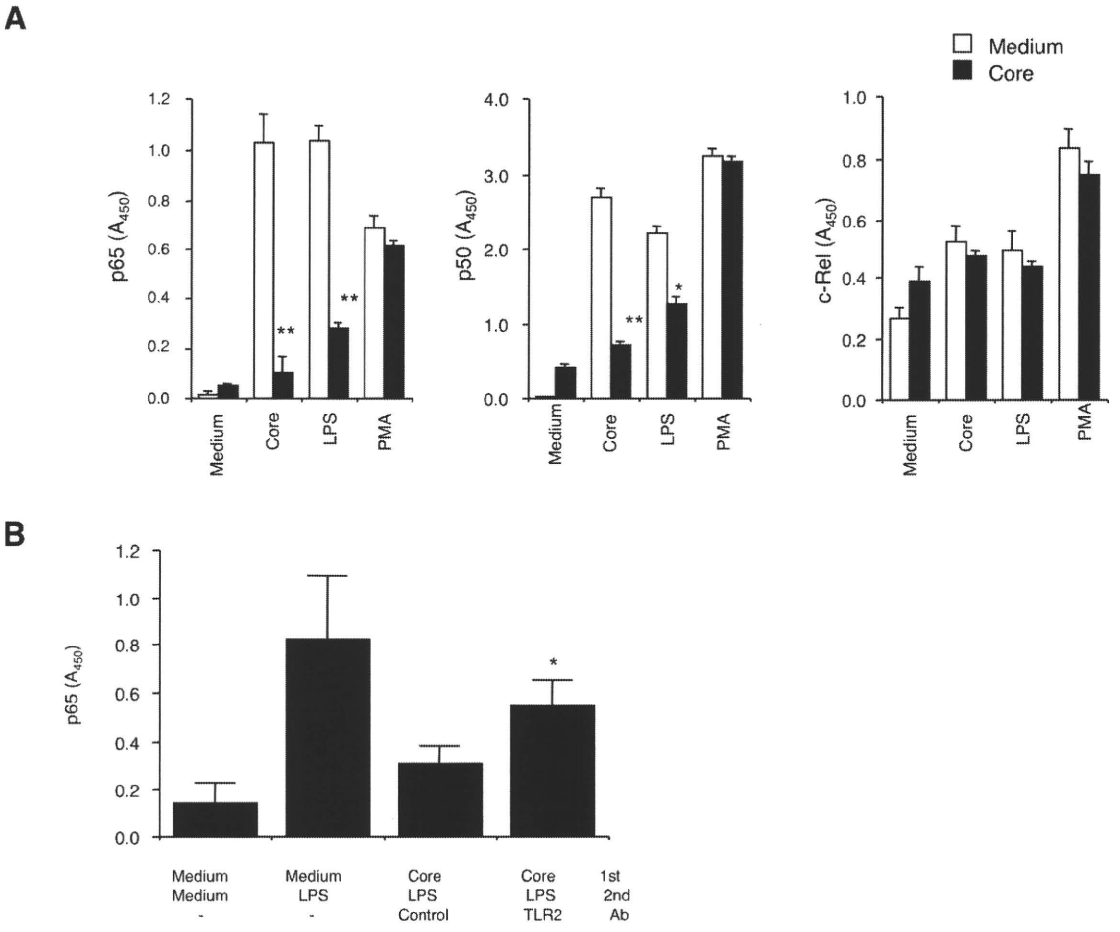

Figure 4. Activation of NF-κB in cells preincubated with hepatitis C virus (HCV) core protein and stimulated with core protein or lipopolysaccharide (LPS). *A*, MM6 cells (1×10^6 cells/mL) were incubated with HCV core protein (10 μg/mL) or culture medium alone for 24 h and then stimulated with HCV core protein (5 μg/mL), LPS (1 μg/mL), or phorbol myristate acetate (PMA) (50 ng/mL) for 1 h. Nuclear extracts were isolated, and the expression of NF-κB subunits was determined. *B*, Nuclear extracts were isolated from MM6 cells treated with an anti-TLR2 monoclonal antibody (mAb), or control antibody (Ab), followed by stimulation with HCV core protein and lipopolysaccharide. The expression of p65 was determined. Results shown are representative of 2 experiments (panel *B*) or 3 experiments (panel *A*) and are expressed as mean ± standard deviation. * $P < .05$, ** $P < .01$ compared with cells preincubated with medium alone (panel *A*). * $P < .05$, compared with cells treated with control Ab (panel *B*).

M at the protein level in core protein-stimulated MM6 cells. Transfection of IRAK-M siRNA led to a significant increase in IL-6 production in core protein–prestimulated MM6 cells after subsequent stimulation with either core protein or lipopolysaccharide, and these effects were associated with restored nuclear translocation of p65 (Figure 5*C* and 5*D*). These studies clearly show that induction of IRAK-M expression is involved in the inhibitory effects mediated by core protein prestimulation.

Production of IL-6 by monocytes from HCV-infected patients. Given the fact that circulating peripheral blood APCs in patients with HCV infection are exposed to core protein in the blood, it is interesting to examine whether core protein modulates the responsiveness of APCs by the mechanisms outlined above. To address this, we stimulated peripheral blood monocytes isolated from patients with HCV infection with core protein and TLR ligands and measured the production of IL-6 and IL-8. The production of IL-6 and IL-8 by monocytes isolated from HCV-infected patients was significantly reduced compared with that of healthy control subjects when cells were stimulated with core protein, peptidoglycan, Pam_3CSK4, or lipopolysaccharide (Figure 6). In contrast, no difference was seen in IL-12p40 production between the 2 populations. Thus, the continuous activation of TLR2 by core protein results in reduced cytokine responses to TLR ligands in monocytes from HCV-infected patients.

Impaired production of IL-17 by $CD4^+$ T cells cocultured with monocytes from HCV-infected patients in the presence of TLR ligands. Because APC-derived IL-6 is essential for Th17 differentiation [27], it is possible that chronic activation by core protein induces development of APCs with limited

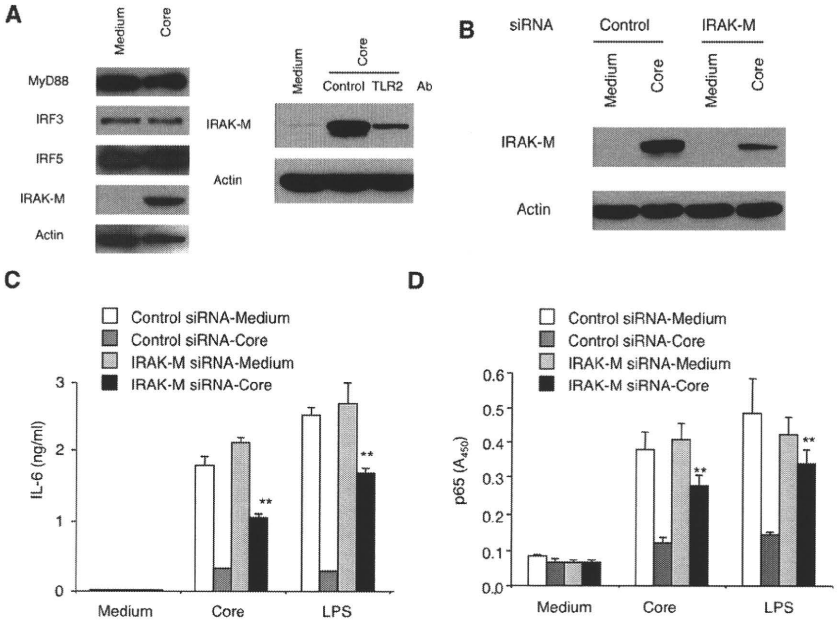

Figure 5. Core protein stimulation is associated with IRAK-M expression. *A*, Whole extracts were prepared from MM6 cells (1×10^6 cells/mL) stimulated with hepatitis C virus (HCV) core protein (10 μg/mL) or culture medium alone for 24 h. Whole extracts were immunoblotted with antibodies against the indicated proteins. The expression of IRAK-M after treatment with anti-TLR2 monoclonal antibody (mAb) (50 μg/mL) or control antibody (Ab) (50 μg/mL) is shown in the right panel. *B*, IRAK-M expression in MM6 cells transfected with IRAK-M small interfering RNA (siRNA). MM6 cells (5×10^5 cells/mL) were transfected with IRAK-M siRNA or control siRNA (25 nmol/L), followed by stimulation with HCV core protein (10 μg/mL) for 48 h. Whole extracts were subjected to immunoblot analysis. *C*, MM6 cells (5×10^5 cells/mL) transfected with IRAK-M siRNA or control siRNA (25 nmol/L) were incubated with HCV core protein (10 μg/mL) for 24 h and then stimulated with HCV core protein (5 μg/mL) or lipopolysaccharide (LPS) (1 μg/mL) for another 24 h. Culture supernatants were analyzed for interleukin 6 (IL-6) production. *D*, Nuclear extracts were isolated from MM6 cells transfected with siRNAs after stimulation with HCV core protein and LPS for 1 h. The expression of p65 was determined. Results shown are representative of 2 experiments (panel *D*) or 3 experiments (panels *A*, *B*, and *C*) and are expressed as mean ± standard deviation. $^{**}P < .01$, compared with cells preincubated with core protein and treated with control siRNA.

ability to drive Th17 differentiation in response to TLR ligands. To address this issue, we examined allospecific adaptive immune responses in naive $CD4^+$ T cells cocultured with monocytes isolated from HCV-infected patients or healthy control subjects in the presence of TLR ligands. Figure 7 shows that IL-17 production is markedly enhanced during antigen presentation by monocytes isolated from healthy control subjects in the presence of core protein, Pam_3CSK4, and lipopolysaccharide. Thus, the stimulation of TLR signaling in monocytes leads to increased production of IL-17 by $CD4^+$ T cells. In contrast, this enhancement of IL-17 production was absent in $CD4^+$ T cells stimulated by monocytes isolated from HCV-infected patients. Therefore, alloantigen presentation by monocytes from HCV-infected patients decreases IL-17 production by T cells in the presence of TLR ligands. Interestingly, IFN-γ production was similarly enhanced by alloantigen presentation by monocytes from both healthy control subjects and HCV-infected patients in the presence of TLR ligands. Furthermore, this reduction of IL-17 production was not due to counter-regulation of immunosuppressive cytokines, because the production of IL-10 or TGF-β was not increased by coculture with monocytes isolated from HCV-infected patients (Figure 7) (data not shown). Therefore, these data suggest that impaired production of IL-6 by monocytes isolated from HCV-infected patients is associated with a defective IL-17 response by $CD4^+$ T cells in the presence of TLR ligands.

DISCUSSION

This study demonstrates that activation of TLR2 by core protein induces not only homotolerance to subsequent TLR2 stimulation but also cross-tolerance to TLR4 stimulation. Consistent with this is the finding that monocytes isolated from HCV-infected patients show defective production of IL-6 after stimulation with TLR ligands, presumably due to chronic exposure to core protein. Impaired production of IL-6 by monocytes from HCV-infected patients is associated with reduced production of IL-17 by allogeneic T cells in the presence of TLR ligands. These results are supported by those of Villacres et al [28], who report a reduced IL-6 response to TLR ligands by PBMCs isolated from patients with HCV infection. They found that IL-6 production by PBMCs from patients with HCV in-

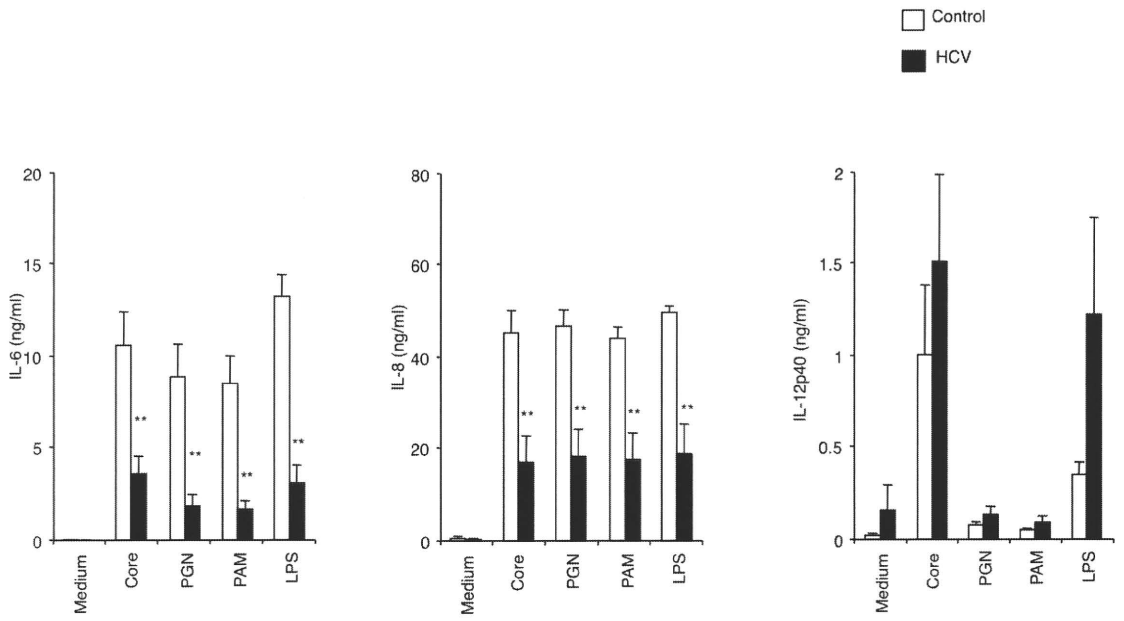

Figure 6. Production of interleukin 6 (IL-6) and interleukin 8 (IL-8) by monocytes isolated from patients infected with hepatitis C virus (HCV). Monocytes were isolated from 10 patients with HCV infection or 10 healthy control subjects. Monocytes (1×10^6/mL) were stimulated with HCV core protein (5 μg/mL), peptidoglycan (PGN) (10 μg/mL), Pam_3CSK4 (PAM) (10 μg/mL), or lipopolysaccharide (LPS) (1 μg/mL) for 24 h. Culture supernatants were analyzed for production of IL-6, IL-8, and interleukin 12p40 (IL-12p40). Results are expressed as mean ± standard deviation. $^{**}P < .01$, compared with monocytes from healthy control subjects.

fection was significantly decreased after stimulation with TLR4 ligands [28]. In addition, another report shows that DCs isolated from HCV-infected patients exhibit an impaired production of TNF-α in response to TLR4 ligands [29]. These results, taken together with our data, show impaired cytokine responses to TLR2 and TLR4 ligands in APCs isolated from HCV-infected patients.

We clearly show that antigen presentation by APCs isolated from HCV-infected patients affects T helper (Th) cell differentiation in the presence of TLR ligands. Chronic exposure to core protein results in the development of APCs with a limited ability to drive Th17 differentiation in the presence of TLR ligands. IL-17 (but not IFN-γ) production by allogeneic naive $CD4^+$ T cells was markedly reduced when T cells were cocultured with monocytes from HCV-infected patients and with TLR ligands. This selective impairment of the adaptive IL-17 response can be explained by profiles of cytokine production by these monocytes. IL-6 production induced by core protein and TLR ligands was significantly reduced in monocytes from HCV-infected patients compared with those from healthy control subjects, whereas IL-12p40 production was comparable in monocytes from both populations. Consistent with the results of the patient study (Figure 6), preincubation of APCs with core protein results in reduced production of IL-6 (but not IL-12p40) after restimulation with TLR ligands (Figure 2). Because IL-6 and IL-12 play an essential role for Th17 and Th1 differentiation, respectively [18, 27, 30], the defective IL-17 response seen in allogeneic $CD4^+$ T cells may be due to impaired IL-6 production by APCs from HCV-infected patients. Thus, chronic exposure to core protein appears to impair the adaptive IL-17 response (through the development of APCs with a limited ability to produce IL-6 after stimulation with TLR ligands) without affecting adaptive IFN-γ or TGF-β responses. However, it should be noted that we cannot exclude the involvement of TGF-β in reduced IL-17 production by $CD4^+$ T cells in the presence of TLR ligands and monocytes from HCV-infected patients. Rowan et al [31] show the indispensable role played by virus-induced TGF-β in the suppression of HCV-specific Th17 cells. This discrepancy regarding the role played by TGF-β may be explained by the differences in target antigens, responses to virus-specific antigens [31] or to allogeneic antigens, or by the difference in types of TGF-β tested—bioactive form [31], cell-surface, [31] or total.

Activation of NF-κB is impaired in APCs prestimulated with core protein after subsequent restimulation with TLR ligands. Nuclear translocation of p65 and p50 is reduced in cells prestimulated with core protein, whereas the translocation of c-Rel is not. Impaired nuclear translocation of p65 and p50 is responsible for a marked decrease in production of IL-6, because transcription of IL-6 is mediated by activation of the p65-p50 heterodimer [32]. In contrast, APCs that were prestimulated with core protein produced comparable levels of IL-12p40,

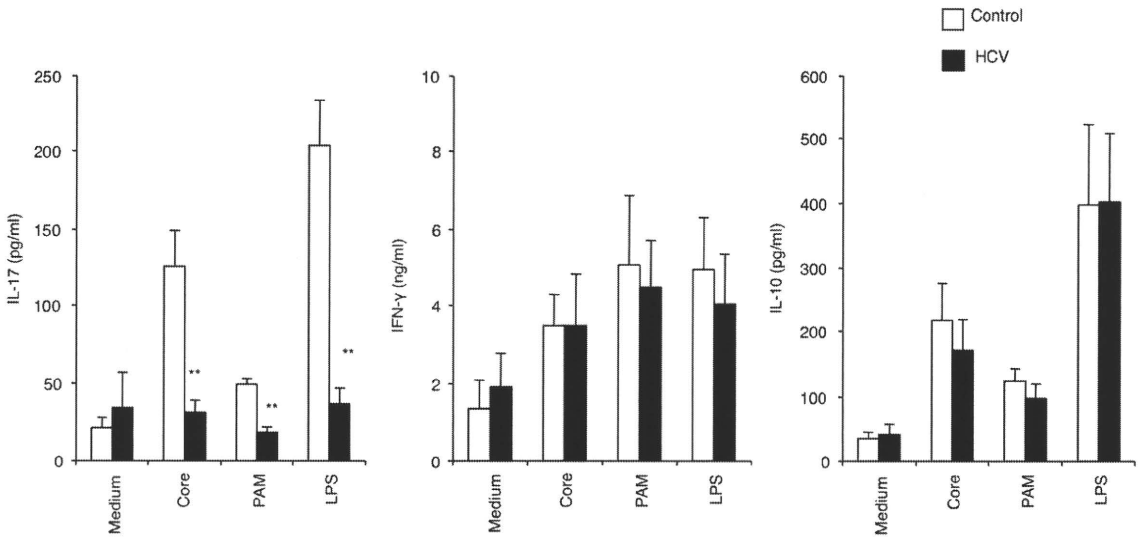

Figure 7. Production of interleukin 17 (IL-17) by allogeneic naive CD4+ T cells in the presence of Toll-like receptor (TLR) ligands and monocytes from patients infected with hepatitis C virus (HCV). Naive CD4+ T cells were isolated from the peripheral blood of healthy control subjects. Naive CD4+ T cells (1×10^6 cells/mL) were cocultured with peripheral blood monocytes (1×10^6 cells/mL) from 8 HCV-infected patients and 8 healthy control subjects in the presence of HCV core protein (5 μg/mL), Pam_3CSK4 (PAM) (10 μg/mL), or lipopolysaccharide (LPS) (1 μg/mL) for 7 days. Culture supernatants were analyzed for production of interleukin 17 (IL-17), interferon γ (IFN-γ), and interleukin 10 (IL-10). Results are expressed as means ± standard deviation. $^{**}P < .01$, compared with culture with monocytes from healthy control subjects.

the transcription of which depends on activation of the c-Rel subunit [21]. Therefore, preexposure of APCs to core protein results in reduced production of IL-6 because of the impaired nuclear translocation of p65 and p50 subunits. Impaired activation of NF-κB by prestimulation with core protein is associated with up-regulation of IRAK-M. Our results show that core protein–mediated activation of TLR2 leads to IRAK-M expression and that knockdown of IRAK-M expression by specific siRNA restores production of IL-6 by APCs prestimulated with core protein. Because IRAK-M is one of the most important negative regulators in TLR signaling [15], these data suggest that IRAK-M expression, induced by core protein–mediated TLR2 activation, modulates the cytokine responses mediated by multiple TLR ligands by the inhibition of NF-κB activation. However, it should be noted that transfection of IRAK-M siRNA did not completely restore the production of IL-6 by APCs prestimulated with core protein. Thus, other mechanisms of negative regulation of TLR signaling may also operate in the induction and maintenance of homotolerance and cross-tolerance by HCV core protein.

The impaired production of proinflammatory cytokines mediated by TLR2 and TLR4 might be involved in persistent infection by HCV. In fact, the activation of TLR2 and TLR4 plays a protective role in the case of respiratory syncytial virus and cytomegalovirus infection [33]. However, the reduction in IL-6 production by monocytes isolated from HCV-infected patients did not correlate with the HCV load in the serum (data not shown). This finding may be explained by the fact that the doses of core protein used in this study are much higher than those in the serum of HCV-infected patients. Indeed, serum levels of IL-6 are comparable between HCV-infected patients and healthy control subjects [34, 35]. Similarly, Shiina et al [36] report that infectious cell culture-produced HCV did not inhibit TLR4-mediated IL-6 production by DCs, which suggests that the dose of core protein in this system is not enough to cause cross-tolerance.

Given the fact that TLR2 and TLR4 play critical roles in host defense against microbial infection [12], and that Th17 cells are involved in host defense against bacterial infection [37], our results suggest that TLR2 activation by core protein may contribute to an increased susceptibility to microbial infection in individuals with chronic HCV infection. However, most patients with HCV infection are asymptomatic, although bacterial infections are more common among HCV-infected patients than among those without HCV infection [9–11]. Thus, impaired proinflammatory responses through TLRs might be compensated by other mechanisms in patients with HCV infection. In this regard, Foster et al [38] report that proinflammatory cytokine responses and antimicrobial effectors are differently regulated by TLR-induced chromatin modifications. Therefore, it is possible that antimicrobial effectors rather than proinflammatory cytokines play an important role in host defense against bacterial infection in HCV-infected patients.

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JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2010;40(Supplement 1)i19–i27
doi:10.1093/jjco/hyq123

Liver Cancer Working Group Report

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Hepatocellular carcinoma is a highly prevalent disease in many Asian countries, accounting for 75–80% of victims worldwide. The incidence of hepatocellular carcinoma varies enormously across Asia, but tends to follow the incidences of hepatitis B infection and liver cirrhosis. The incidence and etiology of hepatocellular carcinoma in Japan are different from the rest of Asia, but similar to that in Western countries because hepatitis C infection is the main etiological factor in Japan. Hepatitis B virus vaccination programs are showing great success in reducing hepatitis B virus-related hepatocellular carcinoma. Screening program improves detection of early hepatocellular carcinoma and has some positive impact on survival, but the majority of hepatocellular carcinoma patients in Asia still present with advanced hepatocellular carcinoma. Long-term outcomes following treatment of even early/intermediate or advanced disease are often unsatisfactory because of a lack of effective adjuvant and systemic therapies. Various clinical practice guidelines for hepatocellular carcinoma have been established and are in use. Clinical diagnosis of hepatocellular carcinoma by imaging diagnosis is replacing diagnosis of hepatocellular carcinoma by pathological confirmation. New imaging and treatment techniques are continuously being developed and guidelines should be updated every 3 or 4 years, incorporating new evidence. New molecularly targeted therapies hold great promise. Sorafenib is the first systemic therapy to demonstrate prolonged survival vs. the placebo in patients with advanced hepatocellular carcinoma. Various other new molecularly targeted agents are currently under investigation.

Key words: liver cancer – epidemiology – etiology – diagnosis – treatment

INTRODUCTION

The Liver Cancer Working Group report was divided into seven topics: (i) epidemiology and etiology in Asian countries; (ii) proportions of early, intermediate and advanced stages of hepatocellular carcinoma (HCC); (iii) surveillance systems and prediction of HCC development; (iv) recent developments in imaging diagnosis; (v) pathological development of early HCC, especially consensus between Asia and the West; (vi) current status of treatment strategies; (vii) future perspectives, especially in regard to sorafenib; and other molecularly targeted agents.

Epidemiology and Etiology

Liver cancer, or HCC, is endemic in Asia. It is expected that around 75–80% of HCC cases worldwide develop in Asia (Fig. 1) (1). In most Asian countries, HCC is ranked from number 1 to number 5 among the leading causes of death. In

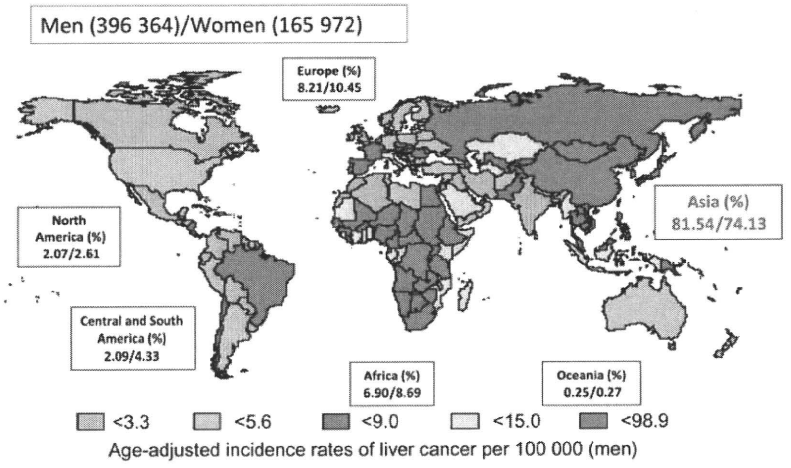

Figure 1. Liver cancer in the world (Curado et al. IARC Press, 2010).

Mainland China and Taiwan, the incidence of HCC has been increasing in the past 30 years, but in Japan, the incidence has been relatively stable during that period (2). In Korea, particularly in the male population, the incidence of HCC decreased slightly in the past 10 years. The primary etiological factor in Asia is hepatitis B. As exemplified by Korea, hepatitis B virus (HBV) accounts for 70–75% of HCC cases and hepatitis C virus (HCV) accounts for 10–15% (3). In Hong Kong, 80% of HCC cases are caused by HBV, and around 7% are caused by HCV. Japan is unique in the etiology of HCC in Asia because almost two-thirds of cases are caused by HCV and only 15% are related to HBV (2,4–6). Taiwan appears to be in between. In the early 1980s, HBV was the dominant cause of HCC in Taiwan, accounting for 88% (4), but in the past 30 years, HCV increased significantly and now accounts for more than 30%. HBV remains the predominant cause, but because of a vaccination program that was started in 1984, Taiwanese younger than 25 years old will have a carrier rate of around 1%. Thirty years from now, HBV-related HCC will decrease dramatically in Taiwan and in other countries that have adopted a nationwide HBV vaccination program (7). Regarding the age distribution of HCC, in all countries in which HBV is the dominant cause, the median age is around 55 years old. Statistics for Japan, which is characterized by HCV, show that the median age is about 10 years older.

In conclusion, HCC in the Asia-Pacific region accounts for 75–80% of victims worldwide. The incidence of HCC is on the rise in some countries, such as mainland China and Taiwan, but it is plateauing and decreasing slightly in some countries, like Japan. Except in Japan, HBV is the major etiology of HCC. The proportion of HCV has increased significantly in the past 30 years in Taiwan. Because of successful vaccination, the incidence of HBV-related HCC will decrease dramatically by 2040 (8).

Proportions of Early, Intermediate and Advanced HCC

There are various staging systems for HCC, with each system having its pros and cons and no consensus regarding which system is the best. The Barcelona Clinic of Liver Cancer, BCLC, system (9,10) is quite widely used in the West and in many clinical trials. The BCLC system stages patients into very early stage, early stage, intermediate stage, advanced stage and end stage according to the tumor size, vascular invasion, the tumor nodule number and the presence of metastasis. The BCLC system also provides a guideline for treatment according to the stage of HCC. Basically, patients with very early-stage or early-stage HCC are considered for curative treatment, either resection, liver transplantation or local ablation. Patients with intermediate-stage HCC, mainly those with multinodular disease, will be eligible for transarterial chemoembolization (TACE), and patients with advanced-stage disease showing portal invasion or distant metastasis will be considered for sorafenib or recruitment to clinical trials.

In addition to the BCLC, the Japanese TNM staging system (11) is quite widely used in Japan and Korea. This staging system takes into account three criteria for the T stage, i.e. whether the tumor is solitary or multiple, the tumor size, $\leq$2 cm or >2 cm, and the presence of any vascular or bile duct invasion. Patients are thus classified as T1, T2, T3 or T4. For N and M, it is similar to other TNM staging systems, based on the presence of lymph node or distant metastasis. By integrating Japanese TNM stage and Child–Pugh grade, Japan Integrated Staging system was developed (12) and widely used in Japan and Korea.

The current distribution of HCC based on the BCLC system is quite similar in Hong Kong and Korea, with about 30–40% of patients having early-stage disease, about 20–30% having intermediate-stage disease and about 30% having advanced-stage disease. In Japan, the proportion of early-stage HCC is very high: about 65%, whereas only 5% of

patients present with advanced-stage disease (5). Japan is thus quite different from the rest of the Asia-Pacific region, probably because of its very well-established surveillance system.

But even within a country, there can be a significant variation between regions, as exemplified by Taiwan. In northern Taiwan, about 58% of patients have early-stage HCC, whereas in the southern part, the rate is only 35.2%. This is probably related to differences in the popularity of surveillance due to cultural, social and economic differences between the populations in the north and south of Taiwan. Data generated in Japan and Korea, using the Japanese TNM staging system, are similar to the BCLC staging results and show that Japan has a higher number of patients with early-stage HCC compared with Korea.

The disease stage obviously affects the treatment modality. For early-stage cancers, curative treatments like surgery or ablation are generally implemented, whereas TACE is performed for intermediate-stage disease and systemic therapy for advanced disease. Comparison between Hong Kong and Japan shows a dominance of ablation and surgery in Japan, whereas in Hong Kong, the percentage of patients amenable to ablation is limited. Even for TACE, the proportion of patients is higher in Japan than in Hong Kong, where a large proportion of patients have advanced disease and receive systemic therapy. For early-stage disease, curative treatment is the first choice, and about 38% of patients in Hong Kong and 65% in Japan are amenable to curative treatments. For intermediate-stage HCC, the rates are 22% in Hong Kong and 30% in Japan, and for advanced-stage disease, the rates are 40% in Hong Kong and 5% in Japan.

BCLC staging has important predictive power for overall survival. Data for more than 3000 patients in Hong Kong show very good stratification of overall survival in terms of the stage. Survival data from Yonsei University (Korea) show a very similar stratification. For patients with early HCC, the 5-year survival rate is now more than 50%, whereas for patients with advanced-stage disease, the 5-year survival is $<5\%$, showing a great difference in the survival outcomes. In some countries, like Korea, evidence points to some recent improvement in the overall survival of HCC patients: comparison between 1993 and 2005 shows that the 5-year survival has improved from 10.7% to 18.9% in the most recent 5-year period.

In conclusion, there is a significant variation in the distribution of early, intermediate and advanced stages of HCC among Asia-Pacific countries, with the highest proportion of early HCC in Japan. Curative treatment for early-stage HCC is associated with the 5-year survival $>50\%$, while the prognosis of advanced-stage HCC remains dismal. These results underscore the importance of early diagnosis by means of surveillance of high-risk patients.

Surveillance Systems and Prediction of HCC

A Hong Kong study proved that a screening program can improve survival by increasing the chance of treatment in the screened group (13). Unfortunately, in Hong Kong, the percentage of patients with HCC diagnosed by screening is low, but it has increased slightly, from 29% in 1991–1997 to 33% in 1998–2004 (14). There is no government-funded surveillance program for HCC in Hong Kong or other parts of China. Korea, however, established a national surveillance program in 2003, with the target population being those over 40 years of age, with liver cirrhosis or an HBV or HCV carrier (15). Taiwan has a similar surveillance program in place, and a different testing interval is applied depending on whether the subject has cirrhosis or not: 3–6 months for cirrhosis, but 6–12 months for non-cirrhosis. There is no age limitation for surveillance of HBV carriers in Taiwan, but in Korea, the government recommends over 40 years. The surveillance program in Japan is slightly different: it selects super high-risk patients, meaning liver cirrhosis B or C, and applies a shorter interval for examination, every 3 or 4 months, and test for more tumor markers (three tumor markers, including AFP, AFP-L3 and DCP) (16,17). The surveillance programs in Korea and China prefer a 6-month interval. Japanese surveillance program also recommends CT or MRI every 6–12 months for improving sensitivity. Thus, there are some differences in HCC surveillance among Asia-Pacific countries, including the candidates for surveillance and the age limit for HBV carriers. As surveillance tools, ultrasonography and AFP are still the standards, but there is a need to know whether more tumor markers will improve the sensitivity. A study investigated whether the surveillance interval is important for improving the survival. The group with a surveillance interval of within 6 months showed better survival than that of more than 6 months.

It is important to predict the development of HCC by quantitative risk estimation. An individualized prediction model is possible by combining multiple risk factors into a comprehensive risk expression. A study identified eight independent risk factors, and a special formula was established to calculate the relative risk factors. This model enables identification of the high- and low-risk groups.

In conclusion, HCC surveillance can detect early tumors and increase the chance of a curative approach. All patients at risk of developing HCC with potentially curative treatment available are recommended for regular surveillance. At present, ultrasonography and the serum AFP test at 6-month intervals are the standard surveillance tools. To improve the detection rate of early-stage HCC, the benefit of additional tests and a shorter surveillance interval should be confirmed by a randomized clinical trial in Asia. The application of individualized prediction model to surveillance programs may improve the cost-effectiveness by focusing on the high-risk group.

Recent Developments in Imaging Diagnosis

Various clinical practice guidelines for HCC are being implemented around the world, including in Europe, Korea, America, Japan and the Asia-Pacific region. In accordance

with those guidelines, the use of dynamic imaging, such as contrast-enhanced ultrasound (US), CT and MRI, is increasing and becoming more important, whereas application of biopsy is decreasing. Angiography and fusion imaging are other imaging tools that are available for the diagnosis of HCC. These tools are based on different imaging techniques. US is the first step for imaging diagnosis of HCC in accordance with the guidelines. If a nodule is found by US examination, the next technique to be used depends on the size of the mass. For a nodule that is <1 cm in diameter, follow-up study is usually recommended. If the nodule is >2 cm in diameter, one further imaging examination, such as contrast-enhanced US, CT or MRI, is sufficient to make a diagnosis of HCC with specific findings. Specific findings consist of a hypervascular nature in the arterial phase of imaging, and a washout pattern in the equilibrium phase. Diagnosis of HCC by dynamic imaging (contrast-enhanced ultrasonography, CT or MRI) is based on the enhancement pattern according to time sequence or phase. Overt HCC shows high attenuation in the arterial phase, indicating the hypervascular nature of the tumor, iso-attenuation in the portal-venous phase and low attenuation in the equilibrium phase, indicating a rapid washout pattern. These comprise very specific findings for the diagnosis of HCC.

In the APASL Guideline 2009 for imaging diagnosis of HCC, US is a screening test, not a diagnostic test for confirmation. US can detect a nodule but cannot characterize it. However, contrast-enhanced US is as sensitive as dynamic CT or dynamic MRI for the diagnosis of HCC (18). When using a US contrast agent for the diagnosis of HCC, the arterial phase and equilibrium phase show a rapid wash-in and washout pattern, which are characteristic findings for overt HCC. Dynamic CT or dynamic MRI is recommended as a first-line diagnostic tool for HCC when a screening test is abnormal. The hallmark of HCC in a CT scan or MRI is the presence of arterial enhancement followed by washout of the tumor in the portal-venous and/or delayed phases. In the diagnostic algorithm for hypervascular masses, typical HCC can be diagnosed by imaging regardless of the size of the detected tumor if a typical vascular pattern—arterial enhancement with portal-venous washout—is obtained on dynamic CT, dynamic MRI or contrast-enhanced US. In the diagnostic algorithm for hypervascular nodules, US is the initial screening method. If a nodule is detected by US, the nodule is then characterized by dynamic CT or MRI. Further characterization is usually performed by Kupffer cell imaging, including Sonazoid-enhanced US, or gadolinium-ethoxybenzyl-diethylene triamine pentaacetic acid (Gd-EOB-DTPA) MRI (Fig. 2) (19). In the diagnostic algorithm for hypovascular masses, nodular lesions showing an atypical imaging pattern, such as iso- or hypovascularity in the arterial phase, or arterial hypervascularity alone without portal-venous washout, should undergo further examination or close follow-up (Fig. 3). Recently, new imaging techniques are being developed, including volume US using various contrast agents, US elastography (20), volume CT, dual energy CT for perfusion CT, diffusion-weighted MRI, MRI elastography, etc. The efficacy of these techniques in diagnosing HCC is being evaluated.

In conclusion, various clinical practice guidelines including diagnostic algorithm for HCC have been established and

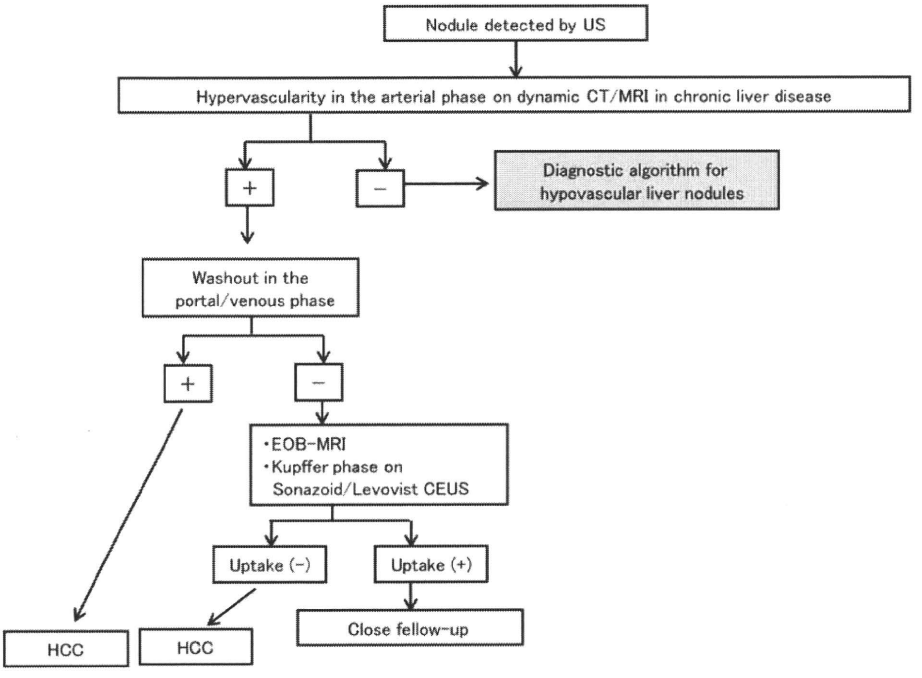

Figure 2. Diagnostic algorithm for hypervascular nodule (APASL Guideline). US, ultrasound; HCC, hepatocellular carcinoma.

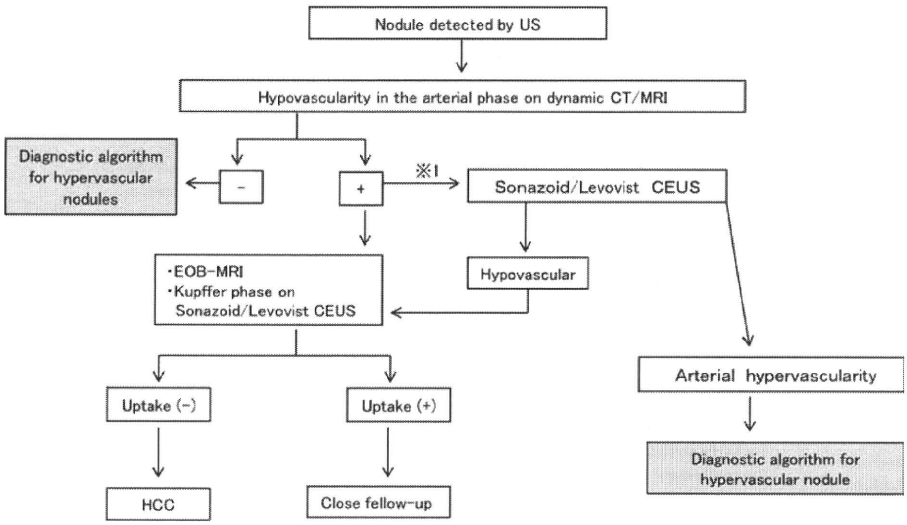

Figure 3. Diagnostic algorithm for hypovascular nodule (APASL Guideline). ※1: When the nodule is hypovascular on dynamic CT or dynamic MRI, Sonazoid-enhanced contrast US is recommended to confirm whether it is truly a hypovascular nodule.

are in use. Use of imaging diagnosis is increasing, whereas the use of biopsy is decreasing. New imaging techniques are continuously being developed. Practice guidelines should be updated to reflect the development of new imaging techniques.

Pathological Diagnosis of Early HCC

In 2009, pathologists from all over the world made great progress by reaching a consensus on the pathological diagnosis of early HCC. A consensus paper was published in the journal, *Hepatology* (21). The main topic of the consensus paper was histopathological definition of early HCC, together with premalignant lesions, dysplastic nodules and progressed HCC. Representative early HCC is a small, well-differentiated tumor, of vaguely nodular type. Microscopically, the border is unclear, and very well-differentiated cancer cells show a replacing growth pattern. They also frequently show stromal invasion, which is quite useful for making a diagnosis of cancer. However, histological atypia or histological alteration is usually very slight in early HCC, which is quite similar to the case of early cancers in other organs. Biopsy diagnosis of early HCC is especially difficult. In an example case, a slight increase in chromatin staining with substantial increase in the nuclear density is seen. Several standard techniques reveal slight changes or alterations in the tumor portion, such as a decrease in reticulin and a slight increase in proliferative activity. However, the use of some new markers, such as heat shock protein (HSP) 70, clearly highlights the tumor portion, making it more easily recognized. Greater use of tumor markers, including glypican 3 and HSP70, is likely and will increase the accuracy of diagnosis of early HCC.

Much has been learned about early HCC, but various problems remain. We know that cancer development is a multistep process, especially when there are cirrhotic changes. Early HCC grows very slowly and has a favorable outcome, whereas progressed, small HCC has a greater likelihood of showing intrahepatic spread and a worse prognosis. It is necessary to recognize that there is a gray zone between precancerous lesion and early HCC. Liver biopsy is recommended for small, equivocal lesions. Also, molecular markers are expected to raise the diagnostic accuracy, especially in the case of biopsy diagnosis of HCC. At the same time, controversy remains regarding which lesions should be examined by biopsy, and there is a risk of overdiagnosis of early cancer.

Current Treatment Strategies

Since 2001, when the Barcelona group published their consensus guideline, at least eight other guidelines have been released worldwide regarding the diagnosis and/or treatment of HCC. In 2003, the Korean guidelines were published, and in 2005, the Japanese guidelines for evidence-based clinical practice (Fig. 4) (16) were released. Clinical practice guidelines should be evidence-based, and they should represent the consensus of expert committees. Sometimes, it is very difficult to reach a consensus in the field of HCC. Guidelines must also take into consideration the socioeconomic status and current daily practice in the country or region. The socioeconomic background and daily practice regarding HCC were compared among Europe and the USA, Asia (Korea) and Japan. The major etiology of HCC is HCV in Europe, the USA and Japan, but HBV in Asia (Korea). A surveillance system has been established in Japan, is being

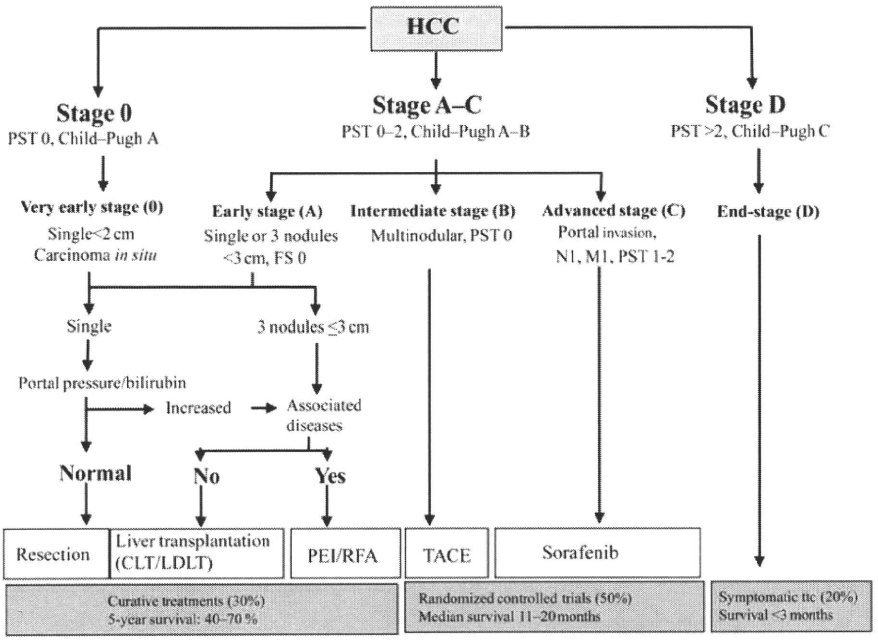

Figure 4. BCLC staging [Llovet et al. (10)]. BCLC, Barcelona Clinic of Liver Cancer; PST, performance status; CLT, cadaveric liver transplantation; LDLT, living donor liver transplantation; PEI, percutaneous ethanol injection; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization.

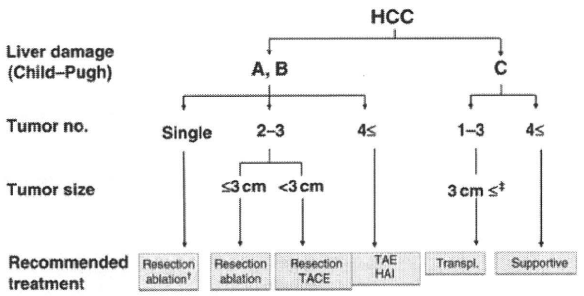

Figure 5. EBM-based algorithm for HCC treatment (J-HCC Guidelines 2009). Resection or transarterial chemoembolization (TACE) may be selected for liver damage A patients with vascular invasion. Chemotherapy may be selected for extrahepatic HCC. LT is only for ≤65 years old. †Recommended for Child B; ‡<2 cm for solitary lesion. HAI, hepatic arterial infusion.

developed in Asia (Korea), but does not exist in the Western countries. As a result, most HCC patients are diagnosed in an early stage in Japan, but at a very advanced stage in Western countries. As tumor markers, only AFP is measured in Western countries, whereas three tumor markers are measured in Japan. The risk of treatment of HCC must also be considered. The mortality of liver resection is as high as 4–5% in Western countries, but only 0.7% in Japan. Brain-dead donors for liver transplantation are very rare in Japan, but common in Western countries (22). These factors must be considered for development of treatment strategies for HCC.

The BCLC guidelines to staging and treatment of HCC are probably the most popular treatment algorithm in Western countries, but not in Asia. The Japanese guidelines were just revised in 2009, are very simple and cover a majority of early- and intermediate-stage HCC patients (Fig. 5). A Japanese consensus-based algorithm for HCC covers even very advanced-stage HCC, including patients with extrahepatic spread and vascular invasion (Fig. 6) (17,19). Sorafenib is recommended for such advanced disease with good liver function, and an ongoing trial is evaluating its use as an adjuvant therapy. The Korean guideline for management of HCC was initially published in 2003, after which they accumulated evidence, held a nationwide forum for revision of the guidelines and created a revision committee. As a result, their updated guidelines were published in 2009 (23). The algorithm for the Korean HCC treatment plan lists hepatic resection, liver transplantation, radiofrequency ablation and ethanol injection as curative treatments. There is no evidence showing which treatment is superior for cure of HCC in each patient, so the guideline recommends that the physician decide which treatment will be used. The APASL Consensus on Treatment of HCC (24) was published in 2010 and may be utilized in the Asian region.

In conclusion, several practice guidelines presenting treatment strategies for HCC in Asia have been developed. They were created based on evidence-based medicine methodology and consensus among experts in the region. They also reflect the socioeconomic status and current daily practice in the region. A number of ongoing clinical trials aim to

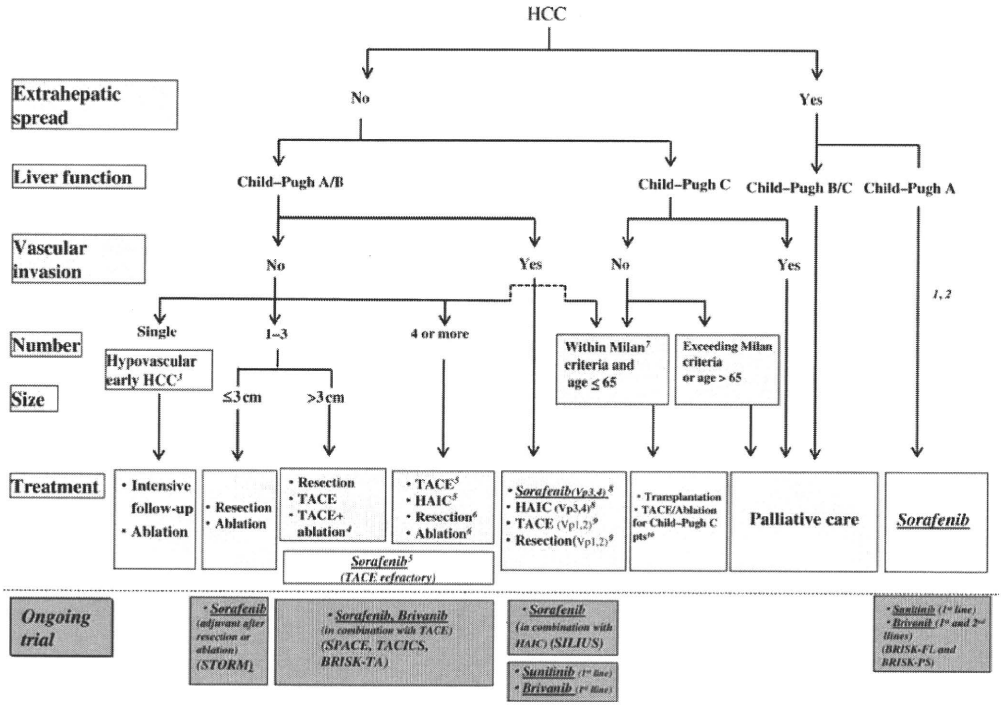

Figure 6. Consensus-based treatment algorithm for HCC proposed by Japan Society of Hepatology (JSH) 2009 revised in 2010. *1*, Treatment should be performed as if extrahepatic spread is negative, when extrahepatic spread is not regarded as a prognostic factor. *2*, Sorafenib is the first choice of treatment 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 case: (i) when the nodule is diagnosed pathologically as early HCC, (ii) when the nodules show decreased uptake on gadolinium-ethoxybenzyl-diethylene triamine pentaacetic acid or (iii) when the nodules show decreased portal flow by CTAP, since 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 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 (5FU + CDDP) or intra-arterial 5FU infusion combined with systemic interferon therapy. Sorafenib is also recommended for TACE refractory patients. *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 frequently recurring HCC patients. *8*, Sorafenib and HAIC are recommended for HCC patients with Vp3 (portal invasion at the first portal branch) or Vp4 (portal invasion at the main portal branch). *9*, Resection and TACE are frequently performed when portal invasion is minimum such as Vp1 (portal invasion at the third or more peripheral portal branch) or Vp2 (portal invasion at the second 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.

generate evidence for a better treatment algorithm. Guidelines should be updated every 3 or 4 years, incorporating new evidence.

Future Perspectives, Especially In Regard To Sorafenib

There was no established systemic chemotherapy for HCC. However, sorafenib has become a standard systemic treatment for advanced HCC. This section addresses the future perspectives for sorafenib and beyond sorafenib. Two randomized control studies have shown the survival benefit of sorafenib in advanced HCC patients with good liver function of Child–Pugh A. The SHARP trial (25), carried out mainly in European countries, and an Asia-Pacific trial (26) both showed that sorafenib provides a survival benefit in advanced HCC patients. Both trials yielded similar hazard ratio of 0.69 and 0.68, respectively, in favor of sorafenib over placebo. Other published reports on sorafenib for HCC include a Phase II trial conducted in Western countries (27), a Phase I Japanese study (28), a Korean study (29) and a Phase 2 Hong Kong study (30). The studies had various differences in patient background, such as involvement of HBV, HCV or others, liver function of Child–Pugh A and B, and the ECOG performance status. Those differences affected the survival outcomes in the four studies like outcomes after other treatment modalities.

Although sorafenib has become a standard systemic treatment for advanced HCC, there are still issues to be investigated with regard to this agent, including its efficacy and safety in patients with Child–Pugh B moderate liver

function, combination therapy with other treatment methods, and the need to identify predictive factors and markers for sorafenib. Various studies are currently attempting to elucidate those issues. The Phase III STORM global trial will evaluate sorafenib as an adjuvant therapy after surgery or radiofrequency ablation. A Japanese Phase II study will evaluate the efficacy and safety of sorafenib in patients with Child–Pugh A and B, with investigation of biomarkers. A global trial of combination of sorafenib with TACE is ongoing, while two Japanese Phase I studies of combination of sorafenib with hepatic arterial infusion are in progress (19). Arterial infusion chemotherapy is a very common and useful treatment in Japan (31), and one of these studies combines sorafenib with cisplatin, whereas the other combines sorafenib with 5-FU and cisplatin. It is anticipated that these trials will lead to Phase III studies.

Other Molecularly Targeted Agents

Sorafenib is the first systemic therapy approved for advanced-stage HCC, and widely used. Sorafenib prolongs time to progression and overall survival in patients with advanced HCC; however, predictive factors are unknown at the present. Good responders show a good response, but how can they be identified in advance? Researchers are currently looking for biomarkers that will identify good responders and lead to modification of the treatment algorithm. Also, a 'good response' has limitations. How can a 'complete response' be attained? Combination therapy and some adjuvant treatment, after palliative or curative treatment, will be needed. There are also many poor responders. How can a poor response be overcome? Second-line agents are necessary, as is combination therapy. Various targeted agents in addition to sorafenib are under development for HCC. They include brivanib, bevacizumab, cediranib, erlotinib, gefitinib, lapatinib, RAD001, sunitinib, thalidomide and TSU-68. These agents have similar yet slightly different mechanisms of action. The results of various clinical studies of these molecular targeted therapy agents were summarized in *Hepatology* (32). The results look good, and many Phase II and Phase III trials are ongoing. The trials can be categorized into three types: first-line or combination studies, second-line studies and adjuvant studies.

First-line or combination studies are being carried out as Phase III trials of sunitinib vs. sorafenib (terminated in 2010 because of severe adverse effect); brivanib vs. sorafenib; lilifanib vs. sorafenib; erlotinib plus sorafenib vs. sorafenib; and erlotinib plus bevacizumab vs. sorafenib. The results of these trials should be available in 2 or 3 years. There are also many first-line Phase II studies. There are two second-line Phase II studies, of brivanib vs. the placebo and RAD001 vs. the placebo, for patients who failed to respond to sorafenib. There are three Phase III adjuvant studies. The STORM study investigates sorafenib vs. placebo after resection or ablation. A second adjuvant study investigated sorafenib vs. placebo after TACE; this is already finished and the results were presented at ASCO-GI in 2010 (33). The third Phase III adjuvant study compares brivanib vs. placebo after TACE. In a first-line Phase II study of brivanib, 46% of the patients showed stable disease, and in the second-line Phase II study, 43% showed stable disease (34,35). These results were promising, and at least three trials are now ongoing for brivanib.

In conclusion, molecularly targeted therapy (MTT) has emerged as a promising approach for advanced HCC. Sorafenib impacted on MTT agents in HCC, but the benefits of sorafenib were reported to be relatively modest. Several MTT agents for first- and second-line treatments are undergoing clinical trials. The advantages of MTT agents are being explored in combination treatments as well as adjuvant therapy with resection, local ablation, radiation, hepatic arterial infusion chemotherapy and TACE.

CONCLUSION

HCC is a highly prevalent disease in many Asian countries and incidence of HCC varies enormously across Asia, but tends to follow incidences of hepatitis B infection and liver cirrhosis. Incidence and etiology of HCC in Japan is different from the rest of Asia, but similar to Western countries since hepatitis C infection is the main etiological factor. Screening program improves detection of early HCC and has some positive impact on survival, but the majority of HCC patients in Asia still present with advanced HCC. Long-term outcomes following treatment of early, intermediate or advanced disease are still unsatisfactory because of lack of effective adjuvant or systemic therapies. Sorafenib is the first systemic therapy to demonstrate prolonged survival vs. placebo in patients with advanced HCC. New molecular targeting therapies hold great promise. Many new agents are under investigation and their results are awaited.

Conflict of interest statement

The author, Joong-Won Park, participated in phase II and phase III clinical studies sponsored by Bristol-Myers Squibb, Pfizer Inc., Bayer Healthcare and Bukwang Pharmaceutical Co. He is also a member of BMS Brivanib study steering committee, Pfizer Sunitinib advisory committee, and Bukwang Pharmacetutical Co. advisory committee.

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Hepatology Research

Hepatology Research 2010; 40: 1043–1059 doi: 10.1111/j.1872-034X.2010.00731.x

Special Report

Report of the 18th follow-up survey of primary liver cancer in Japan

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In the 18th Nationwide Follow-Up Survey of Primary Liver Cancer in Japan, 20 753 people were newly registered as patients with primary liver cancer at 544 medical institutions over a period of 2 years (from 1 January 2004 to 31 December 2005). Of these patients, 94.0% had hepatocellular carcinoma (HCC) and 4.4% had intrahepatic cholangiocarcinoma (ICC). In addition, 30 677 follow-up patients were registered in the survey. Epidemiological and clinicopathological factors, diagnosis and treatment were investigated in the newly registered patients. Compared with the 17th follow-up survey, this follow-up survey in HCC indicated an increase in elder patients and women, a decrease in patients positive for hepatitis B surface antigen and hepatitis C virus antibody, and a decrease in tumor size at the clinical diagnosis. In the local ablation therapy, ratio of radio frequency ablation therapy was increasing. The cumulative survival rates of newly-registered patients between 1994 and 2005 were calculated for each histological type (HCC, ICC, and combined HCC and ICC) and stratified by background factors and treatment. The cumulative survival rates of newly-registered patients between 1978 and 2005 divided into three groups (1978–1985, 1986–1995 and 1996–2005) were also calculated. The data obtained in this follow-up survey should contribute to future research and medical practice for primary liver cancer.

Key words: combined hepatic carcinoma, cumulative survival rate, follow-up survey, hepatocellular carcinoma, intrahepatic cholangiocarcinoma

INTRODUCTION

SINCE 1969, THE Liver Cancer Study Group of Japan (LCSGJ) has conducted 17 nationwide follow-up surveys of primary liver cancer in patients in member hospitals and cooperative institutions in Japan, with the goal of promoting research and clinical treatment of liver cancer.[1–17] The 18th Nationwide Follow-up Survey of Primary Liver Cancer was conducted over a 2-year period from 1 January 2004 to 31 December 2005, and 20 753 patients with primary liver cancer were newly registered at 544 institutions. In addition, 30 677 registered patients were followed up with a valid response rate of 74.2%. Items related to epidemiological and clinicopathological factors, diagnosis and treatment were investigated in the newly-registered patients. Cumulative survival rates of newly-registered patients between 1994 to 2005 were calculated for each histological type and based on background factors and treatment.

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Received 21 June 2010; revision 3 August 2010; accepted 19 August 2010.

METHODS

Basic statistics

THE SUBJECTS WERE 20 753 patients with primary liver cancer who were diagnosed clinically or by autopsy and underwent treatment or autopsy during a 2-year period from 1 January 2004 to 31 December 2005 at 544 institutions in Japan. Doctors in each institution completed a form developed by the Follow-up

Table 1 Classification of primary liver cancer

Diagnosis	Male ($n = 14\,601$)	Female ($n = 6152$)	Total ($n = 20\,753$)
HCC	13 805	5 694	19 499 (94.0%)
ICC	561	344	905 (4.4%)
Combined	119	41	160 (0.8%)
Cystadenocarcinoma	14	13	27 (0.1%)
Hepatoblastoma	5	9	14 (0.1%)
Sarcoma	7	2	9 (0.0%)
Undifferentiated carcinoma	6	2	8 (0.0%)
Others	84	47	131 (0.6%)

Combined, combined hepatocellular and cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma.

Survey Committee of the Liver Cancer Study Group of Japan (chairperson, Masatoshi Kudo). In cases with an inconsistency between the clinical, pathological and autopsy diagnoses, the autopsy and pathological diagnoses were given first and second priority, respectively. Of the 20 753 patients, 94.0% had hepatocellular carcinoma (HCC) and 4.4% had intrahepatic cholangiocarcinoma (ICC) (Table 1). The results in the tables are categorized into HCC, ICC, and combined HCC and ICC, for which more than 100 newly-registered cases appeared in the current follow-up survey. The abbreviations in the tables conform to *The General Rules for the Clinical and Pathological Study of Primary Liver Cancer*, 2nd English edition and *Response Evaluation Criteria in Cancer of Liver* proposed by the Liver Cancer Study Group of Japan.[18,19]

Cumulative survival rate

The cumulative survival rates of newly-registered patients in the 13th to 18th follow-up surveys between 1994 and 2005 whose final prognosis was determined to be survival or death (excluding patients with unknown outcomes) were calculated for each histological type (HCC, ICC, and combined HCC and ICC) and based on different background factors and treatment, including hepatectomy, local ablation therapy and transcatheter arterial embolization. The cumulative survival rates of newly-registered patients between 1978 and 2005 divided into three groups (1978–1985, 1986–1995 and 1996–2005) were also calculated. In this report, patients who had died from either liver-related or liver-unrelated causes were considered to be uncensored cases in estimating cumulative survival rates.

RESULTS

Basic statistics

Causes of death during the study period

FOR HCC, THE mortality of newly-registered patients during the study period was 15.7%: the death rate due to cancer was 55.8% and death rates due to hepatic failure, gastrointestinal bleeding and rupture of esophagogastric varices were 18.8%, 2.1% and 4.1%, respectively. Of the patients who did not survive, 42 died within 30 days after surgery; these patients represented 0.7% of the 5794 patients who underwent surgery. For ICC, the mortality of newly-registered patients during the study period was 35.5% and death rates due to cancer and hepatic failure were 78.5% and 8.3%, respectively (Table 2).

Past history

Of patients with HCC, 76.2% and 60.0% had a past history of chronic hepatitis and liver cirrhosis, respectively, whereas only 19.9% and 9.4% of ICC patients had this history. Interferon therapy had been given to 15.7% of HCC patients due to concomitant chronic hepatitis, and 26.9% and 24.5% of HCC patients and 9.1% and 15.7% of ICC patients had a past history of blood transfusion and habitual alcohol intake, respectively.

Clinical diagnosis

Clinical diagnosis of primary liver cancer in patients with HCC was made at a mean age of 66.4 years in men and 69.9 years in women. For patients with ICC, the corresponding mean ages were 67.2 years in men

Table 2 Causes of death of patients with primary liver cancer

	HCC		ICC		Combined	
Alive	15 885		567		110	
Total deaths of between 2004 and 2005	2 952		312		46	
Cancer death	1 646	(55.8%)	245	(78.5%)	35	(76.1%)
Hepatic failure	554	(18.8%)	26	(8.3%)	7	(15.2%)
Gastrointestinal bleeding	62	(2.1%)	2	(0.6%)	0	(0.0%)
Rupture of esophageal varices	122	(4.1%)	2	(0.6%)	0	(0.0%)
Rupture of tumor	166	(5.6%)	0	(0.0%)	0	(0.0%)
Operative death	42	(1.4%)	4	(1.3%)	0	(0.0%)
Other causes	360	(12.2%)	33	(10.6%)	4	(8.7%)
Unknown	612		22		4	

Combined, combined hepatocellular and cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma.

and 66.6 years in women. The male : female ratios for HCC and ICC patients were 2.41 and 1.67, respectively.

In patients with HCC, the level of liver injury at the time of diagnosis, based on the liver damage classification of the LCSGJ, was class A, B and C in 60.4%, 32.2% and 7.4% of patients, respectively, whereas 71.0%, 23.6% and 5.4% of HCC patients were in the Child–Pugh class A, B and C categories, respectively (Table 3). Of the HCC patients, 37.1%, 36.3% and 26.6% had serum α-fetoprotein (AFP) levels of less than 15 ng/mL, 15–199 ng/mL and 200 ng/mL or more, respectively, and 64.3%, 5.2% and 30.6% of patients with HCC had serum levels of lectin-reactive AFP-L_3 of less than 10%, 10.0–14.9% and 15% or more, respectively. Of the HCC patients, 40.5%, 14.4% and 45.0% had a protein induced by vitamin K absence or antagonist-II (PIVKA-II) level of less than 40 mAU/mL, 40–99 mAU/mL and 100 mAU/mL or more, respectively. In patients with ICC, 60.0%, 13.9% and 26.2% had a carcinoembryonic antigen level of less than 5.0 ng/mL, 5.0–9.9 ng/mL and 10 ng/mL or more, respectively, and 30.5%, 18.0% and 51.4% had a carbohydrate antigen 19-9 level of less than 37 U/mL, 37–99 U/mL and 100 U/mL or more, respectively (Table 3).

Of the patients with HCC, ICC, and combined HCC and ICC, those who were positive for hepatitis B virus surface antigen comprised 15.0%, 6.3% and 18.9%, respectively. The percentages of anti-hepatitis C virus antibody positive patients were 67.7%, 18.8% and 46.7%, respectively (Table 4).

Tumor size was determined using diagnostic imaging. Of patients with HCC, 33.5% and 45.5% had tumors of 2.0 cm or less and 2.1–5.0 cm, respectively. The corresponding numbers for patients with ICC were 9.3% and 48.8%, respectively (Table 5). Of the tumors, 57.7% and 73.7% were solitary in patients with HCC and ICC, respectively. In patients with HCC, 93.2% had a tumor stain, 2.5% exhibited tumor rupture and 40.4% had esophagogastric varices of F2 or RC_1 or higher.

Major treatment

Of patients with HCC, 31.7%, 30.6% and 31.7% had undergone surgery (hepatectomy and liver transplantation), local ablation therapy and transcatheter arterial embolization, respectively. In patients with ICC, 67.1% and 26.5% had undergone surgery (hepatectomy) and chemotherapy, respectively, and in patients with combined HCC and ICC, 63.8% and 13.5% had undergone surgery (hepatectomy) and transcatheter arterial chemoembolization, respectively (Table 6). Among the HCC patients, 74.5%, 23.2% and 2.2% who underwent surgery, 60.6%, 34.7% and 4.7% of those treated with local ablation therapy, and 57.7%, 36.0% and 6.2% of those treated with transcatheter arterial embolization were in liver damage classes A, B and C, respectively.

Surgery

Of patients with HCC, 5646 underwent hepatectomy and 148 received a liver transplantation. Macroscopic analysis of the resected specimens showed that 59.0% of cases were of the single nodular type. Of patients with ICC, 492 underwent hepatectomy and two received a liver transplantation, and 63.1% of these cases were of the mass-forming type.

Table 3 Clinical profile of patients with primary liver cancer

	HCC		ICC		Combined	
Diagnosis	*n* = 35 472		*n* = 1693		*n* = 301	
Computed tomography	15 275	(43.1%)	701	(41.4%)	124	(41.2%)
Magnetic resonance imaging	2 815	(7.9%)	221	(13.1%)	30	(10.0%)
Ultrasonography	9 305	(26.2%)	378	(22.3%)	76	(25.2%)
Selective angiography	6 388	(18.0%)	186	(11.0%)	37	(12.3%)
Histopathological finding	1 504	(4.2%)	162	(9.6%)	29	(9.6%)
Others	185	(0.5%)	45	(2.7%)	5	(1.7%)
performance status	*n* = 16 364		*n* = 741		*n* = 137	
PS0	13 224	(80.8%)	575	(77.6%)	108	(78.8%)
PS1	2 100	(12.8%)	105	(14.2%)	18	(13.1%)
PS2	616	(3.8%)	30	(4.0%)	6	(4.4%)
PS3	273	(1.7%)	14	(1.9%)	4	(2.9%)
PS4	151	(0.9%)	17	(2.3%)	1	(0.7%)
Encephalopathy	*n* = 18 188		*n* = 813		*n* = 146	
None	17 494	(96.2%)	808	(99.4%)	145	(99.3%)
Mild	490	(2.7%)	3	(0.4%)	0	(0.0%)
Coma occasionally	204	(1.1%)	2	(0.2%)	1	(0.7%)
Ascites	*n* = 18 509		*n* = 830		*n* = 154	
Absent	16 135	(87.2%)	769	(92.7%)	138	(89.6%)
Slight	1 474	(8.0%)	19	(2.3%)	7	(4.5%)
Moderate	900	(4.9%)	42	(5.1%)	9	(5.8%)
Serum bilirubin (mg/mL)	*n* = 18 614		*n* = 852		*n* = 153	
0.0–0.9	10 342	(55.6%)	518	(60.8%)	104	(68.0%)
1.0–1.9	6 383	(34.3%)	195	(22.9%)	38	(24.8%)
2.0–3.0	1 140	(6.1%)	32	(3.8%)	4	(2.6%)
≥3.1	749	(4.0%)	107	(12.6%)	7	(4.6%)
Serum albumin (g/dL)	*n* = 18 481		*n* = 825		*n* = 152	
<2.8	1 470	(8.0%)	37	(4.5%)	9	(5.9%)
2.8–2.9	967	(5.2%)	23	(2.8%)	4	(2.6%)
3.0–3.5	5 255	(28.4%)	160	(19.4%)	40	(26.3%)
>3.5	10 789	(58.4%)	605	(73.3%)	99	(65.1%)
ICG R_{15} (%)	*n* = 10 794		*n* = 487		*n* = 106	
≤14	3 875	(35.9%)	341	(70.0%)	62	(58.5%)
15–24	3 286	(30.4%)	103	(21.1%)	31	(29.2%)
25–40	2 409	(22.3%)	32	(6.6%)	11	(10.4%)
>40	1 224	(11.3%)	11	(2.3%)	2	(1.9%)
Prothrombin activity (%)	*n* = 17 538		*n* = 775		*n* = 145	
<40	278	(1.6%)	15	(1.9%)	1	(0.7%)
40–49	372	(2.1%)	7	(0.9%)	1	(0.7%)
50–70	3 876	(22.1%)	70	(9.0%)	19	(13.1%)
71–80	3 900	(22.2%)	119	(15.4%)	31	(21.4%)
>80	9 112	(52.0%)	564	(72.8%)	93	(64.1%)
Platelet count ($\times 10^4/mm^3$)	*n* = 18 374		*n* = 847		*n* = 154	
<3.0	145	(0.8%)	4	(0.5%)	1	(0.6%)
3.0–4.9	942	(5.1%)	5	(0.6%)	0	(0.0%)
5.0–9.9	5 979	(32.5%)	53	(6.3%)	24	(15.6%)
10.0–14.9	5 419	(29.5%)	114	(13.5%)	46	(29.9%)
15.0–19.9	3 119	(17.0%)	216	(25.5%)	36	(23.4%)
20.0–99.9	2 697	(14.7%)	453	(53.5%)	47	(30.5%)
>100	73	(0.4%)	2	(0.2%)	0	(0.0%)

Table 3 *Continued*

	HCC		ICC		Combined	
Liver damage classification by LCSGJ	*n* = 15 574		*n* = 706		*n* = 138	
A	9 400	(60.4%)	596	(84.4%)	100	(72.5%)
B	5 016	(32.2%)	82	(11.6%)	35	(25.4%)
C	1 158	(7.4%)	28	(4.0%)	3	(2.2%)
Child-Pugh classification	*n* = 18 032		*n* = 790		*n* = 149	
A	12 799	(71.0%)	667	(84.4%)	121	(81.2%)
B	4 254	(23.6%)	101	(12.8%)	21	(14.1%)
C	979	(5.4%)	22	(2.8%)	7	(4.7%)
AFP (ng/mL)	*n* = 17 804		*n* = 562		*n* = 145	
<15	6 608	(37.1%)	449	(79.9%)	59	(40.7%)
≤199	6 466	(36.3%)	77	(13.7%)	38	(26.2%)
≤399	1 000	(5.6%)	11	(2.0%)	7	(4.8%)
≤999	994	(5.6%)	7	(1.2%)	11	(7.6%)
≤9 999	1 549	(8.7%)	12	(2.1%)	17	(11.7%)
≤99 999	761	(4.3%)	3	(0.5%)	9	(6.2%)
≥100 000	426	(2.4%)	3	(0.5%)	4	(2.8%)
AFP-L_3 (%)	*n* = 7904		*n* = 126		*n* = 62	
ND	2 661	(33.7%)	71	(56.3%)	14	(22.6%)
<5.0	1 785	(22.6%)	21	(16.7%)	10	(16.1%)
≤9.9	634	(8.0%)	4	(3.2%)	1	(1.6%)
≤14.9	411	(5.2%)	0	(0.0%)	3	(4.8%)
≤19.9	250	(3.2%)	0	(0.0%)	3	(4.8%)
≥20.0	2 163	(27.4%)	30	(23.8%)	31	(50.0%)
PIVKA-II (mAU/mL)	*n* = 16 114		*n* = 389		*n* = 140	
<40	6 531	(40.5%)	311	(79.9%)	61	(43.6%)
≤99	2 327	(14.4%)	32	(8.2%)	17	(12.1%)
≤299	1 998	(12.4%)	12	(3.1%)	18	(12.9%)
≤499	781	(4.8%)	6	(1.5%)	7	(5.0%)
≤999	842	(5.2%)	6	(1.5%)	11	(7.9%)
≤2 999	1 087	(6.7%)	5	(1.3%)	9	(6.4%)
≤9 999	975	(6.1%)	8	(2.1%)	8	(5.7%)
≥10 000	1 573	(9.8%)	9	(2.3%)	9	(6.4%)
CEA (ng/mL)	*n* = 6 192		*n* = 758		*n* = 113	
<2.5	2 329	(37.6%)	236	(31.1%)	38	(33.6%)
≤4.9	2 319	(37.5%)	219	(28.9%)	34	(30.1%)
≤9.9	1 219	(19.7%)	105	(13.9%)	27	(23.9%)
≤19.9	223	(3.6%)	60	(7.9%)	6	(5.3%)
≤49.9	57	(0.9%)	58	(7.7%)	0	(0.0%)
≤99.9	19	(0.3%)	27	(3.6%)	1	(0.9%)
≥100	26	(0.4%)	53	(7.0%)	7	(6.2%)
CA 19-9 (U/mL)	*n* = 4 807		*n* = 737		*n* = 108	
<37	3 023	(62.9%)	225	(30.5%)	49	(45.4%)
≤99	1 224	(25.5%)	133	(18.0%)	26	(24.1%)
≤299	422	(8.8%)	110	(14.9%)	15	(13.9%)
≤999	95	(2.0%)	82	(11.1%)	9	(8.3%)
≤2999	24	(0.5%)	51	(6.9%)	4	(3.7%)
≤9999	12	(0.2%)	64	(8.7%)	2	(1.9%)
≥10 000	7	(0.1%)	72	(9.8%)	3	(2.8%)

AFP, α-fetoprotein; AFP-L_3, lectin-reactive α-fetoprotein; CA 19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; combined, combined hepatocellular and cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; ICG R_{15}, indocyanine green retention rate at 15 min; LCSGJ, Liver Cancer Study Group o Japan; ND, not detectable; PIVKA, protein induced by vitamin K absence or antagonist.

Table 4 Hepatitis B and C virus-associated antigen and antibody

	HCC		ICC		Combined	
HBsAg	*n* = 18 317		*n* = 809		*n* = 148	
Negative	15 550	(84.9%)	758	(93.7%)	120	(81.1%)
Positive	2 754	(15.0%)	51	(6.3%)	28	(18.9%)
Undetermined	13	(0.1%)	0	(0.0%)	0	(0.0%)
HBsAb	*n* = 5 436		*n* = 219		*n* = 62	
Negative	4 293	(79.0%)	181	(82.6%)	46	(74.2%)
Positive	1 107	(20.4%)	38	(17.4%)	16	(25.8%)
Undetermined	36	(0.7%)	0	(0.0%)	0	(0.0%)
HBcAb	*n* = 4 731		*n* = 160		*n* = 55	
Negative	2 200	(46.5%)	105	(65.6%)	28	(50.9%)
Positive	2 515	(53.2%)	54	(33.8%)	27	(49.1%)
Undetermined	16	(0.3%)	1	(0.6%)	0	(0.0%)
HBeAg	*n* = 3 410		*n* = 94		*n* = 42	
Negative	2 829	(83.0%)	91	(96.8%)	38	(90.5%)
Positive	570	(16.7%)	3	(3.2%)	3	(7.1%)
Undetermined	11	(0.3%)	0	(0.0%)	1	(2.4%)
HBeAb	*n* = 3 338		*n* = 84		*n* = 39	
Negative	1 723	(51.6%)	50	(59.5%)	16	(41.0%)
Positive	1 580	(47.3%)	31	(36.9%)	23	(59.0%)
Undetermined	35	(1.0%)	3	(3.6%)	0	(0.0%)
HCVAb	*n* = 18 624		*n* = 828		*n* = 150	
Negative	5 998	(32.2%)	671	(81.0%)	80	(53.3%)
Positive	12 610	(67.7%)	156	(18.8%)	70	(46.7%)
Undetermined	16	(0.1%)	1	(0.1%)	0	(0.0%)

Combined, combined hepatocellular and cholangiocarcinoma; HBcAb, antibody to hepatitis B core antigen; HbeAb, antibody to hepatitis B e antigen; HbeAg, hepatitis B e-antigen; HbsAb, hepatitis B surface antibody; HbsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCVAb, hepatitis C virus antibody; ICC, intrahepatic cholangiocarcinoma.

Macroscopic results from the resected specimens are shown in Table 7. In the HCC patients who underwent hepatectomy, tumors of size 2.0 cm or less, 2.1–5.0 cm and 5.1–10.0 cm were found in 17.7%, 54.9% and 20.2% of patients, respectively, and 74.3% of the tumors were solitary. Vascular invasion in the portal vein, hepatic vein and bile duct were found in 16.2%, 7.3% and 2.7% of the patients, respectively. Regarding findings in non-cancerous parts of the liver, normal liver, chronic hepatitis/liver fibrosis and liver cirrhosis were found in 9.0%, 49.0% and 42.1% of the patients, respectively. The extent of surgical resection was Hr0, HrS, Hr1, Hr2 and Hr3 in 30.7%, 23.4%, 22.6%, 20.8% and 2.5% of the patients, respectively (Table 7).

In patients with ICC, tumors of size 2.0 cm or less, 2.1–5.0 cm and 5.1–10.0 cm were found in 9.3%, 52.1% and 33.9% of patients, respectively, and 83.8% of the tumors were solitary.

Local ablation therapy

Of patients with HCC, 6673 underwent local ablation therapy. Ethanol injection therapy, microwave coagulation therapy and radiofrequency ablation therapy were given to 18.6%, 8.5% and 72.1% of these patients, respectively, suggesting a marked increase in the use of radiofrequency ablation therapy (Table 8). Percutaneous treatment was given in 86.3% of these cases, and of these patients, 71.2% had one tumor, 59.3% had a tumor of size 2.0 cm or less, and 28.5% had a tumor of 2.1–3.0 cm. Treatment outcomes of complete response (CR) and partial response (PR) at 6 months after treatment occurred in 80.3% and 9.9% of patients, respectively.

Transcatheter arterial embolization

Transcatheter arterial embolization was conducted in 8188 patients with HCC. Of these patients, lipiodol