Fig. 6. (a) Increasing the infection rate increased the virus count in equilibrium periods, but the virus count did not change at infection rates of 30% or more. (b) The size of the simulation space increased not only virus count but also the cell count; however, (c) when virus and cell counts were divided by the total number of grids in the space, they were constant for all space sizes. (d) Changing the lifespan and regeneration rate of uninfected cells in opposite directions at the same time makes it possible to change only the cell cycle speed without altering the uninfected cell count. (e) When the cell cycle speed was reduced, the virus count increased toward the right of the graph. This may be because the effect of extending the lifespan of cells exceeds that of reducing their regeneration rate. (a–c and e) Black circles: virus count; line: virus count approximation curve; white bars: uninfected cell count; black bars: infected cell count.

3.7. Infection Rate and Space Size

Increasing the infection rate caused an increase in the virus count, but the change was minimal at an infection rate of 30% or more. The same results were seen for infected cell count, but a decrease in uninfected cell count resulted in a tendency for the infection rate to decrease by up to 60% (Fig. 6a).

The larger the space, higher the increase in both virus and cell counts (Fig. 6b). This increase was proportional to space size, how-

ever, when virus and cell counts were divided by the total number of grids in the space they were all constant (Fig. 6c).

3.8. Cell Cycle Speeds

Running a simulation with the initial virus count set to zero enables only the equilibrium condition for uninfected cells to be simulated. Changing the lifespan and regeneration rate of uninfected cells in opposite directions at the same time makes it possible

to change the cell cycle speed without altering the uninfected cell count (Fig. 6d). We used this technique to investigate how changing the cell cycle speed affected the equilibrium phase. Fig. 6e shows the results. Cell lifespan increases while the cell cycle speed declines. The equilibrium virus count increased in accordance with slower cell cycle speeds.

4. Discussion

In this study, we investigated the models using two agent-based simulation methods to program a simple virus–host chronic infection model. The same model written in two different programming language systems displayed the same results. The transient phase was unlike that seen in a mathematical simulation with no overshoot in virus count, but rather a smooth transition to the equilibrium phase. The virus count at the start of the simulation only had effect on the rate of infection development. Increases in virus lifespan, uninfected cell lifespan, uninfected cell regeneration rate, virus production count from infected cells, and infection rate all led to increased equilibrium-phase virus count. Rises in the infected cell lifespan-shortening ratio, latent period, and cell cycle speed decreased the equilibrium-phase virus count. The size of the space itself had no innate effect on the equilibrium phase, but a speed of movement of the virus that was twice the size of the space produced the maximum virus count.

Reproducibility is the basis for all scientific study, but there are many problems to prove it in computer simulations, such as programming bugs. As agent-based simulation deals with numerous agents individually, it requires vast amounts of calculations. Accumulation of very small change of values leads to large differences of results. In this study, we investigated two programs based on two programming languages to confirm the reproducibility of our simulation results in different programming languages. The results of two simulations were consistent, but in StarLogo, the lifespan parameters had a tendency to be lower than when they were set while simulations were actually in progress. This may be because the number of digits used in calculations was different between the two programs. RePast performs calculations to at least eight decimal places. In StarLogo, the library settings only enable settings to be made up to five decimal places. It is probable that these small differences accumulate during repeated calculations and are reflected in the simulation. Ultimately, we confirmed that the differences in results obtained by using different libraries and programming languages were not innate and by making the parameters consistent during simulation, consistent results were obtained.

Mathematical models using formulae for HIV therapy was published in 1994, the method has since been applied to HBV and HCV (Ho et al., 1995; Nowak et al., 1996; Neumann et al., 1998), and they were thought to be good reflections of the reality. In the mathematical model, viruses and cells are conceived as individuals in the concept itself, but both of them are perceived *en masse* when calculations are performed. However a feature of the agent-based simulation is that it deals with individual viruses and cells as separate agents. By moving each agent individually, it probes the factors influencing overall shifts from the micro viewpoint. When the space is viewed as a whole, it is possible to watch on the screen the collective movement of groups of agents. Recently, models that provide a visual representation of Epstein-Barr virus and HIV infection have been reported, both of which are useful for an instinctive and intuitive understanding (Duca et al., 2007; Shapiro et al., 2008; Castiglione et al., 2007).

In agent-based simulation model, virus count transit smoothly to the equilibrium phase. On the other hand, virus counts overshoot during transient phase in mathematical model. We think this difference is derived from technicality of different modeling. The difference in concepts between mathematical models and agent-based models is the space. The mathematical model has no space in concept, but agents move across the space in the agent-based model. In agent-based models, the densities of virus and cells change overtime especially in the transition phase because of the limited space. These changes of the densities of virus and cells lead to the dynamic change of the encounter rate of viruses and cells. The mathematical model does not make such concept of the density; the encounter rate is constant. This may be the reason for the difference between two models in the transition phase. Since no overshoot of virus counts in transient phase had been reported from in vivo studies of hepatitis C virus and simian immunodeficiency virus (Dahari et al., 2005; Nowak et al., 1997), agent-based model correlates with actual biology in vivo at least for these viruses. The increase of initial virus count at the start of simulation correlates with higher encounter rate of viruses and cells which make the linear increasing of infection forming rate. Mathematical model can only express the infection formation rate as "infected or not".

The importance of viral passing speed in the agent-based model is also explained by the "space". Although the virus actually moves through the blood stream in our body and virus could not decide their moving speeds by themselves, there is most appropriate speed for virus to meet the cells on the simulation space by the highest probability. The effect of cell cycle speed should be mentioned by another affection of the space. A fast cell cycle speed means that the lifespan of uninfected cells is short. Then fast cell cycle speed leads to the short lifespan of infected cells. A higher regeneration rate for uninfected cells results in a higher rate of infection among uninfected cells by viruses, but in situations where viruses and cells are dispersed around the space this is ineffective in increasing the infection rate, as the latter depends on the probability that they will encounter one another. As a result, the infected cell count decreases during the equilibrium phase, as does the virus count.

In this study, we confirmed the reproducibility and usability of agent-based models in expressing the interaction between viruses and cells. A feature of this simulation system is that it uses the concept of space as actual space, which means that the existence of the space becomes an additional controlling factor on the simulation results. This is a concept that is absent from mathematical models. The reality is that we have a spatial existence, and an advantage of the agent-based simulation system is the fact that it accounts for the space. Another feature of the simulation system is that it enables the condition to be perceived in visual terms, making it easy to understand. However it may be affected by computer performance and by the limitations of programming languages or the program itself, this system may offer a powerful tool for the future analysis of real virus–host interaction disease.

Conflict of interest

No conflicts of interest exist for all authors.

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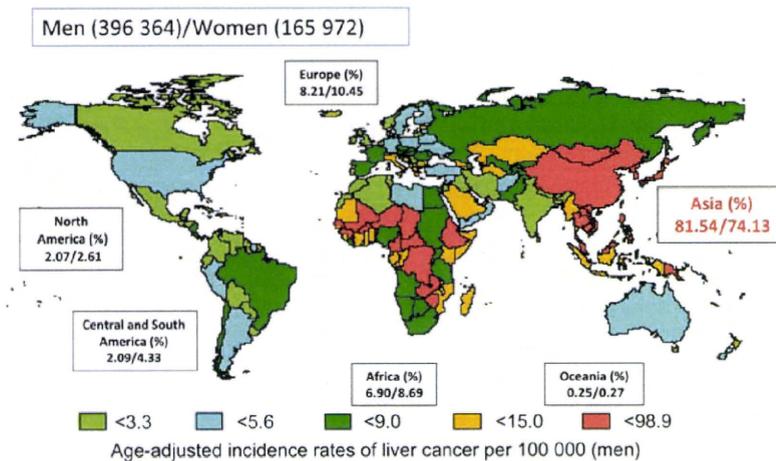
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Figure 1. Liver cancer in the world (Curado et al. IARC Press, 2010).

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, ≤ 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.

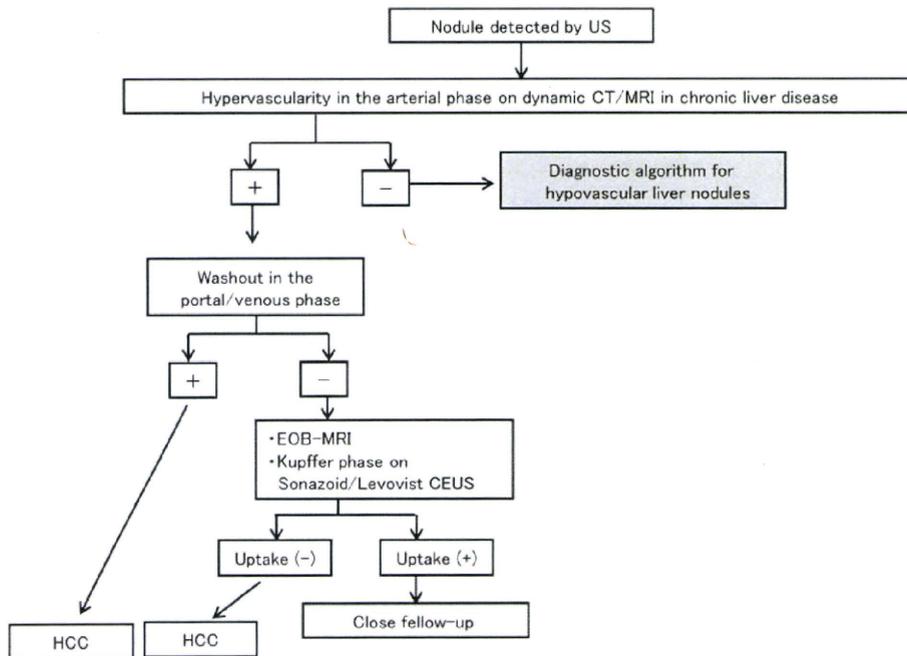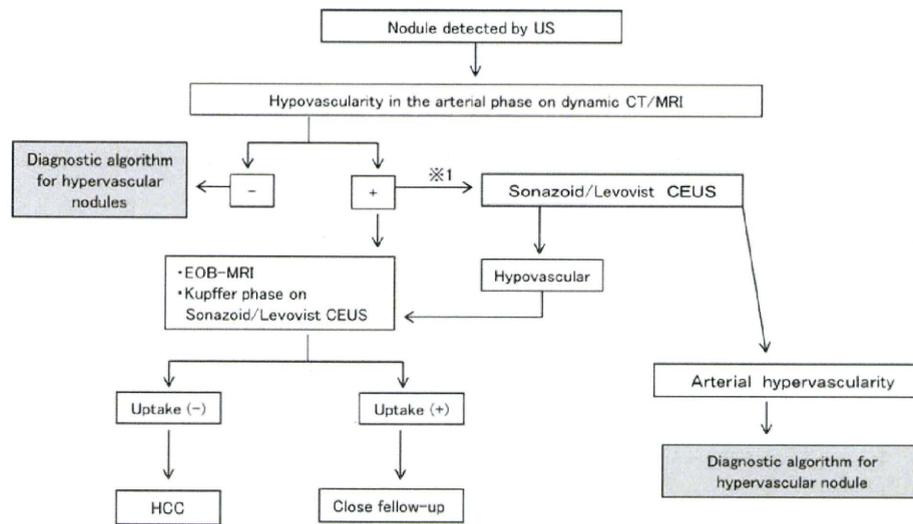In conclusion, various clinical practice guidelines including diagnostic algorithm for HCC have been established and

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 multi-step 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 pre-cancerous 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 over-diagnosis 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

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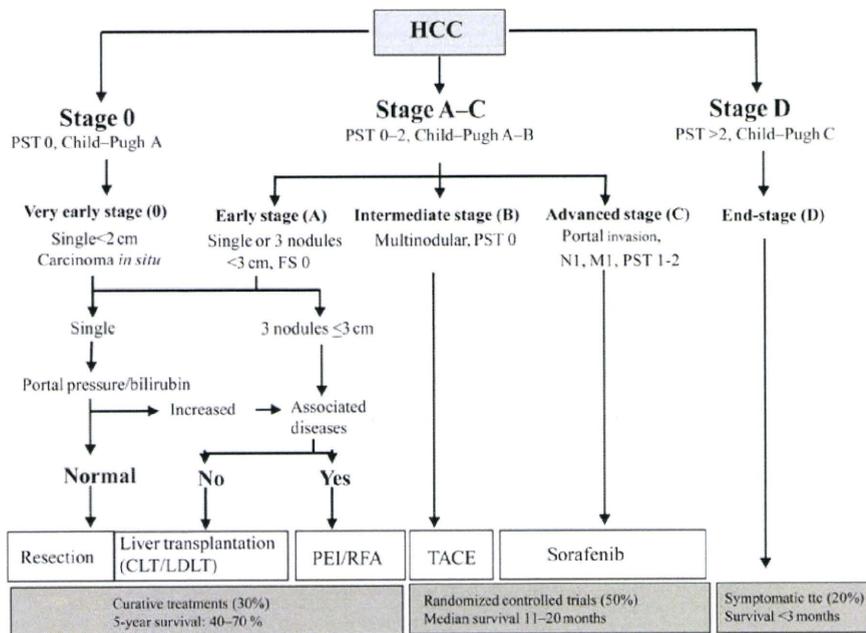
Figure 4. BCLC staging [Llovet et al. (10)]. BCLC, Barcelona Clinic of Liver Cancer; PST, performance status; CLT, cadaveric liver transplantation; LDLT, living donor liver transplantation; PEI, percutaneous ethanol injection; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization.
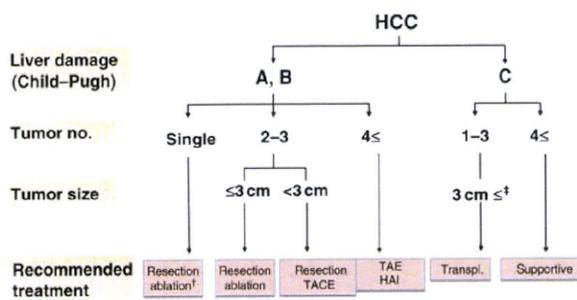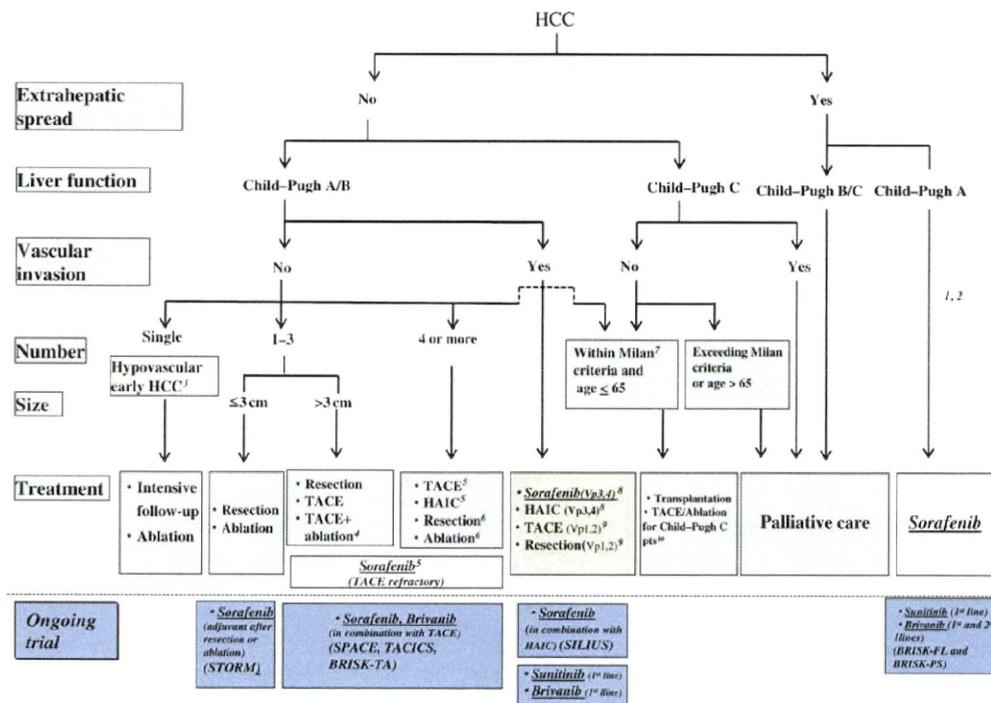

Figure 5. EBM-based algorithm for HCC treatment (J-HCC Guidelines 2009). Resection or transarterial chemoembolization (TACE) may be selected for liver damage A patients with vascular invasion. Chemotherapy may be selected for extrahepatic HCC. LT is only for ≤65 years old. †Recommended for Child B; ‡<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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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Factors predictive of sustained virological response following 72 weeks of combination therapy for genotype 1b hepatitis C

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Abstract

Background Treatment of genotype 1b chronic hepatitis C virus (HCV) infection has been improved by extending peg-interferon plus ribavirin combination therapy to 72 weeks, but predictive factors are needed to identify those patients who are likely to respond to long-term therapy.

Methods We analyzed amino acid (aa) substitutions in the core protein and the interferon sensitivity determining region (ISDR) of nonstructural protein (NS) 5A in 840 genotype 1b chronic hepatitis C patients with high viral load. We used logistic regression and classification and regression tree (CART) analysis to identify predictive factors for sustained virological response (SVR) for patients undergoing 72 weeks of treatment.

Results When patients were separately analyzed by treatment duration using multivariate logistic regression, several factors, including sex, age, viral load, and core aa70 and ISDR substitutions ($P = 0.0003$, $P = 0.02$, $P = 0.01$, $P = 0.0001$, and $P = 0.0004$, respectively) were significant predictive factors for SVR with 48 weeks of treatment, whereas age, previous interferon treatment history, and ISDR substitutions ($P = 0.03$, $P = 0.01$, and $P = 0.02$,

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respectively) were the only significant predictive factors with 72 weeks of treatment. Using CART analysis, a decision tree was generated that identified age, cholesterol, sex, treatment length, and aa70 and ISDR substitutions as the most important predictive factors. The CART model had a sensitivity of 69.2% and specificity of 60%, with a positive predictive value of 68.4%.

Conclusions Complementary statistical and data mining approaches were used to identify a subgroup of patients likely to benefit from 72 weeks of therapy.

Keywords CART analysis · Core protein · Decision tree · ISDR · LDL cholesterol

Abbreviations
HCV   Hepatitis C virus
ISDR   Interferon sensitivity determining region
CART   Classification and regression tree analysis
SVR   Sustained virological response
NR   Non-viral response

Introduction

Chronic hepatitis C virus (HCV) infection is a major global cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma [1–3]. The treatment of chronic hepatitis C has improved with the advent of peg-interferon (IFN) plus ribavirin combination therapy [4–7], but fewer than half of the patients with high viral loads of genotype 1b show a sustained virological response (SVR), defined as testing

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negative for HCV RNA 24 weeks after cessation of the therapy. To overcome this limitation, recent therapeutic regimens have extended the treatment period to 72 weeks [8–11]. This extension is especially effective in patients whose HCV RNA declines relatively slowly [9–11]. Accordingly, recent treatment protocols have recommended extending the treatment period to 72 weeks in patients who become negative for HCV RNA after 12 weeks of treatment but before 24 weeks [10, 11]. This response-guided decision-making approach to therapy has resulted in improvements of the SVR rate [10, 11]. Following this approach, patients with a non-viral response (NR), i.e., patients who show very poor response to the therapy (defined as less than 2-log decline of HCV RNA during 12 weeks of treatment), should be advised to discontinue therapy because SVR is rare in such patients. While response-guided therapy is useful in determining the appropriate duration of treatment for patients who are likely to respond eventually, predictors that can be assessed before the start of therapy will aid in differentiating which difficult-to-treat patients are likely to achieve an SVR with extended therapy and which may be better served by considering alternative therapy options.

To predict NR, recent studies recommend analysis of amino acid (aa) substitutions in the HCV core protein at positions 70 and 91 [12, 13]. The substitution of arginine with glutamine or other amino acids at core protein aa 70 has been reported to be associated with NR, and this finding was confirmed by several other groups [14–16]. Analysis of core aa 70 has also been shown to be useful to predict the outcome of 72 weeks of combination therapy [17]. While many factors have been reported to be useful predictors of the effect of combination therapy [18–26], many of these factors are mutually interdependent. Furthermore, because almost all of these factors have been reported under conditions in which a majority of patients were receiving 48 weeks of treatment, it is necessary to consider the effect of the treatment period.

In this study, we compiled a database of clinical data from 840 patients from 16 national centers in Japan. We used logistic regression and classification and regression tree analysis (CART) to identify factors predictive of SVR for 48- and 72-week therapy and to assess which patients are most likely to benefit by long-term 72-week therapy.

Methods

Study subjects

In this retrospective study, data from 840 patients with chronic hepatitis C treated at 16 different hospitals in Japan were analyzed for predictive factors for SVR based on

Table 1 Patient characteristics for 48- and 72-week treatments

	All patients ($n = 840$)	48-Week therapy ($n = 619$) 73.69%	72-Week therapy ($n = 221$) 25.12%
Age (years)	54.4 ± 10.73	53.8 ± 11.21	56.2 ± 9.03
Gender (male/female)	449/391	357/262	92/129
Body weight (kg)	60.9 ± 10.8	61.3 ± 10.6	59.8 ± 11.4
Height (cm)	162.2 ± 9.1	162.7 ± 9.1	160.7 ± 9.0
BMI	23.0 ± 3.05	23.0 ± 2.92	23.0 ± 3.4
HCV core protein aa 70 (wild/mutant)	539/301	396/223	143/78
HCV core protein aa 91 (wild/mutant)	504/336	369/250	135/86
ISDR (0–1/≥2)	714/126	513/106	201/20
Hypertension (present/absent/ND)	538/113/189	395/78/146	143/35/43
Diabetes (present/absent/ND)	634/47/159	457/38/124	177/9/35
Transfusion (present/absent/ND)	505/227/108	379/162/78	126/65/30
Fibrosis stage (0–2/3–4/ND)	604/128/108	448/90/81	156/38/27
Activity stage (0–1/2–3/ND)	382/343/115	287/245/87	95/98/28
Steatosis (present/absent/ND)	158/344/338	119/250/250	39/94/88
AST (IU/l)	65 ± 49	66 ± 47	63 ± 53
ALT (IU/l)	68 ± 56	68 ± 56	66 ± 55
White blood cell count (/mm^3)	4832 ± 1455	4882 ± 1488	4693 ± 1352
Hemoglobin (g/dl)	14.2 ± 1.36	14.3 ± 1.39	14.1 ± 1.29
Platelets ($\times 10^4$/mm^3)	16.9 ± 5.18	17.0 ± 5.11	16.8 ± 5.35
γGTP (IU/l)	56 ± 59	59 ± 64	49 ± 42
Albumin (g/dl)	4.02 ± 0.348	4.01 ± 0.350	4.03 ± 0.343
Uric acid (mg/dl)	5.41 ± 1.29	5.46 ± 1.27	5.25 ± 1.35
Iron (μg/dl)	147.0 ± 69.65	151.0 ± 75.71	136.1 ± 47.45
Ferritin (μg/l)	173.9 ± 167.9	181.7 ± 175.7	153.0 ± 143.7
Fasting blood sugar (mg/dl)	99.8 ± 19.8	99.3 ± 19.1	101.2 ± 21.5
Alpha-fetoprotein (μg/l)	16.3 ± 50.4	14.2 ± 44.8	22.0 ± 62.7
Total cholesterol (mg/dl)	175 ± 32.3	173 ± 31.8	179 ± 33.4
LDL cholesterol (mg/dl)	100.8 ± 29.8	100.2 ± 30.3	102.5 ± 28.4
HDL cholesterol (mg/dl)	52.1 ± 15.5	51.4 ± 15.0	53.9 ± 16.6
Triglycerides (mg/dl)	103.2 ± 48.8	103.8 ± 46.1	101.7 ± 55.1
HCV-RNA (KIU/ml)	3239 ± 4669	3170 ± 4828	3427 ± 4205
Response to treatment (SVR/TR/NR)	465/246/129	341/164/114	124/82/15

BMI body mass index, *HCV* hepatitis C virus, *aa* amino acid, *ISDR* interferon sensitivity determining region, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *γGTP* γ-glutamyl transpeptidase, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *SVR* sustained virological response, *TR* transient response/relapsers, *NR* non-viral response, *ND* not determined

treatment duration. Inclusion criteria included testing positive for HCV RNA for longer than 6 months and testing negative for both hepatitis B virus surface antigen and anti-HIV antibody. Patients with confounding conditions such as hemochromatosis, Wilson's disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease were excluded. We excluded patients who were lost for follow up and those who did not show a high level of viremia for genotype 1b, as well as patients for whom we failed to determine both core and IFN sensitivity determining region (ISDR) of nonstructural protein (NS) 5A sequences; 385 patients were treatment-naïve. All

subjects gave their written informed consent to participate in the study according to the process approved by the ethics committee of each hospital and conforming to the ethical guidelines of the 1975 Declaration of Helsinki. Patient profiles are listed in Table 1.

All patients initially received weekly injections of peg-IFN-alpha-2b for 48 weeks (60 μg for body weight (BW) 35–45 kg, 80 μg for BW 46–60 kg, 100 μg for BW 61–75 kg, 120 μg for BW 76–90 kg, and 150 μg for BW 91–120 kg). Ribavirin was administered orally, and the dosage was determined based on the patient's BW (600 mg for <60 kg, 800 mg for 60–80 kg, and 1,000 mg

for >80 kg). Ribavirin dosage was reduced when hemoglobin levels were reduced to 10.0 g/dl and stopped if hemoglobin levels reached 8.5 g/dl. Successful treatment was ascertained based on SVR, defined as HCV RNA-negative 6 months after cessation of therapy. Using response-guided therapy, slow viral responders, i.e., patients for whom HCV RNA levels became negative after 12 weeks of therapy but before 24 weeks, and some non-responders were recommended for extension of therapy to 72 weeks.

Biochemical tests were performed at the individual hospitals, and pathological diagnosis was made by pathologists in each hospital according to the criteria of Desmet et al. [27]. Fibrosis and activity data were compared among hospitals to ensure that there were no systematic differences.

Analysis of viral titer and amino acid sequences in the core and ISDR region

The HCV RNA level was analyzed using reverse transcription polymerase chain reaction (RT-PCR)-based methods (Amplicor™ high-range test; Roche Diagnostics, Basel, Switzerland, or TaqMan RT-PCR test; Applied Biosystems, CA). The measurement ranges of these assays were 5–5000 KIU/ml and 1.2–7.8 log IU/ml, respectively. For values exceeding the measurable range, the limit value was used as an approximation. The values obtained by the Amplicor test were converted to logarithmic values [28].

Nucleotide and amino acid sequences of the core and the ISDR region were determined by direct sequencing of cDNA fragments amplified by PCR. Arginine and leucine were considered wild-type for core protein aa 70 and aa 91, respectively [12, 13]. The number of aa substitutions in the ISDR was determined by comparison with the reference sequence reported by Kato et al. [29] using the method of Enomoto et al. [30, 31].

Statistical analysis

Statistical analysis was performed using the R software package (http://www.r-project.org). The χ^2 or Fisher's exact and Mann–Whitney U-tests were used to detect significant associations. All statistical analyses were two-sided, and $P < 0.05$ was considered significant. Simple and multiple logistic regression analyses were used to examine the association between viral substitutions and clinical factors, using $P < 0.05$ as the criterion for inclusion in the initial multivariate model. Multivariate logistic regression analysis was performed using forward/backward stepwise selection based on the akaike information criterion (AIC) score and validated by bootstrapping, using the rms

package in R. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for each factor.

CART analysis

CART analysis was used to generate a decision tree by classifying patients by SVR, based on a recursive partitioning algorithm with minimal cost-complexity pruning to identify optimal classification factors. The SimpleCart classifier in the WEKA data mining package [32] was used with a minimal terminal node size of 4 and trained with the variables listed in Table 1. Performance was assessed using tenfold cross-validation, and the sensitivity, specificity, and precision of the model were calculated. Receiver operating characteristic (ROC) curves were generated and results were compared with the logistic regression model.

Results

Patient characteristics

Patients were partitioned into two groups based on whether they received 48 or 72 weeks of therapy (Table 1). In this study 465 patients achieved an SVR, whereas 375 patients were either non-responders or relapsers, yielding an overall SVR rate of 55.4%. The rate of SVR did not differ significantly between the 48- and 72-week treatment groups (55.3 vs. 56.4%, respectively; $P = 0.81$), but the NR rate was significantly lower in patients who were treated for 72 weeks (18.3 vs. 6.4%; $P = 9.3 \times 10^{-6}$).

Predictive factors for SVR

The association between SVR and individual clinical factors was assessed using logistic regression. A number of factors were significant at the $P < 0.05$ level, including age, sex, viral load, aa70/ISDR substitutions, hypertension, fibrosis, steatosis, prior IFN treatment, low-density lipoprotein (LDL) cholesterol, total cholesterol, white blood cell count, platelet count, hemoglobin, γ-glutamyl transpeptidase (γGTP), and albumin (Table 2). On multivariate logistic regression, only age, sex, core aa70, ISDR, LDL, and γGTP were identified as significant independent predictors of SVR. Although length of treatment was not identified as a significant predictor in this analysis, exploratory analysis suggests the presence of potential interactions between treatment length and age and/or sex that are not captured by the first-order terms in the model. When second-order terms were selected a posteriori, however, a significant interaction was found between sex and treatment length ($P = 0.0034$). When analyzed separately, independent predictive factors for SVR for 48 weeks

Table 2 Factors associated with sustained virological response to combination therapy

Variable	Simple			Multiple			
	n	OR	P	n	OR	(95% CI)	P
Age	840	0.393	3.16×10^{-11}***	517	0.386	(0.27–0.56)	5.08×10^{-7}***
Sex (male vs. female)	840	0.521	3.61×10^{-6}***	517	0.52	(0.35–0.78)	0.001459**
BMI (kg/m^2)	834	0.8	0.1094				
Viral load (Log IU/ml)	840	0.761	0.001828**				
Core aa70 substitution	840	0.537	1.98×10^{-5}***	517	0.507	(0.35–0.74)	0.000521***
Core aa91 substitution	840	0.818	0.1568				
ISDR (0–1 vs. $\geq$2)	840	2.36	5.19×10^{-5}***	517	2.12	(1.19–3.77)	0.01037*
Hypertension	651	0.625	0.02389*				
Diabetes	681	0.794	0.4464				
Blood transfusion	732	1	0.9788				
Fibrosis (F0–1 vs. F2–4)	732	0.674	0.008287**				
Activity (A0–1 vs. A2–4)	725	0.779	0.09567				
Steatosis	502	0.645	0.03413*				
Prior IFN treatment	830	1.37	0.02648*				
HDL cholesterol (mg/dl)	493	0.761	0.1333				
LDL cholesterol (mg/dl)	529	1.46	0.03223*	517	1.61	(1.10–2.38)	0.01521*
Triglyceride (mg/dl)	726	0.913	0.5412				
Total cholesterol (mg/dl)	814	1.25	0.11				
AST (IU/l)	783	0.933	0.6316				
ALT (IU/l)	840	0.972	0.837				
WBC (/mm^3)	836	1.55	0.001831**				
Hemoglobin (g/dl)	838	1.34	0.00276**				
Platelets ($\times 10^4$/mm^3)	838	1.74	7.92×10^{-5}***				
Gamma-GTP (IU/l)	823	0.735	0.0288*	517	0.656	(0.43–0.99)	0.04588*
Albumin (g/dl)	809	1.41	0.01699*				
Ferritin (µg/l)	532	0.898	0.5404				
Treatment period (weeks)	840	1.02	0.6095				

Simple and multiple logistic regression was used to examine the association between SVR and patient and viral factors. Factors with $P < 0.05$ were considered for inclusion in the multiple regression model and the best model selected by backwards stepwise selection using AIC

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

IFN interferon, *OR* odds ratio, *CI* confidence interval, *AIC* akaike information criterion

of treatment included age, sex, viral load, core aa70, LDL, platelets, and white blood cell counts, whereas for 72 weeks of treatment only age, ISDR, and prior IFN treatment were significant, although LDL cholesterol was marginally significant (Table 3).

Among patients who underwent 48 weeks of therapy, 61% of patients with core aa 70 wild-type achieved an SVR compared to only 44% of patients with mutant core aa 70 ($P = 1.8 \times 10^{-5}$, Fig. 1a), whereas for 72-week patients, the ratio was 1:1 (Fig. 3a). Conversely, in the 48-week group, 71% of patients with two or more mutations in the ISDR were able to achieve an SVR compared to 52% with the wild-type ISDR, and in the 72-week group (Fig. 1b), 80% of patients with two or more ISDR mutations achieved an SVR compared to 54% with zero or one ISDR mutations (Fig. 3b). Median baseline viral load was significantly lower in 48-week SVR patients compared to that in non-SVR patients ($P = 0.001$, Fig. 1c), whereas there was no significant difference between viral load and SVR in 72-week therapy patients ($P = 0.625$, Fig. 4c). There was a significant effect of age and treatment outcome among 48-week patients ($P = 9.3 \times 10^{-6}$, Fig. 2), but the difference was not significant among 72-week therapy patients. However, the proportion of patients achieving an SVR tended to decrease with age in both groups, particularly in females over age 70 years in the 72-week group (Figs. 2, 4).

Table 3 Independent factors associated with sustained virological response to 48- and 72-week peg-interferon plus ribavirin combination therapy

Variable	48 Weeks			72 Weeks			
	n	OR	P	n	OR	(95% CI)	P
Age	535	0.642	0.0165*	133	0.4	(0.176–0.91)	0.02877*
Sex (male vs. female)	535	0.481	0.000284**				
Viral load (Log IU/ml)	535	0.738	0.01033*				
Core aa70 substitution	535	0.454	9.95×10^{-5}**				
ISDR (0–1 vs. ≥2)	535	2.75	0.000358**	133	7	(1.35–36.2)	0.02047*
Fibrosis (F0–1 vs. F2–4)	535	0.66	0.03954*				
Prior IFN treatment				133	2.67	(1.22–5.85)	0.01431*
LDL cholesterol (mg/dl)				133	2.04	(0.952–4.35)	0.06673
WBC (/mm³)	535	1.53	0.03342*				
Platelets ($\times 10^4$/mm³)	535	1.54	0.03707*				

Simple and multiple logistic regression analysis was used to examine the association between SVR and patient/viral factors separately for patients receiving 48 and 72 weeks of treatment

** $P < 0.001$, * $P < 0.05$

Fig. 1 Viral factors for 48-week treatment. Relationships between sustained virological response (*SVR*) and **a** core amino acid 70 substitutions, **b** amino acid substitutions in the interferon sensitivity determining region, and **c** baseline viral titers grouped by SVR and non-SVR for patients treated for 48 weeks. *PCR* Polymerase chain reaction
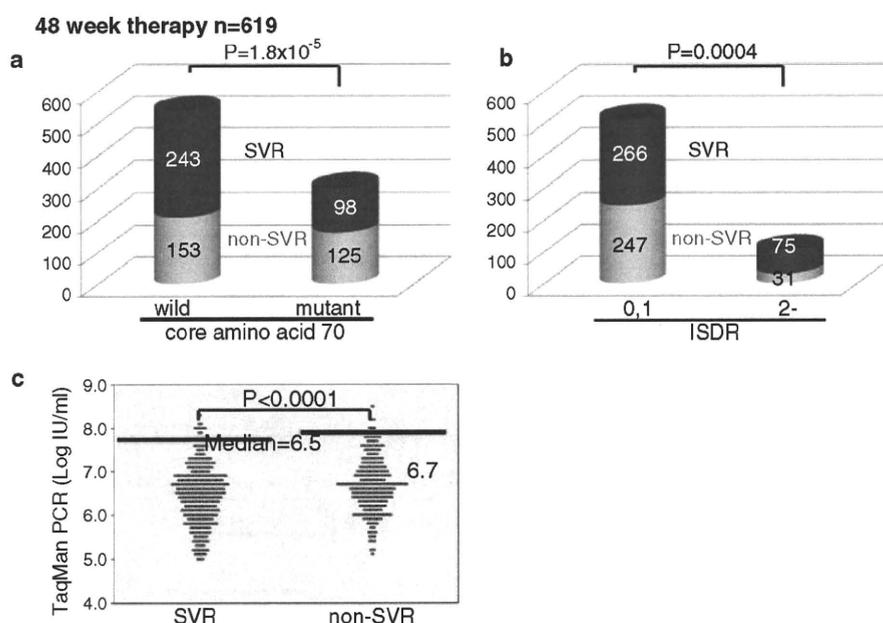
CART analysis

Figure 5 shows the decision tree generated by CART analysis. All variables were included during model construction, and the SimpleCart algorithm generated a tree based on the following fields: age, cholesterol, sex, γGTP, 48 versus 72 weeks of treatment, and aa substitutions in the ISDR and at core aa70. Age was used as the first cutoff, and patients younger than 46.5 years were classified as having a high probability for SVR (78%). Total cholesterol was identified as the next decision point, and patients with cholesterol higher than 211.5 mg/dl were

classified as SVR if they were younger than 62.5 years (84%) and NR (65%) otherwise. Patients with cholesterol lower than 211.5 mg/dl were subdivided next by sex. Females who received 48 weeks of treatment were classified as NR (71%), whereas females receiving 72 weeks of treatment were classified as SVR if they were younger than 58.5 years (71%) or NR otherwise (64%). Males who were infected with aa70 wild-type were classified as SVR (62%), whereas males with aa70 substitutions were classified as NR if total cholesterol was less than 130 mg/dl (97%). Males with ISDR substitutions were classified as SVR (75%), and those with wild-type ISDR were classified

Fig. 2 Relationship between age and response to treatment for 48-week therapy. Treatment outcomes by age in 10-year intervals are shown for **a** all patients, **b** males only, and **c** females only. *NR* non-viral response

48-week therapy n=619

	SVR	TR	NR	total
male (age >50)	125	45	46	216
female (age >50)	79	86	47	212
total	204	131	93	428

$P=9.3\times10^{-6}$

Fig. 3 Viral factors for 72-week treatment. Relationships between sustained virological response and **a** core amino acid 70 substitutions, **b** amino acid substitutions in the interferon sensitivity determining region, and **c** baseline viral titers grouped by SVR and non-SVR for patients treated for 72 weeks. *n.s.*, Not significant
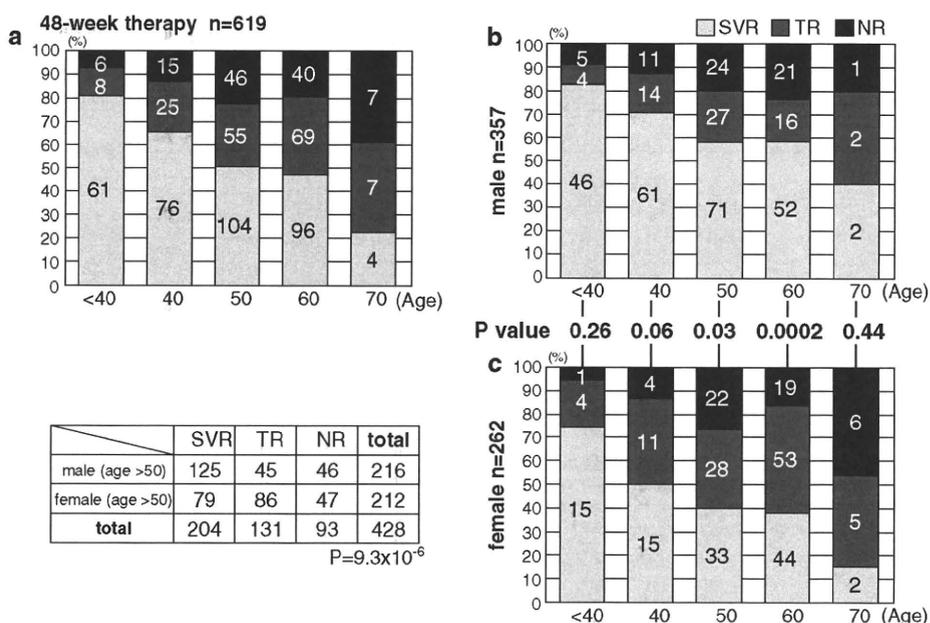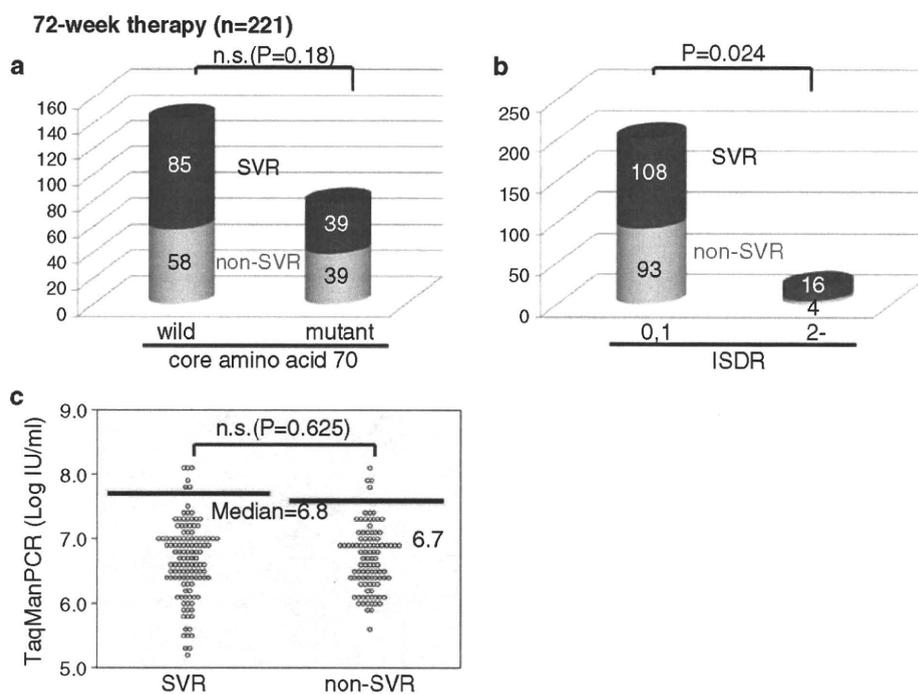

as SVR if γGTP was less than 48.5 IU/l (57%) and NR otherwise (77%).

All factors selected during tree construction were found to be significant in univariate analysis, except for treatment length and cholesterol, and each remained significant in multivariate logistic regression. Although LDL was included in the multivariate logistic model, it was not selected during tree construction. Tenfold cross-validation resulted in 65.2% correctly classified instances with a kappa statistic of 0.29. The true positive rate was 69.2%, the false positive rate was 39.7%, and precision was 68.4%.

To compare the performance of SVR prediction between the logistic and CART models, the WEKA Logistic classifier was used to perform tenfold validation based on the

Fig. 4 Relationship between age and response to treatment for 72-week therapy. Treatment outcomes by age in 10-year intervals are shown for **a** all patients, **b** males only, and **c** females only

	SVR	TR	NR	total
male (age >50)	36	30	4	70
female (age >50)	62	43	6	111
total	98	73	10	181

n.s.(P=0.842)

Fig. 5 Decision tree for SVR prediction. *Boxes* represent branch points based on cutoff values for factors determined by the tree generation algorithm. Each branch contains two choices, and each path ends in a prediction for either SVR or NR with an associated probability. *yrs* Years, *Tx* treatment, *ISDR* interferon sensitivity determining region, *aa* amino acid, *WT* wild-type, *γGTP* γ-glutamyl transpeptidase

multivariate logistic regression model above. The true positive rate for the logistic classifier was somewhat higher, at 73.1%, but with a slightly worse false-positive rate of 48%, and 63.7% correctly classified instances with a kappa statistic of 0.25 and precision 0.65. Receiver operating characteristic (ROC) curves were very similar, and the area under the curve was 0.677 for the CART model and 0.696 for the logistic model.

Discussion

Using two complementary approaches we identified several pretreatment factors predictive for SVR in patients treated for 48 and 72 weeks. Logistic regression and CART analysis both suggest that sex, age, cholesterol, and substitutions at core aa70 and ISDR are associated with SVR in patients with a high viral load of genotype 1b. Based on