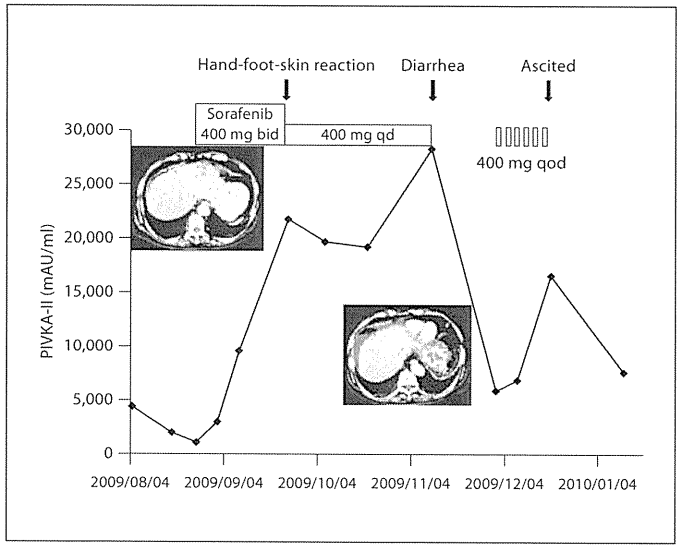

Fig. 1. Discrepancies between the changes in serum DCP levels and clinical findings for a 76-year-old man with advanced HCC. The serum DCP level rapidly increased after starting sorafenib but decreased after reducing or discontinuation of sorafenib therapy. CT showed that the HCC decreased and became necrotic, despite increased DCP levels.

factor receptor (VEGFR)-2, VEGFR-3, platelet-derived growth factor receptor-β (PDGFRβ), Flt-3 and c-KIT [3–7]. Sorafenib-based chemotherapy is used for HCC because HCC is a hypervascular tumor that expresses VEGF and the VEGFR [8], and the survival of tumor cell depends on its vascularity. A meta-analysis has shown that tissue and serum VEGF levels are prognostic factors in HCC [9].

The SHARP study, a phase III randomized trial for advanced HCC, revealed that sorafenib prolonged the overall survival time to progression (TTP) [10]. Based on such findings, sorafenib is now used worldwide. On the other hand, tumor shrinkage was infrequently observed in the SHARP study because the partial response in that study was only 2%. However, stable disease was achieved in 76% of patients, and the overall disease control rate was 78%. The tumoristatic effect of sorafenib contributes to prolongation of overall survival.

Cases of complete response or partial response have frequently been observed since sorafenib was approved in Japan in May 2009. It is thought that the Japanese race has some genetic characteristics that improve the efficacy of sorafenib therapy.

Dynamic computed tomography (CT) and dynamic magnetic resonance imaging (MRI) are often used to evaluate the antitumor effect in the treatment of HCC. However, since the introduction of molecular-targeted therapy, tumor necrosis without tumor regression is often observed. Tumor necrosis is not always induced by a cytotoxic antitumor agent. It was recently suggested that the Response Evaluation Criteria for Solid Tumors (RECIST) [11, 12] and the World Health Organization Criteria [13] are unsuitable for the evaluation of the anticancer effects of molecular-targeted therapy, which inhibits angiogenesis. To overcome the limitations of these criteria, a modified RECIST [14] for HCC was proposed, and evaluates the size of the viable tumor tissue. The validity of the modified RECIST or RECICLE [15] has been discussed, but their utility is still not established. Tumor markers have also been used to evaluate the antitumor effects of therapy. α-Fetoprotein (AFP), the lens culinaris agglutinin-reactive fraction of AFP (AFP-L3), and des-γ-carboxyprothrombin (DCP) are the most widely used tumor markers and are well established for the diagnosis and follow-up of HCC [16–18]. These tumor markers indicate the activity of HCC and are useful for patient follow-up [19]. In particular, tumor markers can be used to examine the efficacy of antiangiogenic molecular-targeted agents.

At our institute, we have experienced discrepancies between changes in DCP levels and clinical findings during sorafenib therapy (fig. 1). Therefore, we retrospectively evaluated the associations between changes in DCP levels and clinical findings.

Patients and Methods

Sixty-two consecutive patients with advanced HCC treated with sorafenib at Kinki University Hospital between May 2009 and April 2010 were included in this study. The criteria for sorafenib therapy were as follows: (1) patients with HCC refractory to TACE or the presence of major vascular invasion or extrahepatic spread; (2) ECOG Performance Status Score of 0 or 1, and (3) Child-Pugh score of ≤7 (Child-Pugh A and some B patients). Patients with laboratory values meeting the following criteria were also eligible for sorafenib: (a) hemoglobin ≥8.5 g/dl, (b) neutrophil count >1,500/mm^3, (c) platelet count >50,000/mm^3, (d) total bilirubin <3 mg/dl, (e) ALT and AST <5 times the institutional upper limits of normal, and (f) serum creatinine <1.5 times the institutional upper limits of normal.

Patients continuously received 400 mg of oral sorafenib (two 200-mg tablets) twice daily. If adverse effects were observed, the sorafenib dose was reduced according to the treatment guidelines.

Tumor response was evaluated by RECIST version 1.1. TTP was constructed by the Kaplan-Meier method and was compared using the log-rank test. Statistical analysis was conducted using SPSS version 11.5.1J for Windows.

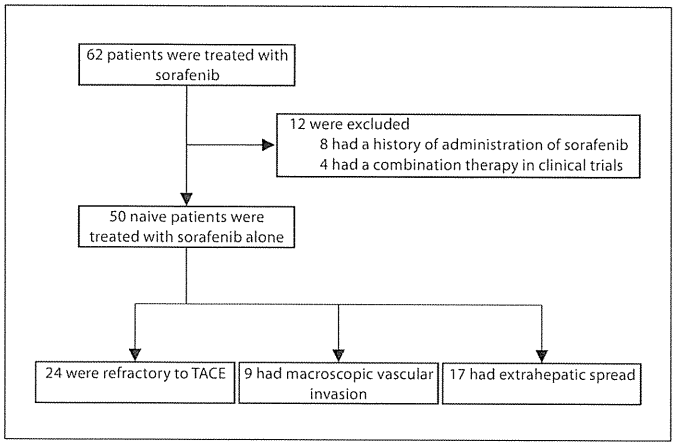

Fig. 2. Patient disposition.

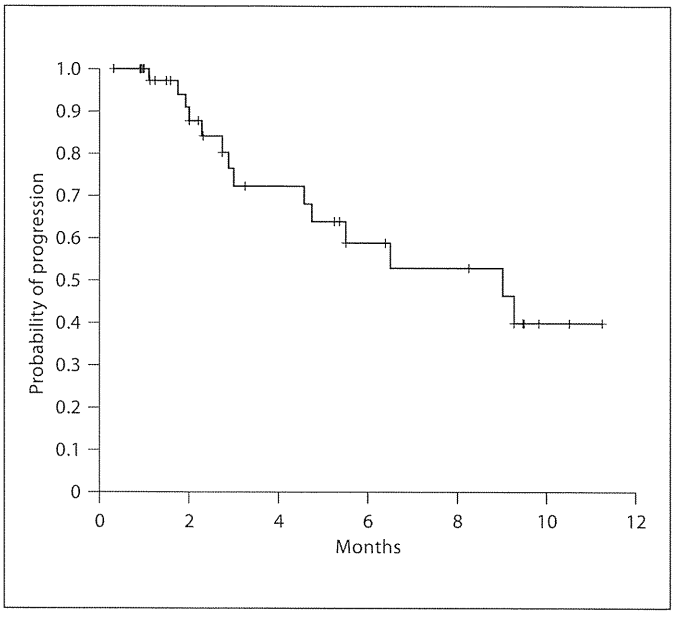

Fig. 3. TTP in 50 patients with HCC treated with sorafenib.

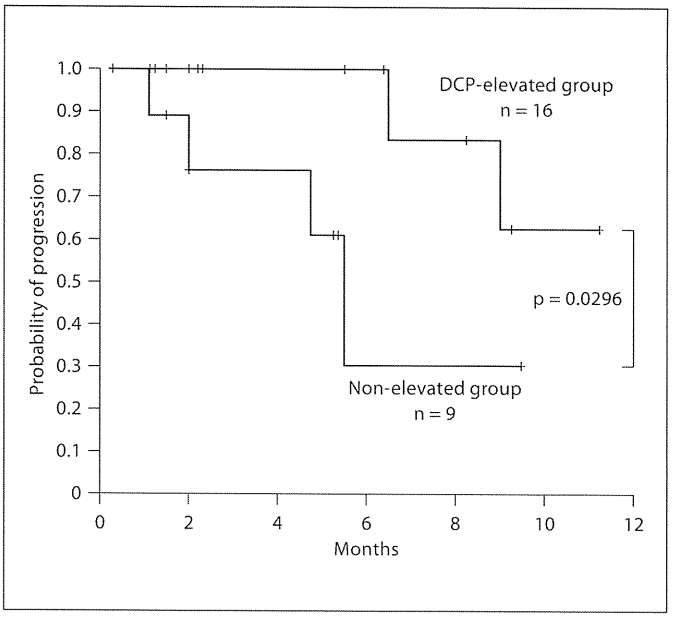

Fig. 4. Comparison of TTP between patients with (elevated) and patients without (non-elevated) an increase in DCP by ≥2-fold at 2 weeks after starting sorafenib therapy compared with pretreatment.

Results

Eight of 62 patients were excluded because they had already been treated with sorafenib before it was approved in Japan. Four patients were excluded because they participated in other clinical trials. Thus, 50 patients were evaluated in this study: 24 were refractory to TACE, 9 had major vascular invasion, and 17 had extrahepatic spread (fig. 2).

The mean duration of treatment was 5.2 months (95% confidence interval (CI) 4.1–6.8 months). The mean dose of sorafenib was 480.0 mg daily, overall survival was 9.5 months (95% CI 8.1–10.8 months), and TTP was 9.0 months (95% CI 4.75–13.25) (fig. 3).

In 25 of the patients treated with sorafenib, the serum levels of DCP were evaluated twice, i.e. before and within 2 weeks after starting treatment. The TTP in the patients in whom the DCP level at 2 weeks was ≥2-fold greater than the pretreatment level was significantly longer than that in patients without elevated DCP (i.e. <2 times the pretreatment levels) (p = 0.0296) (fig. 4). There were no statistically significant differences in other clinical characteristics between the two groups of patients (table 1).

Discussion

Sorafenib shows the significant activity against several receptor tyrosine kinases including VEGFR-2, VEGFR-3, PDGFRβ, Flt-3, and c-KIT, and inhibits angiogenesis. Antiangiogenic activity plays a very important role in HCC therapy because HCC is a typical hyper-

Table 1. Characteristics of patients according to change in DCP

Characteristics	Elevated group (n = 16)	Non-elevated group (n = 9)	p value[1]
Age	73.06 ± 6.03	68.22 ± 6.40	0.065
Male/female	12/4	7/2	0.876
Serum albumin, g/dl	3.44 ± 0.56	3.64 ± 0.32	0.329
Serum bilirubin, mg/dl	0.93 ± 0.51	0.91 ± 0.36	0.803
Prothrombin time, %	86.55 ± 17.65	84.18 ± 10.12	0.978
Child-Pugh score (5/6/7/8)	6/7/2/1	6/3/0/0	0.413
Platelet count	15.90 ± 7.00	16.21 ± 11.18	0.598
Etiology, NBNC/HBV/HCV	4/3/9	4/1/4	0.593
Stage, III/IV	10/6	6/3	0.835
MVI, with/without	3/13	2/7	0.835
EHS, with/without	3/13	4/5	0.170
AFP, ng/ml	977.0 (2–35,014)	304.5 (6–721,260)	0.728
DCP, mAU/ml	2,327.5 (41–34,170)	2,088.0 (46–357,580)	0.846

Elevated: DCP increased by ≥2-fold at 2 weeks compared with pretreatment; non-elevated: DCP increased by <2-fold at 2 weeks compared with pretreatment.
MVI = Macroscopic vascular invasion; EHS = extrahepatic spread.
[1] All values were non-significant.

vascular tumor. In other words, HCC induces angiogenesis to maintain adequate blood supply.

Liebman et al. [20] were the first to report the utility of DCP as a tumor marker for the diagnosis of HCC. DCP, also known as PIVKA-II (proteins induced by vitamin K absence or antagonist-II), is an abnormal prothrombin induced by vitamin K deficiency. Vitamin K-dependent coagulation factors such as prothrombin are synthesized in hepatocytes and contain γ-carboxy-glutamic acid (Gla residues), which can bind calcium. Normal prothrombin contains 10 Gla residues at the amino terminal. However, in the vitamin K-deficient state the Gla residues at the amino terminal are not fully γ-carboxylated. This incomplete prothrombin is known as DCP and is functionally inactive.

It is unclear why DCP is elevated in patients with HCC. Several reports have proposed mechanisms for DCP production, which include: (1) vitamin K deficiency [21]; (2) decreased activity of γ-glutamyl carboxylase in the HCC tissue because of a point mutation in the γ-glutamyl carboxylase gene [22, 23]; (3) abnormal vitamin K metabolism [24]; (4) overexpression of the prothrombin precursor in HCC cells [25, 26], and (5) abnormal uptake of vitamin K into HCC cells. We have focused on the abnormal uptake of vitamin K into the HCC cells. Murata et al. [27–29] reported that hypoxia induces DCP. They explained this phenomenon as follows. The fine filamentous actin network, which plays a crucial role in clathrin-mediated endocytosis of vitamin K, is disrupted in DCP-producing cells because of hypoxia. It is considered that this offers one explanation for the elevated serum DCP level in patients with HCC, for which sorafenib is effective. In this issue, we found that HCC patients with a rapid increase in DCP within 2 weeks after starting sorafenib had a significantly better outcome than patients with no increase in DCP [30]. The CT findings for HCC with rapid DCP elevation tended to include reduced vascularity or presence of necrosis. This indicates that hypoxia was responsible for the change in DCP production. Accordingly, DCP may offer a surrogate marker for hypoxia.

Sorafenib induces hypoxia in HCC by inhibiting angiogenesis. TACE exposes the HCC to hypoxia, as does sorafenib, but this change is very rapid and most of the tumor cells become necrotic. It is thought that not enough DCP is produced after TACE. On the other hand, sorafenib induces tissue hypoxia relatively slowly and many viable HCC cells are exposed to hypoxia. During sustained hypoxia, the tumor cells gradually die and the serum level of DCP subsequently decreases.

During molecular-targeted HCC therapy using sorafenib, we found that the rapid increase in DCP after starting sorafenib does not indicate tumor progression, but rather indicates HCC tissue hypoxia. Therefore, DCP may be a useful predictive marker for the duration of tu-

mor suppression. To our knowledge, this is the first report to show that DCP could be a good biomarker to predict the therapeutic efficacy of sorafenib in HCC.

In conclusion, the serum level of DCP during sorafenib treatment may be a promising biomarker for the therapeutic efficacy of sorafenib therapy for HCC.

Disclosure Statement

The authors have no conflict of interest to declare.

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Digestive Diseases

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Management of Hepatocellular Carcinoma in Japan: Consensus-Based Clinical Practice Guidelines Proposed by the Japan Society of Hepatology (JSH) 2010 Updated Version

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Key Words
Clinical practice guidelines, evidence-based · Clinical practice manual, consensus-based · Hepatocellular carcinoma, prevention · Hepatocellular carcinoma, staging · Hepatocellular carcinoma, surveillance · Hepatocellular carcinoma, diagnostic algorithm · Hepatocellular carcinoma, treatment algorithm

Abstract
Hepatocellular carcinoma (HCC) is one of the leading causes of cancer death not only in Japan but also worldwide. Clinical practice guidelines for HCC were first published in 2001 by the European Society of Study of the Liver (EASL) followed by the American Association for the Study of Liver Disease (AASLD) published in 2005 and updated in 2010. However, these guidelines have proven to be somewhat unsuitable for Japanese patients. In 2005, supported by the Japanese Ministry of Health, Labour and Welfare, evidence-based clinical practice guidelines for HCC were compiled in Japan. In 2009, a revised version of evidence-based guidelines was published. Based on both 'evidence-based' guidelines and the consensus of an expert panel on HCC, the Japan Society of Hepatology (JSH) published the Consensus-Based Clinical Practice Manual in 2007 and updated in 2010. In this article, the 2010 updated version of this manual, especially issues on prevention, surveillance, pathology, diagnosis, staging, and treatment algorithm are summarized.

Introduction

Following the publication by the European Society of Study of the Liver (EASL) in 2001 [1], the American Association for the Study of Liver Disease (AASLD) published the Clinical Practice Guidelines of hepatocellular carcinoma (HCC) in *Hepatology* in November 2005 [2] and updated in 2010 [3].

In Japan, the original Evidence-Based Clinical Practice Guidelines of HCC were published in 2005 [4] and updated in 2009 [5], disclosed on the website of the Japan Society of Hepatology (JSH) [www.jsh.or.jp/], and then widely used for liver cancer treatment in Japan. An ex-

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Table 1. JSH expert panel on Consensus-Based Clinical Practice Manual of the HCC, 2010 revised version (alphabetical order)

Hepatologists	
Norio Hayashi	Kansai Rosai Hospital
Naoki Hiramatsu	Osaka University
Takafumi Ichida	Juntendo University, Shizuoka
Akio Ido	Kagoshima University
Kenji Ikeda	Toranomon Hospital
Tatsuo Inoue	Kinki University
Takao Iwasaki	Tohoku University
Namiki Izumi	Musashino Red Cross Hospital
Shuichi Kaneko	Kanazawa University
Akinori Kasahara	Osaka University
Kazuhiko Koike	Tokyo University
Masatoshi Kudo	Kinki University
Takashi Kumada	Ogaki Municipal Hospital
Yasushi Matsuzaki	Tokyo Medical University
Masahito Minami	Kyoto Prefectural University of Medicine
Yasunori Minami	Kinki University
Takeshi Okanoue	Saiseikai Suita Hospital
Masao Omata	Yamanashi Prefectural Hospital
Yukio Osaki	Osaka Red Cross Hospital
Shuichiro Shiina	Tokyo University
Masatoshi Tanaka	Kurume University Medical Center
Hidenori Toyoda	Ogaki Municipal Hospital
Yoshihide Ueda	Kyoto University
Tatsuya Yamashita	Kanazawa University
Hepato-Biliary-Pancreatic and Transplant Surgeons	
Shigeki Arii	Tokyo Medical and Dental University
Hiroto Egawa	Murakami Memorial Hospital Asahi University
Takumi Fukumoto	Kobe University
Kiyoshi Hasegawa	Tokyo University
Toshimi Kaido	Kyoto University
Seiji Kawasaki	Juntendo University
Norihiro Kokudo	Tokyo University
Yonson Ku	Kobe University
Masatoshi Makuuchi	Japanese Red Cross Medical Center
Morito Monden	Osaka University
Hiroaki Nagano	Osaka University
Tadatoshi Takayama	Nihon University
Ryosuke Tateishi	Tokyo University
Shinji Uemoto	Kyoto University
Shintaro Yamasaki	Nihon University
Pathologists	
Masamichi Kojiro	Kurume University
Osamu Nakashima	Kurume University
Michiie Sakamoto	Keio University
Radiologists	
Osamu Matsui	Kanazawa University
Takamichi Murakami	Kinki University
Kenichi Takayasu	National Cancer Center Hospital
Medical Statistician	
Kenichi Yoshimura	Translation Research Center, Kyoto University

cerpted version has also been published in an English journal by Makuuchi and Kokudo et al. [5–7]. These guidelines were prepared after critical evaluations based on about 100 reports with a high evidence level in each field selected from 7,118 reports on HCC published between 1966 and 2002. In the 2009 revised version, 2,950 articles were reviewed and 532 articles were incorporated into the new version. Since the guidelines were prepared based as much as possible on highly evidenced data, some points may slightly deviate from actual practices related to HCC routinely performed based on the experience and consensus of HCC experts in Japan.

Considering this situation, the JSH summarized HCC treatment as performed in Japan with the consensus opinions of many experts, even though clear evidence was not available, and published a simple manual in 2007 [8] and updated in 2010 [9]. This was an experience- or consensus-based manual based on evidence-based guidelines with respect to the evidence level, and summarized the consensus of expert opinions – widely reflecting the actual state of HCC treatment in Japan.

The manual was prepared in accordance with the Evidence-Based Clinical Practice Guidelines reported by Makuuchi and Kokudo et al. [5–7], and thus contains no conflict with those guidelines. Points that slightly differ are a more detailed explanation of liver cancer treatments based on expert opinions, and a summary of the consensus by the expert panel [10]. Although it may seem unusual that two different guidelines are available and followed in Japan, both have different roles and are not contradictory.

This report introduces the revised version of Consensus-Based Clinical Practice Manual of HCC published by the JSH in 2010, and focuses on prevention, surveillance, pathology, diagnosis, staging, and treatment algorithm. This constitutes a 'practice manual' summarized by the expert panel of the JSH (table 1), and is different from the Clinical Practice Guidelines. The contents of this report may be considered as the current state of the most advanced HCC treatment practices in Japan.

Prevention

Antiviral Therapy

Hepatitis B Virus-Related HCC

Preventive therapy for HCC should be indicated for these patients. In Japan, HBe antigen-positive chronic hepatitis B patients with an ALT level of ≥31 IU/l and an HBV DNA level of ≥5 log IU/ml, HBe antigen-negative

chronic hepatitis B patients with an HBV DNA level of ≥4 log IU/ml, and liver cirrhosis patients with an HBV DNA level of ≥3 log IU/ml are recommended for antiviral therapy.

Previously, a randomized controlled trial (RCT) examined the inhibitory effects of interferon (IFN) therapy on carcinogenesis in patients with chronic hepatitis B. In 1999, Lin et al. [11] randomly divided 101 HBe antigen-positive patients with type B chronic liver disease into three groups: placebo (n = 31), placebo + IFN (n = 34), and prednisolone + IFN (n = 36) groups, and continued follow-up, with a mean follow-up of 8.4 (1.1–11.5) years. HCC was detected in 1 of 67 patients treated with IFN and in 4 of 34 patients receiving a placebo. They reported that carcinogenesis was significantly inhibited in the IFN-treated groups (p = 0.013). However, when investigating only chronic hepatitis patients, excluding 12 with liver cirrhosis, there were no significant differences in the incidence of HCC between the IFN-treated and non-IFN-treated groups.

On the other hand, the incidence of HCC was compared between 233 IFN-treated and 233 untreated patients in a case-control study involving 466 HBe antigen-positive patients with type B chronic liver disease. In the IFN-treated group, carcinogenesis was significantly inhibited (p = 0.011) [12].

Camma et al. [13] conducted a meta-analysis involving seven articles, and examined whether IFN therapy reduces the risk of compensatory liver cirrhosis B-derived carcinogenesis. IFN therapy decreased the absolute risk of liver carcinogenesis by 6.4%. However, the values markedly differed among the studies. A study involving groups in Europe with slight differences reported that there were no differences.

Prevention of Chronic Hepatitis/Liver Cirrhosis B-Derived Liver Carcinogenesis with Nucleoside Analogues

Two RCTs investigated the effectiveness of nucleoside analogue on preventing liver carcinogenesis in patients with chronic hepatitis/liver cirrhosis B. One of these involved 651 patients with marked hepatitis B-related fibrosis or compensatory liver cirrhosis. During the follow-up period (32.4 months), HCC was noted in 17 (3.9%) of 436 patients treated with lamivudine and in 16 (7.4%) of 215 patients treated with a placebo. In the former, carcinogenesis was significantly inhibited [14]. The other trial involving 222 patients with liver cirrhosis B compared lamivudine-treated and additionally adefovir-treated groups with a non-treated group, and reported that HCC incidence was significantly inhibited in the former two groups (p = 0.003) [15]. Furthermore, a non-randomized, comparative study also indicated that lamivudine and additional adefovir treatments significantly inhibited carcinogenesis compared to control group [16]. Thus, antiviral therapy with nucleoside analogues is useful for preventing HCC in patients with chronic hepatitis B or compensatory liver cirrhosis B.

Hepatitis C Virus-Related HCC

Primary Prevention of Chronic Hepatitis C-Derived Liver Carcinogenesis with IFN

The risk of HCC in patients in whom IFN therapy achieved sustained viral response (SVR) was one-fifth of that in untreated patients. In non-SVR group it was significantly inhibited to one-fourth to one-half in comparison with patients with ALT normalization at the end of IFN therapy and biochemical responders (BR) with ALT normalization for ≥6 months after the completion of such therapy [17]. A meta-analysis involving 4,614 patients examined the relationship between the presence or absence of IFN therapy in patients with type C chronic liver disease, including those with liver cirrhosis, and the incidence of HCC indicated that IFN therapy decreased the risk of HCC by 13%. The effects were more marked in BR [18]. These results suggest that IFN therapy inhibits the development of HCC in comparison with untreated patients, and that not only SVR but also BR are related to the prevention of HCC. Furthermore, a retrospective cohort study regarding the inhibitory effects of combination therapy with IFN and ribavirin on HCC in patients with chronic hepatitis C showed that the risk of HCC development was significantly lower in responders to this combination therapy [19]. Based on these findings, it is recommended that antiviral therapy with IFN be performed to prevent HCC incidence in patients with chronic hepatitis C. The primary goal of IFN treatment is virus eradication (SVR). When it is impossible, the liver function should be normalized as much as possible (BR).

Recently long-term follow-up of the HALT-C study confirmed this observation [20].

Two RCTs investigated the effectiveness of IFN therapy for liver cirrhosis C on preventing liver carcinogenesis. Of these, one reported that there was no difference in the incidence of HCC between IFN-treated and non-treated groups. However, the other study indicated that IFN therapy inhibited the development of HCC. Seven non-randomized, comparative studies, in which a non-IFN-treated group was set as a control group, have been

published. In six of these, IFN therapy inhibited the development of HCC in patients with liver cirrhosis C. Two meta-analyses also affirmed the preventive effects of IFN therapy on the development of HCC in patients with liver cirrhosis C. These effects were marked in patients who achieved SVR. Previously, one study examined the inhibitory effects of combination therapy with IFN and ribavirin on HCC in liver cirrhosis C patients, and reported that, in the combination therapy group, the development of HCC was inhibited in comparison with the non-treated group.

Anti-Inflammation Therapy

Glycyrrhizin Preparations

The intravenous administration of glycyrrhizin for chronic hepatitis/liver cirrhosis is commonly performed to improve the transaminase level. No RCT has investigated whether glycyrrhizin preparations inhibit liver carcinogenesis. However, a retrospective cohort study reported that the intravenous administration of glycyrrhizin preparations for chronic hepatitis C decreased the risk of liver carcinogenesis [21]. It is recommended that glycyrrhizin be intravenously administered for prevention of HCC development in patients with chronic hepatitis C when IFN therapy is not effective or indicated.

Ursodeoxycholic Acid

When administering ursodeoxycholic acid (UDCA) to patients with chronic hepatitis C, cytotoxic bile acid may be substituted for UDCA, protecting the hepatocyte membrane. Furthermore, a study suggested that the immunity-regulating and apoptosis-inhibiting actions of UDCA are involved in the protection of the hepatic cell membrane.

To date, no study has reported the preventive effects of long-term UDCA administration on liver carcinogenesis. However, UDCA administration at 600–900 mg/day improved the serum ALT level [22].

Phlebotomy Therapy

Phlebotomy therapy decreases the serum ALT level, suggesting the usefulness of phlebotomy for the treatment of chronic hepatitis C. Kato et al. [23] reported that long-term iron chelation significantly inhibited the development of HCC. In the future, a large-scale comparative study should be conducted.

Consensus Statements

1 Among patients with type B chronic liver disease, the incidence of HCC is high in those with a high HBV DNA level.
2 Nucleoside analogues are useful for preventing HCC in patients with chronic hepatitis B or compensatory liver cirrhosis B.
3 Among patients with chronic hepatitis C, the incidence of HCC is higher in those with marked fibrosis or liver cirrhosis.
4 It is recommended that antiviral therapy with IFN be performed to prevent HCC in patients with chronic hepatitis C. Firstly, virus elimination is important. When it is impossible, the liver function must be normalized.

Surveillance of Hepatocellular Carcinoma

Definition of the Population at High Risk for HCC

Persistent infections with hepatitis B and C viruses (HBV and HCV, respectively) are the highest risk factors for liver carcinogenesis. The carcinogenesis risk for HBV carriers is about 200 times higher than that for non-carriers, and the risk is higher in patients with type C liver cirrhosis than in those with hepatitis B-related cirrhosis. The HCV-associated risk is about 5 times higher than that associated with HBV. The characteristics of HCV-associated carcinogenesis are carcinogenesis in the F4 step in which liver cirrhosis is completed in most cases, and its occurrence in many cases at 60 years of age or older. The yearly carcinogenesis rate of cirrhosis type C is 7–8% in Japan, which is higher than that in Europe or North America; it might be that the mean age of carriers is closely involved. Liver cirrhosis induced by various causes, even though HBV and HCV are negative, is a risk for liver carcinogenesis. Since carcinogenesis occurs in some cases of liver cirrhosis associated with non-alcoholic steatohepatitis (NASH), alcoholic liver disease, primary biliary cirrhosis (PBC), and autoimmune hepatitis (AIH), the course of the disease should be followed paying close attention to carcinogenesis as in cases of viral liver cirrhosis. Alcohol increases the risk of chronic hepatitis B- and C-associated liver carcinogenesis.

Based on the above, patients with chronic hepatitis B and C and non-viral liver cirrhosis are defined as high-risk populations for HCC in both the Consensus-Based Clinical Practice Manual and Evidence-Based Practice Guidelines. Patients with liver cirrhosis types B and C are defined as a super-high-risk population (table 2). Risk factors other than hepatitis virus or liver cirrhosis are also proposed (table 3).

Table 2. Definition of populations at high risk for HCC

A. Super-high-risk population
1. Hepatitis B-related liver cirrhosis
2. Hepatitis C-related liver cirrhosis
B. High-risk population
1. Chronic hepatitis B
2. Chronic hepatitis C
3. Liver cirrhosis (causes other than HBV or HCV)

Table 3. Risk factors other than hepatitis virus infection or liver cirrhosis

Older age
Male gender
Diabetes mellitus
High body mass index (BMI)
High AST
High ALT
Low platelet count (PLT)
Heavy alcohol drinker
High viral load (HBV carrier)

Table 4. Surveillance protocol for early detection of HCC

1. Super-high-risk patients
Every 3–4 months
Ultrasound examination
AFP/PIVKA-II/AFP-L3 measurements
Every 6–12 months
Dynamic CT or dynamic MRI/EOB-MRI
2. High-risk patients
Every 6 months
Ultrasound examination
AFP/PIVKA-II/AFP-L3 measurements

EOB-MRI = Ethoxybenzyl-MRI.

Surveillance Protocol for Early Detection of HCC

For HCC screening, the HCC detection sensitivity of ultrasonography (US) is higher than that of α-fetoprotein (AFP) measurement, but specificities are not markedly different. For liver cirrhosis, a combination of the two methods has been reported to increase detection frequency compared to detection by US or AFP measurements alone.

No clear evidence is available to determine the optimum interval for periodic screening, but HCCs detected in periodic screenings by AFP, a protein induced by vitamin K absence or antagonist-II (PIVKA-II), and AFP lectin fraction (AFP-L3) measurement, and US are solitary and small in many cases, as compared to those detected in symptomatic patients. Thus, the Evidence-Based Clinical Guidelines [4, 5] proposed performing US and tumor marker measurements every 3–4 months in the super-high-risk population and every 6 months in high-risk populations. Based on HCC doubling times, these intervals appear appropriate (table 4). At present, AFP, PIVKA-II, and AFP-L3 are covered under the Japanese national health insurance as HCC tumor markers. Measurement of two or more tumor markers increases the sensitivity, while minimizing the specificity reduction, for small liver cancer, but alternate measurements of the AFP and PIVKA-II combination or the AFP and AFP-L3 combination is proposed according to the coverage under the current Japanese health insurance. For cases with a very rough background liver parenchyma because of cirrhosis and obesity with difficulty for US evaluation, periodic imaging screening by dynamic CT (multidetector-row CT (MDCT)) or dynamic MRI/EOB-MRI (ethoxybenzyl-MRI) every 6–12 months is proposed [9] (table 4), which is identical to the protocol in the Evidence-Based Clinical Practice Guidelines.

Consensus Statements

5 Patients at high risk for developing HCC should be entered into surveillance programs. The high-risk population and risk factors are identified in tables 2 and 3.
6 Surveillance for HCC should be performed using both US and tumor markers.
7 In Japan, three tumor markers (AFP, PIVKA-II, AFP-L3) are covered by the national health insurance in clinical settings for HCC surveillance.
8 Patients should be screened at 3- to 6-month intervals based on their risk of developing HCC.
9 The surveillance interval needs to be shortened for patients at higher risk for HCC, as described in table 4.

Pathology of Hepatocellular Carcinoma

For the diagnosis and treatment of HCC, it is important to understand the pathology of HCC growth/progression pattern. Clinicians should know the entity of early HCC and the association between pathological features of liver cancer growth/progression and malignancy.

The liver does not have any epithelial structure, different from the digestive tract; therefore, it is impossible to

evaluate the invasive stage of HCC based on the grade of infiltration. In addition, simultaneous/metachronal multicentric development is relatively frequent, making the definition of early HCC difficult. However, several studies showed that the pathological morphology and biological malignancy grade of HCC changed with an increase in the tumor diameter, suggesting the presence of lesions corresponding to early cancer of other organs [24, 25].

Definition of Early HCC with Respect to Pathological Morphology

According to the 'General Rule of Clinical and Pathological of Primary Liver Cancer', HCC is macroscopically classified into five types: vaguely nodular with indistinct margin-, simple nodular-, simple nodular type with extratumor growth-, and multinodular confluent type [26]. In addition, macroscopic findings of small HCCs are classified into two types: simple nodular and vaguely nodular type with indistinct margin. Histologically, most simple nodular type lesions are composed of moderately differentiated carcinoma, whereas vaguely nodular type with indistinct margin consist of well-differentiated carcinoma without severe atypia. In addition to findings such as small cells with an increase in the N/C ratio, an increase in the cell density, 2- to 3-thin layer arrangement, and a small pseudo-glandular structure, these lesions include the several original portal areas. At the boundary of the tumor, cancer cells proliferate to replace the normal hepatocellular cords in the non-cancerous region; therefore, macroscopically, the tumor border becomes unclear. Nodules with indistinct margin, which reflect the earliest change of hepatocarcinogenesis that can be clinically diagnosed, are defined as 'early HCC'. In patients with early HCC, vascular invasion is very exceptional, and there is no intrahepatic metastasis [24]. It is often difficult to differentiate early HCC from high-grade dysplastic nodules. However, the presence or absence of the infiltration of cancer cells in the portal area involved (stromal invasion) [27, 28] should be evaluated for differentiation.

Vascular Structure of Early HCC

It is well known that advanced HCC is completely supplied by arteries. However, early HCC is supplied by the portal venous flow at various levels, i.e. early HCC is supplied by both portal and arteries. However, the number of portal regions in cancer tissue accounts for approximately 25% of that in the non-cancerous region. In addition, arterial tumor vessels are undeveloped; portal and arterial blood may be decreased. On the other hand, arterial tumor vessels develop with an increase in the tumor diameter. However, tumors measuring approximately 10 mm in diameter show insufficient development, and vascularization of the tumor stroma, that is, the capillarization, is also insufficient. Therefore, early HCC does not show hypervascularity on angiography or contrast-enhanced CT.

Fatty Change of Early HCC

Although early, small liver cancer is often visualized as a hyperechoic nodule on US, most lesions reflect the fatty change of the nodule. The fatty change of HCC was the most frequent (approx. 40%) in lesions measuring 10–15 mm in tumor diameter, and the incidence decreases with an increase in the diameter and a reduction in the grade of differentiation. Based on this, fatty change is regarded as a morphological characteristic of early HCC. As previously described, with respect to the pathogenesis of such fatty change, portal blood flow and arterial blood flow may reduce via a decrease in the portal area in lesions measuring 10–15 mm in tumor diameter, and cancer may transiently show ischemia due to the insufficient development of arterial tumor vessels, causing fatty change [29].

Diagnostic Imaging of Early HCC

As many lesions of early HCC are hypovascular, they are difficult to demonstrate on CT through a hemodynamic basis; the correct diagnosis rate is not high. Recently, diagnostic imaging of intrahepatic nodular lesions by contrast-enhanced MRI with Gd-EOB-DTPA has been introduced. For Gd-EOB-MRI to be used to evaluate the hepatocellular function, lesions with a decreased intense at the hepatocyte phase are regarded as HCC. The CT diagnosis rate (including CTHA and CTAP) when lesions with a decrease in portal blood flow were regarded as HCC was approximately 60–70%, whereas the diagnosis rate of HCC by EOB-MRI is approximately 90% [30]; MRI may improve the diagnostic accuracy of early HCC. However, the presence of HCC with isointense and dysplastic nodule with low intense on hepatocyte phase of Gd-EOB-MRI has been indicated.

Macroscopic Classification of HCC and Malignancy Grade

The association between macroscopic findings and malignancy grade depends on the grade of tissue differentiation. When investigating resected specimens of HCC

Table 5. Pathology of small HCC: relationship between macroscopic classification, histological differentiation and tumor size (all resected cases, nodule diameter ≤3 cm) [cited from 9, with permission]

	n (%)	Well	Well + mod.	Mod.	Mod .+ poor	Tumor size, mm
SNIM	22	19 (86.4)	3 (13.6)	0	0	13.6 ± 5.4
SN	123	6 (4.9)	24 (19.5)	92 (74.8)	1 (0.8)	22.8 ± 5.6
SNEG	45	0	5 (11.1)	40 (88.9)	0	23.1 ± 5.4
CM	19	0	6 (31.6)	11 (57.9)	2 (10.5)	23.9 ± 5.3

SNIM = Small nodule with indistinct margins; SN = simple nodular type; SNEG = simple nodular type with extranodular growth; CM = confluent multinodular type. Percent values are shown in parentheses.

Table 6. Pathology of small HCC: macroscopic classification and microscopic findings (all resected cases) [cited from 9, with permission]

	fc	fc-inf	sf	vp	vv	im
SNIM	0	0	2 (9.1)	0	0	0
SN	90 (73.2)	79 (64.2)	65 (52.8)	23 (18.7)	3 (2.4)	5 (4.1)
SNEG	38 (84.4)	35 (77.8)	35 (77.8)	20 (44.4)	2 (4.4)	12 (26.7)
CM	1 (5.3)	1 (5.3)	14 (73.7)	12 (63.2)	3 (15.8)	5 (26.3)

fc = Capsular formation; fc-inf = capsular infiltration; sf = septum formatin; vp = portal vein invasion; vv = hepatic vein invasion; im = intrahepatic metastasis; SNIM = small nodule with indistinct margins; SN = simple nodular type; SNEG = simple nodular type with extranodular growth; CM = confluent multinodular type.
Percent values are shown in parentheses.

measuring ≤3 cm, approximately 85% of vaguely nodular type with indistinct margin lesions (early HCC) consisted of uniform, well-differentiated cancer tissue. The remaining 15% contained an area consisting of moderately differentiated HCC tissue, in which dedifferentiation was noted, showing unclear/clear 'nodule-in-nodule lesion' (table 5). In vaguely nodular type lesions, intrahepatic metastasis and portal tumor invasion are extremely rare. The mean tumor diameter is approximately ≤15 mm, and these lesions are significantly smaller than other macroscopic types of nodular lesions. Approximately 75% of simple nodular type lesions are classified as moderately differentiated HCC. Histologically, portal invasion is observed in 20%, and intrahepatic metastasis in 4%, suggesting advanced HCC. Simple nodular type with extratumor growth and multinodular confluent type lesions suggest advanced HCC. Most lesions consist of moderately to poorly differentiated HCC tissues. Portal invasion and intrahepatic metastasis are more frequently seen than in simple nodular type lesions (table 6). The number of intrahepatic metastatic foci and distance from the primary nodular are greater than in simple nodular type lesions [30] (table 7). In other words, lesions with high-level biological malignancy may be macroscopically evaluated as simple nodular type with extratumor growth or multinodular confluent type lesions. Therefore, curative treatment to avoid intrahepatic metastasis and recurrence must be kept in mind in these lesions in comparison with vaguely nodular type and simple nodular type HCCs.

Table 7. Pathology of small HCC: distance between main nodule and intrahepatic metastasis [cited from 9, with permission]

	n (%)	Distance, mm			
		≤2	2.1–5	5.1–10.0	>10.1
SN	9	6 (66.7)	1 (11.1)	0 (0.0)	2 (22.2)
SNEG	75	23 (30.7)	12 (16.0)	17 (22.7)	23 (30.7)
CM	65	27 (41.5)	19 (29.2)	13 (20.2)	6 (9.2)
Total	149	56 (37.6)	32 (21.5)	30 (20.1)	31 (20.8)

SN = Simple nodular type; SNEG = simple nodular type with extranodular growth; CM = confluent multinodular type.
Percent values are shown in parentheses.

Table 8. Pathology of small HCC: rate of portal venous invasion/intrahepatic metastasis and size of nodule (all resected specimens) [cited from 9, with permission]

	Nodule size, cm				
	0–1	1.1–2.0	2.1–3.0	3.1–5.0	5.1–10.0
PVI	0	28.3%	33.3%	49%	58.5%
IM	0	6.7%	17.1%	29.6%	43.9%

PVI = Portal venous invasion; IM = intrahepatic metastasis.

Differentiation and Malignancy Grade of HCC

Most early HCC lesions appear as well-differentiated lesions. Macroscopically, they are detected as nodules with an unclear border. However, the tumor diameter increases with dedifferentiation. Moderately to poorly differentiated HCCs, contained in the well-differentiated cancer tissue after dedifferentiation, are more malignant than the peripheral well-differentiated HCC tissue, showing expansive growth, completely replacing the well-differentiated cancer tissue, and leading to classical HCC with clear margin. When examining small HCC with 'nodule-in-nodule', p53 overexpression is detected in approximately 40% of moderately to poorly differentiated cancer tissues in the internal area. In addition, a Ki-67 labeling index, which reflects the proliferative capacity, indicates that the malignancy is advanced when the peripheral well-differentiated HCC (early HCC) shows 'nodule-in-nodule' pattern. This is consistent with the finding that an increase in the tumor diameter was accelerated with the appearance of 'nodule-in-nodule' during clinical follow-up. 'Nodule-in-nodule' type HCC is recognized as being in the dedifferentiation process from early to advanced HCC; clinical management similar to advanced HCC is necessary.

In 'nodule-in-nodule' type HCC, there is a marked difference in vascularity between the marginal well-differentiated and internal moderately to poorly differentiated HCC tissues. On contrast-enhanced US or CT, the marginal well-differentiated cancer tissue is visualized as a hypovascular area, because the development of arterial tumor vessels and the capillarization is insufficient. However, moderately to poorly differentiated cancer tissues in the internal area are visualized as hypervascular area due to sufficient neovascular development. Briefly, the vascular structure of liver cancer is closely correlated with the grade of differentiation. The malignancy of early liver cancer may be predicted to some degree based on hemodynamic findings.

Nodule Size and Malignancy Grade of HCC

The size of HCC is associated with the macroscopic morphology, grade of histological differentiation, and intrahepatic metastasis/portal invasion rates. Most vaguely nodular type lesions measure ≤ 2 cm, and lesions measuring ≥ 3 cm are rare. However, simple nodular type with extratumor growth and multinodular confluent type lesions become more frequent with an increase in the tumor diameter. Concerning the grade of histological differentiation, the proportion of lesions consisting of uniform, well-differentiated cancer tissue markedly decreases when the tumor diameter exceeds 2 cm. Most lesions consist of moderately to poorly differentiated HCC tissues. The portal invasion/intrahepatic metastasis rates also increase in proportion to the tumor diameter (table 8). Usually, there is a correlation between an increase in the tumor diameter and malignancy grade. However, exceptionally, large, well-differentiated, slowly expanding HCC is present [32].

Consensus Statements

10 Vaguely nodular type HCCs, which are composed of very well-differentiated HCC, are defined as 'early hepatocellular carcinoma'.
11 Early HCC does not show hypervascularity on angiography or dynamic CT/MRI.
12 Fatty change and stromal invasion are regarded as the morphological characteristics of early nodular HCC.
13 In simple nodular type with extranodular growth and multinodular confluent type HCCs, intrahepatic metastasis and recurrence are more frequent than in lesions with vaguely nodular type and simple nodular type HCCs. This must be kept in mind on the curative treatment.
14 'Nodule-in-nodule' findings of very well-differentiated carcinoma (early HCC) reflect higher malignancy grade than early HCC.

Diagnosis of Hepatocellular Carcinoma

Diagnostic Criteria

The diagnosis of HCC is determined by three factors: the background chronic liver disorder, tumor markers, and imaging diagnosis. When the liver has hepatitis B- and C-related cirrhosis, tumor marker levels are increased, and typical imaging findings are detected, HCC can be definitely diagnosed. Typical imaging findings are hypervascularity in the arterial phase and washout in the portal equilibrium phase on dynamic CT or dynamic MRI. Hypervascularity on CTHA and a perfusion defect on CTAP also leads to a diagnosis of typical HCC. How-

ever, HCC cannot be definitely diagnosed based on a combination of tumor markers and chronic liver disorder alone, or on the elevation of tumor markers alone. Moreover, hypervascular nodules in the arterial dominant phase without washout in the portal equilibrium phase are not typical and more precise investigations are necessary. Hypovascular nodules in the arterial dominant phase also require further examination. Cases meeting all A, B, and C criteria in table 9 are definitely diagnosable as HCC in Japan. Cases not accompanied by the typical imaging findings are diagnosed and treated by the examinations detailed in figures 1 and 2.

Table 9. Diagnostic criteria of typical HCC[1]

A. Background liver disease (one positive factor)
Hepatitis B-related liver disease
Hepatitis C-related liver disease
Liver cirrhosis
B. Tumor markers (at least one positive study)
AFP ≥200 ng/ml associated with a rising trend over time
PIVKA-II (≥40 mAU/ml) with a rising trend over time
AFP-L3 (>15%)
C. Typical imaging findings (one positive study)[2]
Arterial phase hypervascularity with portal-venous phase washout on dynamic CT, dynamic MRI or CEUS
Hypervascularity on CTHA with perfusion defect on CTAP

[1] A+B+C, A+C, B+C, C: HCC confirmed A+B, B: HCC highly suspicious, thus, dynamic CT/MRI is required.

[2] Nodules with atypical imaging study, namely, hypervascularity without portal/venous washout or hypovascular nodule on arterial phase should undergo further study (as shown in fig. 1 and 2).

Multistep Development of HCCs and Abnormal Blood Flow

Many cases of HCCs originate from HBV and HCV infections via multistep development. Premalignant lesions and early HCCs are mainly fed by a portal venous flow in contrast to overt HCCs, which are supplied by an arterial flow. Thus, there may be no objection to indicating a hypervascular HCC for treatment.

However, how to diagnose a typical HCC is the most problematic issue when the hemodynamic pattern is not typical for a HCC. Although the Guidelines for Evidence-Based Clinical Practice for the Treatment of Liver Cancer [4, 5] do not suggest any detailed imaging diagnostic criteria for atypical nodules, a more detailed algorithm referring to these issues has been proposed in the new practice manual (fig. 1).

Diagnostic Modalities of HCC

Dynamic CT

Dynamic CT using MDCT acquires images within several seconds during a single respiratory pause if the slice thickness is about 5 mm and is superior for detection of hypervascular HCC. On dynamic CT of the liver, 100–120 ml of contrast medium is rapidly infused within 30 s. The arterial dominant phase is generally acquired at around 30–45 s following the initiation of the injection of contrast medium [33]. A characteristic of HCC is a corona-shaped enhancement in the late arterial phase or portal venous dominant phase [34]. The vascular and extracellular contrast medium concentrations reach equilibrium about 200 s after infusion; scanning at this time point is called the equilibrium or parenchymal phase. In a typical HCC, attenuation decreases in the equilibrium phase.

In MDCT, time resolution is high and acquisitions of the arterial, portal, and equilibrium phases have markedly increased the ability to qualitatively diagnose tumorous lesions. The diagnosis of a typical hypervascular HCC is easy because the lesion is detected as a high attenuation area in the arterial phase, corona enhancement is noted in the late arterial or the portal phase, and this becomes a low attenuation area in the equilibrium phase. However, the frequency of obtaining typical findings varies depending on CT equipment and acquisition conditions. Diagnostic accuracy of hypervascular HCCs by CT has been reported to be 68–91% [35–37].

Contrast-Enhanced US

Sonazoid-enhanced US are classified into the two phases: the vascular and Kupffer phases (fig. 3).

Vascular Phase

The sensitivity of contrast-enhanced US with Sonazoid to detect intranodular blood flow in liver tumors is extremely high. The sensitivity of 4-phase imaging (plain, arterial, portal, and equilibrium phases) by MDCT, as a gold standard, for detecting intranodular blood flow is similar to or superior to that of MDCT. In addition, in most patients in whom there is no intranodular blood flow in the arterial phase on contrast-en-

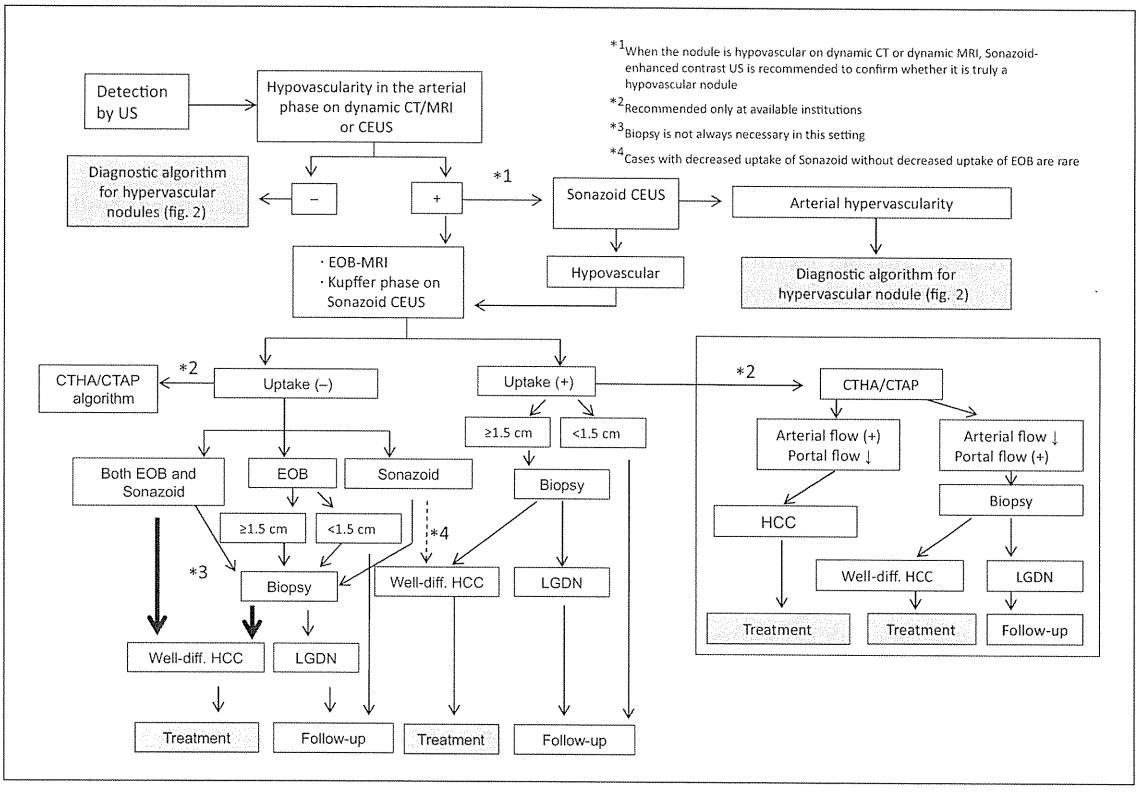

Fig. 1. Diagnostic and treatment algorithms for hypovascular liver nodules (JSH 2010) [cited from 9, with permission].

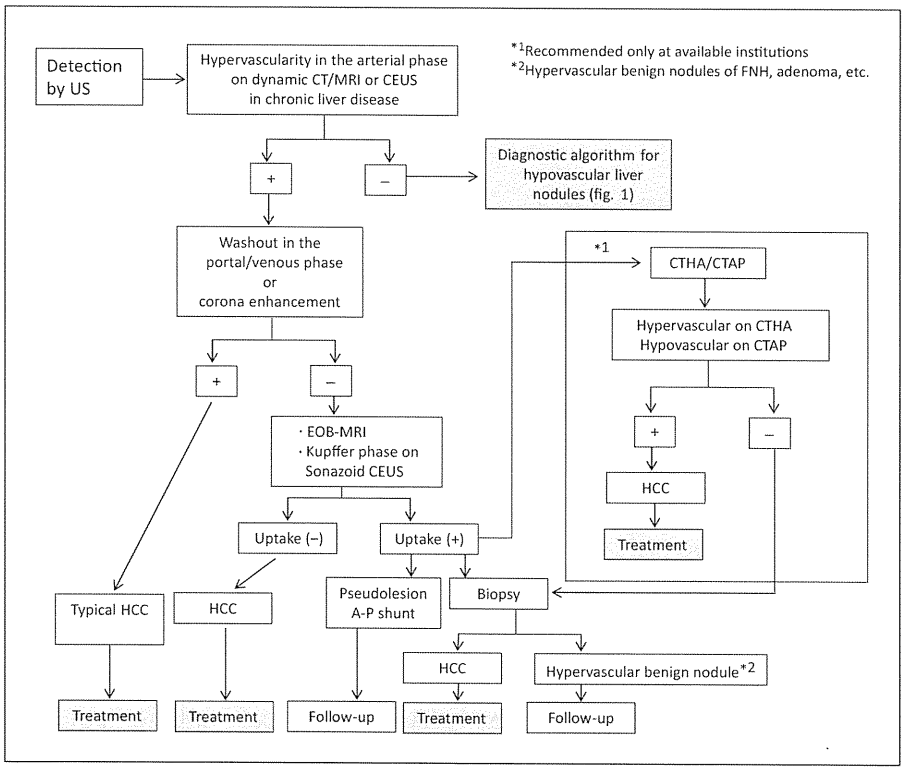

Fig. 2. Diagnostic and treatment algorithms for hypervascular liver nodules (JSH 2010) [cited from 9, with permission].

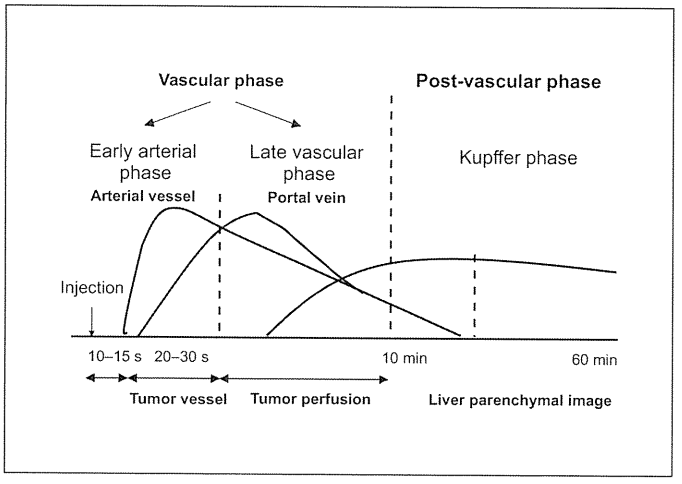

Fig. 3. Phases of contrast-enhanced US with Sonazoid. There are two phases: vascular and post-vascular phase.

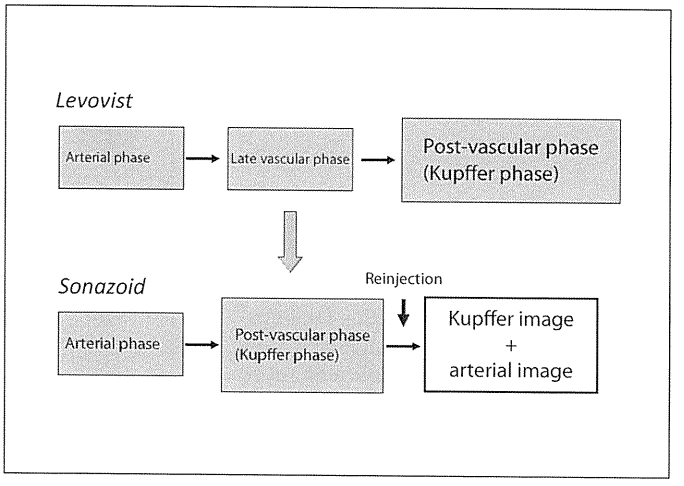

Fig. 4. Basic concept of defect reperfusion imaging: Reinjection at the Kupffer phase plays an important role.

hanced US, contrast-enhanced CT does not show any enhancing area in the arterial phase. However, in some patients without intranodular blood flow on dynamic CT, contrast-enhanced US reveals arterial blood flow. Briefly, contrast-enhanced US may be more sensitive than CT for the detection of intranodular arterial blood flow [38]. Basically, contrast-enhanced US should be performed as a precise examination tool of intranodular blood flow in nodules detected on B-mode US. When using Sonazoid, the Kupffer phase is very stable, differing from that with Levovist, SonoVue or Definity. Therefore, initially, entire liver scanning should be conducted in the Kupffer phase, and, additionally, Sonazoid should be intravenously injected into Kupffer defect sites (defect reperfusion imaging), which facilitates cancer detection to making a definitive diagnosis. In the future, contrast-enhanced US may be applied for screening [39, 40] and staging, which have been considered to be impossible using CEUS with blood pool agents, such as SonoVue or Definity.

Kupffer Phase

The Kupffer phase of Sonazoid is very important.

(1) Most lesions of moderately or well-differentiated hypervascular HCC in which arterial blood flow is abundant show a decrease in Sonazoid uptake or defect in the Kupffer phase.

(2) Among precancerous or early HCC lesions, Sonazoid uptake in the Kupffer phase is similar to that of the surrounding liver parenchyma in which arterial blood flow is reduced, with the influx of portal blood flow is preserved.

(3) For the differential diagnosis between a precancerous lesion, dysplastic nodule (DN), and early well-differentiated HCC (early HCC), the two lesions show iso uptake in the Kupffer phase in many cases; therefore, differentiation is difficult. However, hypovascular nodules in which Kupffer uptake is reduced in the Kupffer phase can be diagnosed as early HCC.

Significance of Defect-Reperfusion Imaging (Double Injection CEUS)

Recently, a new procedure (defect-reperfusion imaging) was developed, in which stable Kupffer images of Sonazoid and real-time blood flow imaging are applied, facilitating the accurate local diagnosis and treatment of typical liver cancer that shows arterial enhancement with venous wash on CT, washout in the late phase, and is not visualized as on B-mode US (fig. 4) [39, 41].

Sonazoid is intravenously injected. Presence or absence of Kupffer defect is evaluated in the Kupffer phase ≥10 min after confirming an arterial enhancement area in the vascular phase. In addition, an entire liver scan is necessary to detect a defect site in the Kupffer phase. If a Kupffer defect is found in the liver, Sonazoid is additionally administered whether or not arterial blood flows in the defect site is assessed (defect reinjection test). Regarding the presence of arterial vascularity in the defect area, typical liver cancer can be diagnosed at a rate of approx-

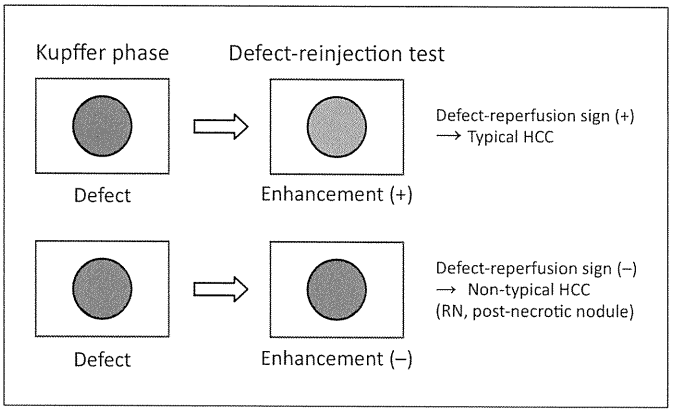

Fig. 5. Value of defect reperfusion imaging in B-mode undetectable nodule. Diagnostic ability of B-mode undetectable HCC by this technique using Sonazoid-enhanced US is 100%.

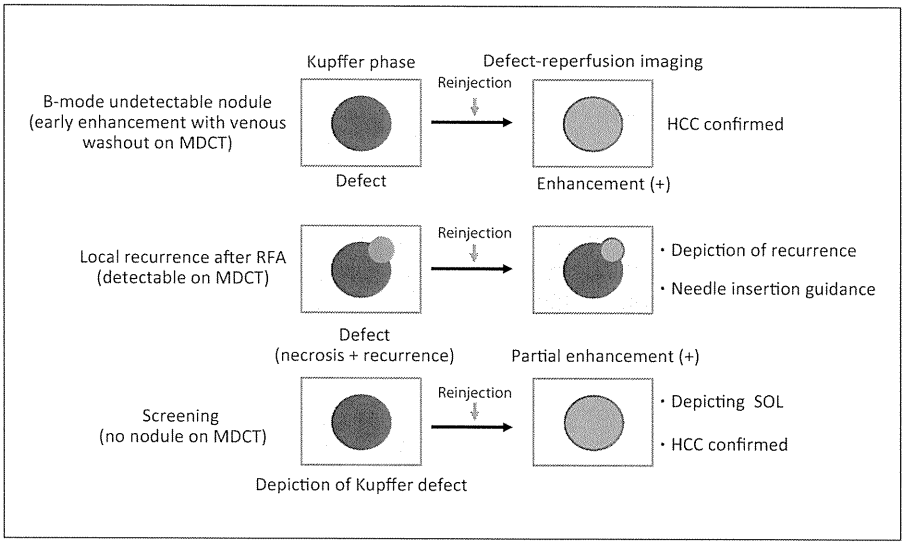

Fig. 6. Defect reperfusion image is useful for confirming B-mode undetectable nodule, local recurrence after RFA and surveillance of HCC in cirrhotic patients.

imately 100% (fig. 5). In addition, concerning nodules that cannot be visualized on B-mode US, it is possible to detect clear defects in the Kupffer phase, although diagnosis is impossible in the vascular phase since the detection of arterial blood flow is impossible to detect. The additional intravenous injection of Sonazoid in this area facilitates the detection of arterial vascularity in the defect site. In this area, Sonazoid contrast-guided radiofrequency thermal ablation (RFA) therapy becomes possible. In this procedure, initially, nodules that cannot be detected on B-mode US are detected as defects in the Kupffer phase. Subsequently, the additional intravenous injection of Sonazoid is performed, and whether or not nodules with defects involve arterial blood flow is evaluated. This procedure may be a breakthrough for diagnostic imaging. This contrast enhancement procedure does not require any specific device or analysis. Concerning typical CT findings, arterial enhancement with venous washout, defects are initially detected in the Kupffer phase, and, subsequently, arterial perfusion in the detect site is confirmed (fig. 5).

The introduction of such an idea facilitates the identification of nodules that show hypervascular typical features on CT, and are unclear on B-mode US, at a probability of approximately 100%. If there are nodules without arterial enhancement by reinjection for defects, they may differ from nodules detected on CT (fig. 6). Therefore, clinically, this procedure is breakthrough as a treatment aid of HCC [42]. In addition, this defect reperfusion imaging method can be applied for various purposes such as HCC screening in liver cirrhosis with rough liver parenchyma, the identification of local tumor progression sites after treatment. In addition, in cases of evaluation of the treatment response after RFA or transcatheter arterial chemoembolization (TACE) and contrast-enhanced guided needle placement it may be also very useful (fig. 6).

Dynamic MRI

The consistency rate of histopathology excised liver after liver transplantation with various imaging findings has been reported, with the sensitivity of dynamic MRI shown to be the highest [43]. In another report, CT, abdominal US, and MRI were compared with regard to evaluations of nodular recurrence after transarterial chemoembolization mixed with lipiodol; again, MRI was the superior modality [44]. However, detectability varies among MRI equipment models, and MRI cannot be readily used in some facilities. Use of MDCT has spread rapidly in Japan, and its usefulness is well established. It may be better to diagnose hypervascular HCC using dynamic MRI or MDCT depending on the conditions found in the institution, but the X-ray exposure problem arising with frequent MDCT images should be kept in mind.

Gd-EOB-MRI

Gd-EOB-MRI Is Superior to SPIO-MRI

A new hepatocyte-specific contrast agent for MRI, Gd-EOB-DTPA (gadoxetate sodium), became commercially available in Japan in January 2008. This contrast agent is taken up by hepatocytes and excreted from the kidney and from the liver through the bile duct. As a result, liver parenchyma is intensely enhanced showing definite hyperintensity in the hepatobiliary phase $\geq$20 min after intravenous injection based on T_1-weighted images, in addition to the diagnosis based on blood supply. Nodules without liver parenchymal cells, such as liver cancer, are visualized as hypointense. This diagnostic imaging method is simple for hepatologists in addition to radiologists specialized in MRI in comparison with SPIO as a black liver agent (black coloration of the entire liver on T_2-weighted images in which the spatial resolution is poor). Gd-EOB-DTPA-enhanced MRI is a breakthrough for diagnostic imaging of the liver and HCC.

Mechanism of Gd-EOB-DTPA Uptake and Findings with Respect to the Grade of Differentiation of HCC

Approximately 50% of the dose of Gd-EOB-DTPA is taken up by hepatocytes and excreted in bile. The remainder is excreted from the kidney. Hepatocellular uptake may be related to passive diffusion mediated by organic anion transporter 1 (OATP1) in the hepatocellular membrane in rat [45]. Furthermore, ATP-dependent active transport related to multi-drug resistance-associated protein 2 (MRP2) may be involved in excretion from hepatocytes into the capillary bile duct [46].

A recent study reported that Gd-EOB-DTPA uptake in humans depended on OATP1B3 (synonymous with OATP8) [47, 48] among various kinds of human OATP families. Based on these reports, concerning the mechanism via which Gd-EOB-DTPA is taken up in the hepatobiliary phase even when well- or moderately differentiated liver cancer is hypervascular HCC, OATP1B3 expression may be detected in some lesions, and visualized as an iso or high signal intensity in the hepatobiliary phase. According to the study, there was no statistically significant association between OATP1B3 expression and the bile-producing capacity (green hepatoma)/grade of differentiation [47, 48]. It is now considered that the expression of OATP1B3 may be decreased in accordance with the elevation of the grade of malignancy of the hepatocellular nodules. According to the recent immunohistochemical study, low-grade dysplastic nodule showed the same or increased OATP1B3 expression relative to the surrounding liver. On the other hand, around half of high-grade dysplastic nodules, around 80% of early HCCs, around 90% of well- and moderately differentiated HCCs and almost all of poorly differentiated HCCs demonstrated decreased expression [Matsui O., pers. commun., submitted]. Therefore, the majority of HCCs may be detected as hypointense nodule in the hepatobiliary phase when liver function is well preserved. However, in approximately 5–10% of patients with hypervascular, well- or moderately differentiated liver cancer, there was definite expression of OATP1B3 and no reduction in the signal intensity in the hepatobiliary phase, probably due to genetic alteration [47, 48].

Actually, in clinical practice, in some nodules detected on US, histopathological investigation with biopsy specimens leads to a diagnosis of well-differentiated liver cancer despite the absence of signal-intensity reduction in the hepatobiliary phase; well-differentiated liver cancer in which OATP1B3 expression is maintained may be present.

Another issue is whether signal-intensity reduction is absent in all dysplastic nodules in the hepatobiliary phase. Concerning this issue, biopsy/pathological findings of nodules with signal-intensity reduction in the hepatobiliary phase suggest dysplastic nodules in some cases. However, a study involving resected specimens (not biopsy materials) reported that all dysplastic nodules showed an iso signal intensity in the hepatobiliary phase. Therefore, there may be few dysplastic nodules with such a decrease in OATP1B3 expression according to pathologists specialized in the liver pathology of early HCC (table 10).

In our experience on the study of resected specimens, dysplastic nodules with a low signal intensity are extremely rare [49]. Unless stromal invasion is detected in the sample despite a pitfall of biopsy diagnosis, sam-

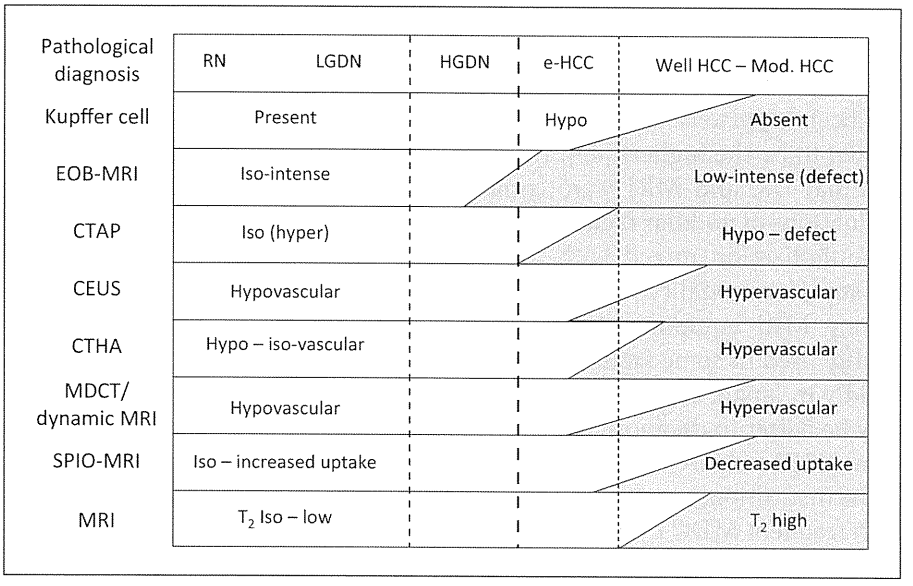

Fig. 7. Imaging findings of hepatocellular nodules in cirrhotic liver [cited from 47, with permission]. Hepatocyte phase of EOB-MRI is the most sensitive technique to detect initial change of multistep hepatocarcinogenesis.

pling errors, or similar cellular/structural atypia, under a biopsy diagnosis of dysplastic nodules, it is sometimes difficult for specialists of liver pathology to make a diagnosis of early HCC. Therefore, biopsy-based pathological diagnosis is limited, and lesions may be underestimated.

Differentiation of Early HCC from Dysplastic Nodules Using Gd-EOB-DTPA-MRI

As described in the above section, blood flow findings are hypovascular in many cases when investigating resected specimens of early HCC. Even when employing CTHA/CTAP, diagnosis of early HCC is difficult. In some patients with early liver cancer, there is a slight decrease in portal blood flow on CTAP. However, CTAP shows iso-perfusion in many patients. Most patients with a reduction in signal intensity in the hepatobiliary phase are diagnosed with early HCC based on resected specimens by specialists of liver pathology [49]. In most patients without a reduction in the signal intensity in the hepatobiliary phase, resected specimens suggest dysplastic nodules. Considering this, functional diagnosis with Gd-EOB-DTPA MRI may facilitate the more sensitive evaluation of initial changes of multistep hepatocarcinogenesis than hemodynamic assessment, SPIO-MRI, Kupffer cell function diagnosis using the Kupffer phase on Sonazoid contrast-enhanced US, or portal blood flow assessment to differentiate early HCC from dysplastic nodules (fig. 7). However the following two issues remain: (1) whether there are dysplastic nodules with a reduction in the signal intensity in the hepatobiliary phase, and (2) whether there are nodules without a reduction in the signal intensity in the hepatobiliary phase in which pathological findings lead to a diagnosis of early HCC. With respect to the two issues, data have now accumulated throughout Japan. However, a consensus has not been reached. To overcome these issues, hypovascular nodules with uptake on SPIO-MRI or in the Kupffer

Table 10. Relationship between the expression of OATP1B3 and findings in the hepatocyte phase

Uptake transporter (OATP1B3 (OATP8))	Hepatocyte phase imaging of EOB-MRI
Dysplastic nodule	
+	Iso-high intensity
– (rare)	Low intensity
Early HCC	
+ (rare)	Iso-intensity
–	Low intensity
Well- to mod. diff. HCC	
+ (5–10%)	Iso-high intensity/iso-high intensity
– (90–95%)	Low intensity
Poorly diff. HCC	
–	Low intensity

OATP = Organic anion transporter polypeptides.

phase of Sonazoid US can be differentiated from hepatocellular nodules. However, in nodules with a reduction in the signal intensity in the hepatobiliary phase of Gd-EOB-DTPA, biopsy findings should be obtained, and the natural course of these nodules must be followed up. It may be important to analyze the grade of malignant potential in a large number of patients.

Angiography

Diagnosis of HCC by angiography including DSA is 69.0%, lower than that (86.9%) of helical CT [50]. Also, detectability more markedly decreases than does CT sensitivity when the tumor size is small. In Japan, very few institutions perform angiography alone for diagnosis, and many facilities routinely perform CT during hepatic arteriography (CTHA) and CT during arterial portography (CTAP) in combination. The rate of detectability of liver cancer by angio-CT is the highest, but specificity is low, and some reports, mainly from Europe and USA, have questioned its diagnostic value. However, observation of corona-like intense staining around liver cancer in the second phase of CTHA (acquisition of images during contrast medium infusion is designated as the first phase, and acquisition after completion of infusion as the second) enables differentiation from pseudo-hypervascular lesions, thus increasing the specificity. The HCC diagnostic ability of arterial injection CT may be highest when second-phase CTHA images are acquired [34]. Sensitivity and specificity of hypervascular HCC diagnoses are highest when both CTHA and CTAP are performed. Since CTHA/CTAP detects nodules <1 cm in diameter, differentiation from pseudo lesions, such as arterial-portal (A-P) shunt or focal nodular hyperplasia (FNH), is necessary.

Diagnostic and Treatment Algorithm of Hypervascular Hepatocellular Carcinoma

For surveillance, abdominal US is a first-line test in many hospitals. As a subsequent examination, MDCT is the most commonly conducted, therefore it was decided as a first-line test in the surveillance algorithm both in the AASLD or JSH guidelines. As dynamic MRI is performed as a first-choice procedure in some hospitals, it should be regarded as similar to MDCT. When the lesion is enhanced in the early arterial phase, a diagnosis of HCC may be made based on washout in the equilibrium phase (table 5). However, FNH and A-P shunt must be ruled out. The number of hepatocytes and Kupffer cells was examined on Gd-EOB-MRI or in the Kupffer phase of Sonazoid contrast-enhanced US. If a defect is confirmed at a low signal intensity in the hepatobiliary phase of Gd-EOB-MRI or in the Kupffer phase of Sonazoid-enhanced US, a diagnosis of HCC can be made (fig. 2).

When low density is noted in the equilibrium phase without an enhancement in the early arterial phase on MDCT, the lesion can be evaluated as hypervascular nodule. CTHA, which is more sensitive, must be performed to examine whether or not it is hypervascular HCC. In institutions where CTHA cannot be conducted, it is necessary to confirm blood flow in the early phase of CO_2 angiography or contrast-enhanced US, or evaluate whether the lesion is hypervascular in the early arterial phase on dynamic MRI.

When abdominal angiography reveals hypovascular tumors, CTHA should be performed if possible, because the sensitivity of the former for the diagnosis of HCC is low. If impossible, CO_2 angiography with high-level spatial resolution should be simultaneously conducted to evaluate the number of hypervascular tumors.

For angiography or CTHA, the possibility of pseudotumors such as A-P shunt must be ruled out. In this case, a diagnosis of hypervascular HCC can be made if there is a decrease in the number of Kupffer cells in the hepatobiliary phase of Gd-EOB-MRI or in the Kupffer phase of Sonazoid-enhanced US. The HCC-diagnosing capacity of Gd-EOB-MRI is similar to that of CTHA/CTAP, therefore these invasive tests may be omitted.

Consensus Statements

15 Typical HCC can be diagnosed by imaging regardless of the size detected if a typical vascular pattern is obtained on dynamic CT, dynamic MRI, CEUS or a combination of CTHA and CTAP. Different from Western guidelines, only one dynamic study showing the typical pattern is sufficient to diagnose HCC even if nodule is <2 cm. The typical imaging pattern includes hypervascularity in the arterial phase and washes out in the portal venous phase.

16 Nodular lesions showing an atypical imaging pattern, such as iso- or hypovascular in the arterial phase or arterial hypervascularity alone without portal venous washout, should undergo the examinations shown in figures 1 and 2. EOB-MRI and Sonazoid-enhanced US play an important role.

17 Elevated AFP (≥20 ng/ml) and PIVKA-II (≥40 mAU/ml) with a rising trend over time and a positive AFP-L3 value (>10%) are highly suggestive of the presence of typical HCC even if US fails to depict an apparent nodule in the liver.

Diagnostic and Treatment Algorithm for Hypovascular Nodules

Among liver cirrhosis-related nodular lesions, hypovascular nodules include low-grade dysplastic nodules (LGDN), high-grade dysplastic nodules (HGDN), which are pathologically regarded as precancerous lesions, early HCC, and nodule-in-nodule type HCC [11, 24, 51, 52]. In this section, the current consensus of diagnosis and treatment algorithms with respect to the treatment of such nodules, especially findings important for the diagnosis and treatment of hypovascular nodules detected on imaging, will be described.

Diagnostic Algorithms for Hypovascular Hepatocellular Nodules (fig. 1)

There has been no diagnostic imaging method to accurately differentiate precancerous lesions such as LGDN and HGDN from early HCC. However, since Gd-EOB-MRI was introduced in 2008, such a situation is rapidly changing.

Among current diagnostic imaging procedures, the following modalities may facilitate the most sensitive tool to detect the initial change of hepatocarcinogenesis: (1) EOB-MRI [49], (2) CTAP [53, 54], (3) contrast-enhanced US [38, 41, 55], (4) CTHA, and (5) MDCT/dynamic MRI, SPIO-MRI (fig. 7). Usually, precancerous lesions show EOB uptake. However, most early liver cancer lesions show a low signal intensity in the hepatobiliary phase of EOB-MRI [49]. Therefore, EOB-MRI may facilitate the earliest, most sensitive assessment of the initial features of early HCC. As the second-most sensitive method, CTAP facilitates the evaluation of initial changes related to hepatocarcinogenesis. However, a partially increased area in intranodular arterial blood flow detected on CTHA or contrast-enhanced US, in which portal blood flow is maintained in the outer area in about two-thirds of early liver cancer nodules, reflects an advanced state of the carcinogenic process. Briefly, hypervascular foci visualized in the nodule (nodule-in-nodule or entirely hypervascular) may biologically reflect small advanced cancer even if histological findings suggest well-differentiated HCC. In some cases, satellite nodules or microvascular invasion is observed at the periphery of the nodule.

According to a study, EOB-MRI shows a decrease in uptake in the hepatobiliary phase in HGDN lesions, therefore a consensus regarding differentiation between benign and malignant tumors has not been reached. Briefly, hypovascular tumors with a decrease in uptake may be basically regarded as early HCC, however DN cannot be ruled out.

Although MDCT and dynamic MRI are relatively sensitive for the detection of arterial blood flow, some hypervascular nodules detected on CTHA or contrast-enhanced US are not visualized as arterial staining, which depends on the timing of imaging, tumor site, and liver function. Hypervascular nodules on MDCT or dynamic MRI may show high intensity on T_2-weighted MRI images.

Based on this, EOB-MRI or Sonazoid contrast-enhanced US should be performed in hypovascular nodules demonstrated on MDCT or dynamic MRI. If there is a decrease in uptake on EOB-MRI or in the Kupffer phase of Sonazoid contrast-enhanced US, malignancy must be initially considered. Furthermore, portal blood flow is reduced on CTAP in some nodules in which EOB-MRI or Kupffer-phase Sonazoid contrast-enhanced US shows uptake, although such cases are rare. When CTHA/CTAP is not conducted, or in institutions in which it is impossible to perform these procedures, biopsy should be conducted in such nodules measuring >1.5 cm, because they may become hypervascular nodules, leading to typical liver cancer. When performing CTHA/CTAP, nodules with increased arterial blood flow or the reduction of portal blood flow are biologically regarded as malignant. In nodules in which arterial blood flow is insufficient in the presence of portal blood flow, biopsy is necessary.

The capability of contrast-enhanced US is dependent on US equipment. However, in institutions in which high-end machines are available, the combination of this procedure and MDCT improves the accuracy of arterial/portal blood flow assessment in comparison with a single method alone. Furthermore, the application of the Kupffer phase and hepatobiliary phase of EOB-MRI makes the prediction of malignancy more accurate.

Concerning nodules that are not visualized as hypervascular nodule on MDCT or dynamic MRI, when both EOB-MRI and Kupffer-phase Sonazoid contrast-enhanced US reveal a reduction in uptake, these nodules should be treated as HCC. In this case, biopsy is not always necessary.

When EOB-MRI shows a reduction in uptake and the nodule size exceeds 1.5 cm, biopsy should be performed if possible. When a diagnosis of typical well-differentiated HCC is made, treatment should be performed. Even when EOB-MRI and Kupffer-phase Sonazoid contrast-enhanced US reveal uptake, CTHA and CTAP may be performed in nodules measuring >1.5 cm. When there is