inhibitors, those with β-catenin mutations had a significantly lower DCR (0% *vs*. 53%) and PFS (2.0 *vs*. 7.4 months) (*6*). The first report of β-catenin mutations in HCC was published in 1998, showing that 20-30% of patients carry a genetic mutation in exon 3, which contains a functionally repressive phosphorylation sequence, and that this mutation suppresses β-catenin degradation and leads to increased function (*7,8*). Comprehensive analysis by next generation sequence has confirmed that it is the third

most frequent genetic mutation after hTERT promoter and TP53 (*9*).

Binding of WNTs to the plasma membrane activates intracellular signaling and β-catenin translocates into the nucleus, where it activates the expression of target genes. In the absence of ligand binding, WNTs are localized in the vicinity of the plasma membrane to form a complex with APCs, which is phosphorylated and then degraded (*10*). It is well known that APC mutations are highly prevalent in colorectal cancer, but β-catenin mutations are more common in HCC.

WNT/β-catenin signaling in hepatic zonation

WNT signaling has been reported to play an important role in the maintenance of metabolic zonation in hepatic lobes and the formation of liver tissue. It is known that WNT/β-catenin signaling is activated in zone 3 around the central vein, and GS (Glutamine synthetase), which is often used as a marker of zone 3, is a representative target gene for this signal, and many metabolic genes involved in hepatocyte function are among its targets. On the other hands, hepatocytes located in periportal area (zone 1) are initially exposed to nutrients and oxygen supplied from portal veins and hepatic arteries, as well as bacterial and viral pathogens (*11*). Hepatic zonation is also critical for hepatic regeneration, and the discussion about location of hepatocyte stem cells has been at the center of the debate. Although hepatocyte stem cells have long been thought to be oval cells in the canals of Hering near zone 1, recent research using lineage-tracing experiments of mice has raised the possible liver progenitors of Axin2-positive cells in zone 3 (*12*). Axin2 is a factor associated with WNT/β-catenin signaling, and it has been shown that WNT ligands from endothelial cells of central vein may be involved in zonation and stem cell maintenance. Recent studies suggest that, while oval cells may be the source of regenerative hepatocytes during liver injury, pericentral cells may be the source of regeneration in the absence of liver injury (*13*).

WNT/β-catenin signaling in sub-classification and clinical features of HCC

Because the constitutively activation of WNT/β-catenin signaling in HCC confers distinct characteristics, HCC with β-catenin mutation is sub-classified as a single cluster by gene expression profiles. Its clinical characteristics include relatively slow progression and good prognosis. In addition, β-catenin mutation may associate with interesting radiologic feature in clinical settings (*14*). Gadoxetic acid-enhanced magnetic resonance imaging (EOB-MRI) are frequently applied to HCC high-risk patients because of the high detectability. In this imaging modality, most of HCC show the decreased uptake of EOB comparing with normal liver tissue in the hepatobiliary phase, because the expression of the transporter, OATP1B3 decreases in cancer cells. Rather, a small portion of HCC nodules are reported to uptake more EOB. According to expression and mutation analysis, cancers with β-catenin mutation increase EOB uptake (*15,16*), and especially increased uptake prove to be due to activation of both HNF4a and β-catenin (*17*).

WNT/β-catenin signaling associated immunosuppressive phenotype

RNA seq data have been used to classify HCC by gene expression signature involved in immunity. Fujita *et al*. classified them into four groups, tumor-associated macrophage (TAM), β-catenin, cytolytic activity (CYT), and regulatory T cells (Treg) (*18*). Shimada *et al*. also divided them to three groups, including mitogenic and stem cell-like tumors with chromosomal instability, β-catenin-mutated tumors displaying immune suppression, metabolic disease-associated tumors (*19*). Both reports classified into a single cluster of HCC with β-catenin mutations as immunosuppressive phenotype. Expression analysis showed less infiltration of immune cells, suggesting that the tumors are immunologically cold and may be related to immune checkpoint inhibitor responsiveness. Expression analysis has also been performed in cancers other than HCC, and analysis of expression data for 31 solid tumors from The Cancer Genome Atlas (TCGA) showed that activation of β-catenin was inversely correlated with the expression signature of T-cell-inflamed tumors (*20*). This has been examined in detail in basic studies in malignant melanoma. Malignant melanoma, the earliest cancer type for which immune checkpoint inhibitors were used clinically (*21*), has also been found in some of these tumors with β-catenin mutations (*22*). In a mouse model of carcinogenesis expressing knock-in activated β-catenin, there was less infiltration of T cells and less response to immune checkpoint inhibitors (*23*). Similarly, in a mouse model of HCC, β-catenin-activated cancers canceled the therapeutic effect of PD-1 antibodies (*24*). These basic findings may explain the clinical investigation in HCC.

An era of genetic mutation profiles coming to add to the HCC treatment algorithm

WNT/β-catenin is activated in normal hepatocyte around central vein in hepatic lobule and some proportion of cancer cells. In contrast, periportal area, which is more likely to be exposed to external pathogens such as bacteria or virus, may be prepared to induce inflammatory and immune cells, which is easily recalled by the pathological findings that inflammation is located in this area in the cases of viral hepatitis (Figure 1). In addition, inflammatory cells in tumor tissue are often involved in the invasion of cancer, and it is possible that such cancers

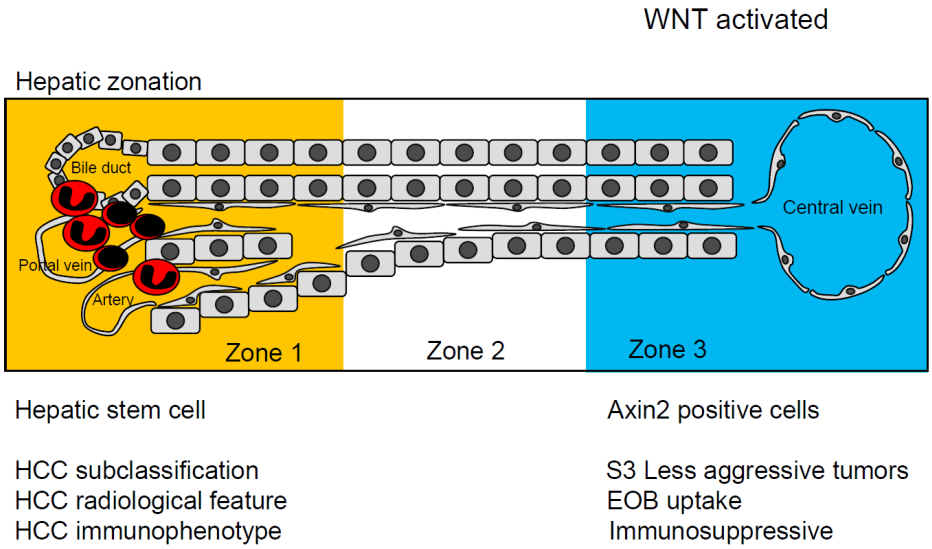

Figure 1. Basic and clinical features associated with WNT activation.

are more aggressive and have a worse prognosis. On the other hand, immune checkpoint inhibitors may be more effective in cancer tissues with abundant inflammatory cell infiltration. If immune checkpoint inhibitors are less effective in β-catenin mutated cancers that are less invasive and have a relatively good prognosis, then the therapeutic indications for local treatment, such as resection or ablation, may be expanded in these cancers. This suggests that an era of genetic mutation profiles may be coming to add to the HCC treatment algorithm.

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Hepatocyte ploidy and pathological mutations in hepatocellular carcinoma: impact on oncogenesis and therapeutics

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Abstract: Hepatocellular carcinoma (HCC) occurs in the chronic liver inflammation such as viral hepatitis, alcoholic and non-alcoholic steatohepatitis. While anti-viral treatment has been significantly improved, the prevalence of HCC remains high and treatment is still challenging. The continuation of hepatocyte death, inflammation, and fibrosis leads to the accumulation of gene alterations, which may trigger carcinogenesis. Hepatocytes are a unique cell type having more than one complete set of 23 chromosomes, termed polyploidy. Due to gene redundancy, hepatocytes may tolerate lethal mutations. Next generation sequencing technology has revealed gene alterations in HCC related to telomere maintenance, Wnt/β-catenin pathway, p53 cell-cycle pathway, epigenetic modifiers, oxidative stress pathway, PI3K/AKT/mTOR, and RAS/RAF/MAPK pathway with or without a chromosomal instability. Some type of driver gene mutations accumulates in hepatocytes and breaks the orchestration of excessive copies of chromosomes, which may lead to unfavorable gene expressions and fuel tumorigenesis. Recently, molecular targeted drugs, developed with the aim of interfering with these signaling pathways, are being used for HCC patients in the clinics. Therefore, a deeper understanding of hepatocyte ploidy and genetic or epigenetic alterations is indispensable for the establishment of novel therapeutic strategies against HCC.

***Keywords*:** hepatocellular carcinoma (HCC), ploidy, mutation, next generation sequence, molecular targeted drug

Introduction

Hepatocellular carcinoma (HCC) is a primary liver tumor which is the fifth leading cause of cancer-related death in Japan. Most HCC is found in patients with liver cirrhosis or chronic liver injury, such as viral infection [hepatitis B virus (HBV), hepatitis C virus (HCV)], alcoholic injury, non-alcoholic fatty liver disease (NAFLD) and autoimmune diseases including primary biliary cholangitis and autoimmune hepatitis. WHO estimated that 53% of HCC occurrence is found in patients with HBV infection, and another 25% in patients with HCV infection. On the contrary, in Japan, approximately 65% of HCC cases were caused by HCV infection and 15% by HBV infection (*1*). The recent multi-institutional nationwide survey in Japan reported that the proportion of non-viral liver cirrhosis caused by alcohol intake or non-alcoholic steatohepatitis (NASH) has increased (*2*). To prevent from chronic injury in liver according to its local circumstances, measures against viral infections control of viral infection, and lifestyle modification including reduction of alcohol consumption, healthy diet and physical exercises are feasible for secondary prevention. Although the direct acting antiviral (DAA) therapy for HCV and the nucleotide analogs for HBV have been widely used, HCC is still one of the few neoplasms showing the greatest increase in mortality in the United States during the past two decades (*3*). Moreover, the recent systematic review, utilizing economic studies published for a decade from 2008, demonstrated that HCC incidence is approximately 100 times higher among patients with chronic hepatitis/cirrhosis and one third of them also diagnosed with advanced disease (*4*).

Chronic injury and inflammation stimulate proliferation of cells and accumulate gene mutations resulting in carcinogenesis, which occurs not only in hepatitis, but also in several inflammatory diseases including pancreatitis, colitis, esophagitis, cholangitis, and gastritis. Many researchers have aimed to elucidate the mutations that drive oncogenesis in the liver for decades. The recent development of next-generation sequencing (NGS) technology provides us with a better understanding of the mutational landscape during liver oncogenesis and the correlation with pathological appearances and clinical prognosis (*5,6*).

The fourth edition of guidelines for HCC treatment in Japan was recently published (*7*). The recommended treatments are determined by liver functional reserve, extrahepatic metastasis, vascular invasion, tumor number, and tumor size. Radiofrequency or microwave ablation, liver resection, or liver transplantation, all

potential curative therapies for HCC, should be the first-line treatments when the tumors are limited to the liver within an early stage criterion. For patients who are not candidates for these above treatments, locoregional treatments, including transarterial chemoembolization (TACE), transarterial radioembolization (TARE), and stereotactic body radiation (SBRT) are recommended. In the guideline, molecular targeted therapy is a newly added recommendation for HCC patients qualified as Child-Pugh A liver functional reserve with extrahepatic metastasis. Sorafenib is the first approved molecular targeted drug for advanced-stage or unresectable HCC which inhibits tyrosine kinase of VEGF receptors, PDGFR, and Raf kinases. The recent guidelines released by the American Association for the Study of Liver Diseases (AASLD), the European Association for the Study of the Liver and European Organization for Research and Treatment of Cancer (EASL-EORTC) , and the Japan Society of Hepatology (JSH) recommend sorafenib as a systemic therapy (*8,9*). Therapeutic developments are also being made in the field of systemic chemotherapy. Several clinical trials using other molecular targeted drugs including tyrosine kinase inhibitor and immune checkpoint inhibitor have been undertaken for HCC treatment. The molecular understanding of HCC may guide in developments of promising cancer therapy for patients with advanced stage HCC. Thus, a molecular understanding of HCC has become more important in clinical practice .

In this review, we briefly outline the recent research on HCC from three perspectives: *i*) biology of hepatocyte heterogeneity (especially ploidy) with adaptive and protective effects for injury and oncogenesis, *ii*) pathological mutations to allow HCC oncogenesis, and *iii*) its therapeutic implications.

Hepatocyte polyploidy and carcinogenesis

A characteristic appearance of hepatocytes is polyploidy, which is an increase in the number of chromosome sets per cell. A population of hepatocytes has two nuclei in one cell with a difference in DNA amount per nucleus. For instance, a tetraploid hepatocyte could have a mono-nucleated tetraploid (4N) nucleus or two bi-nucleated (2N+2N) diploid nuclei (Figure 1). The accumulation of chromosomes happens drastically around weaning and remains during ageing, mainly because of cytokinesis failure. Liver injury, such as surgical resection, toxic stimulation, metabolic iron and copper overload, telomere attrition, chronic viral infection with HBV and HCV, or oxidative stress has been reported to induce polyploidization (*10,11*). Additionally, many age-related diseases including arterial hypertension, hyperthyroidism, metabolic disorders and cancer have an association with polyploid accumulation (*11*). These findings have raised the question of whether the polyploidy of hepatocytes is beneficial or detrimental

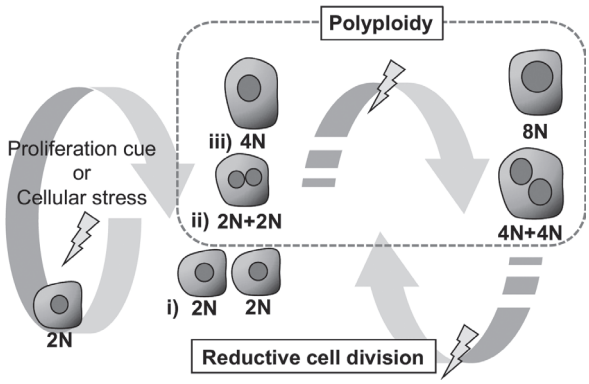

Figure 1. The polyploidization of hepatocyte and normal cell division. Under proliferative demands or cellular stresses, the hepatocyte undergoes duplication of its DNA and generates three different types of ploidy states: *i*) The conventional cell division generates two daughter cells with same DNA content of parent cell, *ii*) Cytokinesis failure results in bi-nucleated hepatocyte with same DNA content in each nucleus, and *iii*) Endoreplication generates mono-nucleated hepatocyte harboring double. Another cue of cell cycle drives accumulation of ploidy or even reductive cell division without DNA synthesis. N means number of haplotypes.

for homeostasis. Aneuploidy especially, including multiplication of complete sets of chromosomes and excessive or deficit parts of chromosomes (*e.g*. trisomy or monosomy), has been frequently identified in cancer cells and is regarded as a risk factor for chromosomal instability. The functions of polyploidy in the liver either as physiological conditions or pathological responses are still largely unknown.

Studies of plants demonstrate that polyploidy contributes to species diversity, which provides an evolutionary advantage in response to its environment (*12*). Mammalian polyploidy is rare and is limited to cardiomyocytes of the heart, trophoblasts of the placenta, megakaryocytes in bone marrow, acinar cells of the pancreas, and hepatocytes of liver under physiological circumstances. The idea that these polyploidy states contribute to cellular diversity which provides a selective advantage in response to injuries is plausible. Aneuploidy itself is not sufficient to generate neoplastic chromosomal instability (CIN), but modulates cellular metabolic and transcriptional programs, such as higher glucose and/or glutamine consumption, changes in proteins involved in the cell cycle, ribosome biogenesis, endoplasmic reticulum, Golgi apparatus, lysosomes, membrane metabolism, the major histocompatibility complex (MHC) proteins, and antigen processing (*13*). Polyploid cells like cardiomyocytes, megakaryocytes and trophoblast giant cells, have been accepted as terminal differentiated and functionally mature and considered less stem cell-like. Interestingly, Duncan *et al*. demonstrated that polyploid hepatocytes could contribute to regeneration through a "ploidy conveyor", in which proliferating polyploid hepatocytes generate a highly diverse population of daughter cells with multiple numerical chromosome

imbalances as well as uniparental origins (*14*). In the other study utilizing fumarylactate hydrolase (Fah) deficient mice, in which the liver is chronically injured by the accumulation of metabolite, hepatocytes with a heterozygous mutation of the homegentisic acid dioxygenase (Hgd) gene, upstream of Fah, show loss of the chromosome with the Hgd gene and escape from toxic metabolite synthesis (*13,15*). This phenomenon clearly demonstrates that the selection of specific aneuploid karyotypes can result in the adaptation of hepatocytes to chronic liver injury.

The impact of polyploidy on tumorigenesis has also been reported. Aneuploidy is mostly caused by deregulation of the spindle assembly checkpoint (SAC). Some mouse models with dysfunction of SAC genes show resistance to tumorigenesis (*13*). These findings support the beneficial role of excessive chromosomes. On the contrary, the study utilizing TP53 null mice, of which tetraploid mammary epithelial cells have more potent malignant tumor formation than diploid, suggested that polyploidy is a gateway to tumorigenesis (*16*). In terms of heterozygosity, multiple copies of a tumor suppressor gene allele would be protective against loss of heterozygosity (LOH) which leads to oncogenesis. Indeed, Zhu and his colleagues demonstrated that a transcriptional factor responsible for cell cycle, E2f8 knockdown in the liver resulted in diploid hepatocytes which became vulnerable to DNA damage by diethylnitrosamine (DEN), and developed liver tumors; while an F-actin binding protein responsible especially for cytokinesis, Anillin knockdown in the liver resulted in higher polyploidy hepatocytes which did not develop tumors (*17,18*).

A clinical study shows a reduced proportion of binucleated hepatocytes in non-tumor liver tissue adjacent to the HCC, and suggests that non-tumoral hepatocytes have a susceptible condition to LOH (*19*). Interestingly, a TP53 mutation has been seen in hyperploid hepatocytes or multinucleated hepatocytes in different studies (*5,19*). Cytokinesis failure and tetraploidization can activate the Hippo tumor suppressor pathway *via* extra centrosomes, which resulted in p53 stabilization and inhibition of cell growth when p53 was intact (*20*). Taken together, polyploid status appears to be protective against oncogenesis until TP53 is disrupted, in which case it becomes promotive toward oncogenesis thereafter. These evidence remind us that the combination of mutations would result in different outcomes. Therefore, a comprehensive understanding for HCC oncogenesis based on the landscape of gene mutations is crucial for treatment.

Genetic alterations in liver cirrhosis and HCC

Many liver cancers exhibit high degrees of genomic instability, which is roughly categorized as mitotic error-mediated chromosome instability (CIN) and DNA metabolism defect-mediated microsatellite instability (MIN). While MIN may have a minor role in hepatocarcinogenesis, CIN is one of the most frequent abnormalities in HCC (*21*). More than half of HCC (58-86%) have been harboring a copy number gain at 1q where five cancer genes, BCL9, ARNT, TPM3, MUC1 and NTRK1, and cell-cycle related genes, CHD1L, CKS1B, JTB and SHC1 located (*22,23*). Chromosome 8q is the second and is seen in half of HCC, which results in amplification of MYC, DDEF1 and MLZE (*24*). These amplifications as gain-of-functions are associated with patients' prognosis.

Micronuclei (MNi) are extra-nuclear bodies that contain damaged chromosome fragments isolated from the parent nucleus after cell division. MNi are considered sensitive markers of genotoxic damage and chromosomal instability in cirrhosis and HCC (*25*). A large number of rearrangements in a restricted region of chromosome known as chromothripsis are processed in these MNi. Therefore, a part of chromosomal instability is a consequence of MNi formation. Recent studies also highlight the high frequency of the chromothripsis throughout malignant tumors including HCC (*26,27*).

The recent intensive studies of whole-exome and whole-genome sequencing have identified mutations responsible for HCC oncogenesis and its pathological character. In these studies, the commonly observed gene mutations are related to telomere maintenance, Wnt/β-catenin pathway, p53 cell-cycle pathway, epigenetic modifiers, oxidative stress pathway, PI3K/AKT/mTOR, and RAS/RAF/MAPK pathway (Figure 2).

TERT promoter

Chronic liver injury leads to shortened telomere length and results in cirrhosis. Telomerase reverse transcriptase (TERT) is an enzyme that elongates telomeres with telomerase RNA component. The reactivation of telomerase is seen in approximately 90% of HCC. TERT promoter mutations have been found in 12-48% of HCCs and more frequently in HCCs from HCV infection and alcohol intake than HBV infection (*28*). However, integration of HBV DNA into the TERT promoter region contributes to overexpression of TERT, resulting in cell immortalization in 15-20% of HBV-associated HCCs (*29-31*). The TERT promoter mutations were also found in 6% of low-grade dysplastic nodules and 19% of high-grade dysplastic nodules in patients with cirrhosis (*32*) and appear to be required for hepatocellular adenoma to carcinoma development in non-cirrhosis patients (*33*). Thus, mutation in the TERT promoter is one of the important early events of HCC oncogenesis as a tumor initiation, and has close interactions with the MYC, Wnt/β-catenin pathway, and NF-κB pathway (*6,34,35*).

Wnt/β-catenin pathway

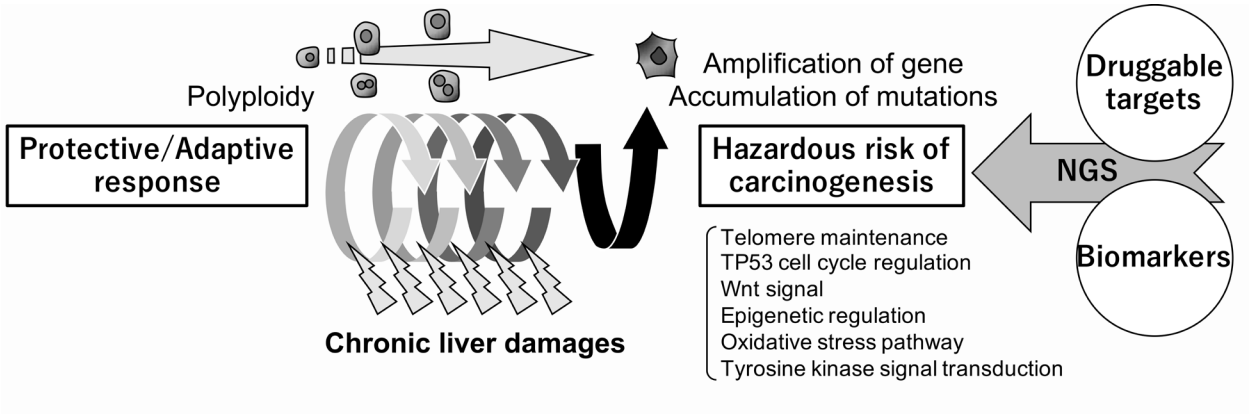

Figure 2. The gene alterations of hepatocytes under chronic liver injury and hazardous mutations of carcinogenesis. The polyploidy of hepatocytes plays a protective role against chronic liver injury. The multiple dysregulation of important genes by accumulation of gene alterations, including mutations and amplifications, could contribute to carcinogenesis. Next-generation sequencing (NGS) has revealed some key mutations in hepatocellular carcinomas. These findings would be helpful for the identification of "druggable" targets and biomarkers.

Wnt signaling is responsible for cell motility, dedifferentiation, and proliferation (*36*). The activating mutation of β-catenin (CTNNB1) and inactivating mutation of AXIN1, one member of β-catenin destruction complex, have been found in 11-37% and 5-15% of HCCs, respectively (*28*). While CTNNB1 and TP53 mutations have been found to be mutually exclusive, the CTNNB1 mutation coincided with TERT promoter mutations in early oncogenesis of HCC that is characterized as a CTNNB1 tumor subgroup, which is usually large and well-differentiated with less inflammatory infiltrates (*5*). The correlation between Wnt/β-catenin pathway and absence of T cell infiltration has been reported in melanoma and recently in HCC (*37,38*).

TP53 cell-cycle pathway

The dysfunction of TP53 resulting from mutations and/or repression by HBx has been detected in approximately 12-48% of HCCs, and with a higher frequency in advanced tumors (*28*). These mutations are characterized as a TP53 tumor HCC subgroup, which is likely to be poorly differentiated, associated with vascular invasion, multinucleated, and pleomorphic (*5*).

Epigenetic modifiers

Mutations in the chromatin remodeling enzyme ARID1A and ARID2 are found in 4-17% and 3-18% of HCCs, respectively (*28*). These mutations are closely related with transcription factor E2F and cyclin-dependent kinase inhibitor p21.

Mutations in the histone methylation MLL, MLL2, MLL3, and MLL4 have also been found. Inactivation of chromatin remodeling enzymes have been detected mostly in HCCs with liver disease from alcoholism (*39*).

Oxidative stress pathway

The mutations repeatedly identified in the oxidative stress pathway, such as NFE2L2 and KEAP1 lead to prolonged cell life and tumor growth (*39*).

PI3K/AKT/mTOR and RAS/RAF/MAPK pathway

Some HCCs show mutations in tyrosine kinase receptor pathways including PI3K/AKT/mTOR and RAS/RAF/MAPK. EGFR, VEGFR and PDGFR, lying upstream of these pathways, are targets of "molecular targeted drugs" such as sorafenib. High level amplification of VEGF signaling has been identified in 7-10% of HCCs (*40,41*). Contrary to rare mutations in KRAS, RAS/RAF/MAPK signaling is activated in half of early and almost all advanced HCC, as a result of Epidermal Growth Factor (EGF), Insulin growth factor (IGF) and MET activation (*42,43*). PI3K/AKT/mTOR pathway is activated in half of HCCs (*44*). IGFR signaling, upstream of PI3K, is activated in 20% of HCC through IGF2R allelic loss, ligand overexpression, or dysfunction of IGF binding proteins (IGFBPs) (*45*).

Others

IL-6/JAK/STAT and TGF-β are seen in 9% and 5% of HCC. Other mutations or copy number variations have been found in FGF19, VEGFA, MYC, CCND1, IGF, Hedgehog, and MET pathways (*46*). PTEN and CDKN2A (P16INK4A) are frequently deleted.

Many other gene alterations are involved in HCC oncogenesis and are closely associated with each other. To simplify these complicated interactions, researchers have classified HCCs based on their mutation signatures, which are aligned with HCC-related risk factors, such as age, sex, race, HBV infection, tobacco and alcohol consumption, and sporadic mutations by aflatoxin B1 in fungal contaminated food or aristolochic acid derived from Chinese herb (*47*). In clinical practice, the 5-gene score, based on combined expression level

of HN1, RAN, RAMP3, KRT19, and TAF6 is proposed to predict the disease-specific survival after resection (*48*). Moreover, for cohorts with different etiology, other studies have proposed prognostic signatures in transcripts of different gene sets, microRNA or DNA methylation (*49-53*). Cholangiocarcinoma, another type of primary tumor that develops in the liver, has also been studied in whole genome sequences, revealing that different gene mutation including KRAS, BRAF, BAP1, SMAD4, IDH1, and IDH2 are involved (*54-56*).

Another gene signature study has also highlighted the heterogeneity of even hepatic cancer stem cell (CSCs) marker EpCAM, CD133, CD24 and triple positive cells with single cell level which can be used for HCC patient survival prediction (*57*). Further analyses with single cell resolution could help establish a model of cancer evolution and identify targets for therapy.

The study on genomic mutations of Mongolian HCC, which reported new driver genes such as GTF2IRD2B, PNRC2 and SPTA1 that are closely associated with hepatitis D viral infections (*58*), is a reminder of the importance of stratification by precise etiological characteristics of cohorts for understanding the interpatient heterogeneity of HCC.

Although studies on gene signatures of non-tumor livers have yielded information on prediction of HCC early recurrence, the question of how and which mutations are accumulated, and in which chronological order before aggressive tumor growth has yet to be fully answered. Recently, a comparative study between the outside and inside of nodule-in-nodule tumors, along with regenerative nodules and non-tumor areas was undertaken. The study showed that mutations, CNV, and epigenetic modification in some of the previously reported HCC-related driver genes, including TERT and TP53, have been found during early stage HCC (*59*). As series of gene alteration accumulated in the liver are shown to closely associate with the phenotype of HCC. Further investigations on both unique chromosomal regulation in hepatocyte and heterogenous accumulation of genetic alterations in HCCs with different etiology are indispensable for better understanding of HCC.

Molecular targeted drugs for HCC: Sorafenib and beyond

Because underlying mutations play important roles in oncogenesis of the liver, inducing deleterious functions in them has become an attractive strategy for cancer therapy. Molecular targeted therapies differ from standard chemotherapy, which are characterized by targeting specific enzymes, growth factor receptors and signal transducers, thereby interfering with a variety of oncogenic cellular processes without adverse effect on normal cells (*60*).

A tyrosine kinase inhibitor, sorafenib, has been confirmed to improve median overall survival in two multicenter RCTs: the Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP) trial, including mostly Caucasians with HCV infection, and the Asia Pacific trial including Asians with HBV infection (*61,62*). The proposed targets of sorafenib broadly covers the RAF/MAPK/ERK pathway, VEGFR, PDGFR, and anti-apoptotic protein Mcl-1 (*63*). Unfortunately, molecular targeted therapies, including sorafenib, still provide insufficient outcome in terms of the overall survival elongation. To overcome this, therapy for HCC should include personalized modification and selection of drugs (*63*). The SHARP trial shows a non-significant trend towards higher survival benefit of sorafenib treatment with high c-kit or low plasma HGF levels. Therefore, the predicting the outcome of sorafenib treatment is not so simple. It requires an identification of poor/outstanding responding patients by using biomarkers and characterization of the HCC with pathological alterations including gene mutation.

The phase 3 STORM trial and BIOSTORM study on the efficacy of sorafenib as an adjuvant therapy following surgical resection or local ablation, revealed a gene signature of 87 poor prognosis genes and 59 good prognosis genes (*64*). In enrichment analysis, sorafenib recurrence free survival (RFS) responders showed downregulation of pathways indicative of poor prognosis such as KRAS, activation of EIF2 signaling, oxidative stress responses, immune-related processes, and upregulation of bile acid and lipid metabolism-related pathways. Of note, all these molecular traits were also present in the non-tumor adjacent tissue. Although VEGF-A gene copy number is suggested as a response predictor by retrospective observation study (*65*), VEGF-A focal amplification correlates with tumor satellites and microvascular invasion but not with recurrence prevention in the study.

The molecular targeted drugs already approved for clinical use interfere with the PI3K/AKT/mTOR and RAS/RAF/MAPK pathways, which are also involved in liver regeneration. Such drugs may lead to unfavorable effects on non-tumor regions of parenchyma damaged by chronic liver injuries. Of importance is not only the identification of suitable cohorts for treatment with the existing drugs, but also the development of new drugs that interact with malignant cells specifically. The proteomic profiling of 110 paired tumor and non-tumor tissues derived from the patients with HBV-related early HCC have identified sterol *O*-acyltransferase 1 (SOAT1) as a potential target (*66*). Using different cohorts, this approach may reveal other candidates as "druggable" targets leading to development of specific therapies for certain cohorts and/or robust therapies for all HCC patients.

Several negative trials have been reported for a decade since sorafenib approval. Regorafenib, an oral multi-kinase inhibitor, has shown significance in overall survival, compared with placebo in a phase

III (RESORCE) study, as a second-line therapy after sorafenib treatment (*67*). Recently in succession, lenvatinib in the REFLECT study, ramucirumab in the REACH-2 study, and cabozantinib in the CELESTIAL study have demonstrated their efficacy for HCC. In Japan, lenvatinib as well as sorafenib are recommended as first-line therapies for unresectable advanced HCCs. Regorafenib is recommended as second-line therapy, after sorafenib, for patients with HCC showing disease progression (*7*). Lenvatinib is an oral multi-kinase inhibitor that targets VEGF receptors, FGF receptors, PDGF receptor α, RET and KIT, and is non-inferior to sorafenib in overall survival as a first-line therapy (*68*). Ramucirumab is a human IgG1 monoclonal antibody that blocks signal transduction of VEGFR2, and shows improved overall survival in sorafenib-treated patients with α-fetoprotein concentrations of 400 ng/mL or greater (*69*). Cabozantinib targets tyrosine kinases including EGFs, MET, and AXL, and shows significantly longer overall survival than placebo as a second line treatment following sorafenib (*70*). As with sorafenib, the other molecular targeted drugs have been subjected to subgroup analyses and molecular assays including genetic sequences, which should provide cues to improve HCC treatment.

Genes involved in HCC chemo-resistance

Drug resistance results from the reduction of drug intake, enhancement of drug efflux and metabolic degradation, as well as mutations in drug targets. Based on the comparison of blood samples from 3 different responder types, such as extreme, strong and poor, six non-synonymous SNVs were found in four ADME (Absorption, Distribution, Metabolism, and Excretion) related genes: ABCB1, FMO3, and SLC15A2 (*71*). These molecules are important in terms of drug resistance to antibiotic and anticancer therapy. ABCB1 codes for one of the super families of ATP-binding cassette transporters. FMO3 codes for a flavin-containing monooxygenase which is a member of an important class of drug-metabolizing enzymes. SLC15A2 codes for a member of a family of proton-coupled peptide transporters. Among these genes, the single nucleotide polymorphism of ABCB1 was also related to sorafenib sensitivity in HCC patients (*72*). The ATP-binding cassette (ABC) transporter superfamily is one of the largest classes of transporters, which translocates many substrates including nutrients, viruses, and waste products through membranes of cells. Members of the ABC transporter family are present in organisms from all kingdoms of life, and play essential roles in maintaining homeostasis. Recent studies also repeatedly identified another member of the ABC transporter family, ABCC1 (MRP2); of which SNPs show altered transport activity for sorafenib, and efflux of paclitaxel and doxorubicin (*73,74*). The solute carrier (SLC) transporter superfamily, one of the counter parts of ABC transporters, imports solutes from the extracellular milieu into the cell depending on concentration gradient. The SLC superfamily, genetically heterogeneous with more than 200 exonic SNVs, is associated with clinical drug response or toxicity (*75*). Another member of SLC transporters, SLC22A1 (OCT1) is also associated with response to antitumor therapy with sorafenib (reviewed by Cabral *et al*. (*76*)). Intracellular drug metabolism Phase I and II enzymes including FMO3, cytochrome P450 (CYPs), and UGT (uridine diphosphate glucuronosyltransferase) play an important role in homeostatic control of lipophilic endobiotics, detoxification of xenobiotics and drug transportation (Phase III). Sorafenib is metabolized by CYP3A4 and UGT1A9, in which polymorphism leads to poor metabolism and is associated with sorafenib-induced severe toxicity (*76*). Awareness of genetic polymorphisms in metabolizing enzymes therefore, is also indispensable for precision medicine.

Future perspectives: towards the stage of immuno-therapy

In the IMbrave 150 phase III trial, combination therapy utilizing two monoclonal antibodies, atezolizumab and bevacizumab, against PD-L1 and VEGF-A respectively, showed a significantly improved survival rate of unresectable HCC compared to sorafenib therapy (*77*). To date, clinically available molecular targeted drugs are limited. Combination therapy including immunotherapy such as blockade of immune checkpoint CTLA-4 and PD-1, neoantigen, and CAR (chimeric antigen receptor) T cell therapy would be feasible alternatives.

Aneuploidy, the harboring of an abnormal number of chromosomes, in several tumors has been reported as a predictive biomarker for efficacy of immune checkpoint inhibitor (*78*). A recently conducted study on the relationship between copy number alterations (CNAs) and immune profiles of HCC, found that higher levels of broad CNAs resulting from aneuploidy show less immune-cell infiltration and are regarded as the primary reason for resistance to immune therapy (*79*). This CNAs and immune-phenotype relationship was seen in dysplastic nodules and early HCC. The enriched copy number loss, including HLA-DQB1, found in high broad CNA tumors appears to be one reason. The study also shows that 44-68% of HCC display polyploid status, which enriches high levels of broad CNAs. These chromosomal abnormalities, along with Wnt/β-catenin and JAK/STAT pathway signatures discoveries, should be prioritized in the development of future immunotherapies for HCC.

Neoantigens expressed specifically in tumors, including proteins derived from viruses or mutated genes, are most attractive targets for cancer immunotherapy. To date, clinical trials targeting AFP,

GPC3, TERT or MRP3 have been conducted as phase I or II (reviewed by Lu *et al*. (*80*)). These limited studies could identify new candidates.

To translate this rapidly growing knowledge for clinical practice, blood samples from patients with HCC have been subjected to genetic analysis. Droplet digital PCR (ddPCR) is highly sensitive and enables detection of small amounts of DNA. Liquid biopsy specimens contain genetic information in circulating tumor cells (CTCs) or cell free nucleic acids (cfNAs) including DNA, mRNA, miRNA and lncRNA. Total cfDNA levels themselves appear to be a biomarker for HCC screening, monitoring of treatment, and prediction of recurrence (reviewed by Bubu *et al*. (*81*)). Establishment of a system for sample collection, isolation and preservation, especially for liver and tumor tissues, is still challenging.

Herein, we review the biology of hepatocyte ploidy, pathological mutations in HCC oncogenesis, and their therapeutic implications. The breakthrough of next generation sequencing has provided a huge amount of information in understanding genetic alteration of cancer. However, such information is fragmented and needs to be pieced together for discovery of missing links. Molecular targeted drugs and immune checkpoint inhibitors may revolutionize the practice guidelines for HCC therapies in the near future.

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Difference in treatment algorithms for hepatocellular carcinoma between world's principal guidelines

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Abstract: Hepatocellular carcinoma (HCC) is the fifth most common cancer and the second leading cause of cancer-related death globally. Clinical guidelines for HCC have been established and revised by many countries and regions. We summarized and compared the treatment algorithms in the updated HCC guidelines established by Japan, China, Hong Kong, the Asian-Pacific Association for the Study of the Liver, the American Association for the Study of Liver Diseases, and the European Association for the Study of the Liver and European Organization for Research and Treatment of Cancer. Variations in treatment algorithms between the guidelines is inevitable, considering the differences in the prevalence and etiology of HCC, local clinical practice, and medical and insurance systems between countries or regions, and this might be confusing for practitioners worldwide. A comprehensive understanding of the guidelines that are globally available might be useful for future improvement of each guideline.

***Keywords*:** surgery, ablation, transcatheter arterial chemoembolization, systemic chemotherapy, transplantation

Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver. It is the fifth most common cancer and second leading cause of cancer-related death globally (*1,2*). Although HCC is predominant in Southeast Asia and Africa, the incidence rate of HCC has been increasing in other regions, particularly in Europe and the United States (*2,3*), which has led to a greater interest in the diagnosis and treatment of HCC worldwide.

In the past two decades, clinical guidelines for HCC were established and revised by many countries including Japan (*4*), China (*5*), Hong Kong (*6*), the Asian-Pacific countries (*7*), European countries (*8*), and the United States (*9*). The guidelines reflect the differences between countries, including the prevalence and etiology of HCC, local clinical practice, and medical and insurance systems, which entail many differences, especially in treatment algorithms. Recently, advancement of HCC treatment especially in systemic chemotherapy has been attracting attention to the revision of the guidelines for HCC.

In this review, we have summarized and compared the treatment algorithms in the updated HCC guidelines established by Japan, China, Hong Kong, the Asian-Pacific Association for the Study of the Liver (APASL), the European Association for the Study of the Liver and European Organization for Research and Treatment of Cancer (EASL-EORTC), and the American Association for the Study of Liver Diseases (AASLD).

Overview of the treatment algorithms of the guidelines

The treatment algorithms in each guideline are summarized in Figures 1-6. The characteristics of the treatment algorithms in each country are as follows.

Japan

The Japanese evidence-based clinical practice guidelines were published in 2005 by the Japan Society of Hepatology (JSH). The guidelines were formulated based on a systematic review of the evidence for hepatocellular carcinoma. The treatment algorithm was simple and clear, and the guidelines were revised three times, in 2009, 2013 and 2017, incorporating growing new evidence and paying more attention to the consensus among the specialists in Japan.

In the early version of the Japanese treatment algorithm, vascular invasion and extrahepatic metastasis were not included because of the lack evidence for treatment. However, to reflect the varieties of clinical practice in the real world, especially regarding non-surgical treatments, a treatment algorithm covering all situations of HCC was requested especially from gastroenterologists. As a result, the latest version of the treatment algorithm in the Clinical Practice Guidelines for Hepatocellular Carcinoma 2017 included vascular invasion and extrahepatic metastasis (Figure 1).

Unlike the treatment algorithms of other countries,

in the 2017 guideline, performance status was not included in the algorithm. Child-Pugh C patients are allocated into liver transplantation or palliative care according to the Milan criteria. The evaluation of liver function was mainly performed by Child Pugh classification, while liver damage classification was used in the earlier versions. Liver transplantation is not indicated for patients with good liver function (Child-Pugh A/B) because of the organ shortage for transplantation and medical insurance system in Japan. In the minor revision of the 2017 Japanese guidelines in 2019, new expanded criteria for LDLT candidates with HCC, the 5-5-500 rule (nodule size ≤ 5 cm in diameter, nodule number ≤ 5, and alfa-fetoprotein value ≤ 500 ng/ml), were established based on a retrospective data analysis of the Japanese nationwide survey (*10*). In the presence of extrahepatic metastasis, systemic therapy is indicated only for Child-Pugh A patients. After evaluation of liver function and extrahepatic metastasis, each local and systemic therapy is indicated according to vascular invasion, tumor number (≤ 3 or > 3), and tumor size (≤ 3 cm or > 3 cm).

China

The Chinese guideline on the management of hepatocellular carcinoma was revised in 2017 from the previous 2011 version (Figure 2). Based on updated evidence and clinical practice, new staging systems and treatment algorithms have been developed that are far more comprehensive and suitable for use in China, focusing on treatment distribution according to respective stage.

First of all, the general condition and liver function of patients are evaluated by performance status and Child-Pugh classification, and patients with performance status 3–4 and/or Child-Pugh C are distributed into palliative care. Patients with performance status 0–2 and Child-Pugh A/B with extrahepatic metastasis are assigned into systemic therapy, transcatheter arterial chemoembolization (TACE), and radiotherapy. After the evaluation of performance status, liver function and extrahepatic spread, each local and systemic therapy is indicated according to vascular invasion, tumor number (solitary, 2-3 or ≥ 3), and tumor size (≤ 5 cm or > 5 cm for a solitary tumor and ≤ 3 cm or > 3 cm for 2-3 tumors).

In particular, surgical resection is widely indicated regardless of vascular invasion, tumor number, or tumor size in the Chinese guidelines. Liver transplantation is indicated in patients with performance status 0–2 and Child-Pugh A/B, and the indication is determined in accordance with the University of California San Francisco (UCSF) criteria.

Hong Kong

The Hong Kong liver cancer staging system with treatment stratification was published in 2014, in order to establish an appropriate prognostic staging system for HCC with treatment guidelines applicable to Asian patients. The Hong Kong guidelines were formulated based on data collected from 3,856 patients with HCC predominantly related to hepatitis B treated at Queen Mary Hospital in Hong Kong (Figure 3).

In the Hong Kong guidelines, HCC is classified into three phases as follows: (1) Early tumor: ≤ 5 cm, ≤ 3 tumor nodules and no intrahepatic venous invasion; (2) Intermediate tumor: *i*) ≤ 5 cm, either > 3 tumor nodules or with intrahepatic venous invasion, or *ii*) > 5 cm, ≤

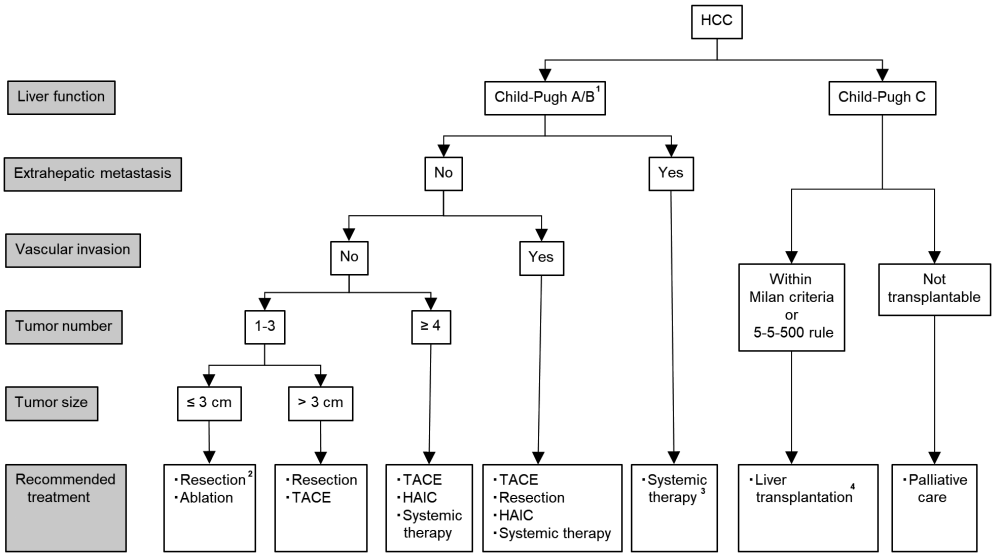

Figure 1. Treatment algorithm of clinical practice guidelines for HCC in Japan (2017), summarized and modified from Kokudo *et al.* (*4*). [1]Evaluation using liver damage classification is recommended when liver resection is indicated. [2]For solitary tumor, liver resection is first-line and local ablation is second-line. [3]Only for Child-Pugh A. [4]Patient age ≤ 65. HAIC, hepatic arterial infusion chemotherapy; HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization.

3 tumor nodules, and no intrahepatic venous invasion; and (3) Locally advanced tumor: *i*) ≤ 5 cm, > 3 tumor nodules and with intra-hepatic venous invasion, or *ii*) > 5 cm, > 3 tumor nodules, and/or with intrahepatic venous invasion, or *iii*) diffuse tumor.

In accordance with the tumor classification system, performance status, Child-Pugh classification, and the presence of extrahepatic metastasis, patients are divided into prognostic stages and treatment is allocated. According to the flowchart, patients with performance

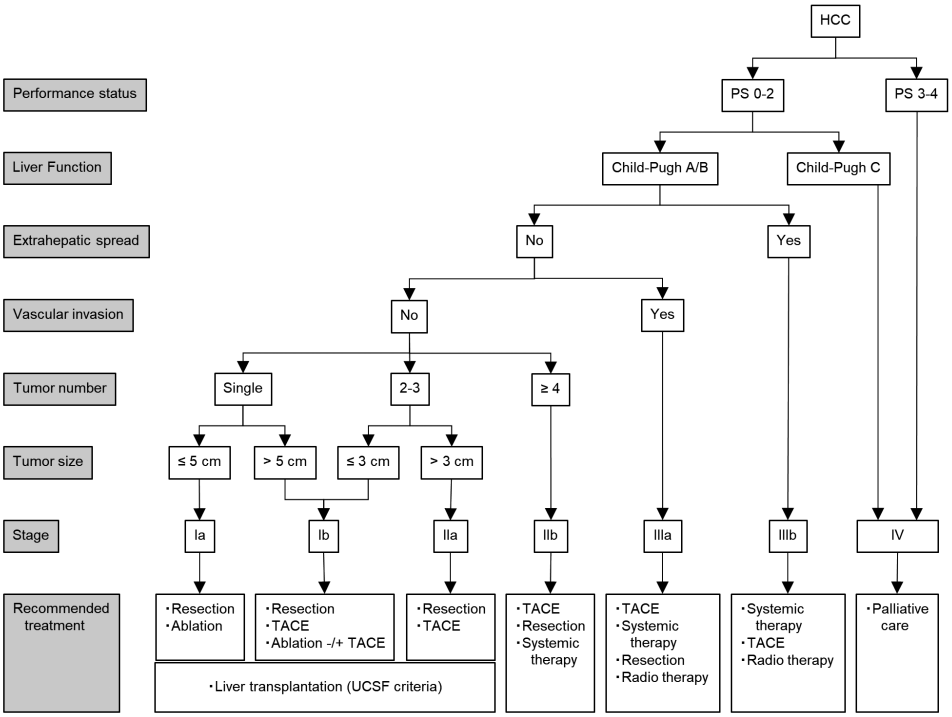

Figure 2. Treatment algorithm of clinical practice guidelines for HCC in China (2017), summarized and modified from Xie *et al.* (*5*). HCC, hepatocellular carcinoma; PS, performance status; TACE, transcatheter arterial chemoembolization; UCSF, University of California San Francisco.

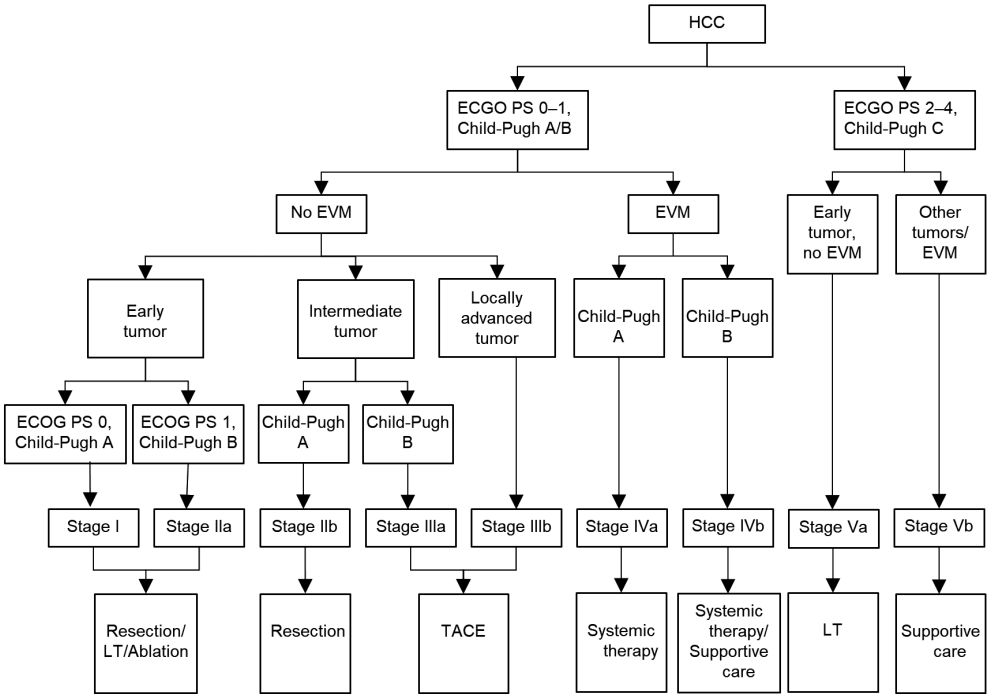

Figure 3. Treatment algorithm of clinical practice guidelines for HCC in Hong Kong (2015), summarized and modified from Poon *et al.* (*6*). ECOG, Eastern Cooperative Oncology Group; EVM, extrahepatic vascular invasion/metastasis; HCC, hepatocellular carcinoma; LT, liver transplantation; PS, performance status; TACE, transcatheter arterial chemoembolization.

status 2–4 and/or Child-Pugh C are allocated into palliative care, but liver transplantation is indicated for early tumor without extrahepatic metastasis. In the presence of extrahepatic metastasis, patients with performance status 0–1 and Child-Pugh A/B are allocated into systemic therapy or palliative care. Each local therapy is indicated for patients with performance status 0–1 and Child-Pugh A/B without extra hepatic metastasis according to tumor phase as follows: Early tumor: resection, liver transplantation, ablation; Intermediate tumor: resection, TACE; Locally advanced tumor: TACE.

APASL

The APASL HCC guidelines were published in 2010 (*11*). The guidelines were revised in accordance with the statement of the "Toward Revision of the APASL HCC Guidelines" meeting held at the 25th annual conference of the APASL in Tokyo on February 23, 2016 (Figure 4). The guidelines are evidence-based and considered generally acceptable in the Asia-Pacific region, which has a diversity of medical environments. The evidence and recommendations in the guideline have been graded according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system (*12*).

Consistent with the Japanese evidence-based clinical practice guidelines, performance status is not included in the algorithm. In the presence of extrahepatic metastasis, the first-line therapy is systemic therapy for Child-Pugh A/B patients and palliative care for Child-Pugh C patients. In the absence of extrahepatic metastasis, liver transplantation or palliative care is indicated for Child-Pugh C patients according to the Milan or UCSF criteria, and each local and systemic therapy is indicated according to resectability, vascular invasion, tumor number (≤ 3 or > 3), and tumor size (≤ 3 cm or > 3 cm).

Notably, resectability is included in the APASL treatment algorithm, which reflects a variety of surgeons' skills and hospital facilities in Asian-Pacific countries (*13*). In addition, resectability is also evaluated from the viewpoint of extended indication of liver resection in the real world due to recent advances in surgical technique and postoperative management (*14*).

EASL-EORTC

The first European joint guidelines for the management of hepatocellular carcinoma were first developed in 2001 by EASL, updated by EASL-EORTC 2012, and then revised in 2018 (Figure 5). The treatment algorithms are mostly based on the Barcelona-Clinic Liver Cancer (BCLC) staging system, which classifies HCC patients into five stages, including very early

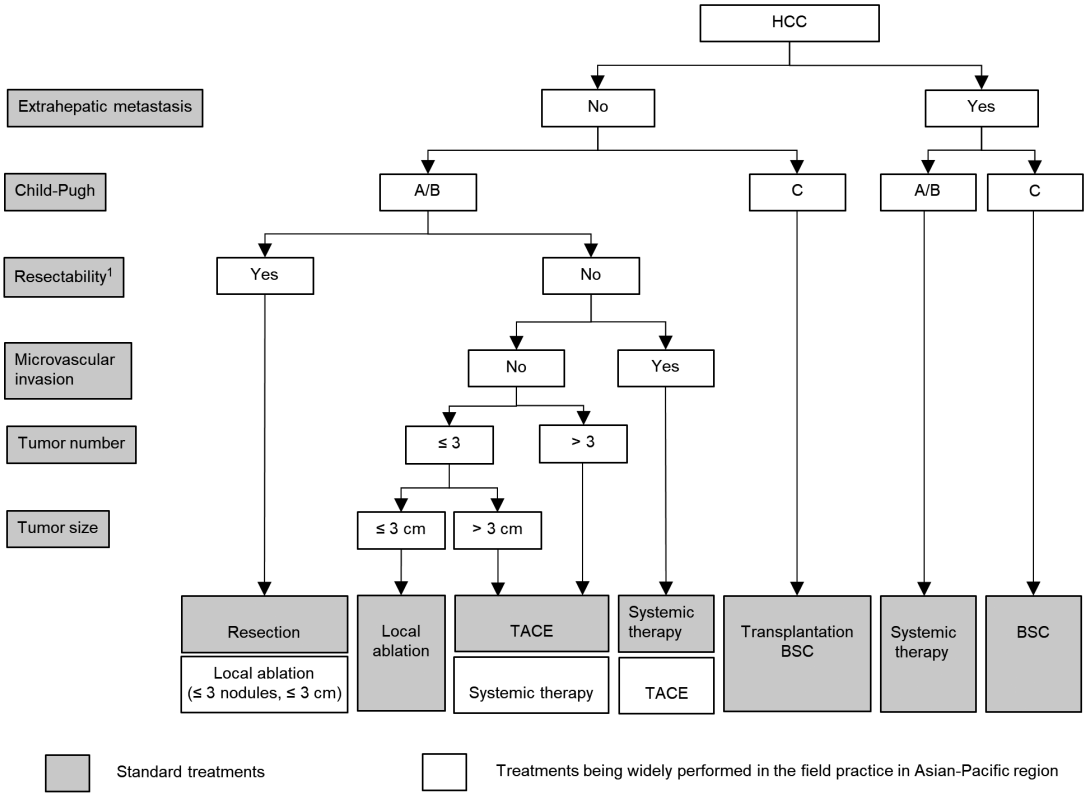

Figure 4. Treatment algorithm of clinical practice guidelines for HCC in APASL (2017), summarized and modified from Shiha *et al.* (*7*). [1]Decisions regarding resectability should be discussed in a multidisciplinary team. BSC, best supportive care; HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization.

stage (Stage 0), early stage (Stage A), intermediate stage (Stage B), advanced stage (Stage C), and terminal stage (Stage D) (*15*).

However, the evaluation of liver function has been slightly changed in EASL-EORTC guidelines. Although the BCLC staging system used Child-Pugh A for Stage 0 and Child-Pugh A/B for Stages A–C, the EASL-EORTC guidelines defined "preserved liver function" as Child-Pugh A without any ascites, and used this criterion to sort treatable stage (Stage 0–C) and terminal stage (Stage D). Therefore, according to EASL-EORTC guidelines, staging based on liver function is stricter than in the BCLC staging system.

The staging of HCC in the EASL-EORTC guidelines is as follows: (1) Very early stage (Stage 0: < 2 cm, single nodule, preserved liver function, and PS 0); (2) Early stage (Stage A: single nodule or ≤ 3 nodules of < 3 cm, preserved liver function, and PS 0); (3) Intermediate stage (Stage B: multinodular, preserved liver function, and PS 0); (4) Advanced stage (Stage C: portal invasion, extrahepatic spread, preserved liver function, and PS 1-2); and (5) Terminal stage (Stage D: Child-Pugh C, and PS 3-4).

All stages except Stage A directly connect to treatment: Stage 0 to ablation or resection, Stage B to chemoembolization, Stage C to systemic therapy, and Stage D to palliative care. In Stage A, the patients are classified into optimal surgical candidates and transplant candidates. Optimal surgical candidacy is based on a multiparametric evaluation including compensated Child-Pugh class A liver function with MELD score < 10, to be matched with grade of portal hypertension, acceptable amount of remaining parenchyma and possibility of adopting a laparoscopic or minimally invasive approach, and transplant candidacy as indicated by the Milan criteria (*16*). Ablation is also indicated for patients in Stage A who are neither optimal surgical candidates nor transplant candidates. Although macrovascular invasion is contraindicated for surgery in the EASL-EORTC guidelines, intervention to distal portal invasion, at segmental or subsegmental level, is considered to deserve investigations within a prospectively designed protocol reflecting on a Japanese report (*17*).

AASLD

The AASLD practice guidelines on the management of HCC were established in 2005 and revised in 2010 and 2018 (Figure 6). In accordance with the EASL-EORTC guidelines, treatment algorithms are based on the BCLC staging system (*15*) with minor modifications. The performance status for BCLC Stages 0, A, and B has been changed from 0 to 0-1 and BCLC stage C from 1-2 to 0-2 in order to better reflect clinical practice in reality (*18*). Therefore, the treatment indication for AASLD guidelines is expanded in terms of performance status compared to BCLC guidelines.

Treatment is allocated to each stage as follows: (1) Stage 0: resection and ablation; (2) Stage A: resection, liver transplantation and ablation, transarterial radio embolization (TARE), TACE, radiotherapy; (3) Stage B: TACE, TARE, and liver transplantation; (4) Stage C: systemic therapy, and TARE; and (5) Stage D: liver transplantation and palliative care. Unlike EASL-

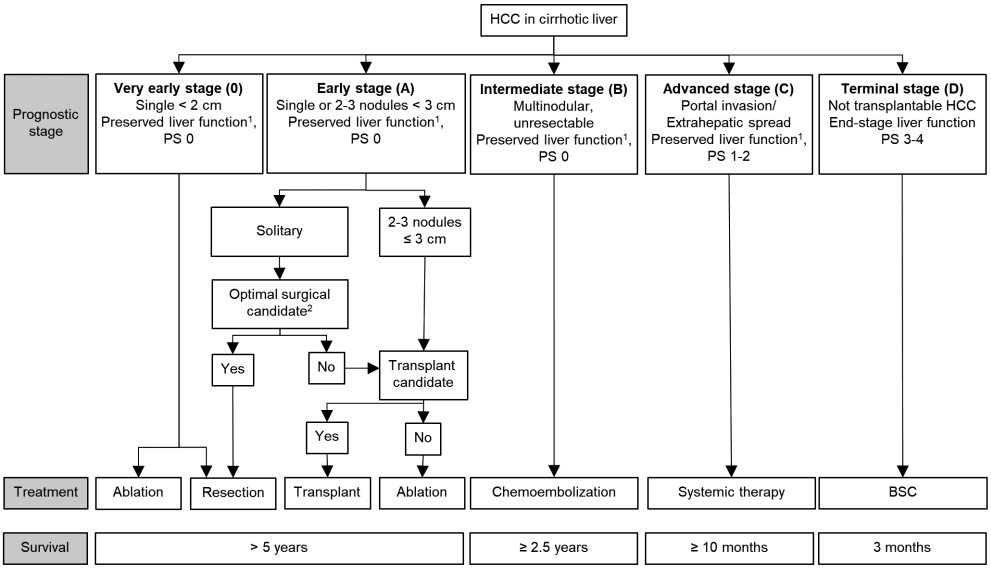

Figure 5. Treatment algorithm of clinical practice guidelines for HCC in the European Association for the Study of the Liver and European Organization for Research and Treatment of Cancer (EASL-EORTC) (2018), summarized and modified from the European Association for the Study of the Liver (*8*). [1]Without any ascites. [2]Optimal surgical candidacy is based on a multiparametric evaluation including compensated Child-Pugh class A liver function with MELD score < 10, to be matched with grade of portal hypertension, acceptable amount of remaining parenchyma, and possibility to adopt a laparoscopic/minimally invasive approach. BSC, best supportive care; HCC, hepatocellular carcinoma; PS, performance status.

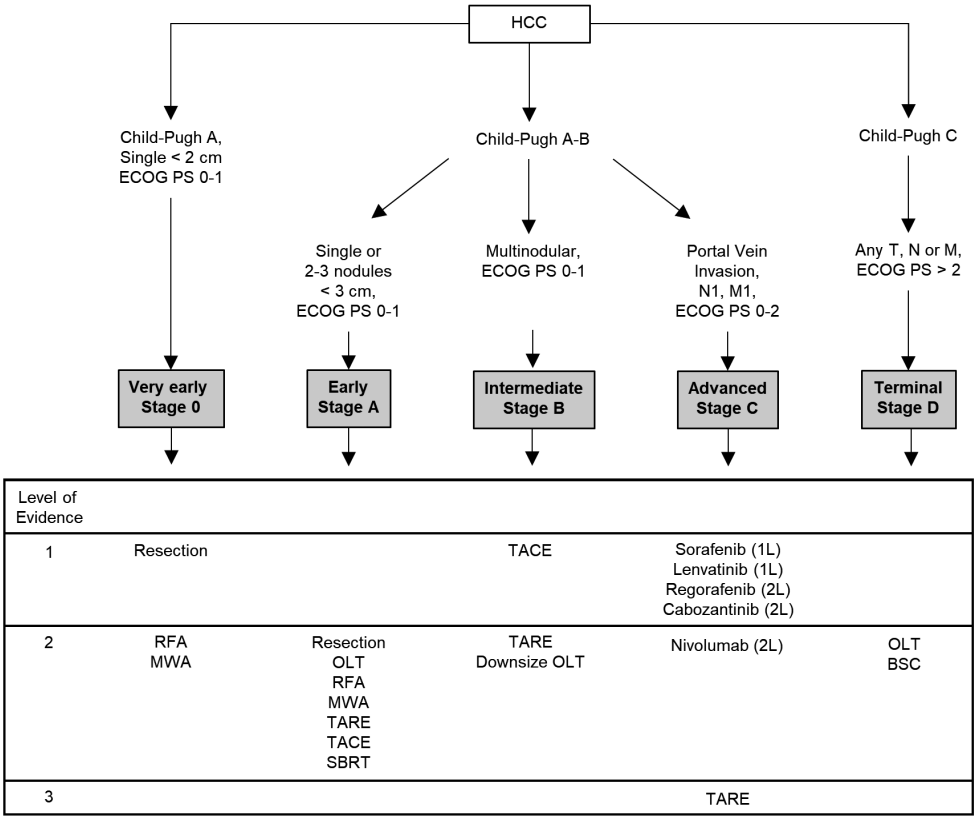

Figure 6. Treatment algorithm of clinical practice guidelines for HCC in the American Association for the Study of Liver Diseases (AASLD) (2018), summarized and modified from Marrero *et al.* (*9*). ECOG, Eastern Cooperative Oncology Group; HCC, hepatocellular carcinoma; MWA, microwave ablation; OLT, orthotopic liver transplantation; PS, performance status; RFA, radiofrequency ablation; SBRT, stereotactic body radiation therapy; TACE, transcatheter arterial chemoembolization; TARE, transarterial radio embolization.

EORTC guidelines, TARE is indicated for Stages A–C, and liver transplantation is also considered for Stages B and D according to the Milan criteria (*16*).

Differences of treatment indications between guidelines

The differences in treatment indications of resection, ablation, TACE, and systemic therapy by tumor condition between guidelines are summarized in Figure 7. Treatment allocation by liver function and performance status is not included, in order to focus on the differences of treatment indications based on tumor condition. The stratification is mainly conducted by treatment in Figure 7a and by country in Figure 7b.

Liver resection

Liver resection is indicated for advanced HCC in terms of tumor burden in the treatment algorithms of Asian countries (*19*). The Japanese treatment algorithm indicates liver resection for any nodule size (within 3 in number). In the Chinese treatment algorithm, surgical resection could be a choice for HCC for any nodule size and number. The Hong Kong treatment algorithm recommends liver resection for any nodule size (within 3 in number and > 3 nodules within ≤ 5 cm in size). Notably, vascular invasion is not a contraindication for surgical resection in the Japanese, Chinese, and Hong Kong guidelines. In contrast, the EASL-EORTC and AASLD guidelines, which follow the BCLC staging classification (*15*), have set narrower indications for liver resection. Liver resection is only recommended for those with single nodules of any size in the EASL-EORTC guidelines, and single nodules of any size and 2-3 nodules within 3 cm in size in the AASLD guidelines. In addition, liver resection is not indicated for HCC with vascular invasion in the EASL-EORTC and AASLD guidelines.

In terms of liver functional reserve, liver resection is an option for patients with Child-Pugh A/B in the Asian guidelines, including Japan, China, Hong Kong, and the APASL. In accordance with the Asian guidelines, patients in Child-Pugh A/B are candidates for surgical resection as per the AASLD guidelines, although a stricter indication (Child-Pugh A without ascites) is set in the EASL-EORTC guidelines. Furthermore, while normal bilirubin and portal pressure are supposed to serve as a prerequisite for resection under the BCLC recommendations, slightly elevated bilirubin or portal hypertension is not a definite contraindication for surgical resection in Asian guidelines (*20*). As for

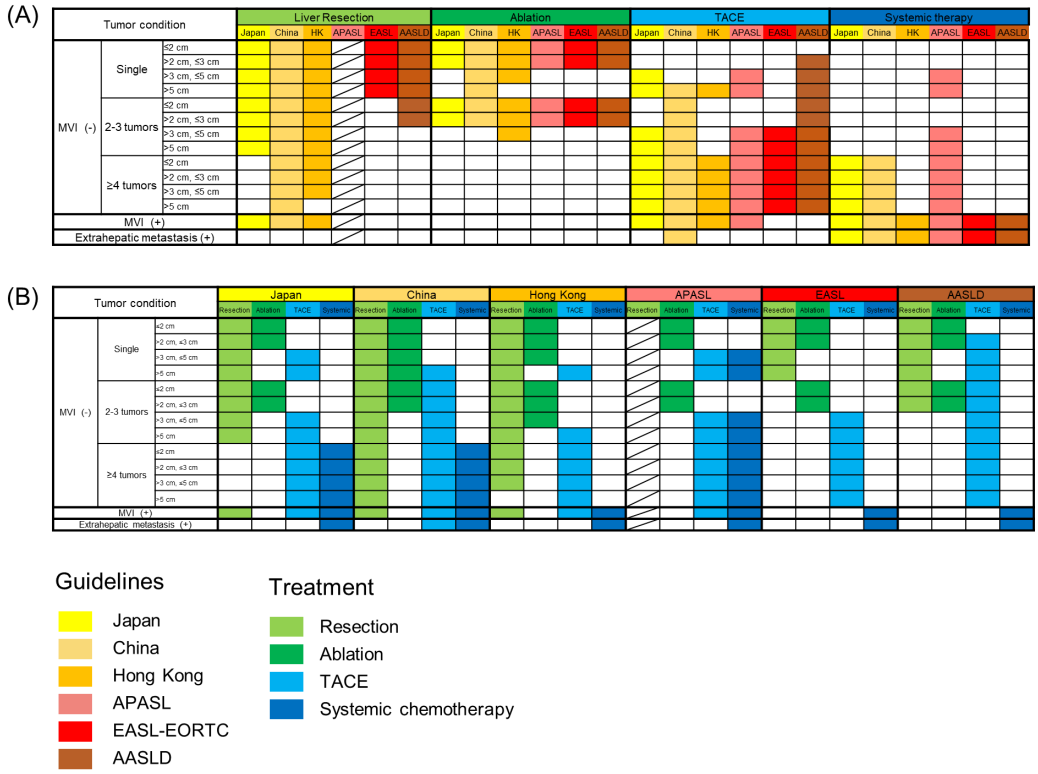

Figure 7. Difference of treatment indications between guidelines. (A) Difference by treatment; **(B)** Difference by country. HAIC, hepatic arterial infusion chemotherapy; HCC, hepatocellular carcinoma; MVI, macrovascular invasion; PS, performance status; TACE, transcatheter arterial chemoembolization; TARE, transarterial radio embolization; UCSF, University of California San Francisco. In APASL guideline, the indication of liver resection is determined by "resectability" which reflects a variety of surgeons' skills and hospital facilities.

portal hypertension, the EASL-EORTC indicates that portal hypertension should always be balanced with the extent of hepatectomy and liver function indicators, such as the MELD score and availability and predicted effectiveness of alternative HCC therapies in decision making for eligibility for liver resection because limited hepatectomy in patients with preserved liver function and moderate clinically relevant portal hypertension (hepatic venous pressure gradient > 10 mmHg) yields competitive survival outcomes (*21*).

However, surgical indications for HCC are decided not only by selection criteria included in each treatment algorithm, as mentioned above, but also by tumor location, estimated liver resection volume, and liver functional reserve. Although there are several algorithms to guide secure hepatic resection, the detailed operative indication and procedure should be determined by well-experienced hepatobiliary surgeons in accordance with the condition of each patient.

Ablation

Image-guided percutaneous ablation therapies mainly mention ethanol injection (*22*), microwave ablation (MWA) (*23*), and radiofrequency ablation (RFA) (*24*). Of these, RFA is recommended first in all guidelines, and ethanol injection is a treatment of choice only in cases in which RFA cannot be performed safely because of either enterobiliary reflux or adhesion between the tumor and the gastrointestinal tract. Recently, MWA has been utilized more frequently because application of higher temperatures in a shorter period of time has led to excellent local tumor control and less concern for heat sink (*25*), and the AASLD guidelines recommend MWA as a choice of local ablation therapy. However, there are no prospective randomized trials comparing RFA with MWA.

The indication of local ablation therapy is almost the same among the various guidelines described above. Local ablation therapy is mainly performed on patients with small HCC, generally in Child-Pugh class A or B patients with three or fewer tumors, each 3 cm or less in diameter. In the Hong Kong guidelines, local ablation is indicated for solitary tumors within 5 cm in size. The combination of ablation and TACE is recommended for solitary tumors measuring 3-7 cm in diameter as per the Chinese guidelines (*26*).

TACE

TACE is recommended as a first-line treatment of HCC for patients with unresectable, large or multifocal HCCs, which do not have vascular invasion or extrahepatic spread, namely equivalent to BCLC stage

B patients (*27*). Therefore, the guidelines published by the EASL-EORTC and AASLD recommend TACE as a first-line, non-curative therapy for BCLC stage B patients, although only systemic therapy is indicated for patients with vascular invasion according to the EASL-EORTC and AASLD recommendations.

On the other hand, TACE is a treatment option for lesions with vascular invasion according to the Asian guidelines. TACE is indicated for lesions with vascular invasion at the peripheral portal branch as per Japanese and Hong Kong guidelines (*28*), and even for lesions with portal vein tumor thrombus (PVTT) at the main trunk in Chinese guidelines as long as collateral circulation is well developed, although temporary liver decompensation and postembolization syndrome were noted to occur frequently (*29*). In APASL guidelines, TACE is recommended as the second-line therapy for tumors with vascular invasion, whereas systemic therapy is indicated as the first-line therapy. In addition, TACE alone or in combination with radiotherapy for patients with extrahepatic metastasis can be an option in China guidelines based on some retrospective observational studies (*30*), although there is insufficient evidence of a recommendation for TACE over systemic therapy for advanced HCC.

For patients with multiple and/or portal invasion, TARE is recommended in the Chinese and AASLD guidelines. In Japan, TARE is not included in national insurance and is therefore not commonly performed. Instead, hepatic arterial infusion chemotherapy (HAIC) is commonly recommended for patients with multiple and/or portal invasion without indication of liver resection and TACE.

Systemic chemotherapy

Basically, systemic therapy is recommended over no therapy for patients with advanced HCC with macrovascular invasion and/or metastatic disease in all guidelines. In addition, systemic therapy is also indicated for multiple tumors (> 3 in number) in the Japanese and Chinese guidelines, and the APASL treatment algorithm recommends systemic therapy for TACE candidates as a second-line treatment according to the concept of conversion from TACE to sorafenib before the appearance of macrovascular invasion or extrahepatic metastasis (*31*). Systemic therapy is also indicated for tumors that are refractory to other locoregional therapy in all guidelines. In terms of liver function, systemic therapy is indicated only for patients with Child-Pugh A in Japan and EASL-EORTC, and for patients with Child-Pugh A and well-selected Child-Pugh B in China, Hong Kong, and AASLD.

First-line agents used for systemic therapy are sorafenib in Hong Kong and APASL, sorafenib and FOLFOX 4 in China, and sorafenib and lenvatinib in Japan, AASLD, and EASL-EORTC. As second-line therapy, regorafenib is recommended in Japan and EASL-EORTC, and regorafenib and nivolmab are recommended in AASLD. The differences among the agents used for systemic therapy should be interpreted in consideration with the recent rapid advance of antitumor drugs for HCC systemic therapy, including molecular targeted agents and immune checkpoint inhibitors.

In a latest report, Finn *et al.* reported the superiority of atezolizumab-bevacizumab to sorafenib in patients with advanced unresectable hepatocellular carcinoma not previously treated with systemic therapy (*32*). Atezolizumab is a programmed death ligand 1 (PD-L1) inhibitor, and bevacizumab is a monoclonal antibody targeting vascular endothelial growth factor. Treatment with the combination of atezolizumab-bevacizumab resulted in significantly longer overall survival at 12 months (67.2% [95% CI, 61.3 to 73.1] with atezolizumab–bevacizumab and 54.6% [95% CI, 45.2 to 64.0] with sorafenib, and progression-free survival (median progression-free survival, 6.8 months [95% CI, 5.7 to 8.3] with atezolizumab–bevacizumab and 4.3 months [95% CI, 4.0 to 5.6] with sorafenib). The confirmed objective response rates were 27.3% (95% CI, 22.5 to 32.5) with atezolizumab–bevacizumab and 11.9% (95% CI, 7.4 to 18.0) with sorafenib. The combination of atezolizumab-bevacizumab might be a new benchmark for first-line therapy in advanced HCC. This evidence will be included in each guideline in the near future.

Liver transplantation

Thus far, the two major accepted criteria for liver transplantation have been the Milan criteria (solitary tumor ≤ 5 cm or within 3 nodules ≤ 3 cm without vascular invasion and extrahepatic metastasis) (*16*) and the UCSF criteria (solitary tumor ≤ 6.5 cm or ≤ 3 nodules ≤ 4.5 cm plus total tumor diameter ≤ 8 cm without vascular invasion and extrahepatic metastasis) (*33*). The Milan criteria have been adopted in Japan, Hong Kong, APASL, EASL-EORTC, and AASLD as the first-line criteria, and the UCSF criteria are used in China as the first-line and in Hong Kong and APASL as the second-line. Although the expansion of the Milan criteria is not recommended by the Japan and AASLD guidelines, the recently updated EASL-EORTC and AASLD guidelines suggest that patients beyond the Milan criteria can be candidates for transplantation after successful down-staging into the Milan criteria (*34*). In the Japanese 2017 guidelines with minor revision in 2019, new expanded criteria for LDLT candidates with HCC, the 5-5-500 rule (nodule size ≤ 5 cm in diameter, nodule number ≤ 5, and alfa-fetoprotein value ≤ 500 ng/ml), were established based on a retrospective data analysis of the Japanese nationwide survey (*10*).

Although each guideline has adopted the Milan or

UCSF criteria, the difference in graft sources between the East and West should be taken into consideration. Briefly, living-donor liver transplantation (LDLT) is the mainstay in Eastern countries, whereas deceased-donor liver transplantation (DDLT) is prevalent in Western countries (*35*). Unlike DDLT, LDLT is not restricted by the nationwide allocation system, and the indication for LDLT in patients with HCC should be decided based on institutional or case-by-case consideration, balancing the burden on the donor, operative risk, and overall survival benefit for the recipient.

Conclusion

In conclusion, the differences in treatment strategy for hepatocellular carcinoma between the updated guidelines in Japan, China, Hong Kong, APASL, EASL-EORTC, and AASLD are summarized. Variations in the treatment algorithms between the guidelines is inevitable considering the differences in the prevalence and etiology of HCC, local clinical practice, and medical and insurance systems between countries or regions, and this might be confusing for practitioners worldwide. The present review provides comprehensive understanding of existing guidelines worldwide and it may be useful for future improvement of each guideline.

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REVIEW
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Effects of volume on outcome in hepatobiliary surgery: a review with guidelines proposal

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Abstract: The positive relationship between volume and outcome in hepatobiliary surgery has been demonstrated for many years. As for other complex surgical procedures, both improved short- and long-term outcomes have been associated with a higher volume of procedures. However, whether the centralization of complex hepatobiliary procedures makes full sense because it should be associated with higher quality of care, as reported in the literature, precise criteria on what to centralize, where to centralize, and who should be entitled to perform complex procedures are still missing. Indeed, despite the generalized consensus on centralization in hepatobiliary surgery, this topic remains very complex because many determinants are involved in such a centralization process, of which some of them cannot be easily controlled. In the context of different health systems worldwide, such as national health systems and private insurance, there are different stakeholders that demand different needs: politicians, patients, surgeons, institutions and medical associations do not always have the same needs. Starting from a review of the literature on centralization in hepatobiliary surgery, we will propose some guidelines that, while not data-driven due to low evidence in the literature, will be based on good clinical practice.

***Keywords*:** volume outcomes, hepatobiliary surgery, surgeon volume, centralization

Introduction

For years, the issue of centralization in liver surgery in specialist 'high-volume' hospitals has been prominent in the debate on improving quality in healthcare. It is well established that high volume, in general, means better outcome, and many studies have shown lower mortality and higher survival rates in high-volume versus low-volume centers (*1-6*). Indeed, in high-volume centers 90-day mortality rate is approximately 3%, with the morbidity rate around 30% (*7-10*). The factors involved seem to be many: better knowledge of the anatomy, more accurate selection of patients, refinements of surgical perioperative medicine techniques, as well as optimization of the management of postoperative complications (*11-15*).

The present review involves all available literature on the relationship between hospital or surgeon volume and postoperative mortality and survival in liver surgery suggesting some guidelines for management and creation of centralized departments.

Review of the literature

Table 1 details review of the literature regarding the relationship between outcome and volume in hepatobiliary surgery. Considering the rapid evolution of liver surgery, we have included articles published in the last 20 years in English. Moreover, we have included only those articles that have declassified hepatobiliary surgery from pancreatic surgery, which are usually considered together (*16-45*). As detailed, almost all the included articles supported a positive relationship between hospital volume and outcome indicating the validity of the union of high-volume and high-quality. In particular, in 2003, Dimick *et al*. (*20*) analyzed more than 2,000 hepatectomies performed in North America and found that those institutions that performed more than 20 resections per year had significantly lower mortality. Although the resulting cut off of 20 resections per year seems too inclusive, objectively the differences were substantial. However, in both groups the mean values outranged the benchmarks even of that period (6.3% *vs*. 15.5%). In 2009 a systematic review and in 2012 a meta-analysis confirmed a reduced mortality risk after liver surgery in high-volume centers (*46,47*).

Few of these articles, investigated how this relationship was mainly based on hospital or organization factors rather than on surgeon factors. In general, the positive relationship was evident both for the hospital and surgeon volumes. Even if this is reasonable, there

Table 1. Review of the literature on the relationship between outcome and volume in hepatobiliary surgery

Author (*Ref.*)	Year	Patients	Importance of hospital volume	Importance of surgeon volume
Begg CB, *et al*. (*16*)	1998	801	+	n/a
Choti MA, *et al*. (*17*)	1998	606	+	n/a
Glasgow RE, *et al*. (*18*)	1999	507	+	+
Gordon TA, *et al*. (*19*)	1999	293	+	+
Dimick JB, *et al*. (*20*)	2003	2,097	+	+
Imamura H, *et al*. (*21*)	2003	1,056	+	+
Fong Y, *et al*. (*22*)	2005	3,734	+	n/a
Hollenbeck BK, *et al.* (*23*)	2007	3,630	+	n/a
Eppsteiner RW, *et al*. (*24*)	2008	2,949	-	+
McKay A, *et al*. (*25*)	2008	1,107	+	+
Nathan H, *et al*. (*26*)	2009	6,871	+	-
Stella M. (*27*)	2009	n/a	-	n/a
Chamberlain RS, *et al*. (*28*)	2011	84	-	+
Giuliante F, *et al*. (*29*)	2012	588	+	n/a
Yasunaga H, *et al*. (*30*)	2012	18,046	+	n/a
Viganò L, *et al*. (*31*)	2013	106	+	n/a
Goetze TO, *et al.*[*] (*32*)	2014	487	+	n/a
Ravaioli M, *et al*. (*33*)	2014	621	-	+
Schneider EB, *et al*. (*34*)	2014	3,695	+	+
Buettner S, *et al*. (*15*)	2014	9,874	+	n/a
Aldrighetti L, *et al.*[**] (*35*)	2015	1,497	+	n/a
Ejaz A, *et al*. (*36*)	2015	9,466	n/a	+
Buettner S, *et al*. (*37*)	2016	5,075	+	+
Gani F, *et al*. (*38*)	2016	27,813	+	n/a
Botea F, *et al*. (*39*)	2017	3,016	+	+
Chapman BC, *et al*. (*40*)	2017	12,757	+	+
Idrees JJ, *et al*. (*41*)	2018	96,107	+	n/a
Bouras AF, *et al.*[***] (*42*)	2019	46	-	n/a
Chen Q, *et al*. (*43*)	2019	4,902	+	n/a
Filmann N, *et al*. (*44*)	2019	110,332	+	+
Chang CM, *et al*. (*45*)	2019	13,159	+	+

[*]Focus on gallbladder cancer; [**]Learning curve not hospital volume; [***]Focus on laparoscopic liver surgery.

are confounding factors that are difficult to separate. In this sense, it is important to note that it is difficult to distinguish when high quality care in complex surgery is a consequence of reaching the plateau of a learning curve or when it is the consequence of a standard volume that is a minimum number of procedures per year. Besides, it is important to note that good outcomes in hepatobiliary surgery are also related to the quality of other hospital services, such as the anesthesiology service and the intensive care unit, which similarly to the surgeons have to reach the plateau of their learning curves. In this sense, further studies should be conducted to better characterize these two phenomena (*i.e*. learning curve versus minimum standard volume). Nathan H *et al*. (*26*) reported that the surgeon volume was not associated with in-hospital mortality, while Chang CM *et al*. (*45*) reported the combined effects of hospital and surgeon volume strongly influenced short-term survival after hepatic resection. In this latter study, the prognosis was adjusted for several different factors such as indication for surgery, quality of the underlying chronic liver disease, and socio-economic status that were found to be important to be recorded and analyzed to strengthen the relationship between perioperative outcome and surgeon and/or hospital volume. Besides, Chang CM *et al*. (*45*) figured out that the combination of high-volume surgeons in high-volume hospitals was associated with higher quality results, while the combination of high-volume surgeons in low-volume hospitals was not. Notably, in this study high-volume hospitals were those institutions performing more than 245 cases per year, while high-volume surgeons were those surgeons performing more than 59 cases per year. Notwithstanding these published studies, the definition of "high-volume center" remains to be elucidated. There is not an established cut-off of liver resections per year to perform (*48*).

Centralization of hepatobiliary surgery

The goal of centralization of hepatobiliary surgery is to provide optimal care of patients affected by hepatobiliary diseases within a given geographical area. This centralization passes through a complex process of assessment, development of dedicated policies, ongoing assurance and support from national government agencies, which should have the competence and authority to promote high quality care, good use of technical and technological tools, good allocation of human resources, and at the same time monitor, minimize and control the probability of unfortunate events. This process should be provided along a space-

time continuum that should warrant quality in all phases of the care of patients affected by hepatobiliary diseases.

These critical issues are very important in particular in liver surgery for several reasons. First, the definition of resectability is not standardized and wide variability is, in fact, observed among expert surgeons (*49*). Second, the complexity of liver surgery is difficult to be classified because several different types of resections requiring an extremely wide range of expertise can be performed. A standard distinction between major and minor hepatectomies is inadequate in the current era of modern liver surgery (*50*). Indeed, there are different technical solutions allowing parenchymal-sparing hepatectomies, much more complex than standard major hepatectomies, that remain in the shadow of the definition of minor hepatectomy. Yet, high quality centers should not be considered those centers performing a high proportion of major hepatectomies. In this sense, a new classification for minor hepatectomy that might help in better reporting minor but complex resections has been recently proposed (*51*). Third, post-operative morbidity and mortality rates have a limited validity to assess quality. Centers selecting only patients operable by performing small limited resections may have lower morbidity rates in comparison with centers routinely selecting patients operable by performing complex resections. Fourth, realistic cutoffs of mortality and morbidity rates after hepatectomy as a benchmark of quality should be defined to avoid the risk of denying the chance of care to those patients with higher complexity due to tumoral presentation or advanced age or because of severe comorbidities. Apart from the specificity of their indications for surgery, which requires being addressed by the local multidisciplinary teams (MDT), risk-adjusted metrics to compare outcomes among institutions are mandatory. Otherwise the risk of unfair comparisons will remain. In this sense, a benchmarking process has been started by merging the comprehensive complications risk (CCI) (*52*), liver failure occurrence, and morbidity and mortality classified according to the Clavien-Dindo classification (*53*). Last but not least, as recently pointed out by Aloia *et al*. (*54*) there are some downsides to the strategy of aiming at zero mortality rates after surgery such as the performance of innovative operations, which at least at the beginning are not compatible with perfection that might be strongly limited in the context of no-mortality. Therefore, the centralization process in hepatobiliary surgery should pass through the development and adoption of a new and modern common language for indications, resectability, terminology of resection, and good quality indicators.

Minimum hospital requirements in hepatobiliary surgery

To date, there are no specific published criteria that a given hospital should have to perform hepatobiliary surgery. Most of the authors that have focused on this topic have reported their personal experiences, which anyway should be taken into consideration at least in the meantime of the reading out of some new studies with data. In 2016 a position paper published on behalf of the Italian Society of Surgery had the merit to feed up the debate and set some standards of reference (*55*). In Italy the current law about hospital standards is detailed by rule n. 70/2015, which divides hospitals into three levels (*i.e*. basic, I, and II levels). Accordingly, hepatobiliary surgery should be performed at least in level I hospitals or even better in level II hospitals, and the surgical team should be dedicated only to hepatobiliary and/or hepatobiliary and pancreatic procedures. This dedication should warrant a high-quality standard.

Moreover, those high-quality hospitals, in which hepatobiliary surgery might be performed, should have the following departments: *i*) Department of Medical Oncology; *ii*) Department of Diagnostic Radiology, which should include some interventional radiologists dedicated to hepatobiliary diseases; *iii*) Department of Hepatology and/or of Internal Medicine with some internists dedicated to hepatobiliary diseases; *iv*) Department of Digestive Endoscopy; *v*) Intensive Care Unit; *vi*) Department of Pathology; *vii*) Department of Nuclear Medicine; and *viii*) Department of Radiation Oncology.

Even stating that the above-mentioned departments should be present in any high-quality hospital certified for hepatobiliary surgery, there might be a case of a given hospital that does not have some of the previous departments. In such a case, strong operative networks between that hospital and another institution should be activated to cover any deficiency. Similarly, in such a case of a given department of hepatobiliary surgery that does not provide liver transplantation another referral center in the same geographical area should be in the network to give consultation for liver transplantation. It should not be any more allowable that a patient with complex hepatobiliary disease hospitalized in a given hospital without the titles of performing diagnosis and/or therapy for that specific disease do not provide the required network of care in the same geographical area.

Multidisciplinary team

Nowadays, it is mandatory to have MDT dedicated to patients affected by hepatobiliary diseases. MDT meetings provide the right global assessment of the patient both for diagnosis as well as for therapy. Any MDT meeting should include at least one member of the previous listed hospital departments with the aim to cover all the inherent aspects. Only physicians dedicated to liver diseases should take part to the MDT meeting, which should be scheduled based on

the case-load but in general once per week. A written report of the MDT should be provided for each patient with the signature of all those members that have contributed to the discussion. It is important to note that the correct functioning of the MDT meeting relies on the proper union between the scientific evidence and the local experience in the diagnosis and cure of a given hepatobiliary disease. A MDT well balanced among specialties represented, and authoritative in all its specialists, provides better patient management resulting in better short- and long-term outcomes (*56-59*).

Hospital volume versus surgeon volume

Ideally, hospital volume and surgeon volume should match while in the real world this is not always warranted. In hepatobiliary surgery, the relative importance of hospital versus surgeon volume is very important because both short- and long-term outcomes are dependent on hospital factors, such as the presence of intensive care unit, and surgeon factors, such as the operative technique. Nathan H *et al*. (*26*) showed that the protective effect of hospital hepatic resection volume persisted after case-mix adjustment for competing risk factors, while that was not the case considering the surgeon hepatic resection volume. Indeed, high- and low-volume surgeons had comparable in-hospital mortality rates after hepatectomy (*26*). There are also other factors inherent in the hospital organization which were not considered and may have biased Nathan *et al*. conclusions: *i.e*. an active MDT meeting discussing each patient as above stated, which was not considered by them and by many other authors as well.

Learning curve or standard volume?

Center volume, surgeon volume, and surgeon experience all appear to impact success rates in liver surgery. A better understanding of how these factors interact to influence outcomes could help to develop specific healthcare strategies for the improvement of the quality of care in patients with hepatobiliary diseases. As said before, it is difficult to distinguish if good outcomes in hepatobiliary surgery are more dependent on the learning curve or to a minimum standard volume. A possible strategy to overcome this infertile dualism might be the introduction of certification for hepatobiliary surgeons. Far from the idea of more bureaucracy, this strategy might include analysis of the training with emphasis on the schools of surgery, and mentors that a given surgeon might have trained under during his or her career to be entitled in performing complex hepatobiliary surgery. As recently pointed out by some authors, this was found to be a good strategy in the field of pancreatic surgery and might work also in other fields of surgery (*31,59*). Besides, it might be the way to reinforce the importance of schools of surgery, which are those named to train young surgeons.

Toward certified hepatobiliary surgeons

A strategy to overcome the difficulty in decoding the dualism hospital volume – surgeon volume might be the introduction of certification provided by a national board of specialists. This board should be an independent, non-profit organization founded for the purpose of certifying surgeons who have met a defined standard of education, training and knowledge. Moreover, this board might work in defining the minimum standard of care in hepatobiliary surgery on an individual basis and might analyze the applicant's training and operative experience as well as his/her professionalism and ethics. Upon successful completion of these analyses, the surgeon might become certified in hepatobiliary surgery. This certification might serve as a prerequisite of good practice in hepatobiliary surgery, which together with the above reported minimum hospital requirements in hepatobiliary surgery, both as a single institution or as an established network between different institutions, might be warranted for high-quality care – independently by a number of procedures. Notably once certified, the hepatobiliary surgeon should undergo a process of maintenance of certification (every 5-10 years) with the aim of demonstrating ongoing professionalism and commitment to continuing medical education in the field of hepatobiliary surgery.

Conclusions

In conclusion, volume and outcome data in hepatobiliary surgery are intrinsically associated with some limitations. The published studies are mostly observational, and retrospective. Besides, the centralization process requires preparatory and preliminary agreements among experts about the development and adoption of new and modern common language for indications, resectability, terminology of resection, and good quality indicators. Without these agreements, hospital as well as surgeon volume act as proxy measures for technical and nontechnical skills. However, such a centralization process remains very important to offer better care for patients suffering from complex hepatobiliary disease.

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REVIEW

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Simulation and navigation liver surgery: an update after 2,000 virtual hepatectomies

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Abstract: The advent of preoperative 3-dimensional (3D) simulation software has made a variety of unprecedented surgical simulations possible. Since 2004, we have performed more than 2,000 preoperative simulations in the University of Tokyo Hospital, and they have enabled us to obtain a great deal of information, such as the detailed shape of liver segments, the precise volume of each segment, and the volume of hepatic venous drainage areas. As a result, we have been able to perform more aggressive and complicated surgery safely. The next step is to create a navigation system that will accurately reproduce the preoperative plan. Real-time virtual sonography (RVS) is a navigation system that provides fusion images of ultrasonography and reconstructed computed tomography images or magnetic resonance images. The RVS system facilitates the surgeon's understanding of interpretation of ultrasound images and the detection of tumors that are difficult to find by ultrasound alone. In the near future, surgical navigation systems may evolve to the point where they will be able to inform surgeons intraoperatively in real time about not only intrahepatic structures, such as vessels and tumors, but also the portal territory, hepatic vein drainage areas, and resection lines that have been planned preoperatively.

***Keywords*:** liver simulation, liver navigation, real-time virtual sonography (RVS), fusion imaging

Introduction

Hepatectomy is one of the most curative treatments for patients with various malignant liver tumors, but the high post-hepatectomy morbidity and mortality rates were once obstacles to the adoption of this choice of treatment. By the contribution of the establishment of surgical criteria (*1,2*) and the development of surgical devices (*3-5*), the surgical outcomes have improved over the last three decades.

Around the year 2000, one such advancement, 3-dimensional (3D) simulation software, was developed and facilitated understanding of intrahepatic structures by visualizing 3D imaging, even for novice hepatic surgeons. In addition, 3D simulation software makes it possible to calculate not only the volume of the liver as a whole but also the volume of future liver remnants or the areas perfused by intrahepatic vessels.

Since 2004, more than 2,000 preoperative simulations have been performed at our institution, and the additional information they have yielded has provided surgeons with new knowledge that has enabled safer surgery. The next step in preoperative simulation, intraoperative navigation may help surgeons intraoperatively to perform liver resections as planned, and it may be necessary to perform safer liver resection.

In this article, we review achievements that have been made with this 3D simulation system and consider the future development of intraoperative navigation systems.

Identification of the shape of liver segments

Couinaud's description of liver anatomy (*6*), which is based on portal vein branching, has been widely used by hepatologists and surgeons. Anatomic liver resection (*7,8*), *i.e.*, removal of portal venous territory, was proposed to spare the future liver remnant without impairing oncological outcomes for patients with hepatocellular carcinoma (HCC). Anatomic liver resection is sometimes required even in patients with colorectal cancer liver metastasis (CRLM) to avoid a large ischemic area when the portal vein adjacent to the tumor is resected. The first step in anatomic liver resection is identification of the boundaries of the segment. Makuuchi *et al.* reported a dye staining technique in which the portal vein is punctured and indigo-carmine is injected under ultrasonographic guidance (*9*), and clamping or dividing the corresponding portal vein makes it possible to visualize

the boundaries of the segment as a demarcation line (*10,11*). However, these methods only allow visualization of the portal territory on the liver surface, and the border deep in the liver parenchyma is unclear. Injection of the objective portal vein with an ultrasound contrast-enhanced agent and ultrasound examination of the area of the parenchyma stained has been reported as a means of identifying the boundary of the liver parenchyma (*12,13*).

After 3D simulation software became available, it became possible to calculate the shape of a segment by considering the portal vein dominant area. Shindoh *et al.* used 3D simulation software and were the first to identify the shape of intersegmental planes and show that intersegmental planes are not always flat (*14*). They found that the right portal scissura is not always flat, but appeared to have a concave shape when viewed from the dorsal side in 54% of the cases examined. Anatomical liver resections may be incomplete, if surgeons do not pay attention to the shape of segmental borders, and the same technical pitfall has been reported in the use of right lateral sector grafts during living donor liver transplantation (LDLT) (*15,16*). The 3D simulation validation re-realized that intersegmental plane always located along the hepatic vein, and exposing the landmark vein was important for accurate resection (*14*). Indocyanine green (ICG) fluorescence imaging has recently been used as a staining method to improve identification of segmental borders (*17-19*). Because the ICG fluorescence persists for a long time during liver parenchyma transection, it is possible to correctly identify the shape of liver segments intraoperatively.

It is especially difficult to identify the 3D shape of segment 1 without using simulation software, because the anatomy of the portal vein perfusing segment 1 is variable, and, usually, any major hepatic vein runs on the border of the territory. Although caudate lobectomy can be performed by using the counterstaining technique to identify the boundary of a segment (*20,21*), additional information regarding the 3D shape of segment 1 obtained preoperatively using 3D simulation software may play a significant role in identifying the shape of segment 1. Maki *et al.* stated that the acaval vein runs between the paracaval portion and segment 7 or 8 in 48% of the cases (*22*). 3D simulation also demonstrated the cranial margin of the paracaval portion, which reached the diaphragmatic surface in 30% of the cases, and it alerted the surgeon to the fact that if the root of the right hepatic vein or middle hepatic vein were exposed during segmentectomy of segment 7 or 8, the paracaval portion might be resected at the same time (*22*).

Volumetry of portal territory

Preoperative volumetry is now recognized as a technique that is essential to ensuring a safe hepatic resection by estimating the volume of the future liver remnant. Makuuchi *et al.* established criteria for maximum resection volume classified according to the ICG retention rate (*1*), and Kubota *et al.* established indications for portal vein embolization according to the volume of the future liver remnant (*2*). Liver volumetry had conventionally been achieved by manually tracing computed tomography (CT) images and dividing the liver parenchyma along the lines formed by the routes of the hepatic veins (*2,23*). This technique allowed limited portal territories, such as the anterior sector, posterior sector, segment 4, and left liver, which are partitioned by hepatic veins or the falciform ligament, to be calculated, but accurate calculation of the volume of Couinaud segments (*6*) is impossible because of the loss of clear dividing lines in the liver (*2,24*).

After the development of 3D simulation software, simply creating 3D images made automatic liver volumetry possible, and it also became possible to measure liver volume corresponding to any branch of the portal vein. Several studies reported finding that the volume of segments calculated with the simulation software correlated well with the weight of the actual resected specimens (*19,25-28*). Mise *et al.* reported a detailed analysis of Couinaud segment volumes based on their study of 107 LDLT donors (*29*). They found that segment 8 was the largest segment, occupying a quarter of the whole liver (almost 26%), that it was followed by segment 7 (almost 17%), and that these volumes were comparable to one section of left liver (*1*). In addition, since the volume of each segment varies significantly, it is important to estimate the volume of the hepatic remnant in each individual case.

Moreover, anatomic resection of the territory of the portal triad branches distal to the Couinaud segments may be optional. The hepatectomy procedure in each patient should be planned according to the balance between the hepatic functional reserve and estimated volume of the hepatic remnant in that patient. Precise volumetry measurements of portal territories with simulation software may aid in planning an accurate operative strategy.

Hepatic vein anatomy and criteria for hepatic venous reconstruction

The postoperative impact of hepatic congestion that results from resecting hepatic veins was unknown until the early 2000s. Although a venous communication may be formed after hepatectomy combined with resection of hepatic vein (*30,31*), it is unclear whether this phenomenon occurs in all cases. It has also been reported that portal regurgitation occurs in congested areas (*32*), and that such areas are susceptible to necrosis (*33*). Akamatsu *et al.*, on the other hand, found that adequate venous drainage during the first month after liver transplantation was important for liver regeneration

(*34*). Mise *et al.* demonstrated safe standards for venous reconstruction criteria (*35*). In their study, hepatic vein reconstruction was required, if non-congested future liver remnant was not maintained at least 40-50% of total liver volume, and it reduced surgical invasiveness without influencing the postoperative course. By following this criteria, we have previously reported performing aggressive but safe hepatectomy combined with hepatic vein resection and reconstruction (*36,37*). These detailed examinations are largely dependent on the ability of simulation software to calculate the area drained by the hepatic vein. Tani *et al.* created a "venous drainage map" that was drawn by using 3D simulation software, and it informed surgeons about the congested volume in the hepatic remnant after hepatectomy combined with hepatic vein resection (*38*). For example, the venous drainage map showed that extended right hemihepatectomy combined with middle hepatic vein resection would be accompanied by partial congestion of segment 4. Simulation software enables preoperative estimations of such congested volumes that would otherwise be impossible.

How to evaluate liver function in congested areas is also unclear. In a study in which Hashimoto *et al.* used near-infrared spectroscopy to detect ICG uptake they showed that sinusoidal perfusion in veno-occlusive regions was reduced by about half that in non-veno-occlusive regions (*39*), and in a study using ICG fluorescent imaging Kawaguchi *et al.* showed that portal uptake function in veno-occlusive regions was approximately 40% of that in non-occlusive regions (*40*). Gadoxetate disodium-enhanced magnetic resonance imaging (MRI) has also attracted attention as a method of evaluating liver function (*41,42*), and differences in transporter expression, which can be considered an indicator of liver function, between congested and non-congested areas has been investigated (*43*). Site by site liver function assessment, such as congested area or non-congested area, may be possible by verifying gadoxetate disodium-enhanced MRI. In the future, objective studies using simulation software, ICG fluorescent imaging, gadoxetate disodium-enhanced MRI, *etc.*, will be necessary to collect more evidence to clarify liver function in congested areas.

Achievements of virtual hepatectomy

Preoperative examination of 3D images containing images of tumors and intrahepatic vasculature assists liver surgeons in their attempt to acquire an anatomical understanding of liver. Lamade *et al.* described the advantages of referring to 3D simulation images as a means of understanding tumor locations and planning resections, even for surgeons in training (*44*). Saito *et al.* found that preoperative simulations were useful in achieving negative surgical margins (*26*). Lang *et al.* have stated that preoperative simulations helped them prevent postoperative liver failure by accurately preserving the vascularity of the hepatic remnant something, which 2D CT is incapable of doing (*45*). Takamoto *et al.* reported that the image of segment border by preoperative simulation was helpful when intraoperative staining was unclear (*28*). Furthermore, Oshiro *et al.* developed new 3D simulation software called "Liversim" that takes liver deformation during liver resection into account, and they have described this new simulation system as a useful means of preoperative imaging (*46,47*). The Liversim software allows simulation of the whole liver transection procedure, from the start of liver transection to removal of the resected specimen, and provides gradually changing images of the transection plane and exposed vasculature.

Mise *et al.* summarized 1,194 preoperative simulation cases focusing on LDLT and hepatectomy for HCC/colorectal liver metastasis (*48*). Preoperative evaluation of liver volume and the area drained by the middle hepatic vein of living liver transplant donors by 3D simulations enabled aggressive harvesting of right liver grafts while maintaining donor safety. Precise segment volumetry by 3D simulation software in HCC cases allows increased anatomical resection in impaired liver function patients, and it may improve long-term outcomes. Similarly, by enabling surgeons to easily understand the relationship between the tumor and vessels, preoperative 3D simulations in CRLM patients has helped surgeons perform aggressive hepatectomy for advanced tumors by means of complex resections.

Application to intraoperative navigation

The preoperative simulation systems described above are useful for preoperative planning of hepatic resection, but they do not provide the surgeon with a navigation tool during the actual operation. To enable surgeons to use systems to navigate during operations, there are several reports that enable reflection of the preoperative simulation in the intraoperative field. For example, a 3D printing model of the intrahepatic vessels prepared on the basis of 3D simulation images has been introduced to help surgeons understand intrahepatic anatomy in 3D intraoperatively (*49*), and Nishino *et al.* recently reported having devised a method of real-time navigation surgery that uses projection mapping (*50*). Their system makes it possible to project ICG fluorescence images onto the surface of the liver during liver transections and differentiate between areas that need to be resected and those that do not need to be resected. Augmented reality (AR) techniques have also been developed and provide 3D images of intrahepatic vessels and tumors by using a projector beam to display the images on the patient's body or organ surface (*51*), or by using stereovision eyeglasses to display on the monitor (*52,53*).

Real-time virtual sonography (RVS)

RVS is a novel fusion imaging technology that has recently been developed and that simultaneously provides ultrasonography images and virtual sonographic images reconstructed from CT or MRI scans by using an electromagnetic system, and RVS is regarded as one of the "navigation" systems that help surgeons perform intraoperatively. In addition to being useful when performing liver surgery, RVS has been reported to be useful when performing radiofrequency ablation (*54,55*) and breast cancer biopsies (*56-59*). Sato *et al.* have used RVS when performing liver surgery and have demonstrated its usefulness for visualizing the relationships between resection lines and tumors (*60*).

Although the ideal navigation system would be easy to use for persons who are unfamiliar with intrahepatic anatomy, the current RVS system requires manual adjustment using bifurcation of intrahepatic vessels which takes time and requires some understanding of intrahepatic anatomy (*61*). Ang *et al.* reported finding that RVS adjustment took a median time of 3 minutes (range 1-12 minutes) (*62*), and our own measurements showed that it took a median time of 105 seconds (range, 51-245 seconds) (*61*). A new RVS equipped with an automated adjustment system that enables quick, easy adjustments is currently being developed (*63*).

RVS has an error between the ultrasonography image and the reconstructed CT image, and we showed that the error is less than 10 mm (*61*) This error is considered permissible for use while comparing the two images alternately. One of the advantages of using this system is the ability to identify hepatic tumors that are difficult to identify by intrahepatic ultrasonography (IOUS) alone. Two representative cases in which RVS technology was effective in detecting small liver tumors are summarized below.

Case 1

A 67-year-old male underwent hepatic resection for HCC; the tumor measured 30 mm in diameter and was located in segment 8. Preoperative late-phase CT images revealed a low-density nodule, 8 mm in diameter, in segment 4 (Figure 1). At laparotomy, B-mode IOUS clearly demonstrated the tumor in segment 8, and a slightly hyperechoic nodule was seen in segment 4. However, the nodule in segment 4 was too small to conclude that it was the same as the nodule that had been detected in the preoperative late-phase CT images. Intraoperative RVS synchronized with preoperative CT was performed to accurately locate the tumor in segment 4. Since the nodule was seen between the two tributaries of the middle hepatic vein in the preoperative CT images, the RVS was adjusted to the bifurcation point of the middle hepatic vein. Meticulous examination by RVS confirmed that the nodule detected by the IOUS was identical to the tumor detected in the preoperative late-phase CT images. Then, RVS in which contrast-enhanced IOUS (CE-IOUS) was performed using Sonazoid (gaseous perflubutane; GE Healthcare, Oslo, Norway) was synchronized with preoperative CT images was performed 15 minutes after the contrast medium injection, and the nodule was ultimately judged to be benign, because it was isoechoic with the surrounding liver (Figure 2, Video S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=6*) (*64,65*). Based on the above findings, only the tumor in segment 8 was resected. Postoperative follow-up CT for two years showed no changes in the size of the nodule in segment 4, thereby confirming that it was not a malignant tumor.

Case 2

A 76-year-old female was diagnosed with rectal cancer and there were numerous cysts in the liver (Figure 3A). The diffusion-weighted MRI (DW-MRI) and positron-emission tomography (PET) findings led to suspicion of a synchronous liver metastasis, 7 mm in diameter, in segment 5 (Figure 3B and 3C). Since contrast-enhanced imaging was not performed because of the patient's underlying renal dysfunction, it was rather difficult to precisely identify the tumor. Low anterior resection of the rectum and synchronous liver resection were

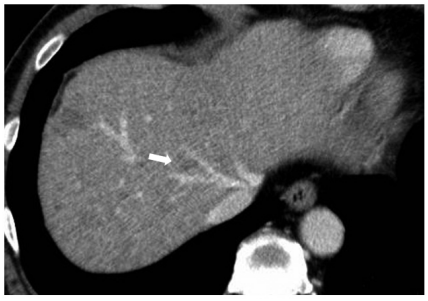

Figure 1. Preoperative CT scan in Case 1. The arrow points to a low-density nodule that was suspected of being a tumor preoperatively.

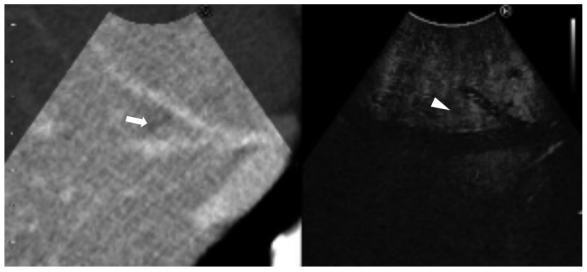

Figure 2. Still intraoperative real-time virtual sonography image in Case 1. Preoperative CT (left side) and contrast-enhanced intraoperative ultrasound (CE-IOUS; right side) were synchronized. The nodule was diagnosed as benign, because there was no hypo-echoic nodule at the same site on the CE-IOUS scan (arrowhead) where the low-density nodule was seen on the CT scan (arrow).

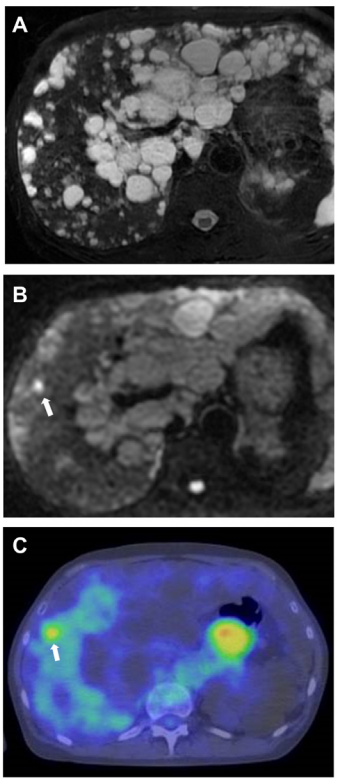

Figure 3. Preoperative magnetic resonance imaging and positron-emission tomography in Case 2. (A) T2-weighted image revealing multiple cysts in the liver. Diffusion-weighted magnetic resonance imaging **(B)** and positron-emission tomography **(C)** were able to detect tumor (arrow).

planned. At laparotomy, the multiple cysts interfered with localization of the tumor in segment 5 both by B-mode IOUS and by CE-IOUS. Intraoperative RVS in which CE-IOUS was synchronized with DW-MRI was performed to locate the tumor, and it demonstrated a hypoechoic nodule at the site where the suspected liver metastasis had been identified by DW-MRI (Figure 4, Video S2, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=7*). B-mode IOUS ruled out the possibility of the nodule being a cyst, because, in contrast to the other cysts, it was isoechoic. The tumor was resected with a negative surgical margin, and histopathology confirmed it to be a liver metastasis from the rectal cancer.

Another fusion imaging system that uses the optical tracking system has also been reported (*66*). In this system, the position and direction of the ultrasound probe are identified using a marker attached on the probe through the optical tracking camera placed above the site of the laparotomy. However, this system is limited by the fact that it will not work if there is an obstruction between the camera and the marker, and during liver surgery the echo probe is sometimes positioned deep in the diaphragm, which obstructs visualization of the marker. Electromagnetic navigation systems, such as the RVS system, on the other hand, can be used in narrow places without worrying about

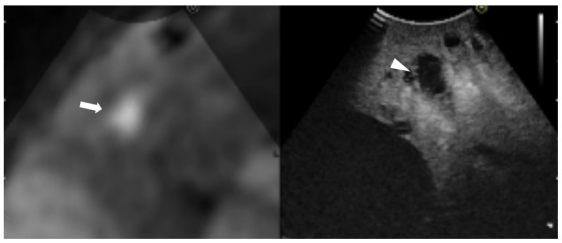

Figure 4. Still intraoperative real-time virtual sonography image in Case 2. Preoperative diffusion-weighted magnetic resonance imaging (DW-MRI; left side) and CE-IOUS (right side) were synchronized. The CE-IOUS image demonstrated a hypo-echoic nodule (arrowhead) at the same site where DW-MRI demonstrated a lesion that was strongly suspected of being a liver metastasis (arrow).

shielding, and can be applied to laparoscopic surgery.

Conclusions

The advent of 3D simulation software has made safer and more aggressive surgery possible. In the future, we need to disseminate new evidence brought by simulation, and expect the development of a new surgical navigation system that will help surgeons performing planned operations.

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Interpretation of guidelines for the diagnosis and treatment of primary liver cancer (2019 edition) in China

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Abstract: Primary liver cancer (PLC) is currently the fourth most common malignancy and accounts for the second most cancer-related deaths in China. Since 2017, a great deal of high-level evidence, and particularly evidence based on Chinese studies and practice, has emerged in terms of diagnosis, staging, and treatment. A new version of the guidelines for the management of PLC specifying the diagnosis and treatment of PLC (2019 edition) has recently been published. The guidelines feature major changes in the techniques for early diagnosis, the combination of surgery, local therapy, and systemic treatment, and the use of traditional Chinese medicine. The guidelines need to be further implemented in clinical practice to demonstrate their validity.

***Keywords*:** hepatocellular carcinoma, diagnosis, treatment

Introduction

According to GLOBCAN 2018 data (*1*), there were 841,080 new cases of liver cancer worldwide annually, of which 392,869 occurred in China, accounting for 46.7% of cases around the world. In China, liver cancer ranks second in cancer deaths and forth in cancer prevalence. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer (PLC), which accounts for 75%-85% of PLC. In terms of prognosis, the overall 5-year survival rate of HCC in China from 2012 to 2015 was 12.1%, the 5-year survival rate of HCC was 14.0% in urban areas and only 11.2% in rural areas (*2*).

The prevalence of PLC in China poses a threat to the health and life of the Chinese people. Since the 2011 and 2017 versions of guidelines for the diagnosis and treatment of PLC were published in China, many new studies have been conducted and more evidence has emerged. China published updated guidelines (2019 edition) to optimize the management of PLC on the basis of the 2017 edition. Here, the recommendations in the 2019 guidelines have been summarized and updates to those guidelines have been interpreted. Consistent with the guideline, PLC refers to HCC in this article. In addition, a comparison of the 2011, 2017, and 2019 editions is shown in Table 1.

Surveillance and diagnostic algorithm

Monitoring and screening

Like the 2017 guidelines, the new guidelines consider patients with a history of chronic liver disease to have a high risk of developing HCC and the guidelines recommend ultrasonography (US) and measurement of alpha-fetoprotein (AFP) for surveillance every 6 months (Figure 1).

Imaging examinations

Once abnormalities are found in AFP/US screening, computed tomography (CT), magnetic resonance imaging (MRI), and contrast-enhanced ultrasound (CEUS) are routine methods with which to definitively diagnose HCC. As imaging technology has advanced, MRI has gradually become a common type of examination for clinical diagnosis of HCC. Therefore, the 2019 guidelines highlight the important role of MRI in the diagnosis and evaluation of HCC, and especially MRI with a hepatocyte-specific contrast agent (Gd-EOB-DTPA). Multimodal MRI is better than dynamic contrast CT in detecting and diagnosing HCC with a diameter of 2.0 cm (*3,4*), and is better than dynamic enhanced CT in evaluating whether HCC has invaded the portal vein or hepatic vein and metastasized to abdominal or retroperitoneal lymph nodes. In addition, MRI with Gd-EOB-DTPA has a higher rate of detecting liver lesions with a diameter of ≤ 1.0 cm (*5-7*).

Serological molecular markers

AFP is the most commonly used serological molecular marker for diagnosis and monitoring the response to treatment. However, normal AFP level may be present

Table 1. Important updates to the 2011, 2017, and 2019 guidelines for diagnosis and treatment of primary liver cancer in China

Version (*Ref.*)	Diagnosis	Staging	Treatment
2011 (*32*)	Specifies the HCC diagnostic criteria.	TNM BCLC	Multidisciplinary integrated treatment.
2017 (*33*)	Pathological diagnosis: "7"point baseline extraction method. MVI	CNLC	Clear root-and-branch LR standards.
2019 (*34*)	Emphasizes the value of MRI Describes new serological molecular markers	CNLC	Treatment of recurrence after liver transplantation: RF ablation, TACE, *etc.*

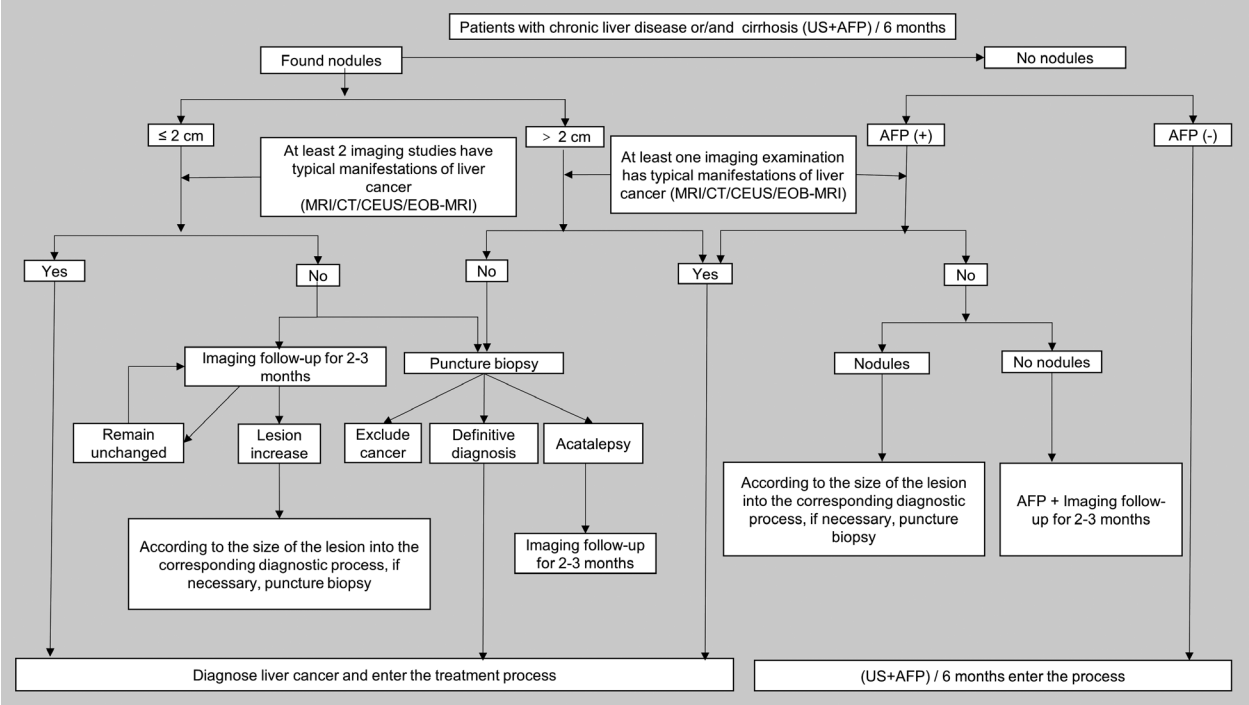

Figure 1. Clinical diagnostic criteria and roadmap for primary liver cancer in China (*34*)**.**

in about 30% of patients with HCC. For serum AFP-negative patients, serum AFP-L3, PIVKA-II, or des-gamma carboxyprothrombin (DCP) and plasma free microRNA are alternatives for early diagnosis or surveillance of HCC. In recent years, liquid biopsy has shown great potential in early diagnosis and evaluation of efficacy.

The 2019 guidelines first describe several new serological molecular markers, such as circulating tumor cells (CTCs), circulating cell-free microRNA, and circulating tumor DNA (ctDNA). Liquid biopsy may have higher sensitivity and specificity than commonly used clinical molecular markers such as serum AFP and PIVKA-II (*8*). A combination of several plasma miRNAs is also highly useful in the early diagnosis of HCC. For example, a model for diagnosis of HCC created using the levels of expression of seven plasma miRNAs can accurately diagnose early HCC (with a sensitivity of 86.1% and a specificity of 76.8%), and its sensitivity is about 30% higher than that of traditional markers. Patients with AFP levels that preclude determination can still be accurately diagnosed with miRNA (with a sensitivity as high as 77.7% and a specificity as high as 84.5%) (*9*). At present, a HCC detection kit based on circulating miRNA has been validated in multi-center clinical trials and is in clinical use in China. miRNA diagnosis is expected to generally facilitate early diagnosis and treatment of HCC and truly benefit patients.

Liver puncture biopsy

Unquestionably, liver puncture biopsy can provide a definitive pathological diagnosis for lesions found in an imaging examination lacking the typical characteristics of HCC. Liver biopsy can provide valuable information on the nature of the lesion, the etiology of liver disease, molecular typing of HCC, guiding treatment, and determining prognosis. However, liver biopsy may cause the rupture of tumor nodules and needle tract

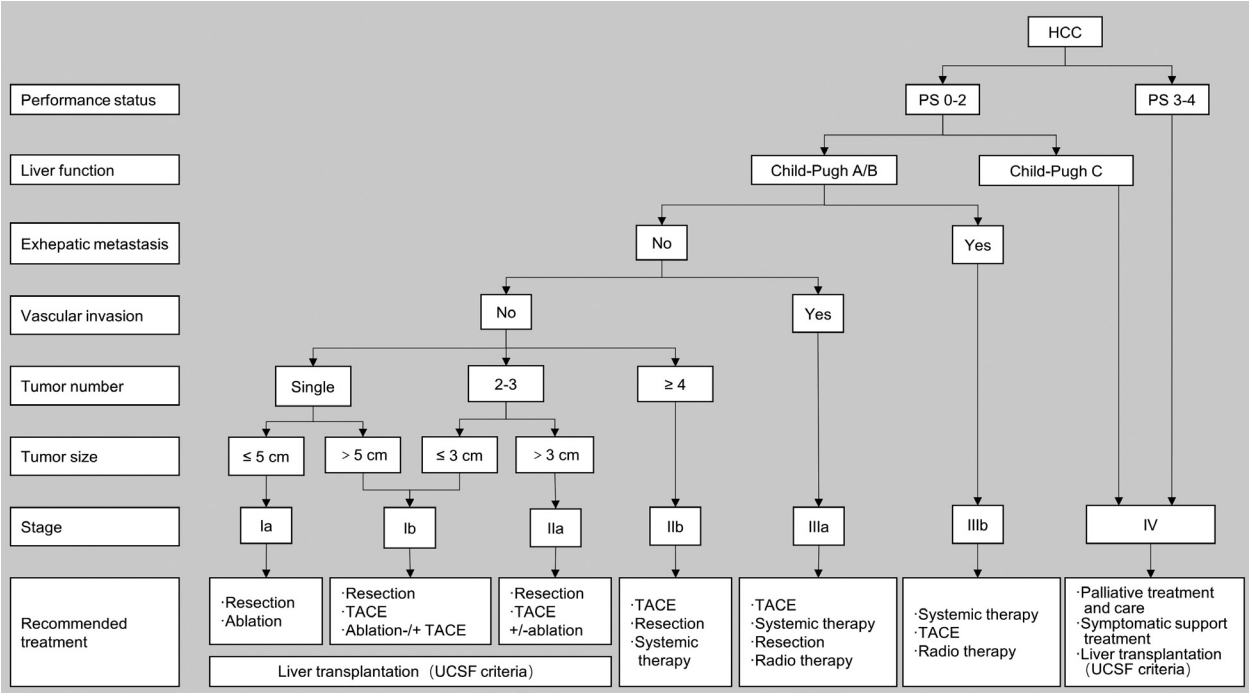

Figure 2. Clinical staging and treatment of primary liver cancer in China (*34*)**.**

implantation, and it can even occasionally lead to a false-negative result. Thus, the 2019 guidelines specify that patients with lesions that have typical imaging features need not undergo a diagnostic liver biopsy.

Staging and treatment algorithm

Identical to the 2017 version, the new guidelines still use the China HCC staging system and treatment algorithm (CNLC) based on the Chinese medical system and Chinese practices and experiences. The algorithm includes the size and number of tumors, performance status (PS), and liver function (Figure 2). The main points of this updated staging algorithm are in systemic therapy and the combination of multiple treatment modalities, indicating that the current model of HCC treatment has entered a new era of comprehensive multidisciplinary treatment.

Surgical resection

Liver resection (LR) is the most effective curative treatment of HCC (CNLC stage Ia, Ib, or IIa cancer), and especially for patients with 1-3 nodules and without metastasis or vascular invasion. There is considerable controversy regarding whether LR is suitable for patients with portal hypertension (PHT). However, most surgeons treating HCC in China do not agree that PHT is a contraindication for LR. Results of several Chinese studies have indicated that PHT does not affect patient prognosis. Therefore, the 2019 guidelines emphasize that selected patients with PHT can still undergo a liver resection after a comprehensive evaluation, and their long-term survival after surgery is superior to other treatments (*10,11*). A more accurate assessment of the degree of PHT can help to select patients eligible for LR (*12,13*).

As surgical resection techniques have made great progress, a lot of new evidence has been incorporated into the new guidelines. For example, preoperative 3D visualization technology can help to design more precise resection margins and approaches to protect the remaining liver (*14,15*). Patients with huge or multiple lesions often need to undergo extensive resection to obtain negative margins. However, an insufficient future remnant liver volume (FRLV) is the main factor hindering the results of radical resection. Transarterial chemoembolization (TACE) and portal vein embolization (PVE) are routine methods to treat these patients. Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) is a complicated surgery that is rarely performed. However, recent studies have found that the long-term survival benefit of patients undergoing ALPPS was significantly better than that of patients undergoing TACE, indicating that ALPPS is a feasible strategy for patients without an insufficient FRLV (*16*). In addition to this evidence, the 2019 guidelines also note that LR with wide margins results in a better long-term prognosis than narrow resection margins, and this is especially true for patients with microvascular invasion (*17,18*). In terms of postoperative treatment, the 2019 guidelines more clearly suggest that an antivirus work-up can reduce recurrence after R0 resection is achieved (*19*). Additionally, two randomized

controlled studies involving patients with a high risk of recurrence have confirmed that TACE reduces recurrence and prolongs survival (*20,21*).

Liver transplantation

The 2019 guidelines are consistent with the University of California San Francisco (UCSF) criteria for liver transplantation (LT), albeit with a modest expansion of the indications for LT. Patients with CNLC stage IV cancer can still undergo LT after an accurate evaluation. In addition, treatment of post-operative recurrence was added to the 2019 guidelines, which focus on multi-disciplinary comprehensive treatment modalities including modification of the immunosuppressive regimen, additional surgery, TACE, local ablation treatment, radiation treatment, or systemic treatment.

Local ablation therapy

Patients in whom HCC is confirmed are often unable to undergo radical surgery due to serious cirrhosis or advanced cancer. Only about 20% to 30% of patients are eligible to undergo surgical resection. Fortunately, local ablation therapy (LAT) causes less damage to liver function, less trauma, and has a high response rate. Patients not eligible for surgical resection can receive radical treatment with LAT. Radiofrequency ablation (RFA) is the most common LAT, and the 2019 guidelines highlight its role in the treatment of early-stage HCC based on a great deal of high-level evidence. For example, patients with early-stage HCC undergoing RFA have a survival benefit comparable to that of patients undergoing surgical resection (*22,23*). For a single lesion ≤ 2 cm in diameter, the survival benefit of undergoing RFA is the same or greater than that of surgical resection, and this is especially true for centrally located liver cancer (*24,25*). Given this evidence regarding RFA, the 2019 guidelines cite RFA as the first-line treatment strategy for patients with early-stage HCC who are ineligible for surgical resection.

*Transarterial chemoembolizatio*n

TACE is commonly used as a non-surgical strategy to treat HCC. It is suitable for patients with CNLC stage IIb, IIIa, or IIIb cancer. It is mostly used as a combination of surgical treatment and ablation treatment. For example, the 2019 guidelines emphasize a combination of ablation therapy, systemic treatment, or antivirus treatment. A randomized controlled phase II trial (TACTICS) has indicated that TACE plus sorafenib significantly improved progression-free survival over TACE alone in patients with unresectable HCC and that TACE can significantly delay the time from disease progression to vascular invasion or extrahepatic metastasis (*26*). In addition, the 2019 guidelines have added a prognostic score called "six-and-twelve" (the sum of the number of tumors and tumor size is used to divide patients into 3 strata: ≤ 6, > 6 but ≤ 12, or > 12) that can individualize prognostic assessment and risk stratification of patients undergoing TACE. Patients in different strata result in significant differences in median survival. Therefore, this prognostic model prior to performing TACE may provide reference values and help patients choose different treatment options (*27*).

Systemic treatment

For patients with advanced HCC that cannot be surgically resected (CNLC stage IIIa and IIIb cancer), systemic treatment may prolong their life and decrease the tumor burden. Sorafenib has already been found to have significant survival benefits for patients with HCC. Before the 2019 version was published, sorafenib was the only molecularly targeted drug for advanced HCC. Recently, many multi-center clinical studies involving new drugs have been conducted around the world, and great progress has been made. A randomized phase III non-inferiority trial (REFLECT) indicated that lenvatinib was not inferior to sorafenib in terms of overall survival for patients with advanced HCC (*28*). Moreover, lenvatinib can also provide better survival benefits for most Chinese patients with HBV-related HCC. Another randomized phase III trial (RESORCE) found that regorafenib is the only targeted drug that benefits patients with cancer progressing despite sorafenib treatment (*29*). Regorafenib provided a median overall survival benefit of 26 months, and this result had already been confirmed by multiple real-world studies worldwide (*30*). Within the context of the great progress made in targeted therapy, the 2019 guidelines have highly emphasized systemic treatment and added lenvatinib as first-line treatment and regorafenib as second-line treatment, thus expanding treatment options for patients. A better protocol combining different targeted drugs and searching for new systemic agents are key ways in which systemic treatment can prolong survival.

Traditional Chinese medicine

The 2017 guidelines noted that traditional Chinese medicine can relieve clinical symptoms, improve the body's resistance, and reduce the adverse effects of radiotherapy and chemotherapy. In the 2019 edition, there is high-level evidence that taking Huaier granules after liver resection can result in lower recurrence and better survival (*31*), demonstrating that traditional Chinese medicine can greatly help the treatment of HCC. Traditional Chinese medicine is considered to have great potential and will be increasingly used in cancer treatment. However, more standardized clinical studies need to be conducted in the future to accumulate

more evidence of the feasibility and safety of traditional Chinese medicine.

Conclusion

The new guidelines place considerable emphasis on multidisciplinary treatment incorporating new evidence-based suggestions, and this will further promote advances in the treatment of PLC in China. Although they are based on Chinese experiences, these guidelines should also help other countries to defeat this condition.

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An overview in management of hepatocellular carcinoma in Hong Kong using the Hong Kong Liver Cancer (HKLC) staging system

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Abstract: In Hong Kong, liver cancer is the fifth most common cancer and the third most common cause of cancer deaths. The prevalence of hepatitis B is high in Hong Kong because of the high rate of hepatitis B virus infection, and chronic hepatitis B has remained the leading cause of hepatocellular carcinoma in the city, accounting for 80% of all cases in the period from 1992 to 2016. In view of the different etiologies of hepatocellular carcinoma around the world, a group of liver experts in Hong Kong developed the Hong Kong Liver Cancer staging system in order to provide more aggressive treatment guidance (predominantly a wider use of surgical resection) for Asian patients of hepatocellular carcinoma. In this article focussing on the Hong Kong Liver Cancer staging system, we briefly reviewed the screening criteria adopted in Hong Kong for liver resection, local ablation, transcatheter arterial chemoembolization, transcatheter arterial radioembolization, stereotactic body radiation therapy, and systemic therapy.

***Keywords*:** HKLC, HCC, SBRT, liver cancer staging, cirrhosis, hepatectomy, ablation, systemic therapy, laparoscopic

Introduction

Globally, liver cancer shows a remarkable distribution in which more than 80% of hepatocellular carcinoma (HCC) cases occur in the East-Asian region (*1*). In Hong Kong, liver cancer is the fifth most common cancer (with a total of 1,834 registered cases) and the third most common cause of cancer deaths (10.8%) (*2*). According to data from the cancer registry, the incidence and mortality of liver cancer are higher in men (fourth and third respectively among all cancers) than in women (eleventh and fourth respectively among all cancers). The prevalence of hepatitis B is high in Hong Kong because of the high rate of hepatitis B virus infection, and chronic hepatitis B has remained the leading cause of HCC in the city, accounting for 80% of all cases in the period from 1992 to 2016 (*3*).

The mechanism of hepatitis B carcinogenesis includes a combination of gradual liver cell necrosis, inflammation, massive fibrosis, and eventual cirrhosis. Overall, the duration between hepatitis B virus infection and development of HCC may take as long as 50-60 years (*4*). Therefore, depending on the degree of malignancy, the severity of neighboring invasion and the adequacy of remnant liver function, treating HCC could be extremely challenging and long in duration. Treatment plans are personalized for maximum benefits for patients. Currently, surgical (liver resection, liver transplantation, local ablation, *etc.*) and non-surgical modalities (transcatheter arterial chemoembolization (TACE), stereotactic body radiation therapy (SBRT), chemotherapy, targeted therapy, *etc.*) are available for HCC treatment in Hong Kong.

In view of the different etiologies of HCC around the world, a group of liver experts in Hong Kong developed the Hong Kong Liver Cancer (HKLC) staging system (Figure 1) in order to provide more aggressive treatment guidance (predominantly a wider use of surgical resection) for Asian HCC patients (*5*). In most Western regions, where the Barcelona Clinic Liver Cancer staging system is widely used, the main risk factors for HCC development are chronic hepatitis C, alcohol-related cirrhosis and non-alcoholic fatty liver disease instead of chronic hepatitis B as in Asia. The HKLC staging system, supported by data from 3,856 patients treated in Hong Kong, has successfully identified patients who are suitable for more aggressive treatment (*6*).

Screening

Alpha-fetoprotein (AFP) is the most widely used biomarker for HCC detection. However, an elevation of AFP level can also be seen in conditions like germ

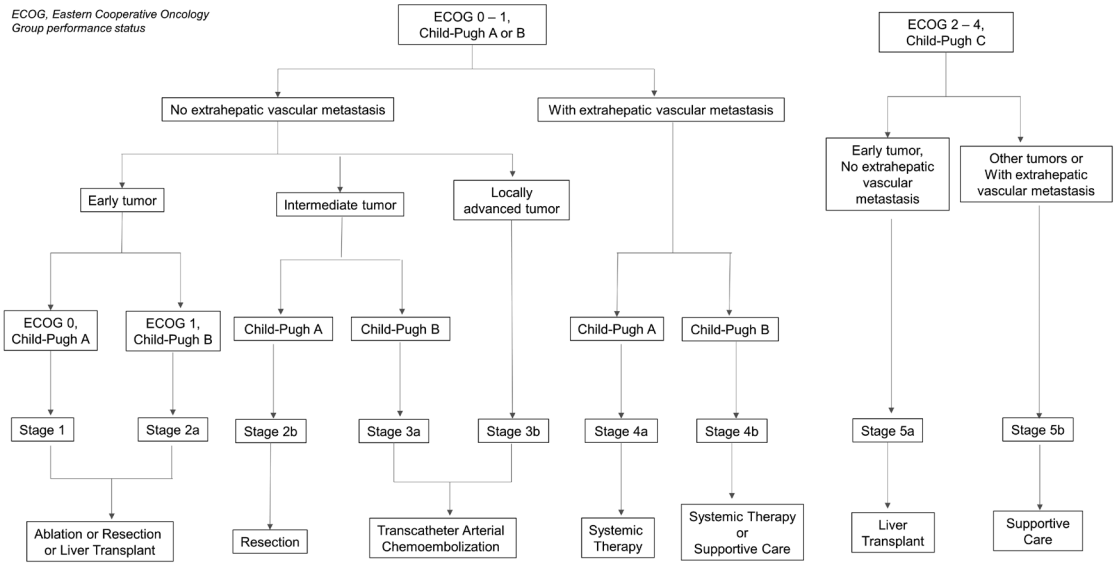

Figure 1. Summary of the Hong Kong Liver Cancer (HKLC) staging system.

cell tumors, liver inflammation (*e.g.* chronic hepatitis), regenerating nodules, and pregnancy. Furthermore, up to 40-50% of HCCs are non-AFP-secreting, further lowering the sensitivity of AFP alone for detection. It was reported that the sensitivity of AFP for detecting early HCC ranged from 39 to 65%, whereas the specificity ranged from 76 to 97% (*7*). Ultrasound can be used in conjunction with AFP for more reliable clinical surveillance. A meta-analysis reported that ultrasound alone had a sensitivity of only 47% in detecting early-stage HCC in cirrhotic patients, while another study reported that the addition of AFP to ultrasound imaging remarkably increased the sensitivity of early HCC detection (*8*). To date, the Cancer Expert Working Group on Cancer Prevention and Screening has made the following screening guidelines after reviewing scientific evidence and local epidemiology (*9*). For persons at average risk, routine screening with AFP and ultrasound is not recommended. Patients with chronic hepatitis B or C or liver cirrhosis are categorized as a high-risk group for HCC development. Routine cancer surveillance (*e.g.* every 6-12 months) with AFP and ultrasound is recommended depending on patients' age, family history, presence of cirrhosis, and other clinical conditions.

The role of surgery

Liver resection

According to the HKLC staging system, liver resection should be considered the first-line treatment for patients with intermediate-stage HCC who have desirable liver function reserve. Indications for liver resection include unilobar disease and absence of invasion of the portal vein and inferior vena cava (*5*). While imaging techniques such as computed tomography and magnetic resonance imaging provide evidence of liver size and tumor position, liver function must be assessed before resection so as to formulate the best surgical procedures. The volume of functional liver reserve should be measured by multiple qualitative and quantitative tests to establish a reliable preoperative assessment. Hence, a tumor larger than 10 cm should not be considered to be a contraindication to liver resection if the liver reserve is satisfactory and a curative resection is expected. This has been supported by a retrospective study comparing surgical outcomes in patients with different tumor sizes. In the study, the median survival of patients with curative resection of solitary HCC larger than 10 cm without macroscopic venous invasion was 38.0 months (*10*). To study the actual functional liver capacity, biochemical parameters are measured and scoring systems (*e.g.* Child-Pugh score and Model for End-stage Liver Disease score) are used to grade the extent of disease. The indocyanine green (ICG) clearance test should be performed and used together with Child-Pugh classification for better assessment of liver function reserve. The cut-off values for a safe major hepatectomy and minor hepatectomy are 14% and 22% respectively (*11*). These values could be pushed higher in relatively young patients and those with a sufficient remnant liver volume. A study of 68 patients in Hong Kong revealed a significant correlation between liver stiffness measurement and ICG retention rate at 15 minutes after injection (ICG-R15). The combination of ICG-R15 and liver stiffness measurement may be used for better prediction of outcomes in potential liver resection candidates (*12*). Aspartate transferase level and alanine transferase level are markers of liver damage and correlate with the extent of hepatocellular necrosis rather than the actual liver function. Albumin

and clotting factors are exclusively synthesized by the liver, and therefore their plasma concentrations can indicate liver function. While plasma bilirubin concentration provides indirect information on the uptake, conjugation and excretion functions of the liver, non-hepatic factors may also influence the plasma bilirubin level. As a result, plasma bilirubin concentration is often used in combination with other laboratory findings and clinical grading systems. Minimally invasive liver surgery initially benefitted patients who had liver metastasis but relatively normal liver function. However, minimally invasive surgical techniques have become much more advanced. Nowadays, it is a common practice to remove HCCs in cirrhotic patients by minimally invasive surgery. It was reported that minimally invasive liver resection resulted in fewer short-term complications but similar long-term oncological outcomes when compared with open liver resection (*13,14*).

Local ablation

In general, liver resection remains the "gold standard" treatment for HCC in Hong Kong if the tumor is operable and satisfactory liver remnant function is expected. Local ablation can be considered an alternative approach to resection for small HCCs (< 3 cm) in Child-Pugh A/B patients. Surgical resection remains the preferable option for resectable tumors (3-5 cm) in patients with good functional liver reserve.

Radiofrequency ablation (RFA) utilizes high-frequency radio waves delivered *via* a needle electrode to cause destruction of tumor by local heating. A local randomized clinical trial comparing treatment outcomes of hepatic resection and radiofrequency ablation in early-stage HCC (solitary tumor no larger than 5 cm; or no more than 3 tumors, each 3 cm or smaller) revealed that they both shared similar clinical data in terms of tumor reoccurrence, overall survival and disease-free survival. In addition, RFA was associated with shorter hospital stay, less blood loss and shorter treatment duration due to its less invasive procedure (*15*). However, another study found that RFA is more likely to result in incomplete clearance of tumor at specific sites of the liver, in which surgical resection may be the more suitable option (*16*). Nevertheless, RFA alone has a tendency to achieve good tumor growth control in small HCC tumours and is recommended for such patients (*17*). RFA has also proved to be safe and effective when adopted in combination with other interventions. This was supported by a retrospective study in which the prognosis and treatment outcomes of patients with multifocal HCC and similar tumors characteristics were reviewed. The group receiving both surgical resection and RFA had fewer major resections (32% *vs*. 62%), less blood loss (400 *vs*. 657 mL), shorter operation time (270 *vs*. 400 min) and shorter hospital stay (7 *vs*. 8.5 d) than that receiving only surgical resection (*18*). For HCC larger than 3 cm, RFA was found to be safe and effective. The study suggested that an overall ablation rate of 91% was achieved for HCCs 3.1-8.0 cm in size with a treatment mortality rate of 3% (*19*). A systematic review and meta-analysis showed that RFA plus TACE was associated with a more significant advantage in recurrence-free survival and overall survival than a stand-alone treatment with RFA (*20*).

Microwave ablation is used as an alternative to RFA. Multiple studies have reported a similar efficacy and safety profile to RFA, with microwave ablation being superior in larger HCC nodules (*21-23*).

High-intensity focused ultrasound (HIFU) ablation is a truly non-invasive tumor ablation technique, which requires an extracorporeal source of ultrasound beams targeting lesions *via* intact skin without surgical technique (*24*). In Hong Kong, HIFU ablation is one of the treatment options adopted as a bridging therapy for HCC patients awaiting deceased donor liver transplantation. HIFU ablation is generally well tolerated in HCC patients with advanced cirrhosis and gross ascites (*25*). This modality is also safe for Child-Pugh A and B patients and even for selected Child-Pugh C patients, offering them a good alternative before transplantation (*26*). Figures from a retrospective study showed that patients with unresectable HCC receiving HIFU ablation had a significantly better rate of complete response and long-term survival rates compared to that receiving TACE as primary treatment (*27*).

Non-surgical treatments

Transcatheter arterial chemoembolization (TACE)

TACE involves the intra-arterial administration of chemotherapeutic drugs carried by iodized poppy seed oil, Lipidol, through the feeding artery of the tumor to achieve cytotoxic effects. This effect is potentiated by simultaneous delivery of an embolic agent such as Gelfoam, achieving tumor ischaemia, which delays the wash out rate by blood flow from the tumor vascular bed (*28*). In Hong Kong, TACE has emerged as a recommended treatment for unresectable HCC with a good liver reserve, no vascular invasion and absence of extrahepatic spread. A local randomized controlled trial gave evidence of its safety and effectiveness, in which TACE has shown an excellent tumor response with one-year survival rate of 57% compared to 32% when conservative management was given (*29*). Furthermore, advanced age is not a contraindication of TACE treatment. Another study has confirmed the comparable efficacy and tolerance to TACE treatment for advanced HCC in both young (≤ 70 years) and elderly (> 70 years) patients, indicating a reliable palliative treatment

for unresectable HCC (*30*). In addition, a recent retrospective study suggested that preoperative TACE was associated with an improved overall survival and recurrence free survival after resection of huge HCC (≥10 cm) (*31*).

TACE with drug-eluting beads is an option when conventional TACE has failed but evidence of its superiority over conventional TACE is still lacking. In TACE with drug-eluting beads, exertion of drug function and embolization occur simultaneously, whereas in conventional TACE, the embolic agent is applied after drug injection (*32*). A recent retrospective study in Taiwan reported that TACE with drug-eluting beads provided better long-term benefits than conventional TACE did (*33*). Although several studies showed the benefits of this new TACE option over conventional TACE, the method is still very controversial in clinical practice.

Current guidelines in Hong Kong state that TACE should be repeated every 2-3 months based on tumor status and liver function closely monitored by computed tomography or magnetic resonance imaging. TACE should be stopped when there is liver impairment or other serious complications. When there is no viable tumor, TACE should also be discontinued and only repeated when residual tumor or new tumor growth has been detected.

Transcatheter arterial radioembolization (TARE)

TARE is a useful bridging therapy as a tumor downstaging treatment for suitable liver transplantation candidates. It also offers a second chance for intermediate HCC patients who have failed to respond to conventional TACE. Radioembolization with yttrium-90 (^{90}Y) mainly induces tumor death by local close-distance radiation instead of embolization in TACE. This treatment has gradually gained support as more studies have provided robust evidence to prove its efficacy and ability to prolong unresectable HCC patients' survival (*34-38*).

A single center study in Hong Kong has found that patients were able to enjoy a longer progression-free survival and overall survival, which was supported by the duration of AFP response ≥ 6 months after radioembolization. Besides, this study also found that radioembolization had positive effects when presented with portal vein thrombosis in their HCC, which is usually regarded as a contraindication to hepatectomy or liver transplantation (*35*). A retrospective study at the same center and a publication from an expert panel also echoed that TARE is a good choice for treatment in patients diagnosed with HCC plus portal vein thrombosis. Encouraging results were seen in this study because patients with major vascular invasion undergoing TARE had a median survival duration of 12 months and a 2-year survival rate of 15.6% (*36,37*).

Stereotactic body radiation therapy (SBRT)

In Hong Kong, most HCC patients are inoperable at the time of diagnosis. Treatment options may become limited due to unfavorable factors including tumor size, location and complications such as portal vein thrombosis. The development of SBRT has lowered the risk of radiation-induced liver disease, a long-time barrier of traditional radiation therapy that results in limited use for treating HCC. This is achieved by the delivery of high-dose radiation localized to only the diseased portion of the liver guided by real-time stereotactic 3D tracking of tumor position. This minimizes excessive radiation to normal liver parenchyma and surrounding healthy tissues.

Currently, the typical patient criteria for SBRT in Hong Kong includes Child-Pugh score of B8 or below, up to 5 lesions, uninvolved liver volume ≥ 700 mL and platelet count ≥ 50×10^9/L. A consensus made by a board of Asian experts confirms that SBRT is a safe and effective therapeutic option for patients with small-sized HCC, and offers substantial local control, improved overall survival, and low toxicity (*38*). Another study comparing outcomes following SBRT for Child-Pugh B and C patients with HCC also found that SBRT is suitable for patients with small HCCs and modestly impaired (Child-Pugh B7) liver function (*39*). SBRT can also be used as an alternative to HCC treatment in close proximity to major blood vessels and biliary duct, which is usually a contraindication to ablation techniques. While a retrospective study suggested that a combination of TACE and SBRT provides a survival benefit in patients with HCC tumors of ≥ 3 cm (*40*), and another study found no significant differences in the survival and adverse effects in patients with small HCCs who underwent SBRT with or without TACE (*41*). SBRT can also be used as a bridging therapy before liver transplantation thanks to its effective function of downsizing and stabilizing tumors prior to liver transplantation with minimal side effects (*42*). Another retrospective study on long-term outcomes of SBRT as a bridging therapy showed that 27% of the patients had achieved complete tumor necrosis according to explant pathology (*43*). Not only did all patients remain on the transplant wait list, no post-transplant recurrences were reported. All these encouraging findings suggested that SBRT could enable patients to remain on the transplant wait list longer especially in Hong Kong, where organ donation rates are low.

Systemic therapy

Sorafenib is used as a first-line treatment for advanced HCC with Child-Pugh A liver function patients who are not suitable for resection, locoregional ablation therapy and transarterial therapy. The Sorafenib HCC Assessment Randomized Protocol trial (SHARP) was a

multicenter, phase-3, double-blind, placebo-controlled trial in advanced HCC patients with Child-Pugh class A liver function (*44*). SHARP concluded that median survival was approximately 3 months longer for patients in the Sorafenib group than those in placebo group.

Regarding the Asia-Pacific region, a few studies have supported the use of Sorafenib as an effective treatment (*45-47*). A randomized controlled trial including 271 Asian patients indicates a 2-month prolongation in terms of median overall survival when Sorafenib was given, comparable to the results of SHARP. It was also found to be well tolerated, with common adverse effects being hand-foot skin reaction, diarrhea and fatigue (*46*). The subset analyses of this study suggested that Sorafenib consistently demonstrates a desirable efficacy and safety profile, irrespective of disease etiology, baseline tumor burden and prior therapy (*47*). In the SHARP trial, treatment was continued until both radiological and symptomatic progression or unacceptable toxicity occurred (*48*). Regorafenib is a second option when patients have developed progressive disease to Sorafenib treatment. In a phase-3 placebo-controlled trial (RESORCE), Regorafenib displayed a survival benefit in Sorafenib-refractory HCC patients; median survival was 10.6 months for Regorafenib compared with 7.8 months for placebo. The improvement in overall survival was consistent in all subgroup analyses (*49*). A retrospective analysis in Korean patients has illustrated results that are consistent with RESORCE; confirming the efficacy and safety outcomes for advanced HCC patients after disease progression on Sorafenib (*50*).

Conclusion

Management of HCC has evolved in the past decades because of pioneering research and innovative medical advances. In Hong Kong, where the prevalence of hepatitis-B-related HCC is relatively high, surgical resection is the first-line treatment for suitable HCC patients, as evidence has shown that more aggressive treatment is effective and safe in the Asia-Pacific region. The "left shift" of HCC treatment is to ensure maximum survival benefits, and careful patient selection for the most appropriate treatment should be enforced.

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Early hemodynamics of hepatocellular carcinoma using contrast-enhanced ultrasound with Sonazoid: focus on the pure arterial and early portal phases

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Abstract: To clarify the early hemodynamics of hepatocellular carcinoma (HCC), we defined the early portal phase of contrast-enhanced ultrasound (CEUS) and examined the reliability of this modality for determining HCC differentiation. Starting in 2007, we performed Sonazoid CEUS in 146 pathologically confirmed hepatic nodules; 118 HCC (8 poorly [Pd], 73 moderately [Md] and 37 well-differentiated [Wd]) and 28 benign nodules. We focused on the pure arterial and early portal phases up to 45 seconds after Sonazoid injection, and then the subsequent phase up to 30 minutes. We calculated covariance-adjusted sensitivities for nodule enhancement combinations of these three phases. Nodule enhancements were divided into hypo, iso and hyper. A positive predictive value of 100% was obtained for the following patterns: iso-iso-hypo, hypo-iso-iso, and hypo-hypo-hypo for Wd, hyper-iso-hypo and hyper-hypo-hypo for Md, hypo-hyper-hypo for Pd, and hyper-hyper-hyper for benign nodules. In Wd HCC (early HCC), there were seven enhancement patterns, thought to be characterized by various hemodynamic changes from early to advanced HCC. Two patterns allowing a diagnosis of Wd HCC were hypo in the pure arterial phase. Subsequent iso-enhancement in the early portal phase indicated a portal blood supply. Decreased enhancement in the early portal phase allows a diagnosis of Md HCC. However, gradual enhancement observed from the pure arterial to the early portal phase allows a diagnosis of Pd HCC. Therefore, even in the early portal phase, hemodynamic changes were visible not only in Wd but also in Md and Pd HCC. In conclusion, with division of the early phase hemodynamics into pure arterial and early portal phases, CEUS can provide information useful for determining the likely degree of HCC differentiation and for distinguishing early stage HCC from benign nodules.

***Keywords*:** histological differentiation, early HCC, well-differentiated HCC, contrast-enhanced ultrasonography

Introduction

Contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI) are widely used for examining liver tumors. As physicians are primarily responsible for the practical implementation of contrast-enhanced ultrasound (CEUS), the number of patients receiving CEUS is limited. However, due to high sensitivity for identifying target tumors, CEUS is a potentially appropriate imaging modality for detailed evaluation of liver tumors. The 2011American Association for the Study of Liver Diseases (AASLD) guidelines do not recommend CEUS for the diagnosis of hepatocellular carcinoma (HCC) (*1*). The Contrast Enhanced Ultrasound Liver Imaging Reporting and Data System (CEUS LI-RADS) from 2017 does, however, categorize CEUS findings for the differential diagnosis of liver tumors (*2*). In Japan, CEUS plays an important role in the diagnostic algorithm for hepatic tumors (*3*).

As for the ultrasound contrast agent used in Japan, Sonazoid was approved in 2007. The incidence of adverse reactions is low. Unlike SonoVue and Definity, which are used mainly in western countries, Sonazoid is characterized by producing distinct enhancement of the liver parenchyma in the post-vascular phase (Kupffer phase) (*4*). Thus, Sonazoid CEUS is widely used in HCC cases for tumor detection, differential diagnosis, as well as ultrasound-guided treatment navigation and evaluation of treatment responses in post-vascular phase images (*5*). However, few reports have focused on early phase hemodynamics of HCC (*6-8*).

CEUS is superior to other imaging modalities for

visualizing tumor blood flow, though conventional observation is insufficient for detailed evaluation of early phase images characterized by rapid changes in blood flow. Inflow-time mapping has been developed, allowing arterial and portal flows to be displayed separately (*9*). We aimed to investigate the diagnostic ability of CEUS for HCC differentiation using this method. Thus, we observed early hemodynamics on CEUS by dividing the arterial phase into the pure arterial and early portal phases and compared the obtained findings with histopathological features.

Materials and Methods

Patients

Among patients in whom pathologically confirmed hepatocellular nodules (primary HCC and benign nodules, ≤ 5 cm) were obtained during the period from 2007 to 2013 in the Tokyo Women's Medical University, 146 patients with 146 nodules in which Sonazoid-enhanced ultrasound was performed and contrast agent inflow had been identified throughout the tumor, intrahepatic artery, and portal vein in a single section were enrolled in this study. These 146 nodules were comprised of 118 HCC nodules (8 poorly differentiated [Pd], 73 moderately differentiated [Md], and 37 well-differentiated [Wd]) and 28 benign nodules (4 hepatocellular adenoma [HCA], 18 focal nodular hyperplasia [FNH], 3 alcoholic hyperplastic nodules, and 3 large regenerative nodules). All Wd HCC included in this study, except for two nodules obtained by biopsy, corresponded to early HCC (*10*) with stromal invasion (*11*).

Methods

Based on the enhancement differences between the nodules and the surrounding liver parenchyma, nodule enhancements were classified into three levels: hypo-, iso-, and hyper-enhancement. The degree of enhancement was analyzed as one of these three levels, regardless of whether the entire or only part of the nodule showed the enhancement changes.

The imaging phase during CEUS was defined as follows: the pure arterial phase indicated by microbubbles initially appeared in the intrahepatic artery and persisted up to the time immediately before intrahepatic portal flow visualization; the early portal phase was from the first visualization of microbubbles in the portal vein up to 45 seconds after contrast injection; and the subsequent phase was from one minute after injection up to 30 minutes. Based on CEUS patterns obtained from these three imaging phases and four histopathological findings (*i.e.*, Pd, Md, Wd, and benign nodules), Bayes' theorem was applied to calculate the positive predictive value for each of pathological types.

CEUS examination

CEUS was performed by a single ultrasound specialist (with 20 years of experience) within 2 weeks before surgery. Sonazoid was intravenously administered (0.01 mL/kg) through a 21-gauge cannula and flushed with 5 mL of normal saline at a speed of 1 mL/s. We continuously observed tumor enhancement during the initial period of 1-50 seconds, and then again at 1, 2, 3, 5, 10, 20, and 30 minutes (several seconds each). All CEUS images were stored digitally to a hard disk. Two ultrasound specialists (with more than 15 years of experience), both of whom were blinded to the clinical and histopathological findings, reviewed the stored images.

The inflow of microbubbles into the intrahepatic artery (mainly the second branch), followed by that into the portal vein, and increased nodule enhancement as compared with the surrounding liver parenchyma were examined with cine-clip replay. The blood flow distributions within the tumor were observed as required using the inflow-time mapping, in order to assess the arterial and portal blood supplies in the nodules. Inflow-time mapping can demonstrate intensity changes of individual pixels. When saturation intensity reaches 80%, each pixel has a color and an inflow-time map is produced. The blood flow changes during the pure arterial and early portal phases are easily displayed separately by applying different colors representing the inflows into the hepatic artery and portal vein. An additional time-intensity curve was prepared for some nodules to confirm the arterial and portal flows.

The ultrasound device used was a Prosound α-10/F75 with extended Pure harmonic detection, which was operated at a frequency of 1.88 MHz, mechanical index (MI) of 0.18-0.24, dynamic range of 43-50 dB, and frame rate of 15-20 Hz, and Ascendus with wideband pulse inversion mode, which was operated at a frequency of 1.8 MHz, MI of 0.16-0.18, dynamic range of 45-50 dB, and frame rate of 13-20Hz (Hitachi Ltd., Tokyo, Japan).

Histopathological examination

In total, 146 nodules (82 obtained from liver resection and 64 from liver tumor biopsy) of patients enrolled in this study were fixed with formalin and then embedded in paraffin for preparation of 2 or 4 μm sections. The sections were principally stained with hematoxylin-eosin, silver, and Victoria-blue (in some cases, also with CK7 and CD34 stains) for the diagnosis of Wd HCC. Additionally, Hep-Per1, CK-19, and epithelial membrane antigen were stained to differentiate HCC from intrahepatic cholangiocarcinoma. For benign nodules, immunohistochemical staining with specific

tumor markers was performed to make a definitive diagnosis at the pathology department of Teikyo University Hospital (*12*). Two liver pathologists (with more than 20 years of experience) were involved in confirming the diagnosis.

Statistical analysis

The nodule enhancements observed in each of the three different imaging phases were categorized into three levels: hypo, iso, and hyper. In each nodule, each level was given a value of 1 when the nodule was judged to reach the categorized level and 0 otherwise, and then converted into multi-dichotomous values. We calculated crude sensitivities, *i.e*., positive rates, for each category of each imaging phase, simply by calculating the sum of these values divided by the number of nodules as the mean values. The covariance-adjusted sensitivities were calculated, because values which differ between imaging phases are not independent. The covariances for three variables were calculated as the sum of products of the difference between each value, 0 or 1, and the sensitivity, divided by the number of cases (*13*). The covariance-adjusted sensitivity was calculated as $s_1s_2s_3 + s_1cov_{s2s3} + s_2cov_{s1s3} + s_3cov_{s1s2} + cov_{s1s2s3}$, where s stands for the sensitivity, cov stands for the covariance, and the subscript numbers correspond to each of the imaging phases.

In order to calculate the positive predictive value or the posterior probability, we set equal prior probability values, *i.e*., 0.25 for each of the four diagnostic categories: benign nodule, Wd, Md, and Pd HCC. We calculated joint probabilities, the product of sensitivity and prior probability, for each of the three imaging phases and a predictive value as a proportion of each joint probability of the sum of the joint probabilities. We used R (*https://cran.r-project.org/*) for the above calculations with scripts that we devised for our research.

Results

Patient and nodule characteristics

The median age of the enrolled patients was 67 years (33-86 years). There were 107 males and 39 females. The numbers of tumors located in segments 1/2/3/4/5/6/7/8 were 1/11/14/19/22/18/19/42, respectively. The mean tumor sizes were 30 mm (15-50 mm) for Pd, 24 mm (10-50 mm) for Md, 16 mm (10-25 mm) for Wd HCC, and 25 mm (10-50 mm) for benign nodules.

The median times required for microbubbles to reach the intrahepatic artery and the portal vein after injection of Sonazoid were 17 sec (9-27 sec) and 24 sec (14-36 sec), respectively.

Predictive values of enhancement patterns for pathological diagnosis

Analysis of the enhancements of all nodules in each phase revealed 11 enhancement patterns, and seven of these showed a positive predictive value of nearly 100% for the pathological diagnosis (Table 1). For nodules with hyper-enhancement in the pure arterial phase, the hyper-hyper-hyper pattern was estimated to be benign. Meanwhile, the hyper-iso-hypo and hyper-hypo-hypo patterns were estimated to be Md. Nodules showing the iso-iso-hypo pattern were estimated to be Wd. For nodules with hypo-enhancement in the pure arterial phase, the hypo-iso-iso and the hypo-hypo-hypo pattern were estimated to be Wd. Another pattern, hypo-hyper-hypo, was considered to indicate Pd. The remaining four patterns corresponded to two (benign ≥ Wd, or Pd > Wd) or four pathological diagnoses. The hyper-hyper-hypo pattern, which was the most common, was seen at all histological grades as well as in benign nodules.

The covariance-adjusted sensitivity gave the exact posterior probability, and the sum of the positive predictive values for the four different diagnoses was 1 (100%). When different prior probalilities were used, some combinations of patterns resulted in a change of the most probable diagnosis (data not shown).

Findings in the early portal phase according to the HCC differentiation

In Wd HCC (early HCC), there were seven enhancement patterns, thought to be characterized by various hemodynamic changes, as compared to only three patterns for Md or Pd. Among the three patterns allowing a diagnosis of Wd HCC, two were hypo in the pure arterial phase. Subsequent iso-enhancement in the early portal phase indicated a portal flow supply which was recognizable with inflow-time mapping (Figure 1). Another early HCC, showing hyper-enhancement, is displayed in Figure 2.

In Md HCC, all three patterns were hyper in the pure arterial and hypo in the subsequent phase. If a nodule was hyper or iso/hypo in the early portal phase, the respective likelihoods of being Md HCC were 38% and 100%. Thus, decreased enhancement in the early portal phase allows a diagnosis of Md HCC (Figure 3).

Of the three patterns observed in Pd HCC, two showed the hypo finding in both the pure arterial and the subsequent phase. If the hypo-enhancement changed to hyper or iso in the early portal phase, the probabilities of these two patterns with a diagnosis of Pd HCC were 100% and 65%, respectively. More specifically, a diagnosis of Pd HCC was possible based on gradual enhancement observed from the pure arterial to the early portal phase. This finding, which depended on arterial flow, was confirmed by the time-intensity curve (Figure 4).

All benign nodules, with the exception of three large regenerative nodules, showed hyper-enhancement in both the pure arterial and the early portal phase,

Table 1. Enhancement patterns for pathological diagnosis of HCC

Nodule enhancement pattern				Pathological diagnosis			
Pure arterial phase	Early portal phase	Subsequent phase	(*n*)	Poorly differentiated (*n* = 8)	Moderately differentiated (*n* = 73)	Well-differentiated (*n* = 37)	Benign nodule (*n* = 28)
Hyper	Hyper	Hyper	9				9 (100%*)
Hyper	Hyper	Iso	14			4 (23.2%)	10 (76.8%)
Hyper	Hyper	Hypo	46	4 (40.5%)	34 (37.7%)	2 (4.4%)	6 (17.4%)
Hyper	Iso	Hypo	16		16 (100%)		
Hyper	Hypo	Hypo	23		23 (100%)		
Iso	Iso	Iso	7			4 (50.2%)	3 (49.8%)
Iso	Iso	Hypo	2			2 (100%)	
Hypo	Hyper	Hypo	2	2 (100%)			
Hypo	Iso	Hypo	7	2 (64.9%)		5 (35.1%)	
Hypo	Iso	Iso	14			14 (100%)	
Hypo	Hypo	Hypo	6			6 (100%)	

*The positive predictive values are in parentheses.

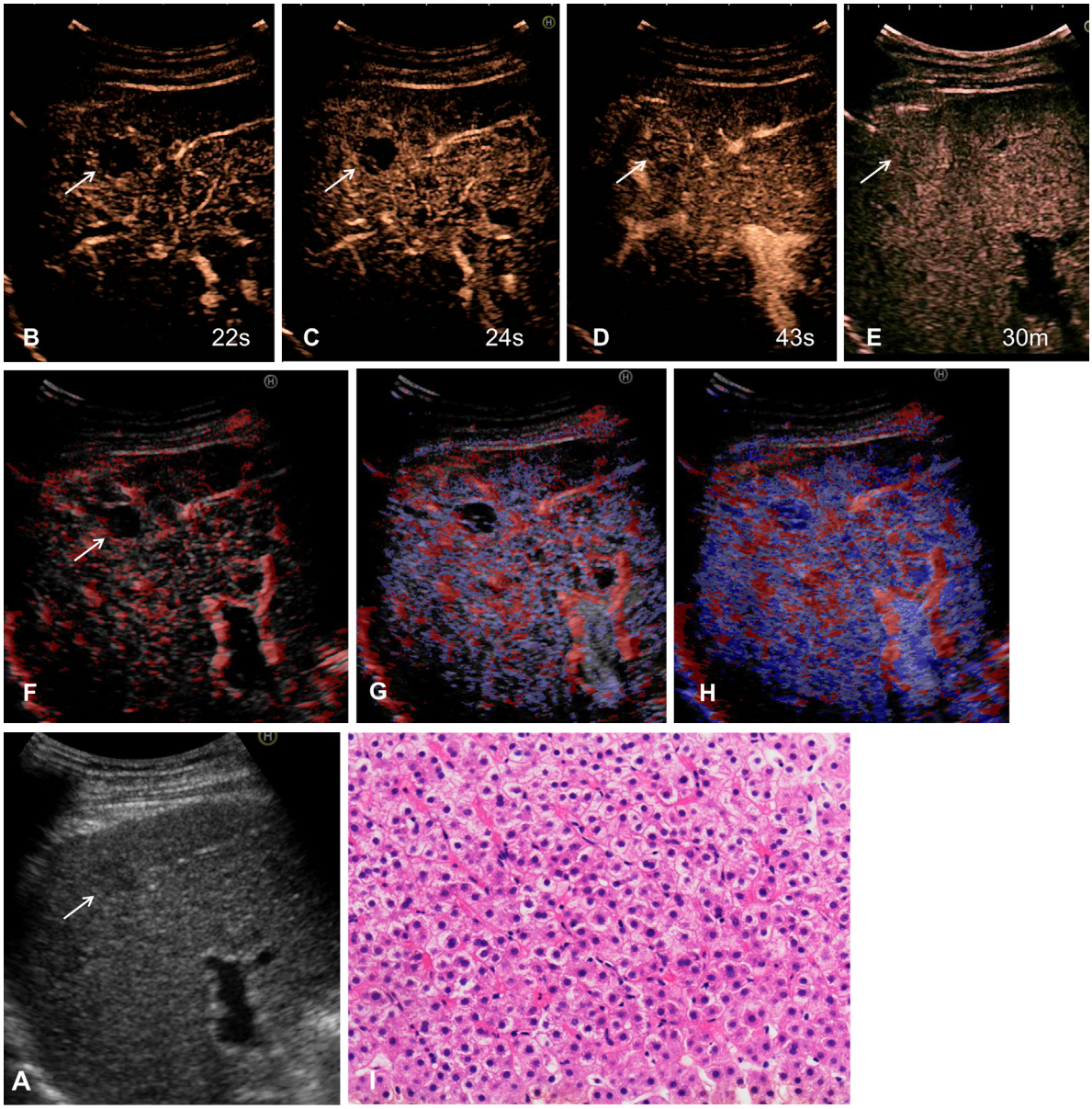

Figure 1. Hypovascular well-differentiated HCC findings on Sonazoid CEUS. A hypoechoic 1.2-cm tumor with an unclear margin on B-mode US **(A)**. In the pure arterial phase, the tumor remains hypo **(B)**, followed by hypo to iso enhancement in the early portal phase **(C, D)**. Iso-enhancement persists at 30 min **(E)**. Inflow time-mapping (ITM) shows red pixels in the artery and liver parenchyma, while there is no arterial flow inside the tumor **(F)**. After the portal flow is detected, the tumor vessels and tumor are enhanced appearing blue **(G, H)**. The biopsy specimen showed well-differentiated HCC **(I)**.

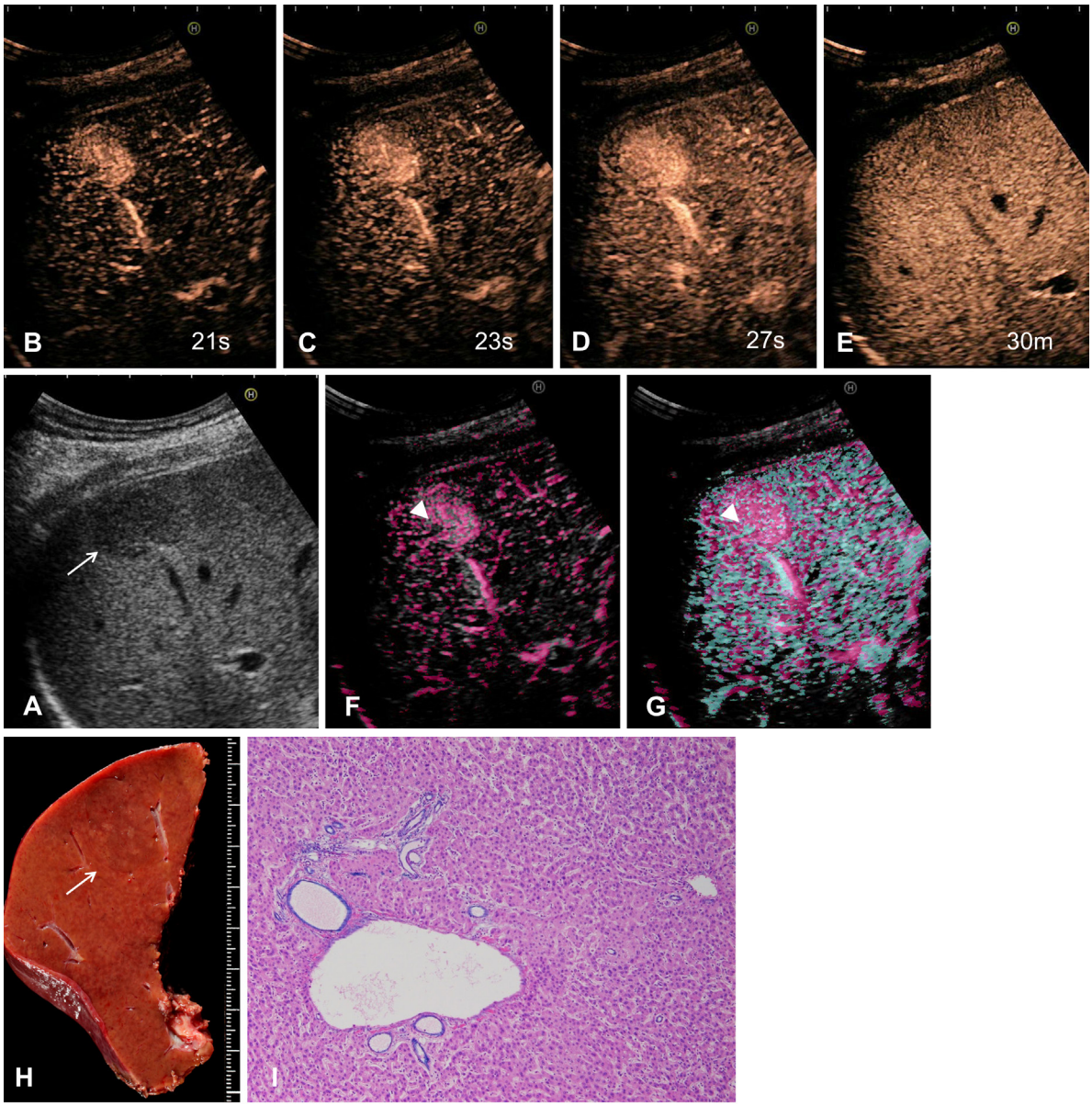

Figure 2. Hypervascular well-differentiated HCC findings on Sonazoid CEUS. A 1.7-cm hypoechoic nodule with ill-defined margins on B-mode US **(A)** shows hyper-enhancement in the pure arterial phase **(B)** and continued peripheral enhancement in the early portal phase **(C, D)**. Mild hypo-enhancement changes at 30 minutes **(E)**. On ITM, central enhancement is pink in the pure arterial phase **(F)**, and a mixed green periphery is clearly demonstrated in the early portal phase **(G)**. Residual portal vein (arrow head) is noted inside the tumor. Early HCC was confirmed by histopathological examination of the resected specimen **(H, I)**.

i.e., no remarkable change in the early portal phase. Hyper-enhancement in all three phases leads to a definitive diagnosis of benign nodules. However, hypo-enhancement change in the subsequent phase requiring differentiation from HCC was observed in all HCA, FNH, and alcoholic hyperplastic nodules.

Discussion

Imaging phases during CEUS can be divided into the vascular phase including the arterial, portal or portal venous and late phases, and the post-vascular phase (*5,14*). Changes in nodule enhancement after injection of contrast agent, observed based on these phases, are applicable to the diagnosis of liver tumors, although the optimal times are actually variable. The arterial phase is observed up to 30 seconds, 45-50 seconds or within one minute (*5*) after injection of a contrast agent, followed by the portal or portal venous phase observations made mainly after one minute. However, considering the multi-step carcinogenesis process of HCC development, ascertaining early hemodynamic changes is important. Particularly for the diagnosis of early HCC, an accurate determination of portal flow involvement is essential. Kudo *et al*. confirmed the pure arterial phase using the time-intensity curve and examined arterial and portal supplies in cases with early HCC, reporting the importance of pure arterial imaging (*15*). In this study, we divided the arterial phase (up to 45 seconds) into the pure arterial and the early portal phase and identified

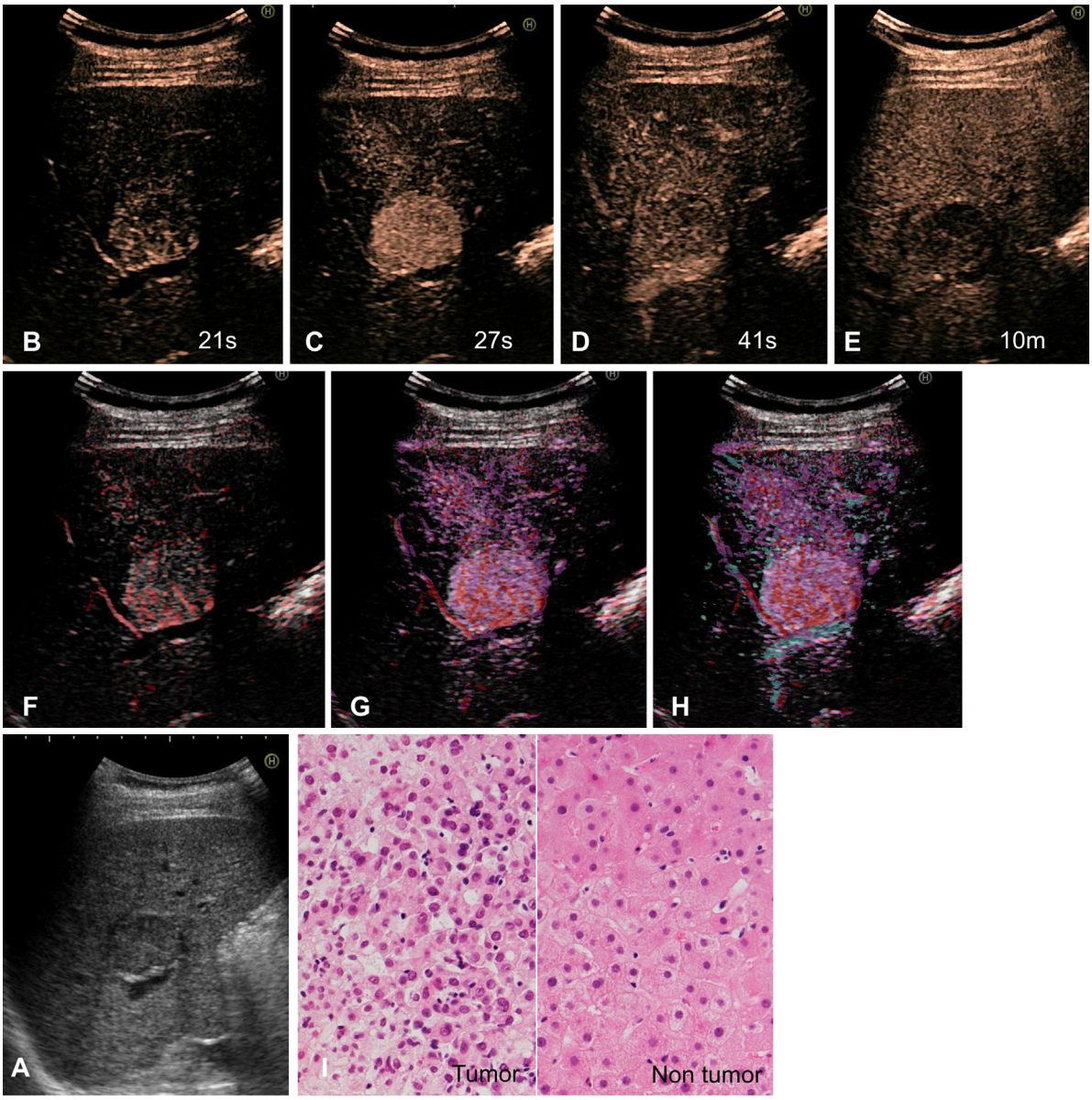

Figure 3. Moderately differentiated HCC findings on Sonazoid CEUS. A 2.5-cm hypoechoic nodule on B-mode US **(A)** showed hyper-enhancement in the pure arterial phase **(B, C)**, slight hypo-enhancement changes in the early portal phase **(D)**, and subsequent hypo-enhancement **(E)** on CEUS. On ITM, the feeding artery and nodule both appear red, then the periphery violet as time elapses in the pure arterial phase **(F, G)**. The portal flow appears green, and the parenchyma maps as a mixture of red and green in the early portal phase. There is no portal flow to this tumor because it never appears green **(H)**. The biopsy specimen confirmed the diagnosis of Md HCC **(I)**.

early hemodynamic changes in these two phases with the aim of diagnosing the histological grade of HCC.

HCC differentiation is determined in the arterial phase based on morphology and the degree of enhancement (*6,16,17*), as well as the timing of washout in the portal venous phase or the post-vascular phase (*18,19*). In particular, Sonazoid is taken up by the Kupffer cells of the liver, thereby producing stable images in the post-vascular phase. Several reports describing the advantages of CEUS for determining the degree of HCC differentiation focused on the post-vascular phase (*20,21*). Although the reported results vary somewhat depending on the imaging phase observed, most are relatively consistent. Wd or early HCC is hypo-iso vascular, while Md and Pd are hyper-vascular, although Pd shows earlier washout than Md HCC (*22*). Wang *et al*. found early HCC to show iso-enhancement in the post-vascular phase, allowing it to be differentiated from Pd or Md in post-vascular phase images. They stated that more attention should be paid to findings in the portal and the post-vascular phases rather than those in the arterial phase (*23*).

However, early stage HCC, unlike other tumors, is characteristically affected by the dual blood supply. Matsui *et al*. demonstrated these sequential blood flow changes in early stage HCC by CT (*24*). We defined the early portal phase to determine whether a diagnosis of early HCC is possible by focusing on the pure arterial

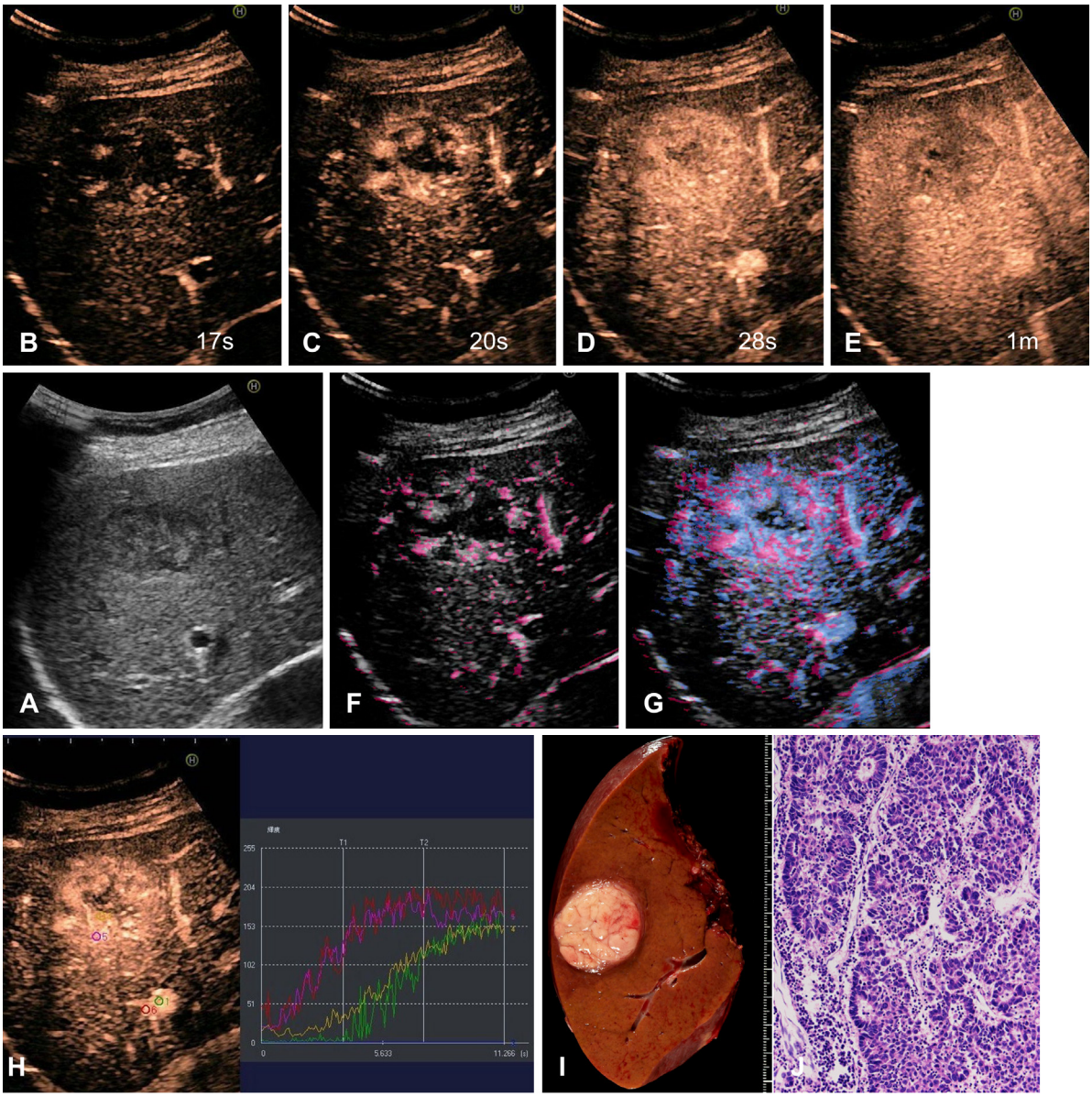

Figure 4. Poorly differentiated HCC findings on Sonazoid CEUS. A 3-cm tumor has an irregular shape and uneven internal echo on B-mode **(A)**. Gradual enhancement is seen during the pure arterial **(B)** and early portal phases **(C, D)**, followed by hypo-enhancement change at 1 minute. **(E)**. ITM shows red pixels in the tumor periphery initially **(F)**, with blue gradually appearing inside the tumor during the early portal phase **(G)**. These color changes are entirely due to arterial flow. The intensity curve for the internal portion of the nodule (yellow line) is similar to that of the periphery (violet line) **(H)**. The diagnosis of Pd HCC was confirmed by hepatic resection **(I, J)**.

and early portal phase hemodynamics. We identified various hemodynamic changes, corresponding to seven distinct enhancement patterns, in cases with early HCC. These patterns may have reflected blood supply changes from early to advanced HCC, *i.e.*, the portal vein disappears due to portal tract invasion (stromal invasion) (*11*) followed by gradual arterial proliferation. In addition, in all but one of the patterns, enhancement changes were slight (hypo- to iso- or iso- to hyper-enhancement). The same tendency was reported based on enhancement patterns of three phases (arterial, portal/portal venous, and post vascular phases) on CEUS (*8*). Our study focused only on the early phase hemodynamics and the subsequent phase, *i.e.* that after one minute which includes all except the arterial phase, to simplify the results. Finally, we obtained three patterns achieving a positive predictive value of 100% for the diagnosis of Wd HCC (early HCC).

Meanwhile, even in Md or Pd HCC supplied mainly by arterial flow, enhancement changes occurred in the early portal phase. For more than half of Md nodules, decreased enhancement was found in the early portal phase, whereas half of Pd nodules showed gradual enhancement from the pure arterial phase to the early portal phase. Early washout (within one minute) is reportedly observed in 5% of HCC (*2*). Detailed information on early hemodynamics as visualized by CEUS is thus provided by this study.

In summary, Sonazoid-enhanced ultrasound was performed to determine enhancement changes in the pure arterial, early portal, and subsequent phases for determination of the degree of HCC differentiation. A positive predictive value of 100% was obtained for the following seven patterns: three (iso-iso-hypo, hypo-iso-iso, and hypo-hypo-hypo) for Wd, two (hyper-iso-hypo and hyper-hypo-hypo) for Md, one (hypo-hyper-hypo) for Pd, and one (hyper-hyper-hyper) for benign nodules. Even in the early portal phase, hemodynamic changes were visible not only in Wd HCC but also in the Md and Pd HCC.

The major limitation of this study was small sample size because of the limited number of resection and biopsy cases, which may have given rise to bias.

Conclusion

With division of the early hemodynamics of CEUS into pure arterial and early portal phases, this modality can provide information useful for determining the likely degree of HCC differentiation and for distinguishing early stage HCC from benign nodules, and for considering the subsequent biopsy and treatment strategies.

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Ethics statement

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for inclusion in this study.

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Conflict of Interest: The authors have no conflict of interest to disclose.

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Definition of the caudate lobe of the liver based on portal segmentation

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Abstract: Models of liver corrosion were developed by injecting colored Mercox, epoxy resin, silicon rubber and other materials into the portal vein, hepatic artery, bile duct and hepatic vein of autopsied livers. The glissonean or venous branches that obstructed the view of the caudate lobe of the liver were subsequently removed. The detailed anatomy of the caudate vessels was studied and the three parts of the caudate lobe (Spiegel lobe, paracaval portion and caudate process) were defined based on portal segmentation. Caudate portal branches should be defined as dorsal branches arising from the main trunk, or from the first order branches of the portal vein covering the hepatic region in front of the inferior vena cava. The hepatic region, where the internal branches from segment eight cover the front of the inferior vena cava, should be defined as segment eight, and not as the paracaval portion. Prof. Couinaud defined the right side of the caudate lobe as segment IX based on the spatial position; however, this classification of the caudate section seemed to lack consistency with that of other hepatic segments, which were defined based on portal segmentation. We have sustained the dogma that any hepatic segment should be defined based on portal segmentation, and our classification of the definition and boundary of the caudate lobe, which was published in 1985, has sufficient consistency to be used as an international standard.

***Keywords*:** caudate lobe, paracaval portion, liver surgery, anatomy of the liver, liver cast, portal segmentation

Introduction

The caudate lobe of the liver is located deep in the liver, in front of the inferior vena cava (IVC) behind the three major hepatic veins, and cranial to the hilar plate. Thus, the diagnosis and treatment of liver cancer arising in the caudate lobe requires close attention owing to its unique location. However, the definition and boundary of the caudate lobe remains controversial.

The famous anatomist, Prof. Couinaud (1922-2008) classified the liver into eight segments (*1*); however, he later withdrew his idea that the caudate lobe should be classified into segments I and IX (*2*). After his death, there has been no international consensus on the definition of the caudate lobe of the liver.

At the beginning of the 1980's, when anatomical resection of 2-3 sections of the liver was started with acceptable safety, detailed anatomy of the caudate lobe was unclear. At that time, staff surgeons in the Department of Liver Surgery, National Cancer Center Hospital, Tokyo, began to consider that the hepatic area in front of the IVC and the cranial aspect of the portal bifurcation might also belong to the caudate lobe. Knowledge of the caudate lobe anatomy was very limited. One of the authors (MK), who had already started to make liver casts at that time, began to focus on the anatomy of the caudate lobe.

The purpose of the present study was to make a rational definition of the caudate lobe based on portal segmentation, which could be used as an international standard.

Materials and Methods

The first author (MK) prepared 75 human liver casts between July 1, 1981 and October 2, 1990 (*3,4*). The candidates included a fetus of the 17th week and old men of uncertain age. The methods used to make casts, including the selection of the injecting materials, were unique to the authors, since there was currently no standard method. There was a wide range of variety in the quality of the casts, and most of them were not suitable for use in further studies; this was primarily because of the fact that the task was difficult for one

person to perform alone.

Colored resin was injected into the portal vein (blue), hepatic artery (red), bile duct (yellow), and hepatic vein (black) of the whole liver. The specimens were fixed in water and not left on the desk, to preserve the natural hepatic shapes, as they would be inside the body. Although the liver casts-manufactured using Mercox resin were fragile, they were suitable for making casts from fetuses. When an excess of Mercox resin was injected, the hepatic sinusoid overflowed with resin making the casts as hard as stone, and it became impossible to observe the inside of the liver. Liver casts manufactured using silicon rubber were not tolerant of age-associated deterioration for more than 30 years, and many of them were transformed and/or had degenerated (*5*). Liver casts manufactured using epoxy resin had poor plasticity, were well controlled, and showed no deformities. Of these, we selected several casts with a good shape, an adequate amount of injected resin, a good state of preservation, and those in which the IVC was preserved over a long distance. Then, we dissected the casts intensively, examined the anatomy of the caudate lobe in detail, and took stereoscopic photographs of the liver specimens.

After fixation, the Glissonean and venous branches obstructing the caudate lobe were removed. During the dissection of the liver casts, we used forceps with fine tips and extracted the small Glissonean and venous branches, gently, piece by piece. Large vessels, such as the middle hepatic vein (MHV), were totally or partially resected when they obstructed the view of the deep structure of the liver. After these meticulous steps, we could expose the vascular structure of the caudate lobe and capture stereoscopic photography.

This study was approved by the National Center for Global Health and Medicine Research Ethics Committee/ Institutional Review Board (approval number: NCGM-G-004020-00).

Results

The anatomy of the caudate lobe was examined and classified into three parts as follows, *i*) Spiegel lobe, *ii*) paracaval portion and *iii*) caudate process. The caudate portal branches should be defined as dorsal branches from the main trunk, or from the first order branches of the portal vein covering the hepatic region in front of the IVC. In cases with a trifurcated type portal vein, the caudate portal branches should be ramified from the main portal trunk of the portal vein, and not from the anterior or posterior portal vein.

Spiegel lobe and the portal branches

The Spiegel portal branches were defined as dorsal portal branches ramified to the left-side caudate lobe from the main trunk or from the first order portal branches. A relatively large portal branch often ramified from the left portal vein was distributed in the Spiegel lobe. The Arantius ligament lies on the boundary between the left lateral section and the Spiegel lobe, and was easily detected and helpful for dividing the anatomical sections.

Paracaval portion

The paracaval portal branches were defined as dorsal cranial portal branches ramified from the main trunk, or from the first order portal branches, including branches having a common trunk with Spiegel branches, but excluding ventral branches. The dorsal cranial portal branches from the root of the anterior or posterior portal vein should not be included in the paracaval branches. Our study was conducted based on the concept of portal segmentation.

Defining the boundary between the caudate lobe and the anterior section in a case having an internal branch from segment eight

In a case where an internal branch from segment eight supplied the hepatic region in front of the IVC, we added a detailed dissection of the caudate lobe to reveal the boundary between the paracaval portion and segment eight of the liver. The whole liver was set to observe the paracaval portion from the cranial side (Figure 1A), and the tiny branches around the MHV were removed to reveal the root of the MHV (Figure 1B). The MHV was then divided at its root (Figure 1C). Further dissection of the paracaval portion revealed the main branch in the paracaval portion accompanied by internal branches from segment eight (Figure 1D, Figure 2A). Removal of the tiny branches in the paracaval portion revealed short hepatic veins (SHVs) draining into the IVC and the tips of the caudate process branches (Figure 2B). The cranial aspect of the portal bifurcation was exposed following removal of the caudate lobe of the liver (Figure 2C).

These SHVs were located at the boundary between the paracaval portion of the caudate lobe and segment eight, suggesting that intersegmental hepatic veins can be identified along the boundary among other hepatic segments.

Thus, the right-sided boundary of the paracaval portion of the liver could be defined based on portal segmentation, but not by its spatial position.

Caudate process

The caudate process is connected to the posterior section of the liver along the Rouviere's sulcus, and the portal branches in the caudate process were always definite. The branches of the caudate process were defined as dorsal caudal portal branches ramified from the main

How to watch a stereoscopic photography:
1). It is possible to watch the photo sterically with naked eyes using a cross method. 2). When you use the stereoscope to watch the pictures, you may often find left and right reversed images. When the images are reversed, cut off the wave line in the center of the two pictures, and reverse the positions of the two pictures.

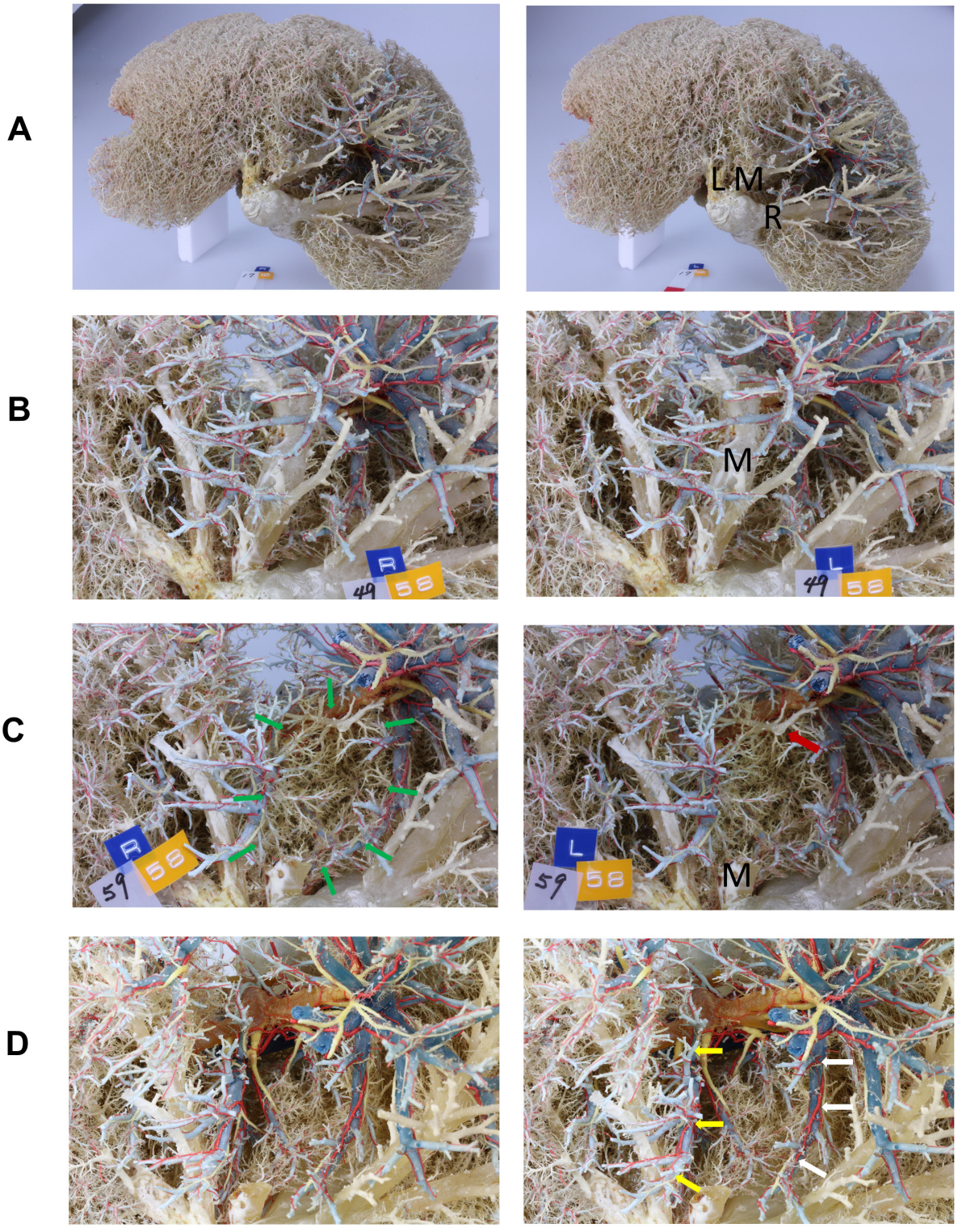

Figure 1. (A) Cranial view of the accomplished liver cast. This view of the cast almost coincides with the intraoperative view of the liver from the position of the anesthesiologists. L, left hepatic vein; M, middle hepatic vein; R, right hepatic vein. **(B) Cranial view of the caudate lobe just before the division of the middle hepatic vein.** M, middle hepatic vein. **(C) Cranial view of the caudate lobe just after the division of root of the middle hepatic vein.** A numerous number of small caudate branches are seen just behind the middle hepatic vein (green arrows). Red arrow indicates hepatic veins located at the ventral side of the caudate lobe draining into the back of the middle hepatic vein. M, middle hepatic vein. **(D) Glissonean branches distributing in the caudate lobe.** The peripheral branches obstructing the view have been removed step by step. White arrow indicates the internal branches in the anterior section, and yellow arrow indicates the main trunk of the portal vein in the paracaval portion.

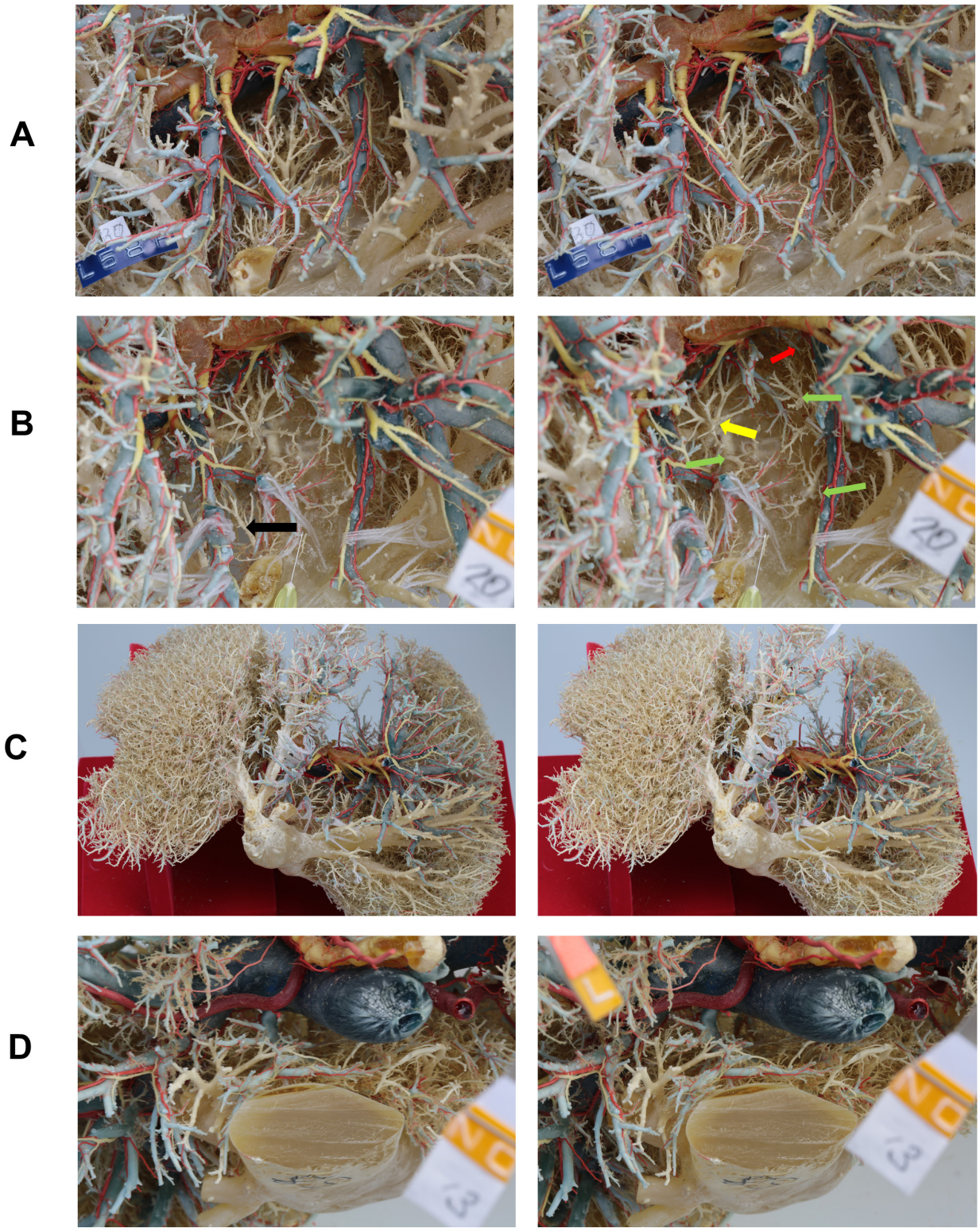

Figure 2. (A) Relationship between the inferior vena cava and the caudate lobe. The outline of the inferior vena cava (IVC) has been gradually exposed. Along with the exposure of the IVC, the cellular space in front of the IVC which was described by Couinaud and colleagues are revealed. This cellular space coincides with the space where a tape is placed to lift the liver during Belghiti's hanging maneuver (*18*). **(B) Cranial view in front of the inferior vena cava. Green arrows indicate the short hepatic veins (SHVs). Yellow arrow indicates the SHV drained from the Spiegel lobe.** Black arrow indicated the venous branches draining into the middle hepatic vein. Red arrows indicate the branches in the caudate process. The size of the needle was 30G (= 300 μm) and the needle is put on the stump of the middle hepatic vein. **(C) View of the bifurcation of the portal vein after complete removal of the caudate branches.** Cranial view similar with that in Figure 1A. The cranial aspect of the portal bifurcation can be seen for the whole length. **(D) Caudal view of the caudate process.** Many communicating veins are seen.

trunk or from the first order branches of the right portal vein. This is because there were either fewer caudate process branches or many variations in cases with posterior independent type of poral branching (Figure 2D).

Discussion

In the present study, we clearly defined the caudate lobe of the liver based on portal segmentation. The concept of diving the caudate lobe into three portions may be classical, but there has been no international consensus of the definition on the caudate lobe especially on the right-sided boundary of the paracaval portion of the liver. Our definition of the caudate lobe based on portal segmentation resolves the longtime puzzlement of Prof. Couinaud, and provides a simple and international consensus of the definition on the caudate lobe.

In the history of the anatomical study of the hepatic segments, the caudate lobe was first described as the "lobus exiguous" by Adrian van der Spiegel in 1622. However, the detailed anatomy of the caudate lobe was not revealed until the recent era. The definition and boundary of the caudate lobe differ slightly depending on the researchers and there has been no international consensus on this topic.

Healy and Schroy's caudate lobe

Healy and Schroy first classified the liver into four sections, the lateral, medial, anterior and posterior segments, and divided each section into cranial and caudal parts, consisting of eight segments (*6*). They defined the caudate lobe as areas that did not belong to either the left or right liver, and further classified the caudate lobe into three portions, the right portion, left portion, and the caudate process. They studied more than 100 liver casts with cholangiography and demonstrated the paracaval biliary branch, which was named the right caudate duct. They also named the Spiegel branch as the left caudate duct.

Change in the definition of Couinaud's caudate lobe

Prof. Couinaud classified the liver into eight segments from segment I to VIII, in 1954 (*1*), and this classification has gradually become more common since the development of hepatobiliary surgery. In 1954, he named the dorsal liver as segment I, and in 1989, he divided segment I into segment Il and segment Ir (*8*) (Figure 3A); however, in 1994, he replaced segment Ir with segment IX. By this time, he proposed the idea of dividing segment IV into subsegments b, c and d, but the concrete spatial areas of these subsegments were not drawn on the axial schema (*8*) (Figure 3B). In 1998, he showed a schematic view of subsegments b, c, and d on the axial view of the liver (*9*) (Figure 3C).

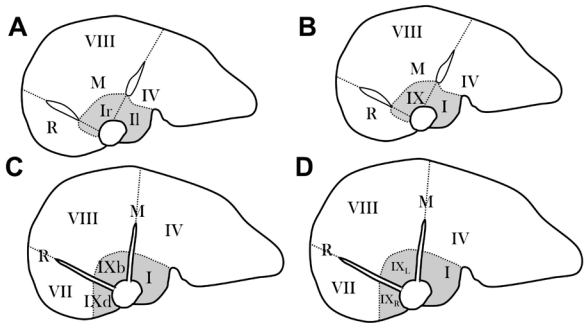

Figure 3. Historical change of definition of the caudate lobe by Prof. Couinaud. (A), In 1989, the caudate lobe was divided into segments Il and Ir based on the spatial position. The boundary between the two segments is the plane connecting middle hepatic vein and inferior vena cava (*7*). By this time, Couinaud had proposed the idea to divide segment Ir into subsegments b, c and d, but the schema of segmentation was not shown. **(B)**, In 1994, segment Ir was replaced by segment IX (*8*). By this time, Couinaud had proposed the idea to divide segment IX into subsegments b, c and d, but the schema of segmentation was not shown. **(C)**, In 1998, the subsegments of IX were shown on the figure. The right hepatic vein runs between subsegments b and d (*9*). Subsegment IXc is located beneath the right hepatic vein. **(D)**, In 2000, the subsegments b and d were replaced by subsegments IXL and IX_R (*10*). The figures were modified from the original ones.

In a manuscript by Filipponi, he changed segment IXb and segment IXd to segment IXL and segment IXR, respectively, while maintaining the concept of segments I and IX (*10*) (Figure 3D). Finally, he abandoned the concept of segment IX in the manuscript by Abdalla in 2002 (*2*).

Paradox of Couinaud's caudate lobe

As is apparent from the transition of the nomenclature and definition of the caudate lobe by Prof. Couinaud, it was difficult to define the range of the caudate lobe, especially on the right-sided boundary. Prof. Couinaud himself noted the following comments in his book of surgical anatomy (*1*): On the right, in front of the IVC, the exact nature of the pedicles is difficult to state. Are they caudate veins? Do they belong to the right liver? How far does the caudate lobe extend to the right? After a thorough investigation of the right portion of the caudate lobe and, the posterior extremity of segments VII and VIII, a special territory was observed in front of the IVC, posterior to the right portal pedicle, inferior to the plane right superior-middle hepatic veins. This territory is supplied by small and ascending posterior branches of various origins, extending to the right territory of the caudate veins, but is obviously different from the caudate lobe.

This led to the concept of a posterior or dorsal liver (designated as sector I) with a left dorsal segment (caudate lobe or segment Il) and a right dorsal segment (or segment Ir). These designations replace the former segment I.

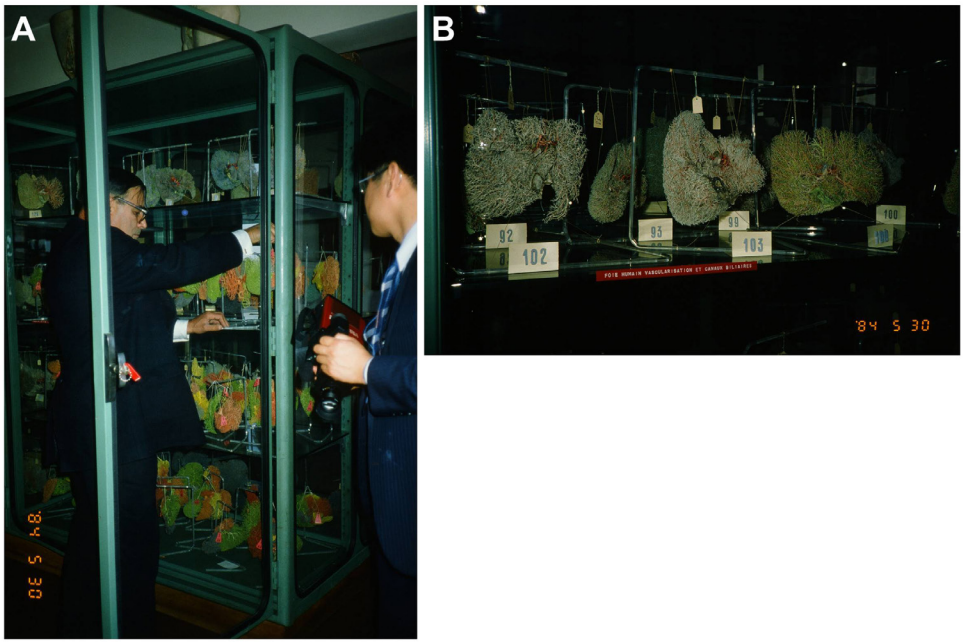

Figure 4. Memorial photos of Prof. Couinaud and his liver casts. (A), Prof. Couinaud gave us the explanation about liver casts of a various animals at the museum of which he played the director. Dr. Nakatani listens to him. (Department of Surgery, Shinshu University, at that time. This picture was taken by KM.) **(B)**, Liver cast manufactured by Prof. Couinaud. The liver cast is compressed back and forth, which suggests that the cast may be manufactured on flat plates.

Discussion with Prof. Couinaud

We fully understand why Prof. Couinaud found defining the boundary of the caudate lobe so difficult after observing plenty of small vessels in the caudate lobe (Figure 1C). We removed all of the Glissonean branches and hepatic veins obstructing the anatomy of the caudate lobe, and made sure to carefully observe the caudate branches in the hepatic casts. We believe that detailed observation of the roots of the small caudate branches off the first order branch of the portal vein, and the tiny branches covering the caudate lobe would be impossible until we could achieve the abovementioned meticulous dissection.

The first author (MK) remembers that Couinaud's cast (Figure 4) seemed to be manufactured with less resin than that used in their own casts, although the final appearance was quite similar. This suggests that the difference in the definition of the caudate lobe between the two researchers is due to the difference in whether the obstructing hepatic parenchyma and vessels were removed or not.

The first author (MK) asked Prof. Couinaud directly about the right-sided margin of the caudate lobe, at which time, his answer was "I have no answer". He did not comment on the invisible area of the caudate lobe because then, he had no method to observe the vessels that were deeply located in the caudate lobe. We assume that he not only adopted the typical portal branching pattern but also the four atypical variations of the portal branching to the dorsal liver (segment IX_L and IX_R). His classification might mislead the researchers that there were four types of portal branches, and that the caudate portal branches always reached the level of the roots of the right and middle hepatic veins running along the IVC. His schema of the caudate lobe presenting the bifurcation of the caudate branches from the portal vein appears unclear; this suggests that it is important to remove the obstructing branches other than those of the caudate lobe, to grasp the bifurcation pattern of the caudate lobe located in the deepest parts of the liver casts. In MK's casts, no branches were found to be running very close to the IVC in the caudate lobe (Figure 1D, Figure 2A).

Definition of the caudate lobe by other researchers

Prof. Nimura is a world authority in the treatment of perihilar cholangiocarcinoma, and first advocated the importance of combined resection of the caudate lobe to increase the curability for the surgical treatment of perihilar cholangiocarcinoma. He stated that the concept of Couinaud's segment IX is difficult to understand, and instead defined the caudate lobe as follows (*11*): The left caudate lobe should be located on the left side of the Arantius ligament between the root of the left hepatic vein and the umbilical portion, while the right caudate lobe should be located on the right side of the left caudate lobe in front of the IVC, and on the left side of the root of the right posterior portal vein (P-point). The tip of the right caudate lobe extends the phrenic surface of the liver between the middle

and right hepatic veins. The caudate process is the protruding portion of the liver caudal to the right portal vein.

Prof. Nimura's definition of the caudate lobe is similar to that of Couinaud's. Prof. Nimura states that the starting point of the caudate lobe should be determined first, followed by the corresponding spatial area of the caudate lobe. This concept is not based on portal segmentation.

Ryu and Kogure (*12*) have published many papers, but it seems that they did not define the anatomical boundary of the caudate lobe.

Takayama primarily defined the caudate lobe mostly based on the definition proposed by Couinaud (*13*), and later became the first surgeon to successfully perform isolated caudate lobectomy without dividing other hepatic segments (high dorsal resection). To this end, he injected blue dye into the liver parenchyma in the caudate lobe, beside the right and middle hepatic veins (tattooing) and determined the right-sided or ventral boundary of the caudate lobe with an aid of the pooled dye. He also injected the dye in the posterior portal branch and stained the posterior section of the liver; this allowed visualization of the boundary between the posterior section and the caudate lobe, and was named as the "counter-staining technique". However, this technique was not used to visualize the boundary between the anterior section and caudate lobe; if the counter-staining were to be used to visualize the ventral boundary of the caudate lobe, it would be more useful. In this manner, the area supplied by the inner branches of the anterior portal vein would be visualized, which would prove that these branches belong to the anterior section, and not to the caudate lobe. Visualization of the right-sided boundary of the caudate lobe using the counter-staining technique should be based on the concept of portal segmentation, which is incompatible with Couinaud's segmentation of the caudate lobe. Notably, none of the above studies on the caudate lobe have been against Couinaud's segmentation.

At the beginning of the 1990's, Prof. Makuuchi described that the caudate lobe only indicated the Spiegel lobe of the liver, missing the caudate process and paracaval potion, in other countries (*14*).

Importance of definition of the caudate lobe based on portal segmentation and the difficulties of Couinaud's definition

In 1985, we defined that dorsal cranial branches derived from the main trunk or from the first order branch of the poral vein as paracaval branches. In addition, the portal venous branches that ramified from the anterior portal branch supplying the liver parenchyma surrounded by the roots of the right and middle hepatic veins were defined as portal branches in segment eight.

The concept of classifying the caudate lobe into three portions has been accepted in many papers as referred to by Abdalla & Couinaud (*2*). However, our idea that the liver area supplied by the branches from the anterior portal vein should be defined as part of the anterior section, and not of the caudate lobe has never been sited or understood.

In the current study, we carefully dissected the liver casts by extracting the small peripheral Glissonean and hepatic venous branches obstructing the caudate. Several questions relating to Couinaud's definition of the caudate lobe were raised as a result of the findings obtained from the dissection of the caudate lobe using our method.

1). The extent of the caudate lobe varied according to the definition of the caudate lobe. For example, "behind the right hepatic vein" might suggest the ventral boundary of the right portion of the caudate lobe, but it was unclear whether this was the main trunk, or the center, right edge or left edge of the right hepatic vein.

2). Prof. Couinaud introduced the concept of the dorsal liver based on the spatial region, and not on portal segmentation; however, he classified other segments (segments II-VIII) based on portal segmentation. Thus, his methods lacked consistency.

3). The nomenclature of the portal branches was complicated and confusing. *i*) Prof. Couinaud declared that the nomenclature of the portal vein was defined based on the distribution to each segment, and not on the origins of the ramification from the main portal trunk. For example, an internal portal branch belonging to segment seven could be named as "caudate portal branch" or "internal portal branch of segment seven". *ii*) Prof. Couinaud stated that each portal branch could "borrow" a name depending on the situation. However, it was unclear how the name was "borrow" from other segments. From the route of each portal segment? Or did it "borrow" the name when it entered the caudate area, while also keeping the original branching name? Or did it restore the original name when it went out of the caudate area? This was unclear. Cho (*15*), Matsui (*16*), and Maki (*17*) performed a meticulous and detailed analysis of the imaging study and reported that the peripheral branches of the caudate lobe could reach the phrenic surface of the liver. How would Prof. Couinaud name these peripheral portal branches?

4). Prof. Couinaud defined the dorsal liver as consisting of *i*) the Spiegel lobe, *ii*) the paracaval lobe and *iii*) the caudate process. This classification perfectly coincides with that of Kumon; thus, we believe that Prof. Couinaud understood and accepted Kumon's classification. However, Prof. Couinaud abandoned segment IX which he advocated. Namely, he found the paradox of his theory and accepted Kumon's classification.

Herein, we have listed the above four paradoxes of Couinaud's theory. We cannot and should not accept his

definition of the caudate lobe as it stands. It is required to establish an international standard definition of the caudate lobe as soon as possible.

Toward an international definition of the caudate lobe of the liver

The author (MK) first proposed the anatomical definition and boundary of the caudate lobe of the liver in 1985, and Couinaud accepted the rational of our concept, as described above. Couinaud's classification has contradictions and cannot be used as a world standard. Although Nimura's definition is fundamentally similar to ours, it is not based on the concept of portal segmentation. Kumon's definition of the caudate lobe published in 1985 is based on the concept of portal segmentation, and is both simple, and easy to understand. Thus, this definition can be used as the world standard in clinical settings, because the landmark and boundary of the caudate lobe is described clearly, and will help to elucidate the right-sided boundary of the caudate lobe in the future.

It is possible that some researchers may be worry if the definition of the caudate lobe is settled by our concept, as opposed to the traditional definition outlined by Prof. Couinaud's. However, we believe that a solution is possible if an internationally unified definition is set up with sufficient evidence.

Conclusion

We studied the vascular and biliary structures of the caudate lobe using liver casts to elucidate the definition of the caudate lobe. Since we could observe the whole circumference of the left and right portal vein, and the first order brances of the portal vein, we could resolve some inconsistencies that Prof. Couinaud could not. We also found that the definition of the caudate lobe by Prof. Couinaud was fraught with issues, and consequently, we determined that a new definition was required. Kumon's classification is based on portal segmentation, and we believe that it has the potential to be easily accepted internationally. However, the proposal of a new definition of the caudate lobe will inevitably be met with opposing opinions, since the highly regarded Prof Couinaud has proposed and applied the concept of the dorsal liver to the caudate lobe. However, let us make an international and simple definition of the caudate lobe beyond these opinions. We aim to show the outline of the caudate lobe according to the types of portal ramification in a future issue, with the aim to further understand and spread the anatomy of the caudate lobe.

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Liver resections between 2014 and 2020 in the Lausanne University Hospital, Switzerland

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Abstract: Lausanne University Hospital is in the Francophone part of Switzerland and services a catchment population of about 1 million people. We recorded and analyzed baseline characteristics and surgical outcomes for 400 consecutive patients who underwent liver resection there between January 2014 and February 2020. Their pathological results were primary liver cancer (including hepatocellular carcinoma and intrahepatic cholangiocarcinoma): 21.8%, extrahepatic cholangiocarcinoma (including perihilar cholangiocarcinoma and gallbladder cancer): 5.3%, liver metastases: 51.8%, echinococcosis: 10.8%, adenoma: 3.0%, and other diagnoses: 7.5%. Global morbidity rate (Clavien-Dindo classification ≥ 1) was 45.5% with major complication (Clavien-Dindo classification ≥ 3) identified in 81 patients (20.3%). Of the 400 patients, two died within 30 days of surgery (0.5%) and five died within 90 days (1.3%). The 2017-2019 subgroup had a significantly greater percentage of patients aged ≥ 75 years (20.5%) than did the 2014-2016 subgroup (10.9%; $p = 0.011$) and a higher percentage of laparoscopic procedures than the earlier subgroup (2014-2016: 9.2%, 2017-2019: 32.5%; $p < 0.001$). We conclude that as the patient population ages, preoperative management and surgical techniques should be constantly improved.

***Keywords*:** liver cancer, hepatocellular carcinoma, liver metastases, liver resection

Introduction

Switzerland is a relatively small country, with 8.54 million inhabitants. It has a high longevity rate. Lausanne University Hospital is located in the Francophone part of Switzerland, with a catchment population of about 1 million people.

Overall age-standardized cancer mortality is about 140/10,000 in men and 85/10,000 in women. Among causes of cancer-related deaths worldwide, liver cancers rank fifth in men and ninth in women (*1*). Colon, rectum and anal cancers have high mortality rates in both men and women in Switzerland (*1*), approximately 30% of patients present with synchronous or metachronous liver metastasis during their disease course. Both primary liver cancer and liver metastases are typically treated with liver resection.

Switzerland is also an endemic area of alveolar echinococcosis (AE), a zoonotic tapeworm disease caused by *Echinococcus multilocularis* (*2-4*). Carnivores (mainly red foxes in urban areas) serve as definitive hosts for adult tapeworms and their herbivorous prey (mainly rodents) acts as intermediate hosts for metacestodes. Humans are generally not directly involved in the transmission but can become accidental hosts. As the parasite growth pattern resembles a malignant tumor, treatment of AE often requires interventional radiology, liver surgery and antiparasitic chemotherapy (*5,6*).

As surgical techniques, multimodal strategies and perioperative management have improved, liver surgery has become safer. Mortality rates have decreased from 10-20% to near zero in the past two decades (*7-11*), which has allowed safer resections even in elderly patients (*12,13*). In particular, laparoscopic liver resections have dramatically increased in the past decade (*14,15*).

Here, we report the characteristics and results of liver resections between 2014-2020, in the Department of Visceral Surgery at Lausanne University Hospital, Switzerland.

Methods

This retrospective study was approved by the local ethics committee and registered (registration number CER-VD 2020-00968). Written informed consent was obtained from all patients.

Patient selection

We recorded and analyzed baseline characteristics and surgical results of the 400 consecutive patients who underwent liver resections at Lausanne University Hospital between January 2014 and February 2020.

Preoperative management

Preoperative assessment included routine clinical and laboratory examinations (hematology, clinical chemistry, liver function tests, tumor markers, coagulation), volumetric computed tomography (CT) to manage surgical strategy (including need for preoperative portal vein embolization), and characterize the future remnant liver.

Chest and abdominal contrast-enhanced CT and magnetic resonance imaging with Gd-EOB-DTPA (Bayer Schering Pharma, Berlin, Germany) were also routinely performed. Therapeutic strategies were discussed in weekly multidisciplinary tumor board meetings. Major hepatectomies were defined as resections of three or more Couinaud's segments. Since July 2013, all patients scheduled for liver resections in our institution were enrolled in our enhanced recovery after surgery (ERAS) program (*16*).

Postoperative data and patient follow-up

We defined 1- and 3-month morbidity and mortality as postoperative complications and death within 30 days and 90 days after surgery, respectively. Postoperative complications were staged using the Clavien-Dindo classification (*17*). Patients were examined at the outpatient clinic at 1 and 3 months after surgery. Subsequent follow-up was performed either by the patient's general practitioner or in our institution. Follow-up included clinical examination, tumor markers levels, serological tests, and imaging. Recurrence was diagnosed based on imaging findings, clinical data, and/or histopathological studies.

Statistical analysis

Categorical variables were expressed as *n* (%) and were compared between groups using Fisher's exact test or the chi-square test, as appropriate. Continuous variables were expressed as median (interquartile range [IQR]) and were compared using Wilcoxon's rank test. Overall survival (OS) and disease-free survival were calculated from the initial liver resection. Survival curves were determined using the Kaplan-Meier method and compared using the log-rank test. Values of $p < 0.05$ were considered significant. Statistical analyses were performed using JMP 13.2.0 software (SAS Institute Inc., Cary, NC, USA).

Table 1. Baseline characteristics

Variables	Number (%) *n* = 400
Age, year	
Median; IQR	64; 54-71
Gender	
Male	237 (59.2%)
Female	163 (40.8%)
BMI, kg/m^2	
Median; IQR	24.8; 22.2-28.0
ASA PS classification	
1	12 (3.0%)
2	274 (68.5%)
3	113 (28.3%)
4	1 (0.3%)
Diagnosis	
Primary liver tumor	87 (21.8%)
Extrahepatic cholangiocarcinoma	21 (5.3%)
Liver metastases	207 (51.8%)
Echinococcosis	43 (10.8%)
Adenoma	12 (3.0%)
Others	30 (7.5%)
Preoperative treatment	
None	221 (55.3%)
Neoadjuvant chemotherapy	162 (40.5%)
Radiotherapy	4 (1.0%)
Radio-chemotherapy	13 (3.3%)

Data are presented as median (IQR) or *n* (%). ASA PS classification, American Society of Anesthesiologists physical status classification; BMI, body mass index.

Results

Patients

We reviewed the records of 400 consecutive patients who underwent liver resections between January 2014 and February 2020. Patients' characteristics, including demographic, clinical, and pathological data, are summarized in Table 1. Their median age was 64 years (IQR: 54-71 years); 28.6 % had high American Society of Anesthesiologists physical status classification ≥ 3. Their pathological results were primary liver cancer (including hepatocellular carcinoma and intrahepatic cholangiocarcinoma): 21.8%, extrahepatic cholangiocarcinoma (including perihilar cholangiocarcinoma and gallbladder cancer): 5.3%, liver metastases: 51.8%, echinococcosis: 10.8%, adenoma: 3.0%, and other diagnoses: 7.5%. About 21% of patients had liver resections for benign lesions. Among the liver metastases, 87.0% were of colorectal origin (Table 2). Preoperative treatments (for 44.7%) included neoadjuvant chemotherapy (40.5%), radiotherapy (1.0%), and radio-chemotherapy (3.3%).

Over time, we saw increasing percentages of elderly

Table 2. Details of liver metastases

Liver metastases	Numbers (%) *n* = 207
Colon cancer	120 (58.0%)
Rectal cancer	60 (29.0%)
Intestinal cancer	4 (1.9%)
Breast cancer	5 (2.4%)
GIST	2 (1.0%)
NET	2 (1.0%)
Others	14 (6.8%)

Data are presented as *n* (%). GIST, gastrointestinal stromal tumour; NET, neuroendocrine tumor.

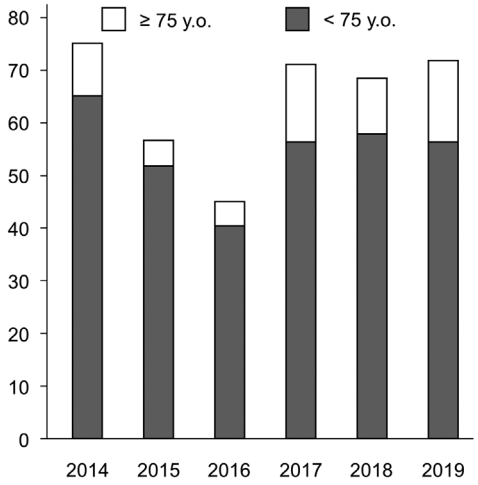

Figure 1. Percentage of hepatectomy in elderly patients. For the later 2017-2019 period, percentages of patients aged ≥ 75 years were 21.4% in 2017, 16.2% in 2018, 22.5% in 2019; and 20.5% in 2017-2019 overall, which was significantly greater than for the earlier 2014-2016 period (10.9%; $p = 0.011$).

patients – *i.e.*, ≥ 75 years old – were receiving liver resections (Figure 1). For the later 2017-2019 period, percentages of patients aged ≥ 75 years were 21.4% in 2017, 16.2% in 2018, 22.5% in 2019; and 20.5% in 2017-2019 overall, which was significantly greater than for the earlier 2014-2016 period (10.9%; $p = 0.011$; Figure 1).

Surgical outcomes

Intra- and post- operative outcomes are summarized in Table 3. Median surgical time was 275 minutes (IQR: 190-353 minutes); median estimated blood loss was 600 mL (IQR: 300-1,000 mL). Major and minor hepatectomies were performed in 207 patients (51.8%) and 193 patients (48.2%), respectively. Laparoscopic approaches were used in 22.0% of procedures overall, but were used significantly more in the 2017-2019 period (32.5%) than in the 2014-2016 period (9.2%; $p < 0.001$; Figure 2). Overall morbidity (Clavien-Dindo classification ≥ 1) was 45.5%, with major complications (Clavien-Dindo classification ≥ 3) identified in 81 patients (20.3%), including 32 patients (8.0%) who needed another surgery within the same hospitalization because of biliary fistula (after biliodigestive anastomosis), bilioma, or surgical site infection. Clinically significant post-hepatectomy liver failure (International Study Group of Liver Surgery [ISGLS] grade ≥ B) occurred in 10 patients (2.5%). Two patients (0.5%) died within 30 days, and five (1.3%) died within 90 days after surgery. Median length of hospital stay was 8 days (IQR: 6-14 days).

Overall survival of patients with malignant lesions

Among patients with one or more malignant lesions, the median follow-up period was 15.4 months (IQR: 11.3-29.7 months) for intrahepatic primary tumors, 13.8 months (IQR: 11.9-52.1 months) for liver metastases, and 12.0 months (IQR: 10.0-13.9 months) for extrahepatic cholangiocarcinoma (including

Table 3. Intra- and post- operative outcomes

Variables	Number (%) $n = 400$
Intraoperative outcomes	
Procedure	
Major hepatectomy	207 (51.8%)
Minor hepatectomy	193 (48.2%)
Hepatico-jejunostomy	41 (10.3%)
Venous reconstruction	23 (5.8%)
Approach	
Laparotomy	312 (78.0%)
Laparoscopic	88 (22.0%)
Operative time, min.	
Median; IQR	275; 190-353
Estimated blood loss, ml	
Median; IQR	600; 300-1000
RBC Transfusion	41 (10.3%)
Postoperative morbidity	
Morbidity	182 (45.5%)
Clavien-Dindo classification	
I	14 (3.5%)
II	81 (20.2%)
IIIa	42 (10.5%)
IIIb	17 (4.3%)
IVa	14 (3.5%)
IVb	8 (2.0%)
Clavien-Dindo classification ≥ 3	81 (20.3%)
Re-operation	32 (8.0%)
ISGLS B/C	10 (2.5%)
Mortality	
30-day	2 (0.5%)
90-day	5 (1.3%)
Postoperative length of stay, days	
Median; IQR	8; 6-14

Data are presented as median (IQR) or *n* (%). ISGLS, the posthepatectomy liver failure defined by the International Study Group of Liver Surgery; RBC, red blood cells.

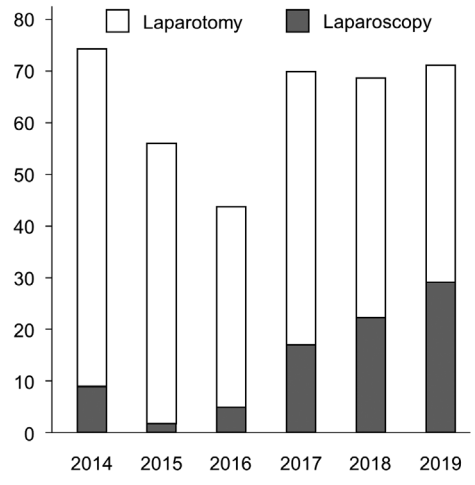

Figure 2. Percentage of laparoscopic/open approach. Laparoscopic approaches were used in 22.0% of procedures overall, but were used significantly more in the 2017-2019 period (32.5%) than in the 2014-2016 period (9.2%; $p < 0.001$).

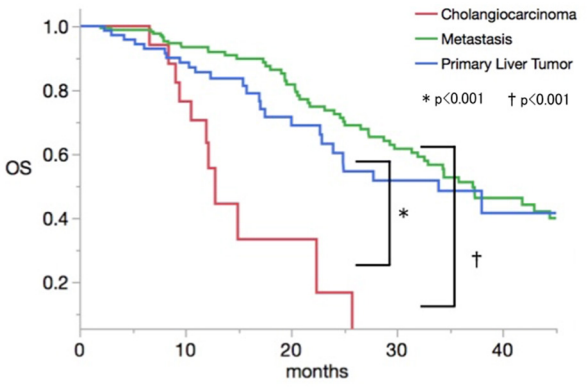

Figure 3. Overall survival of patients with malignant lesions. Extrahepatic cholangiocarcinoma had significantly worse overall survival than did intrahepatic primary liver tumor or liver metastases ($p < 0.001$ for both).

perihilar cholangiocarcinoma and gallbladder cancer); these follow-up periods did not significantly differ ($p = 0.080$). The respective 12-, 24-, and 36-month OS rates were primary liver tumor: 85.6%, 57.5%, and 48.5%; liver metastases: 93.4%, 72.5%, and 51.2%; and cholangiocarcinoma (perihilar cholangiocarcinoma and gallbladder cancer): 63.5%, 16.7%, and 0%. Extrahepatic cholangiocarcinoma had a significantly worse OS than did intrahepatic primary liver tumor or liver metastases ($p < 0.001$ for both; Figure 3).

Discussion

We evaluated results of liver surgery performed at our institution for the period 2014-2020. The major complication rate was 20.3%, and mortality was 0.5% at 30 days and 1.3% at 90 days after surgery – rates similar to those of other European countries.

Globally, hepatocellular carcinoma is the fifth-most common cancer and the second-most common cause of cancer-related death (*18,19*). In parallel, colorectal cancer (CRC) is a growing cause of cancer-associated death, about 30% of patients with CRC develop liver metastases (*20-22*). Although chemotherapy regimens have improved in recent years, liver resection is the main curative treatment for liver malignancies. However, liver failure remains the most feared postoperative complication, and is associated with high mortality (*23-25*). In the present study, significant post-hepatectomy liver failure (ISGLS grade ≥ B) was 2.5% and mortality rate was 0.5% at 30 days and 1.3% at 90 days after surgery in the Lausanne University Hospital. To decrease mortality, many countries have refined their selection criteria, surgical techniques and perioperative management (*26-28*). In our institution, indications for liver resection are mainly based on Makuuchi's criteria (*29*). In other European countries, the Barcelona Clinic of Liver Cancer guideline is most widely used (*30*), and recommends liver resection only for patients without portal hypertension. However, several reports of liver resection in patients with portal hypertension have been published in recent years (*31-34*). Our institution has refined its selection guidelines to avoid excluding patients solely because of portal hypertension. In assessing preoperative liver function and future liver remnant volume and function, we routinely use ICG tests, CT-scan volumetry, portal pressure measurement, and ^{99m}Tc-labeled mebrofenin hepatobiliary scintigraphy (*35,36*). Since 2016, if functional volumetry does not portend a safe liver resection, our group has routinely performed ipsilateral hepatic vein embolization simultaneously with portal vein embolization ("liver venous deprivation"), which we have reported to be safe, as it induces greater and faster future liver remnant hypertrophy than portal vein embolization alone (*37*). We are now studying the relationship of indocyanine green retention rate, ^{99m}Tc-labeled mebrofenin heaptobiliary scintigraphy and portal vein hypertension.

ERAS programs have been shown to improve postoperative outcomes of abdominal, orthopedic, urological and gynecological surgeries (*38,39*). Our institution has used ERAS protocols for liver surgery since July 2013, and had reported financial benefits of ERAS in liver surgery in 2016 (*40,41*). However, in a 2020 systematic review, we concluded that cost-reduction benefits from liver surgery ERAS were still unclear because of the small number of studies and high compliance variability (*42*). As more hospitals use ERAS programs, their utility should become more demonstrable.

We saw significantly more patients aged ≥ 75 years and more laparoscopic resections in the 2017-2019 period than in the earlier 2014-2016 period. Life expectancy continues to increase, in Switzerland and around the world, and surgery in elderly patients is increasingly common as perioperative management and surgical techniques have improved. Although major hepatectomy is no longer contraindicated in this age group (*43*), patients with diabetes have a higher risk of major complications and should be closely monitored in the postoperative course. As the patient pool continues to age, hepatobiliary surgeons will be challenged to improve preoperative evaluation/preparation techniques and preoperative management further, and to develop specific minimally invasive techniques (laparoscopic and/or robotic surgery).

Conclusion

Liver resection has been consistently and safely performed in our institution. As our patient base ages, preoperative management and surgical techniques should be constantly improved. Many innovations and improvements are awaited, especially for evaluation of future liver remnant function and preoperative preparation.

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Conflict of Interest: The authors have no conflict of interest to disclose.

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Kokudo N, Hara T. "History, Tradition, and Progress": The ceremony of 150th Anniversary of the National Center for Global Health and Medicine held in Tokyo, Japan. BioSci Trends. 2019; 13:105-106.

Example 2 (Sample journal reference with more than 15 authors):

Darby S, Hill D, Auvinen A, *et al*. Radon in homes and risk of lung cancer: collaborative analysis of individual data from 13 European case-control studies. BMJ. 2005; 330:223.

Example 3 (Sample book reference):
Shalev AY. Post-traumatic stress disorder: Diagnosis, history and life course. In: Post-traumatic Stress Disorder, Diagnosis, Management and Treatment (Nutt DJ, Davidson JR, Zohar J, eds.). Martin Dunitz, London, UK, 2000; pp. 1-15.

Example 4 (Sample web page reference):
World Health Organization. The World Health Report 2008 – primary health care: Now more than ever. *http://www.who.int/whr/2008/whr08_en.pdf* (accessed March 20, 2019).

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