smaller lesions. The emergence of new contrast agents such as gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) are expected to reveal suspected HCC nodules, including early HCC at approximately 1 cm in size. Tumor biopsy should then be performed to confirm the diagnosis of early cancer before it can progress to overt HCC. It is also expected that the increase in therapeutic options will increase the need for more detailed information of the tumor characteristics, such as tumor differentiation and immunophenotype reflecting tumor aggressiveness, which can only be determined by tumor biopsy.

PROGNOSTIC STAGING SYSTEM

IN TERMS OF estimating the prognosis of HCC, there are currently insufficient evidence-based data; therefore, no definite recommendations can be made, unlike other fields of HCC management. It is well known that the prognosis of HCC is defined by the behavior of the HCC itself, and by host factors such as hepatic functional reserve. The major questions that still need to be answered in terms of estimating the prognosis of HCC are: (i) whether an integrated staging system is necessary for the management of HCC; (ii) what is the best integrated staging system; and (iii) should the integrated staging system be included in the algorithm for HCC treatment?

Tumor staging (TNM staging)

There are two major classifications used for tumor staging of HCC. One is the tumor–node–metastasis (TNM) stage, developed by the American Joint Committee on Cancer (AJCC). This classification can also be applied to liver transplant recipients. However, the cut-off value for tumor diameter of 5 cm is too large to define small HCC, which are frequently found in Japan.

The other is the TNM stage proposed by the Liver Cancer Study Group of Japan (LCSGJ). The cut-off of 2 cm is very appropriate for patients in countries such as Japan, where small HCC are often found in an established nationwide screening system. However, in this system, the weighting of the strongest prognostic factor, vascular invasion, is equal to that of other factors used to estimate prognosis, which might not be adequate.

Staging for hepatic functional reserve

There are two major classifications for estimating liver functional reserve. One is the Child–Pugh classification, which is widely used worldwide, but is difficult to apply for decision making for hepatectomy. The other is the Liver Damage Classification scheme proposed by the LCSGJ, which is useful for hepatectomy. However, this scheme is not widely accepted because of the need to perform the indocyanine green retention at 15 min test ($ICGR_{15}$).

Integrated staging system for HCC

The combined classification of TNM stage and liver function stage, namely, an integrated staging system, is extremely important to estimate patient prognosis and guide decision making for patient management. The integrated staging system contributes to: (i) estimate patient prognosis; (ii) select the best treatment option for each patient; (iii) compare different treatment modalities; and (iv) compare treatment outcomes among different institutions.

Since the Okuda classification in 1985,[10] several integrated staging systems have been reported, including the Cancer of the Liver Italian Program (CLIP) score,[11] the Barcelona Clinic Liver Cancer (BCLC) stage[12] and the Japan Integrated Staging (JIS) score.[13] The Okuda classification scheme is simple and has been found to be suitable in the past, but does not seem to be suitable at the present time, now that relatively small HCC can be detected. The CLIP score is popular in Western countries, but its discriminating power is weak for small HCC, particularly at higher scores of 4–6, and over 50% of Japanese HCC patients are classified as score 0. The BCLC staging is thought to be useful as an integrated staging system and for guiding treatment. Therefore, it is recommended as an integrated treatment algorithm by the European Association for the Study of the Liver and the American Association for the Study of Liver Disease (AASLD). However, it is not suitable for the estimation of patient prognosis, and a large number of variables are used. In contrast, the JIS score essentially consists of the Child–Pugh score and the LCSGJ TNM stage, and is widely accepted in Japan. The discriminating power for relatively small HCC is excellent, and is particularly suitable for countries such as Japan, where many small HCC are detected.

In terms of a comparison of these integrated staging systems, Cillo *et al.*[14] reported that the BCLC was the best system among the Okuda, CLIP, BCLC and French classifications. Meanwhile, Tateishi *et al.*[15] reported that the Tokyo score was superior to BCLC staging and comparable to the CLIP score in predicting prognosis after hepatectomy and ablation. Kudo *et al.*[16] reported that the JIS score was better than the CLIP score, particularly in terms of discriminating power for each subgroup. Similarly, Chung *et al.*[17] reported that the JIS score was

the most excellent staging system among the BCLC, Tokyo and JIS staging systems. Therefore, JIS score is currently considered to be the best integrated staging system in Japan. Regarding other integrated staging systems, modified JIS score has been reported[13,18] to be useful for patients undergoing hepatectomy. Biomarker combined JIS score has also been reported to be useful in discrimination in patients with good prognosis.[19] However, the usefulness of these new staging systems will remain unclear until they are assessed in a range of patient sets with HCC.

Regarding the estimation of HCC prognosis, most hepatologists recognize the importance of an integrated staging system rather than applying the TNM stage and hepatic functional reserve scales individually. Furthermore, the JIS score is considered to be the best integrated staging system for current clinical practice. However, it is still difficult to incorporate the integrated staging systems, such as the JIS score, into algorithms for HCC treatment.

Recommendation 3. Integrated staging system should be used to assess the prognosis of patients with HCC, instead of individually applying scales for TNM stage and liver function stage.

Recommendation 4. The JIS score is the best staging system to estimate the prognosis of patients with HCC.

Informative Statement 1. Integrated staging systems, such as the JIS score, are not yet suitable for inclusion in algorithms for HCC treatment.

SURVEILLANCE AND DIAGNOSIS

Surveillance programs

IT IS WELL known that HCC mainly occurs in cases with chronic liver disease, particularly cirrhosis. Several cohort studies have shown that the surveillance of high-risk patients with hepatitis B virus (HBV)- or hepatitis C virus (HCV)-related chronic liver disease improves the rate of early detection and the rate of curative treatments.[20–27] For this reason, UK[28], European[29] and American[3] practice guidelines for HCC recommend routine surveillance of HCC among individuals with viral hepatitis or cirrhosis. Almost all gastroenterologists in Japan conduct surveillance programs using a combination of tumor markers such as AFP, the *lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3%) and DCP, and by ultrasound (US).[30] However, no consensus has been reached in terms of the optimal surveillance strategy. Thompson *et al.* calculated the number of people who need to be under surveillance to prevent either a single death from HCC or a single premature death (defined as death before the age 75 years) and showed the effectiveness of surveillance programs.[31] In the absence of surveillance, approximately 20% of the mixed etiology cohort died as a result of HCC.

Recommendation 5. Surveillance with US and three tumor markers including AFP, DCP and AFP-L3 should be performed for early detection of HCC in patients with HBV- and HCV-related chronic liver disease, particularly cirrhosis.

Tumor markers

In Japan, AFP, AFP-L3 and DCP are widely and routinely used as serological tumor markers for the surveillance, diagnosis and prognostic estimation of HCC. The Evidence-Based Clinical Practice Guidelines of HCC published in 2005[1] recommended that AFP, AFP-L3 and DCP should be measured at intervals of 3–4 months for very high-risk patients (defined as HBV- or HCV-related liver cirrhosis), and at 6-month intervals for high-risk patients (defined as HBV- or HCV-related chronic liver disease or other causes of liver cirrhosis).[32] Although AFP is the most widely used tumor marker for HCC, the levels of AFP are also increased in patients with liver diseases other than HCC, including viral hepatitis, with a prevalence of 10–42%.[33–35] In contrast, AFP-L3 and DCP are very specific for HCC, compared with AFP alone. The combination assay for AFP, AFP-L3 and DCP should be performed for the early detection of HCC.[36,37] The specificity and sensitivity of the combination assay of AFP and DCP were 83% and 84%, respectively, to detect small HCC of less than 3 cm in diameter.[38] The specificity and sensitivity of the combination assay of DCP and AFP-L3 were 41.7–66.7% and 89.5–89.8%, respectively, to detect small HCC of less than 3 cm in diameter.[39,40]

Recommendation 6. Periodical measurement of more than two kinds of tumor markers (particularly AFP and DCP) is recommended for the early detection of HCC in high-risk and very high-risk patients.

Recommendation 7. The surveillance interval needs to be shorter in very high-risk patients than in high-risk patients.

Imaging modalities

Periodic follow-up of chronic liver disease by US, multidetector row computed tomography (MDCT) and magnetic resonance imaging (MRI) allows relatively

easy detection of small HCC.[41–43] However, it is sometimes difficult to characterize small hepatic nodular lesions detected by these imaging modalities. Definitive diagnosis requires invasive methods such as US-guided liver biopsy. Hemodynamic evaluation of the nodule is also important to assess the biological behavior of HCC. The recent advances in MRI and computed tomography (CT) procedures, such as CT during hepatic arteriography (CTHA) and CT during arterial portography (CTAP), have enabled the detailed hemodynamic evaluation of small hepatic nodules.

Recently, liver-specific contrast agents such as superparamagnetic iron oxide particles (SPIO), which are taken up by Kupffer cells, and Gd-EOB-DTPA, which is taken up by hepatocytes, are frequently used in MRI for early diagnosis of HCC. Gd-EOB-DTPA is a superb agent because it provides dynamic and liver-specific MR images.[44–46] This contrast agent is highly liver specific; approximately 50% of the injected dose is taken up by functioning hepatocytes and is excreted in bile, compared with just 3–5% for gadobenate dimeglumine.[46] Early studies comparing Gd-EOB-DTPA-enhanced dynamic MRI with dynamic MDCT showed that Gd-EOB-DTPA-enhanced MRI is significantly more accurate, sensitive and specific than dynamic MDCT for the diagnosis of HCC in patients with cirrhosis.[47,48] In addition, Gd-EOB-DTPA-enhanced MRI has a high detection rate for early stage HCC nodules that are not enhanced in dynamic studies. However, although the differentiation of early HCC from dysplastic nodule by hepatobiliary phase images of Gd-EOB-DTPA MRI is promising, more data are still needed.

Informative statement 2. Gd-EOB-DTPA-enhanced MRI provides dynamic and hepatocyte-specific images and is more accurate than dynamic MDCT or SPIO-MRI for the detection and characterization of small HCC, including early HCC.

ABLATION THERAPIES

IMAGE-GUIDED PERCUTANEOUS ablation therapies have long played important roles in the treatment of HCC. Percutaneous ethanol injection has been used for unresectable, small HCC since the early 1980s[49–51] and offers us the potential to treat HCC using non-surgical means. Percutaneous microwave coagulation therapy became popular in Japan in the late 1990s.[52] However, since the introduction of radiofrequency ablation (RFA) into clinical practice around 1999, there has been a dramatic shift from ethanol injection or microwave coagulation to RFA.[53] RFA for HCC has been covered by public health insurance since April 2004 in Japan. Although more than 1700 institutions have experienced RFA in Japan, RFA is estimated to be performed routinely in approximately 1000 institutions throughout Japan at the present.

Radiofrequency ablation often seems to be performed with less than adequate treatment planning or preparation compared with surgical resection. RFA appears to be a very simple procedure. Thus, some physicians may perform RFA without adequate training or experience. In addition, RFA does not require expensive equipment. Thus, several hospitals have introduced RFA into clinical practice without high-performance US and CT.

However, RFA is indicated for malignant tumors and inadequate outcome should be avoided. Thus, only physicians with sufficient experience and appropriate skill should perform the procedure. Furthermore, only well-equipped hospitals should perform RFA because the outcomes of RFA are strongly influenced by the performance of the CT and US equipment available at each institution. It is crucial to offer consistent outcomes for RFA at all institutions and for all operators.

More importantly, before commencing RFA, the tumors should be evaluated by US, contrast-enhanced CT or MRI to determine tumor size, shape, number, presence or absence of extracapsular invasion, presence or absence of satellite lesions, location relative to Glisson's capsule or other critical structures, and to determine the optimal route to approach the tumor.

Within 1–3 days after RFA, contrast-enhanced CT or MRI is essential to objectively assess the treatment response. If the tumor is completely ablated with a sufficient safety margin, the treatment may be considered complete. However, if there is any residual cancer tissue or an insufficient safety margin, RFA should be repeated until complete tumor destruction with a sufficient ablative margin is achieved. The following recommendation was supported by 94% of the experts.

Recommendation 8. Imaging should be performed within 1–3 days after RFA to evaluate treatment response. It is essential that RFA is repeated until entire tumor destruction with a sufficient ablative margin is achieved.

For accurate tumor evaluation, CT and MRI performed before and after RFA should be done using a thin slice interval. The following recommendation was agreed by 94% of the experts.

Recommendation 9. CT and MRI before and after RFA should be done using a slice thickness and interval of 5 mm or less; slice thickness and interval of 10 mm or more is not adequate.

A histopathological study has revealed that, in cases with incomplete necrosis, viable cancer tissue remains around the main tumor, in portions isolated by the septa, or along the edge of the tumor after ablation therapies.[54] There may also be extranodular growth, satellite nodules or portal vein invasion, which cannot be detected by imaging modalities.[55,56] The incidence of satellite nodules and portal vein invasion is associated with the gross appearance of the main tumor. The single nodular type with extranodular growth and the confluent multinodular type both show satellite lesions more frequently than early HCC (vaguely nodular-type HCC showing preservation of the preexisting liver structure) and the single nodular type. Thus, it is important to determine the gross appearance of the tumor by imaging. It is also essential to ablate beyond the tumor border to achieve complete tumor necrosis and prevent local tumor progression (ablative margin or safety margin). Sonazoid-enhanced US in the Kupffer phase is useful to determine the gross tumor appearance.[57] The width of the safety margin should be modified based on the gross appearance of the tumor, the number of tumors, the initial tumor or recurrent tumor, the duration of time between the previous treatment and recurrence in recurrent cases, tumor location (particularly in relation to the Glisson's capsule), liver function, comorbid conditions and the patient's age.

Furthermore, the accuracy of contrast-enhanced CT or MRI for evaluating the extent of necrosis is limited because of the partial volume effect.[58] The following recommendation was agreed by 94% of the experts.

Recommendation 10. A safety margin completely surrounding the lesion should be achieved in cases in which RFA is performed as a locally curative treatment (level 6, grade A).

Ablation therapies, including RFA, are widely accepted as the preferred treatment for unresectable small HCC. On the other hand, it has been strongly debated whether ablation therapies can provide a treatment option for resectable HCC since the introduction of ethanol injection. Although the number of patients treated by RFA has steadily increased, the Clinical Practice Guidelines for Hepatocellular Carcinoma in Japan recommends surgery rather than ablation.[1] Their scientific statement recommends the following: "(i) if only one tumor is present, liver resection is recommended irrespective of the diameter of the tumor. Ablation therapy may also be selected if the severity of liver damage is class B and the diameter of the tumor is no more than 2 cm; (ii) if two to three tumors with diameters of no more than 3 cm are present, liver resection or local ablation therapy is recommended". This scientific statement is based on a cohort study of patients at clinical stage I (fair liver function), with a solitary tumor of less than 2 cm in diameter, patients across all clinical stages with a solitary tumor greater than 2 cm, and patients of clinical stage II (moderately impaired liver function) with two tumors greater than 2 cm. In that cohort, those who underwent hepatic resection showed higher survival rates than those who received non-surgical interventions.[59]

However, those findings were not based on randomized controlled trials (RCT) and the different survival rates may be subject to bias arising from the background characteristics of the patients. Of note, the hepatic resection group was younger than the ethanol injection group. Furthermore, even among patients at clinical stage I, most patients with normal liver or chronic hepatitis seemed to undergo resection while many with cirrhosis seemed to receive ethanol injection. This might reduce the recurrence rate because of multicentric carcinogenesis and less frequent development of liver failure in the resection group. Moreover, the trend that patients with severe comorbid conditions, such as cardiopulmonary diseases and others, received ethanol injection rather than resection might explain some of the disparity in survival. By contrast, in one RCT the recurrence and survival rates were comparable between surgical resection and ethanol injection.[60] In addition, other non-randomized trials have reported similar or better overall survival after ethanol injection than after resection.[61–63]

In addition, the findings described above only compared resection with ethanol injection. For example, our RCT showed that RFA had higher survival and lower recurrence rates than ethanol injection while the adverse events were similar between the two therapies.[64] Similarly, other RCT have shown that RFA is superior to ethanol injection in terms of treatment outcomes for HCC.[65–67] Another RCT has shown that there was no difference between resection and RFA in terms of overall and disease-free survival, while post-treatment complications occurred more frequently and were more severe after surgery.[68]

Hence, it is inappropriate to generalize the findings for ethanol injection to other percutaneous local ablation therapies such as RFA, and it should not be concluded that hepatectomy is recommended over percutaneous local ablation.

Further trials are needed to determine whether RFA can become a preferred treatment for "resectable HCC". In such trials, the primary end-point should be overall

survival.[69] The AASLD practice guideline clearly states the following: "although a treatment might be less active against the tumor than another treatment and thus result in a higher recurrence rate after initial treatment, the overall survival might not differ or may even be better".[3]

Recurrence-free survival can be misleading and should not be considered as a surrogate end-point for overall survival. In HCC, unlike other solid tumors, recurrence can still be treated, and the first recurrence does not cause death in most cases. Furthermore, surgery theoretically offers better disease-free survival than RFA because it removes larger liver tissue. However, the better curability associated with hepatectomy could be cancelled out by the surgical invasion and the potential deterioration in liver function. The following recommendation was agreed by 84% of the experts.

Recommendation 11. Overall survival should be the end-point to compare results between ablation and hepatectomy.

SURGICAL TREATMENT: RESECTION AND TRANSPLANTATION

A NATIONWIDE SURVEY by the Japanese Liver Transplantation Society found that a total of 4725 cases of living-donor liver transplantations (LDLT) were reported in Japan as of the end of 2007 since its initiation in 1989. By contrast, during the same period, only 46 cases of deceased-donor liver transplantation (DDLT) were documented. At the end of 2006, 778 patients with HCC had undergone an LDLT in Japan.[70] Because of the severe shortage of brain-dead donors and the extremely long waiting time for such organs, DDLT is not a realistic treatment option for HCC patients in Japan.

Algorithm for the treatment of patients with HCC in Japan

Figure 1 shows the treatment algorithm presented in the Japanese evidence-based guideline for the diagnosis and treatment of HCC.[1] Liver transplantation is recommended for HCC patients with liver damage C (similar to Child–Pugh C), but only when the patients meet the Milan criteria proposed by Mazzaferro.[71] In the revised version of the guidelines published at the end of 2009, an age limit of 65 years was added to the criteria for liver transplantation.

Can the indications for liver transplantation be expanded beyond the Milan criteria?

Until the mid-1990s, HCC was considered a contraindication for liver transplantation because of the extremely poor outcome of early series.[72,73] This pessimistic view was reversed by Mazzaferro *et al.* who conducted a prospective cohort study to identify subgroups of HCC patients who may benefit from DDLT. They presented clear eligibility criteria for transplantation, as follows: the presence of a solitary tumor of 5 cm or less in diameter and no more than three tumor nodules, each 3 cm or less in diameter, in patients with multiple tumors, and the absence of vascular invasion or extrahepatic disease. In their series, the overall and recurrence-free survival rates

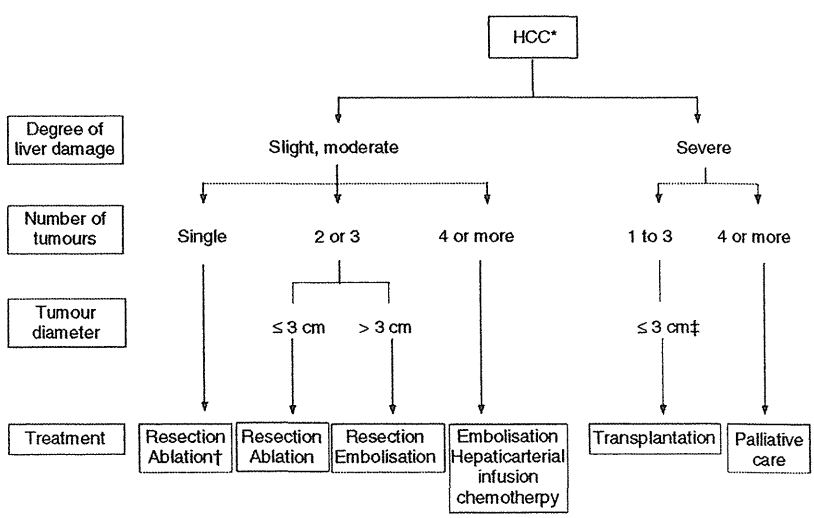

Figure 1 Japanese evidence-based treatment algorithm. HCC, hepatocellular carcinoma.

at 4 years for 35 patients who met the above criteria were as high as 85% and 92%, respectively. These criteria were named the "Milan criteria" and became the gold standard for patient selection for liver transplantation. The Milan criteria were also validated for LDLT using data from a nationwide survey in Japan.[74] Since 2004, LDLT for HCC has been covered by social medical insurance in Japan when the preoperative imaging studies indicate that the patient's condition meets the Milan criteria.

The Milan criteria have encouraged transplant surgeons to increase the number of liver transplantations performed in HCC patients, and the United Network for Organ Sharing (UNOS) has incorporated the Milan criteria as conditions for listing HCC patients. During the extensive application of liver transplantation for HCC, transplant surgeons have noticed that the outcomes of some patients who slightly exceeded the Milan criteria were also favorable. To expand the indications for liver transplantation, several groups from different countries have challenged these restrictive criteria (Table 1).[75–79] Yao *et al.* at the University of California at San Francisco (UCSF) proposed criteria consisting of a single tumor of less than 6.5 cm in diameter or two lesions of less than 4.5 cm in diameter, with a total tumor diameter of less than 8 cm; these criteria are known as the "UCSF criteria".[76] The utility of the UCSF criteria was subsequently confirmed by the University of California at Los Angeles.[80]

Regarding the indications for LDLT in HCC patients, several proposals from Asian centers have extended the eligibility criteria (Table 1). For example, a group at the University of Tokyo proposed the "5-5 rule", which allows up to five nodules with a maximum diameter of 5 cm.[77] The 3-year recurrence-free rate of 72 patients who met the Tokyo 5-5 rule was as high as 94%, which was comparable with that of patients within the Milan criteria. A group at the University of Kyoto subsequently proposed a further expansion of the criteria, increasing the upper limit of the number of tumors to 10.[79]

Because LDLT is not governed by an organ-sharing system, some authors have argued that the indications for LDLT in patients with HCC could be further extended. One might say that "If the patient (recipient) and his/her family (donor) strongly wish to undergo LDLT even in cases of very advanced HCC with full knowledge of potential for poor outcomes, there is no reason for transplant surgeons to reject their wish. The family members may accept the poor outcome after LDLT without doing any harm to the community." However, we should always remember that, while LDLT does not require a donor from the community, it does require extensive medical resources, including a large workload for surgeons and other hospital staff members, medical supplies, drugs and blood products. Furthermore, the premature death of the recipient is well known to cause severe emotional trauma to the living donors and their family members.

Based on an answer-pad vote at the consensus meeting of 45th JSH congress, 84% of the experts supported keeping the Milan criteria for DDLT, but only 25% supported keeping these criteria for LDLT. Although any expansion of the criteria should be modest, no consensus exists as to the extent to which the criteria can be extended.

Recommendation 12. For DDLT, the HCC status of the recipients should meet the Milan criteria.

Recommendation 13. For LDLT, the HCC status of the recipients does not need to be within the Milan criteria.

Which is better, liver resection or transplantation, for HCC patients who are eligible for either treatment?

Because liver transplantation replaces the whole liver, removing the highly carcinogenic background and the cirrhotic liver can avoid multicentric or de novo cancer recurrence.[80] In contrast, liver resection is associated with a very high risk of tumor recurrence. Even after curative liver resection in patients with good liver function, the 5-year recurrence rate is as high as 70–79%.[80] Roughly half of these recurrences are multicentric or de novo recurrences. For this reason, liver transplantation

Table 1 Summary of proposed criteria for indication of liver transplantation for HCC

Criteria	Conditions	References
Milan criteria	Up to 5 cm for single nodule or up to 3 nodules with a maximum diameter of 3 cm	70
UCSF criteria	Up to 6.5 cm for single nodule or up to 3 nodules with a maximum diameter of 4.5 cm	76
Tokyo 5-5 rule	Up to 5 nodules with a maximum diameter of 5 cm	77
Asan criteria	Up to 6 nodules with a maximum diameter of 5 cm	78
Kyoto criteria	Up to 10 nodules with a maximum diameter of 5 cm and PIVKA-II <400 mAU/mL	79
Up-to-seven criteria	Up to seven as the sum of the size of the largest tumor [in cm] and the number of tumors	75

may be recommended for HCC patients with good liver function who are also eligible for liver resection, as in Western countries.

Another issue is the operative risk of the two treatments. In Japan, the operative mortality rates for LDLT and liver resection are estimated to be 4–10% and 0.8–1.2%, respectively. This striking difference in operative mortality rates might preclude LDLT for patients with good liver function.

Using two databases at the National Cancer Center Hospital in Japan and the University of Pittsburgh Medical Center in the USA, Yamamoto *et al.* compared the long-term outcome of liver resection and transplantation in cirrhotic patients with HCC.[81] The overall survival of Child–Pugh A patients who underwent liver resection was similar to that of the patients without vascular invasion or lymph node metastases who underwent transplantation (most cases with Child-Pugh C). The recurrence rate was significantly lower in the transplantation group. For cases in which either treatment can be performed, the outcome of liver transplantation might be better than that of hepatic resection, particularly in cases with only a few small lesions.[81,82] In cases with large lesions, superior outcomes are achieved with hepatectomy. Because some patients may withdraw from treatment during the pre-transplantation period,[83] the outcomes with resection are better than those for liver transplantation based on intention-to-treat analysis of patients who meet the criteria for resection.

The evidence-based guideline[1] recommends the following: considering the occurrence of dropouts during the pre-transplantation period, the outcome of resection is better than that of liver transplantation among patients who meet the criteria for resection (grade B).

According to a question and answer-analyzer vote at this consensus meeting, 83% of the HCC experts selected LDLT for Child–Pugh C patients meeting the Milan criteria, whereas only 15–19% of the audience selected LDLT for Child–Pugh A or B patients.

Recommendation 14. LDLT should not be recommended for HCC patients with Child–Pugh A or B liver function.

PALLIATIVE TREATMENTS: TRANSARTERIAL CHEMOEMBOLIZATION AND CHEMOTHERAPY

PALLIATIVE TREATMENTS FOR HCC include transarterial chemoembolization (TACE), hepatic arterial infusion chemotherapy (HAIC) and systemic chemotherapy.

Transarterial embolization/TACE

Transcatheter arterial embolization (TAE)/TACE is one of the treatment options to treat hypervascular HCC. The theoretical basis of embolization is to induce ischemic tumor necrosis by acute arterial occlusion in hypervascular classical HCC. Embolization may be done alone (TAE) or in combination (TACE) with antineoplastic agents such as doxorubicin, epirubicin or cisplatin and a contrast agent, lipiodol. TACE is more effective and, thus, more widely used than embolization alone.

The technique for TACE is well established. The subsegmental artery or a peripheral artery near the target tumor is selected by a micro-catheter technique, followed by selective injection of antineoplastic agents mixed with lipiodol (lipiodol emulsion). The artery is then selectively obstructed with gelatin sponge particles. For bi-lobular multiple HCC with moderately impaired hepatic function (Child–Pugh B), TACE might need to be performed twice with an interval of several weeks to avoid hepatic decompensation.

The survival benefit of TAE/TACE was controversial until the publication of two RCT in 2002, which showed that TACE improved the survival of selected patients (Child–Pugh A with no vascular invasion) compared with conservative treatment.[84,85] A subsequent meta-analysis of seven RCT comparing TAE/TACE as a primary treatment for HCC in comparison with conservative management and/or suboptimal therapies showed a significant improvement in the 2-year survival, favoring TAE/TACE (odds ratio [OR] = 0.53; 95% confidence interval [CI] = 0.32–0.89, $P = 0.017$).[86,87]

According to the Nationwide Follow-up Survey of Primary Liver Cancer in Japan, one-third of all patients with primary HCC were treated by TAE/TACE (Fig. 2). Thus, TAE/TACE, hepatic resection and local ablation therapy are commonly used in Japan. TAE/TACE is the most widely used treatment for unresectable HCC.

In two Japanese treatment guidelines for HCC, evidence-based[1,30,88] and consensus-based guidelines,[89] TACE is recommended for patients with the severity of the liver damage categorized into A or B, in whom there are two or three tumors with a diameter greater than 3 cm, or four or more tumors.

In early stages of HCC, TACE is not indicated as first-line treatment because the outcome review of the Nationwide Follow-up Survey by the LCSGJ reported worse results for TACE than surgery or percutaneous ablation. This survey revealed that the 5-year survival rates for resection, ablation and TACE were 59.2%,

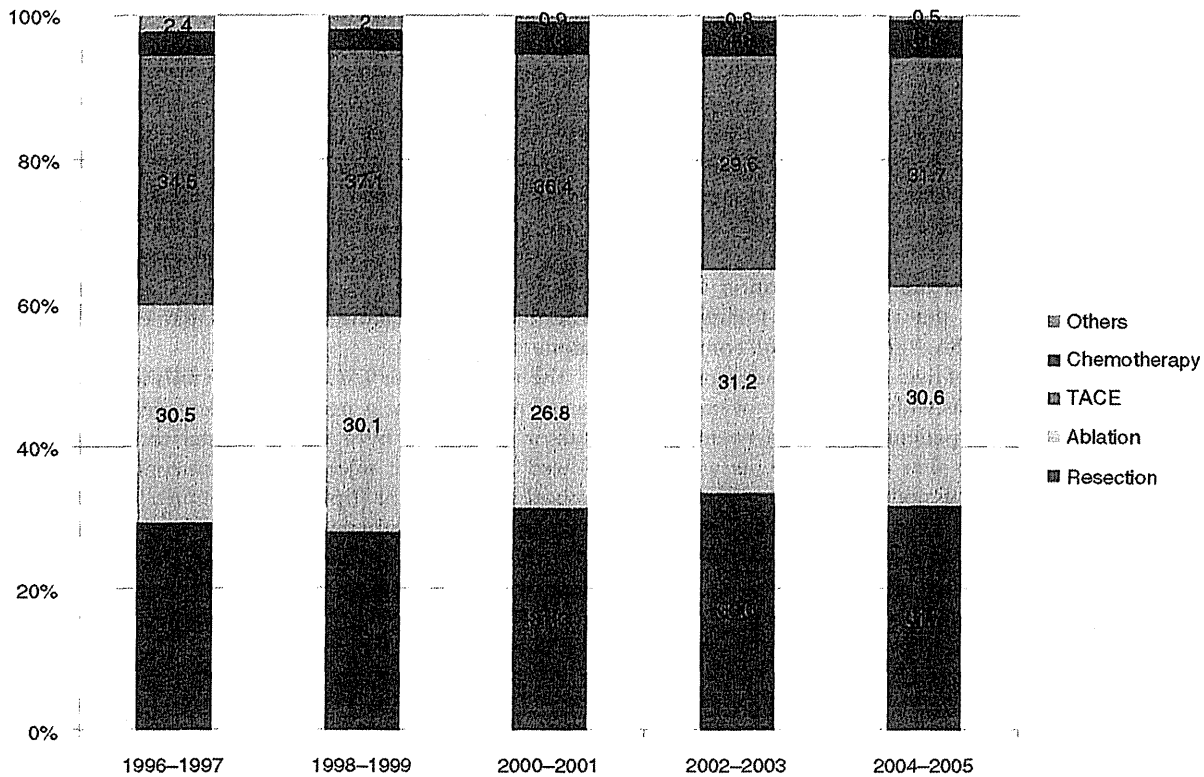

Figure 2 Change of treatment method for hepatocellular carcinoma in Japan. TACE, transcatheter arterial chemoembolization.

48.4% and 29.7%, respectively, for single tumors, and 46.4%, 37.3% and 23.0%, respectively, for two tumors.[90]

In contrast, in a large prospective cohort study of 8510 patients who received TACE for unresectable HCC, according to the LCSGJ, the median survival was 34 months with 1-, 2-, 3-, 5- and 7-year survival rates of 82%, 63%, 47%, 26% and 16%, respectively.[91] In patients with early stage HCC, single tumors of 2 cm or more and preserved liver function (clinical stage I and liver damage A according to the LCSGJ),[92] the median survival was 62 months with 1-, 2-, 3-, 5- and 7-year survival rates of 98%, 92%, 73%, 52% and 38%, respectively.[91] These results for TACE with early stage HCC seem comparable with those for surgery or ablation. Thus, although curative therapies are highly recommended for patients with early stage HCC, TACE can be applied in these patients contraindicated for curative therapies.

Transcatheter arterial chemoembolization can be used in combination with percutaneous ablation, including RFA. A meta-analysis of four RCT comparing combination therapy (TACE plus percutaneous ethanol injection [PE]) or RFA) versus monotherapy (TACE alone, PEI or RFA alone) showed a significant decrease in mortality favoring combination therapy versus monotherapy in patients with small (<3 cm) or large (>3 cm) HCC (OR = 0.534; 95% CI = 0.288–0.990; $P = 0.046$).[93]

In RFA treatment, as the tumor size increases, the therapeutic response decreases because of the limited volume of coagulation necrosis induced by the electrode. Blood flow also promotes heat loss to result in insufficient necrosis; therefore, reducing blood flow during RFA increases the ablation volume. Therefore, it seems to be reasonable to perform RFA after reducing blood flow by preceding RFA with TACE. Several cohort studies have shown that performing TACE before RFA is feasible and safe, and offers a useful treatment in compensated cirrhosis (Child–Pugh A or B) with relatively small HCC nodules (20–50 mm).[94–97] RFA in combination with preceding TACE is already recommended in the consensus-based treatment algorithm proposed by the JSH[89].

In the current consensus meeting, for hypervascular HCC of 2 cm in size, 51% of the experts used TACE

before RFA treatment. By contrast, for hypervascular HCC of 3 cm in size, 81% of the experts performed TACE before RFA. This is theoretically reasonable because the possibility of incomplete ablation is greater for tumors of 2–3 cm in size, compared with tumors of less than 2 cm in size, based on the limited volume possible with a single ablation procedure. Additionally, the accumulation of lipiodol in the tumor should facilitate the decision on whether additional RFA treatment is required following the response evaluation by dynamic CT scan. However, the survival benefit of TACE in combination with RFA should be verified by well-designed RCT.

Transcatheter arterial chemoembolization is performed in various stages in the clinical management of HCC, not only for the initially detected HCC, but also for recurrent HCC. TACE has been shown to be valuable for improving the overall survival of HCC patients, although it is difficult to assess its clinical efficacy as second- or third-line therapy.

Informative Statement 3. TACE performed before RFA is favorable for the curative treatment of hypervascular HCC of 2–3 cm in size.

Recommendation 15. TACE performed before RFA is recommended for curative treatment of hypervascular HCC larger than 3 cm in size.

Chemotherapy

Chemotherapy for HCC is divided into two types according to the route of administration; the first is systemic chemotherapy and the second is hepatic arterial infusion chemotherapy (HAIC). Systemic chemotherapy can also divided into two types: intravenous and oral chemotherapy.

According to the Nationwide Follow-up Survey of Primary Liver Cancer by the LCSGJ, chemotherapy is used in 3.4–5.5% of primary HCC patients (Fig. 2). HAIC is theoretically more favorable for HCC than systemic chemotherapy because hepatic arterial infusion of anticancer drugs enables the delivery of high doses of drugs directly to the hypervascular HCC. In addition, HAIC provides a lower systemic level of the drugs than systemic administration, because the first-pass effect in the liver, and thus reduces toxicity and side-effects. Because of these advantages, HAIC is frequently used in Japan for intrahepatic advanced HCC with portal vein tumor thrombosis and/or intrahepatic multiple HCC. A recent report from the Japanese Nationwide Survey revealed that almost 90% of the chemotherapeutic regimens for HCC are done by hepatic arterial infusion. Thus, HAIC has become widely used in Japan, despite there being no solid evidence for a survival benefit of HAIC compared with systemic chemotherapy or best supportive care (Fig. 3).

Recommendation 16. HAIC is recommended for advanced HCC with major portal vein tumor thrombi with preserved liver function.

Various anticancer drugs and treatment regimens are used for HAIC in Japan. Two regimens in particular are widely used for HAIC. The first is interferon (IFN) in combination with 5-fluorouracil (5-FU); the second is low-dose cisplatin (CDDP) in combination with 5-FU. For IFN plus 5-FU, the response rate was reported to be 52.6%, with 16.4% achieving complete response (CR) and 36.2% achieving partial response (PR) among 116 patients with tumor thrombosis of the major portal vein or first branches of the portal vein. The survival rates at 6, 12 and 24 months were 53%, 34% and 18%, respectively, with a median survival of 6.9 months, compared with survival rates of 40%, 15% and 5%, respectively, in the historical control group.[98] The survival was significantly different between the two groups ($P < 0.01$). For low-dose CDDP plus 5-FU, the response rate was 48%, including 8% with CR and 40% with PR among 48 patients with portal vein tumor thrombosis. The 1-, 2-, 3- and 5-year cumulative survival rates were 45%, 31%, 25% and 11%, respectively, with a median survival of 10.2 months.[99]

In a review of previously reported small-size phase II studies of HAIC for advanced HCC,[10,17,98–108] the response rate varied from 14% to 71%. The mean survival duration also varied from 2.6 months to 32.4 months. However, few reports have compared systemic chemotherapy or HAIC using cytotoxic agents with placebo or best supportive care (Table 2).

The results of a randomized placebo-controlled double-blind phase III study with the multikinase inhibitor sorafenib were recently reported, representing a breakthrough in the chemotherapy for advanced HCC. Sorafenib is an oral drug that inhibits the platelet-derived growth factor (PDGF)-R, vascular endothelial growth factor (VEGF)-R, c-Kit-R and raf signaling pathways in tumor cells and in surrounding endothelial cells. In that study, 602 patients with advanced HCC, who were not indicated for other loco-regional treatments such as hepatic resection, who had not received prior systemic treatment and who had good liver functional reserve (Child–Pugh A) were randomized to sorafenib (400 mg b.i.d.) or placebo. Sorafenib was well tolerated and yielded a statistically significant improvement (44%) in overall survival. The median survival increased from 7.9 to 10.7 months (hazard ratio, 0.69;

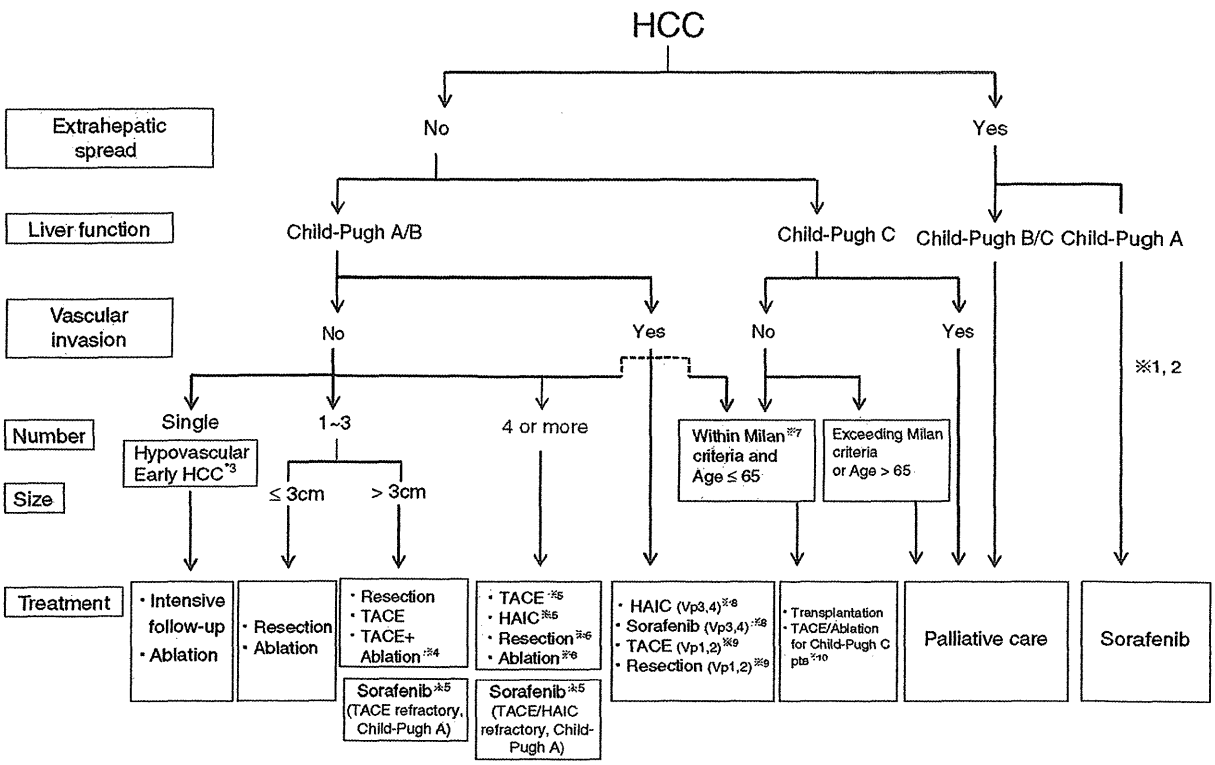

Figure 3 Consensus-based treatment algorithm for hepatocellular carcinoma proposed by the Japan Society of Hepatology (JSH) revised in 2010. (1) Treatment should be performed as if extrahepatic spread is negative, when extrahepatic spread is not regarded as a prognostic factor. (2) Sorafenib is the first choice of treatment in this setting as a standard of care. (3) Intensive follow-up observation is recommended for hypovascular nodules by the Japanese Evidence-Based Clinical Practice Guidelines. However, local ablation therapy is frequently performed in the following cases: (i) when the nodule is diagnosed pathologically as early hepatocellular carcinoma (HCC); (ii) when the nodules show decreased uptake on gadolinium ethoxybenzyl magnetic resonance imaging (Gd-EOB-MRI); or (iii) when the nodules show decreased portal flow by computed tomography during arterial portography (CTAP), because these nodules are known to frequently progress to the typical advanced HCC. (4) Even for HCC nodules exceeding 3 cm in diameter, combination therapy of transcatheter arterial chemoembolization (TACE) and ablation is frequently performed when resection is not indicated. (5) TACE is the first choice of treatment in this setting. Hepatic arterial infusion chemotherapy (HAIC) using an implanted port is also recommended for TACE refractory patients. The regimen for this treatment is usually low-dose FP (5-fluorouracil [5-FU] + cisplatin [CDDP]) or intra-arterial 5-FU in fusion combined with systemic interferon therapy. Sorafenib is also a treatment of choice for TACE/HAIC refractory patients with Child–Pugh A liver function. (6) Resection is sometimes performed even when numbers of nodules are over 4. Furthermore, ablation is sometimes performed in combination with TACE. (7) Milan criteria: tumor size ≤ 3 cm and tumor numbers ≤ 3; or solitary tumor ≤ 5 cm. Even when liver function is good (Child–Pugh A/B), transplantation is sometimes considered for relatively younger patients with frequently or early recurring HCC after curative treatments. (8) HAIC or sorafenib is recommended for HCC patients with Vp3 (portal invasion at the 1st portal branch) or Vp4 (portal invasion at the main portal branch). Sorafenib is only recommended for HCC patients with Child–Pugh A liver function. (9) Resection and TACE is frequently performed when portal invasion is minimal such as Vp1 (portal invasion at the 3rd or more peripheral portal branch) or Vp2 (portal invasion at the 2nd portal branch). (10) Local ablation therapy or subsegmental TACE is performed even for Child–Pugh C patients when transplantation is not indicated when there is no hepatic encephalopathy, no uncontrollable ascites and a low bilirubin level (<3.0 mg/dL). However, it is regarded as an experimental treatment since there is no evidence of its survival benefit in Child–Pugh C patients. A prospective study is necessary to clarify this issue. Even in Child–Pugh A/B patients, transplantation is sometimes performed for relatively younger patients with frequently or early recurring HCC after curative treatments.

Table 2 Response rates and survival periods in studies of intrahepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma

	Drugs	No. of Patients	Response rate (CR + PR, %)	Median survival time (months)	References
Single	Doxorubicin (IHAC)	72	60	7.0	Tzoracoleftherakis *et al.*[102]
	Doxorubicin (systemic)		44.1	6.5	
	CDDP	67	37	10.7	Court *et al.*[103]
Multiple	CDDP, 5-FU (low FP)	52	71	ND	Okuda *et al.*[104]
	CDDP, 5-FU (low FP)	48	48	10.2	Ando *et al.*[99]
	CDDP, 5-FU (low FP)	37	56.3	32.4	Sumie *et al.*[101]
	CDDP, 5-FU (low FP)	38	47	6.2	Tanioka *et al.*[105]
	CDDP, 5-FU	41	22	12.0	Park *et al.*[106]
	CDDP, Mitomycin C, 5-FU, LV	53	28.3	13.2	Lin *et al.*[100]
	IFN, CDDP	68	33	4.4	Chung *et al.*[107]
	CDDP		14	2.6	
	BSC			1.2	
	IFN, CDDP, 5-FU, MTX, LV	34	45	ND	Kaneko *et al.*[108]
	IFN, 5-FU	116	52	6.9	Obi *et al.*[98]

IHAC, intrahepatic arterial chemotherapy; CDDP, cisplatin; 5-FU, 5-fluorouracil; low FP, 5-fluorouracil + cisplatin. BSC, best support care; IFN, interferon; LV, leucovorin; MTX, methotrexate.

95% CI = 0.55–0.87). Side-effects included hand–foot skin reaction, diarrhea and fatigue, but sorafenib was not found to be toxic to the liver.[109] Similar findings were reported in a subsequent Asia–Pacific RCT.[110]

Based on the results of these RCT, sorafenib has become the first-line therapy for advanced HCC worldwide. Some Japanese experts for HCC are claiming low response rates, although the survival was significantly prolonged compared with placebo. This phenomenon could be explained by a longer period with stable disease with sorafenib than with placebo, or the necrotic change in the tumor is present without size reduction.

In Japan, sorafenib was approved for the treatment of HCC on 20 May 2009. In the consensus meeting held in June, 35% of the Japanese experts agreed that sorafenib should be selected as the first-line therapy for advanced HCC considered unsuitable for resection, RFA or TACE. A further 36% of the experts were undecided because they did not have enough experience with using sorafenib.

Informative Statement 4. Sorafenib is the first-line therapy for advanced HCC with major vascular invasion and/or extrahepatic spread and good liver function. However, further studies are needed to compare the overall efficacy of HAIC and sorafenib.

TREATMENT ALGORITHM

TO TREAT HCC, the most appropriate therapeutic option needs to be selected among the available treatment modalities, including resection, percutaneous ablation, TACE and transplantation, but few evidence-based guidelines have been developed to aid decision-making.[1,28,29,88,89,111] Recently, two treatment algorithms for HCC have been proposed in the Japanese guidelines. The profile of these algorithms is briefly described here, in addition to the results of two questions and answers at the JSH Consensus Meeting for HCC at Kobe.

Evidence-based treatment algorithm

The Clinical Practice Guidelines for HCC was established in 2005 based on evidence-based methodology, and covers six topics including prevention, diagnosis, surgery, chemotherapy, TACE and percutaneous ablation. To develop these guidelines, a systematic review of the English medical published work was performed and a total of 7118 articles on HCC were identified, mainly from MEDLINE (1966–2002), of which 334 were selected based on the evidence level to form 58 pairs of clinical questions and recommendations.[1,88] For convenience in clinical use, two algorithms were created for the surveillance and treatment of HCC. A full English version was uploaded to the website of the JSH (www.jsh.or.jp/) in 2006.

The treatment algorithm for HCC was made on the basis of three independent factors: degree of liver damage, tumor number and tumor size. For the resulting six patients' subgroups, the first- and second-line therapies were recommended as objectively as possible (Fig. 1). The degree of liver damage is a modified system based on the Child–Pugh classification: "encephalopathy" was replaced by $ICGR_{15}$, to provide an accurate evaluation of liver functional reserve, particularly in surgical candidates.

Patients with mild (class A) or moderate (class B) liver damage are subject to the following recommendations: (i) in patients with a single tumor, liver resection is recommended, irrespective of the tumor size (percutaneous ablation may be performed if liver damage is of class B and the tumor is no more than 2 cm in size); (ii) for patients with two or three tumors smaller than 3 cm, resection or ablation are recommended; (iii) for patients with two or three tumors larger than 3 cm, resection or TACE are recommended; and (iv) for patients with more than four tumors, TACE or HAIC is recommended. The recommendations for patients with severe (class C) liver damage are as follows: (v) in patients with tumor(s) meeting the Milan criteria, liver transplantation is recommended; and (vi) for patients with more than four tumors, palliative treatment is recommended. For patients with extrahepatic metastasis, chemotherapy may be performed.

The rationale for selecting resection or ablation in patients with class A or B liver damage is based on the outcome of the largest multicenter study involving 12 888 patients in Japan.[59] The recommendation for TACE is based on the findings of two RCT showing a significant improvement in the survival of patients with multiple tumors and class A or B liver damage.[84,85] The indication for liver transplantation is derived from a prospective cohort study using the Milan criteria,[71] and a nationwide survey of Japan justifying the criteria in living donor transplantation.[74]

Consensus-based treatment algorithm

An expert panel of the JSH established a consensus-based treatment algorithm based on the therapeutic policies that are widely used in Japan.[89,111] This algorithm categories the patients on five clinical variables (extrahepatic spread, liver function, vascular invasion, tumor number and tumor size), and it divides the treatment options into resection, ablation, TACE, HAIC, liver

transplantation and palliative treatment (Fig. 3).[89,111] Because of the recent introduction of sorafenib in Japan, this consensus-based treatment algorithm was further revised and approved by the experts at the consensus meeting.[111,112]

Essentially, the consensus-based algorithm follows the evidence-based algorithm, but the treatments widely used in Japan were included by consensus, even though the evidence may be weak. The major differences in the consensus-based algorithm include: (i) ablation is sometimes performed in patients with a single, hypovascular early HCC; (ii) sorafenib is recommended for use in Child–Pugh A patients with vascular invasion, TACE failure or extrahepatic spread of HCC;[109,112] and (iii) liver transplantation is recommended, even for Child–Pugh A/B patients, if the Milan criteria are met.

The consensus-based algorithm based on the consensus of a large number of specialists, and a treatment strategy for management of HCC in Japan is important, and should be revised based on prospective trials for aspects of the algorithm lacking sufficient evidence.[111,112]

Informative statement 5. RFA might be recommended as a first-line treatment option in patients with a single, hypervascular HCC of less than 2 cm in size and with preserved liver function (Child–Pugh A or Liver Damage Class A). However, there was a discrepancy between surgeons and non-surgeons for this statement. This statement is strongly supported by non-surgeons (68%), whereas 80% of the surgeons favor resection rather than RFA. Recommendation 17. Resection should be considered as the first-line treatment option for patients with a single, hypervascular HCC of 3 cm or more in size and with preserved liver function (Child–Pugh A or Liver Damage Class A).

The revised version of the consensus-based treatment algorithm for HCC proposed by the JSH (Fig. 3) should aid decision-making at every stage in clinical practice. By sharing the information contained within the treatment algorithm chart, the physicians can offer recommended treatment options to the patient who can then choose one based on their preference (Fig. 3).

CONCLUSIONS

THIS CONSENSUS STATEMENT is a conclusion of the consensus meeting of HCC, which was held at the 45th JSH meeting, Kobe, Japan on 4–5 June 2009 (Congress President: Professor Masatoshi Kudo). This manuscript and recommendations largely reflect the daily practice in the real world carried out throughout Japan. The biggest difference of Japan's HCC practice from Western countries are pathological assessment issue, prognostic staging system, surveillance and diagnostic strategy, treatment strategy including role of HAIC, and method of RFA procedure, and treatment algorithm shown in Figure 3.

We believe every reader of this manuscript will well understand the real Japanese HCC practice much better than the other already published arterial articles. It is needless to say that consensus statements like this article should be regularly revised every 3–4 years because solid evidence or new diagnostic and treatment tool/drug or concept will be published and then established in clinical practice every year.

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Original Article

Evaluation for clinical utility of GPC3, measured by a commercially available ELISA kit with Glypican-3 (GPC3) antibody, as a serological and histological marker for hepatocellular carcinoma

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Aims: We evaluated the clinical utility of glypican-3 (GPC3), which has been proposed as a potential novel tumor marker for hepatocellular carcinoma (HCC), as a serological and histological marker for HCC.

Methods: The serum GPC3 level was compared between 200 patients with HCC and 200 patients with chronic liver disease (CLD). In addition, the expression of GPC3 was examined with immunohistochemistry on 38 resected specimens from patients with HCC. A commercially available GPC3 antibody was used for these analyses.

Results: The median values of serum GPC3 in patients with HCC and with CLD were 924.8 pg/mL and 1161.6 pg/mL, respectively. We found no elevation of serum GPC3 level in patients with HCC in comparison with those with CLD; rather the level was higher in patients with CLD ($P < 0.0001$). In immunohistochemical analysis, 14 of 38 (36.9%) HCC tissues were positive for GPC3, whereas no corresponding noncancerous tissue was positive. The positivity for GPC3 tended to increase with pathologic decreased differentiation of HCC.

Conclusions: We did not find serum GPC3 level, measured by a commercially available ELISA kit with GPC3 antibody, to be useful in the diagnosis of HCC. However, we did observe increased GPC3 staining in HCC tissue with moderate or poor differentiation, suggesting that GPC3 is produced by HCC tumors. This lack of utility could have been due to the measuring procedure used in the present study. Further evaluation of GPC3 in HCC with other measuring procedures is needed.

Key words: ELISA, glypican-3, hepatocellular carcinoma, immunohistochemistry, tumor marker

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most prevalent malignancies worldwide. It is the sixth most common cancer, and the third most common cause of cancer-related death, in the world.[1] In Japan, HCC is the third most common cause of death from cancer in men, and the fifth most common in women.[2] The most important risk factor for the development of HCC is liver cirrhosis, regardless of etiology.[3] In addition, chronic infection with hepatitis viruses such as hepatitis B virus (HBV) and hepatitis C virus (HCV), as well as high alcohol intake, increase the risk of HCC.[4–7]

Alpha-fetoprotein (AFP),[8–11] Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3),[12–14] and des-gamma-carboxy prothrombin (DCP)[15–17] have been reported to be useful as serological tumor marker for HCC in cases of HCC surveillance and diagnosis, and in the evaluation of patient prognosis.[18] Nevertheless, all tumor markers have limitations and therefore the identification of additional tumor markers for HCC with high sensitivity and specificity is necessary.

Glypican-3 (GPC3) is a member of the glypican family of glycosyl-phosphatidylinositol-anchored cell-

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surface heparan sulfate proteoglycans.[19–21] It has been suggested that GPC3 might be a useful histological[22–24] and serological[25–27] marker for HCC. However, there has not been sufficient agreement on its clinical utility, and the relationship between the expression of GPC3 in tissue and GPC3 level in the serum of patients with HCC has not been fully characterized.

In the present study, we evaluate the clinical utility of GPC3 as a serological and histological marker for HCC, and compare histological results with serological ones. In addition, we compare the utility of GPC3 with other serological markers for HCC, such as AFP, AFP-L3, and DCP.

METHODS

Patients and controls

A TOTAL OF 434 consecutive patients with HCC visited the Department of Gastroenterology at Ogaki Municipal Hospital during the period from January 2000 to December 2004. Two hundred and three patients underwent hepatic resection or radiofrequency ablation (RFA) as treatment for HCC. Stored serum samples that had been obtained before the therapy were available for 200 of these 203 patients; these constituted the subjects of the present study. Written informed consent was obtained from all patients for the analyses of their serum or tissue samples.

Diagnosis of HCC was based on histologic examination of tumor tissue taken from resected specimens in 120 patients who underwent hepatectomy, 29 of the 80 patients (36.3%) treated by RFA were diagnosed with HCC based on specimens by fine-needle biopsy. The remaining 51 patients were diagnosed based on clinical criteria:[28,29] a pertinent clinical background (association with liver cirrhosis or viral hepatitis) and typical imaging findings. Typical imaging features of HCC include a mosaic pattern with a halo observed with B-mode ultrasonography; hypervascularity on angiographic images; and a high-density mass on arterial phase dynamic computed tomography (CT) images together with a low-density mass on portal phase dynamic CT images obtained with a helical or multidetector row CT scanner. When findings typical of HCC were not obtained by means of dynamic CT or angiography, CT during hepatic arteriography and CT during arterial portography or T1- and T2-weighted imaging associated with superparamagnetic iron oxide-enhanced magnetic resonance imaging (MRI) were performed.

Serum samples from 200 HCC patients were obtained at the diagnosis of HCC and before therapy. As controls, serum samples from patients with CLD but without HCC that had been obtained during the same period as the serum samples from HCC patients were selected. We selected samples from patients in whom the lack of HCC development had been confirmed by ultrasonography, CT or MRI at serum sampling and for 3 years after the date of sampling. This was to avoid the inclusion in the control group of patients with occult HCC that could not be detected by imaging modalities at the time of serum sampling. Among them, we made random selection and finally selected 200 samples as controls.

Measurement of GPC3, AFP, AFP-L3 and DCP

GPC3, AFP, AFP-L3, and DCP were measured from the same serum samples. GPC3 was measured using a commercially available ELISA kit (BioMosaics, Burlington VT) according to the manufacturer's instructions. Total AFP and percentage of AFP-L3 were measured by a liquid-phase binding assay with the Wako LiBASys Autoanalyzer (Wako Pure Chemical Industries, Osaka).[30,31] DCP level was determined by sensitive enzyme immunoassay (Eitest PIVKA-II kit; Eisai Laboratory, Tokyo) according to the manufacturer's instructions.[32]

Immunohistochemical staining

Immunohistochemical staining for GPC3 was performed on 38 resected HCC tissue specimens using a commercially available kit (BioMosaics) according to the manufacturer's instructions. Briefly, 4-μm sections from formalin-fixed, paraffin-embedded tissue blocks were deparaffinized, rehydrated and treated with 3% hydrogen peroxide for 15 min to inhibit endogenous peroxidase. Following water bath-based heat-induced epitope retrieval in 0.1 M citrate buffer at 95°C centidegree and pH 6.0 for 40 min, slides were incubated with blocking solution for 20 min at room temperature. After blocking, slides were incubated with a mouse monoclonal antibody specific for GPC3 (1:200 dilution, clone 1G12; BioMosaics) for 6 hours at room temperature. After washing, detection was performed with biotin-free horseradish peroxidase-labeled polymers using the ChemMate EnVision System (Dako Real EnVision: Dako, Carpinteria CA). Staining was visualized using 3,3'-diaminobenzidine substrate-chromogen solution and a hematoxylin counterstain.

The intensity of staining was graded according to the percentage of the stained area and the intensity of staining as: 0, no staining or partial staining of cytoplasm in <25% of cells; 1+, weak/barely perceptible cytoplasm stain in >25% of cells; 2+, moderate stain of the complete cytoplasm in >25% of cells; or 3+, strong stain of

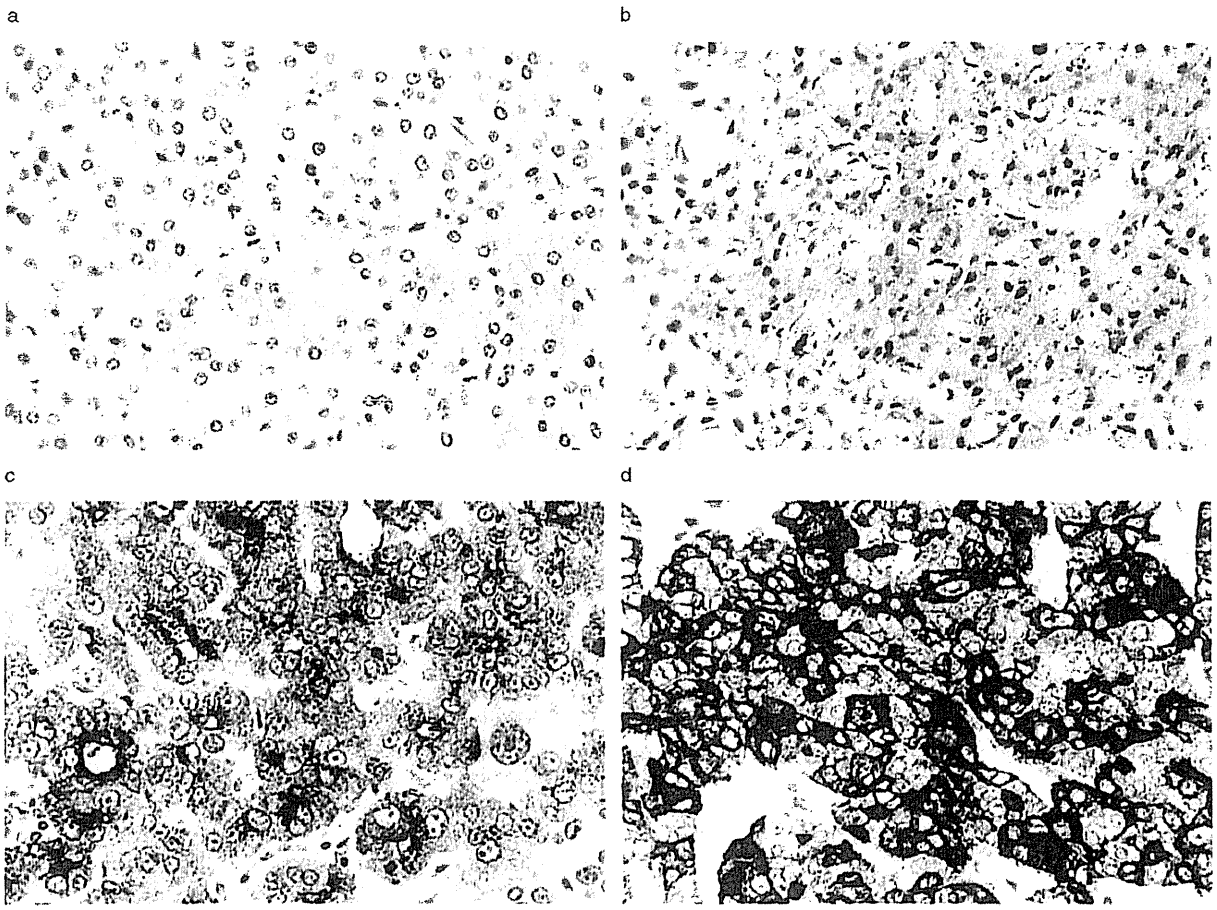

Figure 1 The degree of immunohistochemical staining for glypican-3. (a) No staining, (b) light staining, (c) moderate staining, (d) heavy staining.

the complete cytoplasm in >25% of cells (Fig. 1). HCC with 2+ or 3+ staining was considered to be positive for GPC3. Microscopic findings were evaluated by two authors independently, in comparison with negative and positive controls from the same immunohistochemistry series. Final evaluations of ambiguous cases (fewer than 20% of the samples) were made on a conference microscope with other authors.

Statistical analysis

Data are expressed as the mean ± SD or median and range. Differences in the proportions of patients between groups were analyzed by chi-square test. Differences in quantitative values were analyzed by Mann–Whitney *U*-test and Kruskal–Wallis test. All *P*-values were derived from two-tailed tests, and $P < 0.05$ was accepted as statistically significant. All analyses were performed using JMP6 statistical software (SAS Institute Japan, Tokyo).

RESULTS

THE DEMOGRAPHIC CHARACTERISTICS of the patients included in the analysis are summarized in Table 1. Patient with HCC comprised 153 males (76.5%) and 47 females (23.5%), with a mean age of 67.2 ± 8.5 years. Control patient comprised 112 males (56.0%) and 88 females (44.0%), with a mean age of 61.5 ± 11.8 years. The percentage of patients without cirrhosis, which was clinically evaluated according to typical US findings (e.g. superficial nodularity, a coarse parenchymal echo pattern, and signs of portal