After identifying HIV/HBV-coinfected patients, medical records including laboratory data of these patients were reviewed between the date of the oldest available record for these patients and the final date of the record acquired by the end of the study. The laboratory data at the diagnosis or first recognition of HBV infection and the latest data in the study period were compared for analysis unless otherwise noted. HBV genotypes (A through D) were determined serologically by enzyme immunoassay (EIA) using commercial kits (HBV GENOTYPE EIA; Institute of Immunology, Tokyo, Japan) on the basis of the pattern of detection using monoclonal antibodies of a combination of epitopes on preS2-region products, each of which was specific for each genotype [22, 23].

### Ethical issues

The respective ethics committees of the six hospitals approved the study. Informed consent was obtained from each study participant.

### Statistical analyses

For the comparison of means of collected data, Student's *t* test (paired *t* test) was performed unless otherwise specified. The chi-square test was performed to determine the independence of clinical parameters.

## Results

Two hundred and fifty-two patients were identified to have HIV/HBV coinfection. The mean age was 39.5 years, and the proportion of male patients was very high (243 of 252; 96.4 %). The main presumed transmission route of HIV was male homosexual contact (186 of 252; 73.8 %), followed by heterosexual contact. Among those HIV/HBV-coinfected patients, 21 of the 252 (8.3 %) acquired acute hepatitis during the study period (Table 1).

**Table 1** Clinical background of HIV/HBV-coinfected patients

| | |
|---|---|
| Number (male:female) | 243:9 |
| Age (year) | 39.5 ± 9.6[a] |
| Presumed Transmission Route | |
| Transfusion | 14 |
| Homosexual contact | 186 |
| Heterosexual contact | 24 |
| Injection drug use | 2 |
| Others | 4 |
| Onset as acute hepatitis | 21 |

[a] Mean ± standard deviation

The HBV genotype was determined in 77 patients. Among them, genotype A HBV was the most frequent (58 of 77; 75.3 %), followed far behind by genotype C (7 of 77; 9.1 %), which is the predominant genotype in the entire chronic hepatitis B population in Japan. Genotype B, which is also common in Japan, was found only in three patients (3.9 %). Genotype A was detected almost exclusively in homosexual patients (57 of 58; 98.3 %) (Fig. 1).

At the end of the study period, 113 patients (44.8 %) received some type of anti-HBV drug such as interferon, lamivudine, adefovir, or entecavir, not as part of anti-HIV treatment. Ninety-seven (38.5 %) patients were still taking anti-HBV drugs by the end of the study period. The median ALT level was 30.0 IU/l (5th percentile, 11.1; 95th percentile, 128.9), suggesting the existence of some liver injury. Liver function was normal in most HIV/HBV-coinfected patients. The mean serum albumin level was 4.1 ± 0.6 g/dl, and the median serum total bilirubin level was 0.8 mg/dl (5th percentile, 0.3; 95th percentile, 3.8). The mean platelet count was 21.0 ± 6.1 × $10^4$/ml. The hepatitis B e antigen (HBeAg) was detected in 84 patients, and the HBV DNA level was high (higher than 100,000 IU/l) in 55 patients (Table 2). Three of the 252 (1.1 %) HIV/HBV-coinfected patients developed advanced chronic liver diseases, such as cirrhosis with the complication of ascites and/or hepatic encephalopathy, or hepatocellular carcinoma. Although we tried to retrieve information on alcohol consumption of the patients, it was available for only a limited number of patients (26 of 252); among the 26, only 2 patients had a habit of taking more than 60 g alcohol per day. The remaining 24 patients took alcohol only on social occasions. The antiretroviral agents used for these study patients are listed in detail in Table 3. Among those who had a known history of ART, 158 of 252 (62.7 %) received regimens that include anti-HBV drugs at least once previously, whereas 42 (16.7 %) did not, and no information is available for the remaining 52. The most common drug combination for HIV/HBV-coinfected patients was ATV/r + FTC/TDF (22 of 172; 12.8 %) (Table 4). FTC/TDF, composed of two drugs active against HBV, is recommended for HIV/HBV-coinfected patients

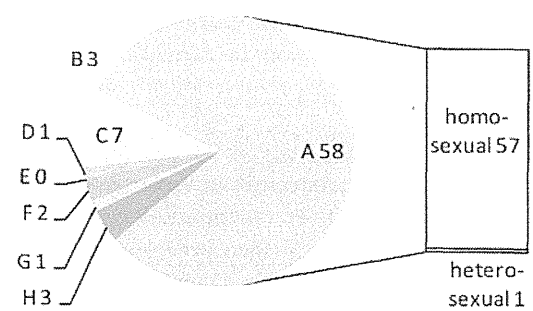


**Fig. 1** Hepatitis B virus (HBV) genotype

**Table 2** Liver function and related parameters of HIV/HBV-coinfected patients

| | |
|---|---|
| Albumin (g/dl) | 4.1 ± 0.6 |
| Bilirubin[a] (mg/dl) | 0.8 (5th percentile, 0.3; 95th percentile, 3.8) |
| ALT[a] (IU/l) | 30.0 (5th percentile, 11.1; 95th percentile, 128.9) |
| WBC ($\times 10^3/\mu l$) | 5.2 ± 1.6 |
| Platelet ($\times 10^4/\mu l$) | 21.0 ± 6.1 |
| HBeAg (positive:negative) | 84:68 |
| HBV DNA (high:low)[b] | 55:127 |

[a] Median and percentiles are provided instead of mean and standard deviation because of the nonnormality of the distribution

[b] HBV DNA level of 100,000 IU/l or higher is categorized as "high"

as one of the preferred NRTI backbones of the ART regimen [2–4].

We compared the clinical characteristics between patients who received the full ART and those who did not. Regarding the baseline statistical data, the observation period was longer for patients on ART, and there were more patients with AIDS in the ART group (10 of 64 vs. 52 of 162) (Table 5a). No significant difference was observed between the non-ART and ART groups in male/female ratio, age, transmission route, HBV markers, or advanced liver disease. Liver-related death was not observed, but hepatic failure with ascites and/or hepatic encephalopathy developed in 2 patients on ART and hepatocellular carcinoma developed in another patient.

Comparison between the ART group and the non-ART group revealed that the baseline liver function was worse in the ART group. At the beginning of the study period, the ART group showed a significantly lower CD4+ T-cell count than the non-ART group. The total white blood cell count and platelet count were also lower in the ART group. Although it is not statistically significant, the serum albumin level and prothrombin time (PT) index were lower in the ART group. However, at the end of the observation period, these parameters improved significantly in the ART group. The difference in CD4+ T-cell count between the ART and non-ART groups became marginal and became statistically insignificant (Table 5b).

Changes in the liver function of HIV/HBV-coinfected patients may not be fully explained by the changes in HBV activity because some parameters relevant to the estimation of liver function showed paradoxical changes. To clarify this observation, we compared the changes in liver function among HIV/HBV-coinfected patients on ART with respect to protease inhibitor (PI) use.

The mean serum total bilirubin level in patients on ART with PI use (PI group) at the beginning of the observation period was 1.1 mg/dl, whereas that in patients without PI use (non-PI group) was 0.8 mg/dl. The means at the end of the study period were 1.6 mg/dl in the PI group and 0.7 mg/dl in the non-PI group. Because the sample distribution of serum total bilirubin level did not follow the normal distribution by logarithmic transformation, we compared the means statistically. At the beginning, the difference in the mean between the PI group and the non-PI group was not significant ($p = 0.257$). At the end of the observation period, a statistically significant difference ($p = 0.001$) was observed. We then calculated the

**Table 3** Antiretroviral treatment of HIV/HBV-coinfected patients

| Antiretroviral drugs | Number of patients |
|---|---|
| NRTIs | |
| Zidovudine (AZT) | 34 |
| Didanosine (ddI) | 9 |
| Ddl / enteric coated | 7 |
| Zalcitabine (ddC) | 1 |
| Stavudine (d4T) | 4 |
| Lamivudine[a] (3TC) | 84 |
| Abacavir[3] (ABC) | 38 |
| Tenofovir[3] (TDF) | 27 |
| Emtricitabine (FTC) / TDF[a] | 57 |
| NNRTIs | |
| Nevirapine (NVP) | 10 |
| Efavirenz (EFV) | 34 |
| Delavirdine (DLV) | 1 |
| PIs | |
| Indinavir (IDV) | 4 |
| Ritonavir (RTV) | 50 |
| Nelfinavir (NFV) | 8 |
| Lopinavir (LPV) | 3 |
| Ritonavir-boosted LPV (LPV/r) | 40 |
| Atazanavir (ATV) | 39 |
| ATV/r | 6 |
| Fosamprenavir (FPV) | 13 |

*NRTI* nucleoside reverse transcriptase inhibitor, *NNRTI* non-nucleoside reverse transcriptase inhibitor, *PI* protease inhibitor

[a] Agents with anti-HBV activity

**Table 4** Antiretroviral regimens used for HIV/HBV-coinfected patients

| Antiretroviral regimen | Number of patients |
|---|---|
| ATV/r + FTC/TDF | 22 |
| LPV/r + 3TC + TDF | 8 |
| LPV/r + FTC/TDF | 7 |
| EFV + FTC/TDF | 6 |
| ATV/r + 3TC + TDF | 5 |

**Table 5** Comparison of changes in clinical parameters of HIV/HBV-coinfected patients with or without antiretroviral therapy (ART)

a. Baseline statistical data

| | Natural course[a] (without ART) | With ART | *p* value (with vs. without ART) |
|---|---|---|---|
| Number (male:female) | 84:6 | 159:3 | 0.105[†] |
| Age (year) | 37.0 ± 10.3 | 39.0 ± 9.1 | 0.362 |
| Observation period (month) | 34.5 ± 55.5 | 50.9 ± 43.9 | 0.022* |
| Presumed transmission route | Blood products:homosexual contact:heterosexual contact:injection drug use:other | | |
| | 5:60:12:2:3 | 9:126:12:0:1 | 0.052[†] |
| Recognized acute hepatitis | 10 | 11 | 0.243[†] |
| HBeAg (positive:negative) | 42:18 | 100:40 | 0.394[†] |
| HBV DNA (high:low) | 29:18 | 83:37 | 0.356[†] |
| HBV genotype | A:B:C:D:F:G:H | | |
| | 17:0:1:1:1:0:1 | 31:3:6:0:1:1:2 | 0.372[†] |
| Ascites | 1/56 | 2/144 | 1.000[†] |
| Hepatocellular carcinoma | 0/62 | 1/159 | 1.000[†] |
| Acquired immunodeficiency syndrome (AIDS) | 10/64 | 52/162 | 0.012*,[†] |

b. Comparison of clinical parameters between pre- and post-ART among patients with and without ART

| | Natural course (without ART) | With ART | *p* value (with vs. without ART) |
|---|---|---|---|
| CD4 count (per μl) | | | |
| Start[b] | 402.9 ± 180.1 | 242.5 ± 187.6 | 0.000* |
| End[c] | 406.4 ± 212.4 | 398.1 ± 195.9 | 0.883 |
| *p* value (start vs. end) | 0.893 | 0.000* | |
| Albumin (g/dl) | | | |
| Start | 4.1 ± 0.4 | 3.8 ± 0.8 | 0.292 |
| End | 3.9 ± 0.8 | 4.2 ± 0.4 | 0.025* |
| *p* value | 0.473 | 0.001* | |
| Bilirubin[d] (mg/dl) | | | |
| Start | 0.7 (0.30, 4.26) | 0.5 (0.30, 2.62) | 0.138 |
| End | 0.5 (0.25, 1.30) | 0.9 (0.36, 4.32) | 0.000* |
| *p* value | 0.046* | 0.000* | |
| ALT[d] (IU/l) | | | |
| Start | 46.0 (15.0, 1418.2) | 34.0 (12.8, 1,068.8) | 0.120 |
| End | 27.0 (9.9, 229.9) | 31.5 (12.73, 89.3) | 0.713 |
| *p* value | 0.003* | 0.000* | |
| Prothrombin time index (%) | | | |
| Start | 89.4 ± 13.1 | 78.8 ± 23.0 | 0.650 |
| End | 78.8 ± 27.3 | 84.2 ± 16.3 | 0.531 |
| *p* value | 0.377 | 0.218 | |
| WBC ($\times 10^3$/μl) | | | |
| Start | 6.1 ± 2.4 | 4.8 ± 2.1 | 0.000* |
| End | 5.4 ± 1.4 | 5.1 ± 1.6 | 0.404 |
| *p* value | 0.044* | 0.247 | |
| Platelet ($\times 10^4$ /μl) | | | |
| Start | 22.2 ± 6.5 | 19.3 ± 6.3 | 0.010* |
| End | 21.2 ± 6.5 | 20.8 ± 6.1 | 0.649 |
| *p* value | 0.204 | 0.001* | |

* $p < 0.05$

[†] Chi-square test was performed

[a] Two patients with habitual alcohol intake were included in this group

[b] Start of observation period

[c] End of observation period

[d] Means were compared by log transformation because of the nonnormality of the distribution; median and percentiles (5th percentile, 95th percentile) are provided

difference in serum total bilirubin level between the beginning and the end of the observation period [Dbilirubin level = (bilirubin level at the end) − (bilirubin level at the beginning)] in individual patients and compared it between the PI group and the non-PI group. The mean Dbilirubin level in the PI group was 0.5 ± 3.4 mg/dl and that in the non-PI group was −0.2 ± 1.6 mg/dl ($p = 0.250$). The Dbilirubin level in a patient in the PI group who was coinfected with HCV besides HIV/HBV as well was −27.4 mg/dl. Excluding this single outlier, the mean Dbilirubin level was significantly different between the PI and non-PI groups (mean Dbilirubin level 0.8 vs. −0.2; $p = 0.01$).

## Discussion

We have summarized here the data from our comprehensive survey of HIV/HBV coinfection in Japan, focusing particularly on the clinical features of the patients and the effect of ART on liver function. As we reported earlier, HIV/HBV coinfection was observed in 6.3 % of Japanese HIV-positive patients [7]. Certain considerations for HBV coinfection are important in HIV patient care.

The major transmission route of HIV was male homosexual contact, which accounted for the infection in about 80 % of the patients; thus, male patients were the majority in the present cohort. The most frequently found genotype of HBV was genotype A, which is infrequent in HIV-negative patients in Japan. Genotype A is often found in the United States, Europe, India, and the west coast of Sub-Saharan Africa [25]. Although the data on HBV subgenotypes were not available in our study, some reports showed that most genotype A strains detected in HIV/HBV-coinfected individuals are of genotype Ae [26]. These findings suggest that HBV infection among Japanese HIV carriers is not caused by the spread of indigenous HBV, such as transmission in the perinatal period, but rather specific strains are circulating among the homosexual population in Japan. Genotypes B and C accounted for more than 96 % of the entire Japanese chronic HBV infection [27, 28]. These findings are compatible with the report that the presumed transmission route of HBV in HIV/HBV-coinfected patients is not from Japanese female partners but from male partners, as shown by Koibuchi et al. [29].

Seventy-five percent of HIV/HBV-coinfected patients received ART with two agents against HBV, and its efficacy against HBV as well as HIV is considered to be high. As recommended by the United States Department of Health and Human Services (DHHS) and the Japanese guidelines on HIV treatment, the initiation of ART with NRTIs with anti-HBV activity as the backbone is indicated for HIV/HBV-coinfected patients regardless of HIV viral load or CD4+ T lymphocyte count [30]. Nucleoside analogues can improve liver function in HBV-monoinfected patients [31]. Our study shows that ART decreased the levels of ALT and albumin in HIV/HBV-coinfected patients. It is noteworthy that the regimen used in ART includes multiple drugs with anti-HBV activity such as lamivudine plus abacavir, which is unusual for HBV-monoinfected patients.

When we compared the characteristics of patients on ART with those not on ART, there were some notable differences in their immune status and liver function. At the beginning of the observation period, patients on ART showed a lower CD4+ T-cell count and poorer liver function. Our study is a retrospective observation, and patients were not grouped randomly. These observations are rather understandable because those who had a low CD4+ T cell count were more likely candidates for ART. Additionally, patients on ART had a longer observation period and were more likely to develop AIDS. These findings are also understandable because the longer the duration of HIV infection, the more likely is the immune system of the patient to deteriorate. Moreover, once ART is started, patients need to visit clinics or hospitals regularly for a long period; in reality, for the rest of their life. Following current recommendations for the initiation of ART for HIV infection, patients with worse immune status are more likely to receive the treatment. These findings can explain our observation.

Our data show that the serum albumin level and platelet count improved in the patients who were on ART. As the regimen of ART usually contains two drugs against HBV, ART suppresses HBV replication, which may lead to an improved liver function, as observed in HBV-monoinfected patients treated with nucleoside analogues [31]. Long-term treatment with lamivudine was shown to regress the fibrosis of the liver [32, 33] and decrease the proportion of patients with hepatocellular carcinoma complication [34]. In view of these findings, ART for HIV/HBV-coinfected patients may markedly improve the prognosis of patients. In our study, only a small number of patients with advanced liver diseases associated with HBV infection such as cirrhosis or hepatocellular carcinoma were observed, which could be attributable in part to the short observation period and the short duration of HBV infection. If we had a longer observational period, we would be able to clarify the difference in clinical course between the ART and non-ART groups, and the actual significance of ART for HIV/HBV-coinfected patients should become clearer.

We found that some parameters related to liver function changed paradoxically, particularly in the ART group. Although the mean serum albumin level, ALT level, and platelet count improved, the mean serum bilirubin level worsened, from 0.5 to 0.9 mg/dl. On the other hand, the serum bilirubin level in the non-ART group decreased. Both changes are statistically significant, which suggests

that the observed hyperbilirubinemia was not associated with HBV activity. The increase in serum bilirubin level is presumably caused by PIs. Hyperbilirubinemia following PI administration was previously reported [35]. Although it is unclear whether hyperbilirubinemia itself may lead to liver injury, PIs should be used carefully particularly for patients with advanced liver diseases.

Our present study has one major limitation; that is, the effect of alcohol on liver function was not analyzed because the history of alcohol consumption could not be obtained in the majority of the studied patients. Excessive alcohol consumption has been found to be an important risk factor for the development of severe hepatic injury in HIV-infected patients with [5] or without HCV coinfection [5]. Our present study showed that among the 26 patients whose history of alcohol consumption was available, only 2 patients were habitual drinkers. The results suggested that the effect of alcohol on liver function is small in HIV/HBV-coinfected patients in Japan.

In conclusion, ART with anti-HBV drugs may retard the progression of liver diseases and prevent liver-related death in HIV/HBV-coinfected patients. Multiple agents with anti-HBV activity seem essential for the efficacy. PIs should be carefully used particularly for patients with advanced liver diseases.

**Acknowledgments** We thank Ms. Ogawa for assistance in the survey. This work was supported in part by Health Sciences Research Grants from the Ministry of Health, Labor, and Welfare of Japan (Research on HIV/AIDS). We thank the hospitals in the HIV/AIDS Network of Japan for cooperation in this survey.

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**Table 1. Characteristics of the Randomized Cohorts and SVR Rates of Heterozygous Genotype rs12979860CT With Additional Genotyping of rs8099917**

| Random Sample Size | Sample Number | Mean Age ±SD | Male | HCV RNA ≥400.000 IU/mL | Severe Fibrosis | SVR rs12979680CT/ rs8099917TT | SVR rs12979680CT/ rs8099917TG | P-value |
|---|---|---|---|---|---|---|---|---|
| 10% | 96 | 47±11 | 58% | 69% | 55% | 48% | 36% | 0.408 |
| 20% | 192 | 48±11 | 59% | 80% | 43% | 43% | 32% | 0.379 |
| 30% | 295 | 48±11 | 60% | 72% | 48% | 50% | 38% | 0.154 |
| 40% | 396 | 47±11 | 63% | 66% | 55% | 57% | 39% | **0.012** |
| 50% | 474 | 47±11 | 60% | 68% | 53% | 56% | 37% | **0.003** |
| 60% | 588 | 48±11 | 58% | 71% | 52% | 57% | 35% | **0.0001** |
| 70% | 654 | 47±11 | 58% | 72% | 52% | 56% | 39% | **0.002** |
| 80% | 754 | 48±11 | 58% | 70% | 51% | 55% | 39% | **0.002** |
| 90% | 835 | 48±11 | 59% | 71% | 52% | 56% | 40% | **0.001** |
| 100% | 942 | 48±11 | 59% | 70% | 52% | 55% | 40% | **0.001** |

SD, standard deviation; IU, international units; SVR, sustained virological response; $P < 0.05$ considered to be statistically significant.

fibrosis stage on the SVR rates of genotype rs12979860CT/rs8099917TT and rs12979860CT/rs8099917TG (Supporting Table 1). Again, it becomes obvious that the impact of additional genotyping of rs8099917 on the prediction of SVR is improved in patients with heterozygous genotype of rs12979860 who have high baseline HCV RNA levels ($P = 3.7 \times 10^{-5}$), HCV subtype 1a ($P = 3.3 \times 10^{-5}$), or severe fibrosis stages ($P = 0.001$), being female ($P = 0.023$), or of younger age ($P = 0.029$). Thus, the different patient characteristics most likely explain the differences in the SVR rates.

From that, one possibly may conclude that two SNPs are good in large cohorts but not relevant for clinical practice. However, the idea of large studies is to inform individual clinical practice. Our results derived from a large cohort suggest that algorithms and models that include both rs12979860 and rs809917 as well as baseline parameters and viral factors are informative to guide therapeutic decision making.[3]

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*View this article online at wileyonlinelibrary.com.*
*DOI 10.1002/hep.25923*
*Supported by the German Competence Network for Viral Hepatitis (Hep-Net), funded by the German Ministry of Education and Research (BMBF Grant No. 01 KI 0437, Project No. 10.1.3 and Core Project No. 10.1 Genetic host factors in viral hepatitis and Genetic Epidemiology Group in viral hepatitis), by the EU-Vigilance network of excellence combating viral resistance (VIRGIL, Project No. LSHM-CT-2004-503359), and by the BMBF Project: Host and viral determinants for susceptibility and resistance to hepatitis C virus infection (Grant No. 01KI0787). Parts of the work were supported by an Australian Research Council Linkage Project Grant (LP0990067), a National Health and Medical Research Council Grant (1006759) and the Robert W. Storr Bequest to the Sydney Medical Foundation, University of Sydney.*

## Plasma Lysophosphatidic Acid Levels and Hepatocellular Carcinoma

To the Editor:

We read with interest the article by Mazzocca et al.,[1] showing that serum lysophosphatidic acid (LPA) levels are increased in hepatocellular carcinoma (HCC) patients correlated with tumor burden, while not enhanced in cirrhosis patients. However, we think that their LPA values in serum samples need to be carefully evaluated, because of some technical issues in the measurement of LPA levels in blood samples. First, because LPA is released from platelets, LPA has been measured in plasma but not in serum when evaluating its clinical significance.[2,3] Second, as we previously demonstrated,[4] LPA levels in plasma samples are markedly increased after sample preparation unless the temperature is kept under strict control, potentially because the synthetic enzyme autotaxin (ATX) and the substrate lysophosphatidyl choline coexist in plasma samples to abundantly produce LPA. LPA was once reported as a biomarker of ovarian cancer,[2] but contrary data were later demonstrated, in which a distinct sampling of plasma may explain this discrepancy.[3] Indeed, LPA levels in serum reported by Mazzocca et al. were approximately 10 times higher than the previously reported LPA levels in plasma.[2,3] If their LPA values in serum were increased after sampling similarly in each sample, plasma LPA levels might be correlated with HCC burden as reported. To clarify this, we have newly measured plasma LPA levels in HCC patients,

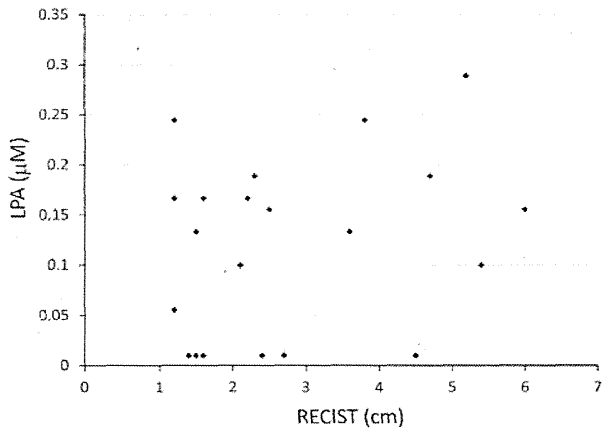


Fig. 1. Plasma LPA levels and HCC burden. Plasma LPA levels, measured in 21 HCC patients (13 males and 8 females; 2 patients with chronic hepatitis B, 15 with chronic hepatitis C, and 4 with non-B non-C chronic liver disease), were not significantly correlated with HCC burden as evaluated by RECIST (Response Evaluation Criteria in Solid Tumors; Spearman rank, $r = 0.158$, $P = 0.4937$). This study was approved by the Institutional Research Ethics Committee and informed consent was obtained for the use of the samples.

and found that they were not correlated with tumor burden, as shown in Fig. 1. Moreover, plasma LPA levels in HCC patients ($0.12 \pm 0.09$ mM, mean $\pm$ SD, n = 21), were not different from the previously reported levels in non-HCC patients with chronic hepatitis C ($0.10 \pm 0.05$ mM).[5] Although Mazzocca et al. reported no enhancement of serum LPA levels in cirrhosis patients, we[5] and others[6] previously showed that plasma LPA levels and serum ATX activity were increased in chronic liver diseases in association with fibrosis and cholestatic pruritus, from which HCC frequently arises. Collectively, a role of LPA in HCC should be cautiously analyzed.

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*View this article online at wileyonlinelibrary.com.*
*DOI 10.1002/hep.25886*
*Potential conflict of interest: Nothing to report.*

## Reply:

Ikeda et al. remark that platelets are a main source of lysophosphatidic acid (LPA) and therefore the interpretation of LPA serum concentrations deserves careful attention. However, the same authors previously reported[1] an inverse correlation between plasma LPA concentrations and the number of platelets in patients with chronic C hepatitis. Therefore, it is possible that in physiologic conditions platelets remain the main source of LPA, while in chronic inflammation such as hepatitis C, liver cirrhosis, or hepatocellular carcinoma (HCC), the platelet contribution to LPA production may likely become less relevant. In our study we analyzed sera for LPA detection in healthy donors, liver cirrhosis, and HCC patients, performing well-standardized procedures of collection for each sample. Thus, the contribution of platelets to the LPA concentration was, in reality, normalized. On the contrary, the authors should consider that even in plasma or whole blood, platelet activation is an extremely difficult problem to deal with and control. For example, prolonged tourniquet application, or twisting of the needle in the vein, are major factors interfering with the function of platelets during blood withdrawal, as reviewed by Ruggeri.[2] Unfortunately, these limitations are common for a number of molecules involved both in cancer and in blood cell biology.[3]

Moreover, Ikeda et al. investigated patients with chronic hepatitis C, in whom the inflammatory response is a key component of the tissue microenvironment. In their study, the fibrotic status was also questionable, due to their choice of statistical method (comparison among groups should be done with Kruskal-Wallis tests), and because of the very limited number of patients (14), further stratified into four different groups, which means the conclusions were affected by low power.[1] In our study,[4] we compared liver cirrhosis versus HCC. In the former case, the inflammation is reduced while the fibrotic response is increased, consequently inducing a different microenvironment response.[5] This could explain why patients with liver cirrhosis display relatively low levels of LPA. In addition, it is conceivable that when HCC develops in cirrhotic liver, LPA levels rise once more, as in cases of active inflammatory states (i.e., viral hepatitis). Another key point is patient selection. Ikeda et al. do not provide any information with regard to the clinical features of the patients, i.e., etiology, BCLC stage, previous therapy, etc., as well as how they calculated the size of the tumor in patients with multifocal disease, for instance. Finally, some differences between Caucasian and Asian patients with HCC are to be expected, since the natural history is completely different in Western and Southeast Asian countries.[6] In our study,[4] we demonstrated that LPA has a role in promoting tumor progression and we did not attempt to speculate about the use of LPA as a clinical biomarker. To validate LPA as a potential biomarker for HCC a different study design is required, as well as first considering the power of the study. The enhancement of serum LPA levels reported by Watanabe et al.[1] referred to a relatively small number of patients with chronic hepatitis C. In addition, the

Liver INTERNATIONAL
OFFICIAL JOURNAL OF THE INTERNATIONAL ASSOCIATION FOR THE STUDY OF THE LIVER

Liver International ISSN 1478-3223

CLINICAL STUDIES

# Percutaneous ethanol injection for hepatocellular carcinoma: 20-year outcome and prognostic factors

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**Keywords**
ablation – hepatocellular carcinoma – percutaneous ethanol injection – prognostic factor – recurrence – survival – treatment outcome

**Abbreviations**
AFP-L3, lectin-reactive AFP; AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; CI, confidence intervals; CT, computed tomography; DCP, des-γ-carboxy-prothrombin; HBs-Ag, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

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Received 4 April 2012
Accepted 22 May 2012

DOI:10.1111/j.1478-3231.2012.02838.x

## Abstract

*Background:* Ethanol injection is the best-known image-guided percutaneous ablation for hepatocellular carcinoma (HCC) and a well-tolerated, inexpensive procedure with few adverse effects. However, there have been few reports on its long-term results. *Aims:* We report a 20-year consecutive case series at a tertiary referral centre. *Methods:* We performed 2147 ethanol injection treatments on 685 primary HCC patients and analysed a collected database. *Results:* Final computed tomography demonstrated complete ablation of treated tumours in 2108 (98.2%) of the 2147 treatments. With a median follow-up of 51.6 months, 5-, 10- and 20-year survival rates were 49.0% [95% confidence interval (CI) = 45.3–53.0%], 17.9% (95% CI = 15.0–21.2%) and 7.2% (95% CI = 4..5–11.5%) respectively. Multivariate analysis demonstrated that age, Child–Pugh class, tumour size, tumour number and serum alpha-fetoprotein level were significant prognostic factors for survival. Five-, 10- and 20-year local tumour progression rates were 18.2% (95% CI = 15.0–21.4%), 18.4% (95% CI = 15.2–21.6%) and 18.4% (95% CI = 15.2–21.6%) respectively. Five-, 10- and 20-year distant recurrence rates were 53.5% (95% CI = 49.4–57.7%), 60.4 (95% CI = 56.3–64.5%) and 60.8% (95% CI = 56.7–64.9%) respectively. There were 45 complications (2.1%) and two deaths (0.09%). *Conclusions:* Ethanol injection was potentially curative for HCC, resulting in survival for more than 20 years. This study suggests that new ablation therapies will achieve similar or even better long-term results in HCC.

Hepatocellular carcinoma (HCC) is the fifth most common malignant neoplasm in the world. Only 20% of HCC patients are candidates for resection (1). Furthermore, recurrence is frequent even after curative resection. Liver transplantation is restricted by donor shortage. Thus, various non-surgical therapies have been introduced (2). Among these, image-guided percutaneous ablation is considered best for early-stage HCC.

The most studied percutaneous ablation is ethanol injection. Ethanol injection is a well-tolerated, inexpensive procedure with few adverse effects and has been considered the standard against which any new ablation therapy should be compared (2). Although ethanol injection was introduced into clinical practice in the 1980s (3, 4), few reports of its long-term results have been published (5–8). We report here a 20-year consecutive case series at a tertiary referral centre. This study documents the largest number of ethanol injection treatments at a single institution. Findings in this 20-year experience may be extrapolated to other ablation therapies, such as radiofrequency ablation, in which such long-term outcomes are not yet available (9).

## Patients and methods

### Indications for ethanol injection

Ethanol injection was performed in patients satisfying the following criteria: (i) ineligible for resection or transplantation, or had refused surgery; (ii) no extrahepatic metastasis or vascular invasion. Exclusion criteria were as follows: (i) tumour was not visualized

by ultrasonography or not accessible percutaneously; (ii) total bilirubin level $\geq 3.0$ mg/dl; (iii) platelet count $<40 \times 10^9$/L; (iv) prothrombin activity <35%; (v) refractory ascites. In general, we performed ethanol injection on patients with Child–Pugh class A or B, with 3 or fewer tumours $\leq 3$ cm in diameter. We performed ethanol injection on patients beyond these conditions, however, who were likely to benefit from the procedure for possible cure or prolongation of life. No patients were excluded solely because of tumour location (10). Informed consent was obtained from each patient. This study was conducted according with the Helsinki Declaration of 1975 and approved by the Institutional Review Board.

### Patients

In this cohort study, we analysed a prospectively collected computerized database. Between 1985 and 2005, 2735 HCC patients were admitted to the Department of Gastroenterology, University of Tokyo (Fig. 1). At initial hospitalization, 1615 had primary HCC and the remaining 1120 had recurrent HCC. The recurrent HCC patients had undergone therapies other than ethanol injection for primary HCC.

Of the 1615 patients with primary HCC, 1459 (90.3%) underwent percutaneous ablation as the initial treatment, including ethanol injection. The remaining 156 patients received other therapies: transarterial chemoembolization for 123 patients with multinodular or large tumours that could not be treated by ablation therapies; hepatic resection for 18 with good liver function who consented to an operation; chemotherapy for four with vascular invasion or extrahepatic metastasis; and best supportive care for 11 with decompensated cirrhosis or poor general condition.

Of the 1459 patients treated by percutaneous ablation, 685 underwent ethanol injection, 122 underwent microwave ablation, and the remaining 652 radiofrequency ablation. The type of percutaneous ablation performed varied with the date of treatment. We started ethanol injection in December 1985, microwave ablation in October 1995 and radiofrequency ablation in February 1999 (11). Between October 1995 and February 1999, both ethanol injection and microwave ablation were performed. Microwave ablation was chosen for patients who had better liver function and whose tumour was located in a position where the electrode could be inserted and held safely. Since February 1999, both ethanol injection and radiofrequency ablation have been performed. Between April 1999 and January 2001, 232 patients with three or fewer tumours, each $\leq 3$ cm in diameter, and Child–Pugh class A or B were entered into a randomized controlled trial (12). Patients outside these inclusion criteria were mostly treated by radiofrequency ablation. After this trial, radiofrequency

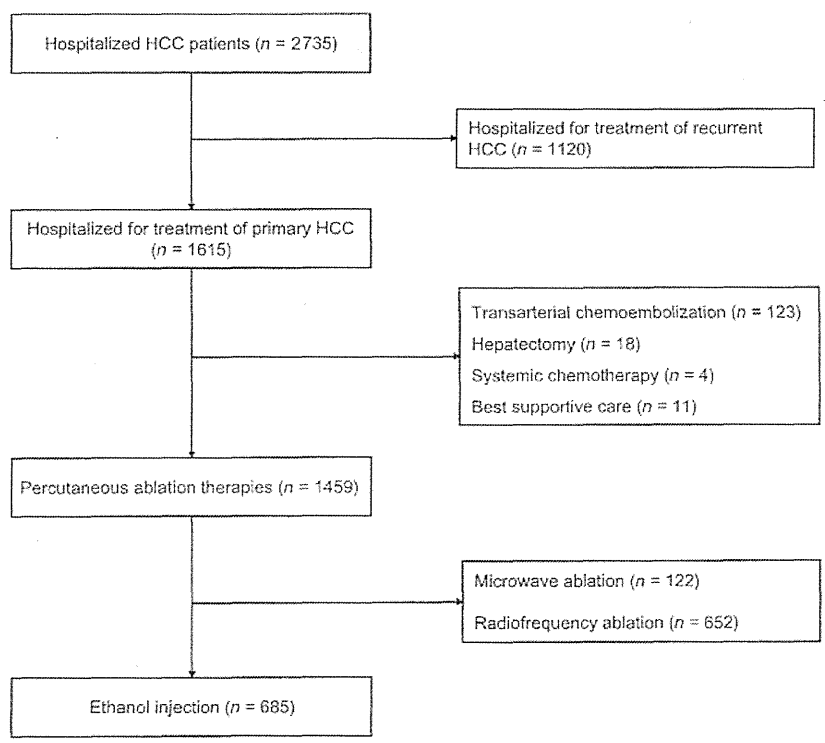


**Fig. 1.** Flow of patients in this study. HCC, hepatocellular carcinoma

ablation was generally the treatment of choice, and ethanol injection was used only in those unsuitable for radiofrequency ablation: those with either enterobiliary reflux or tumour adhesion to the gastrointestinal tract.

Hepatocellular carcinoma was diagnosed based on typical imaging findings of early phase enhancement and late phase contrast washout on computed tomography (CT) (13). HCC diagnosis was also confirmed by biopsy in 630 (92.0%) of the 685 patients with primary HCC treated by ethanol injection. A total of 587 (85.7%) were diagnosed as having cirrhosis.

In general, chemoembolization was combined with ethanol injection in patients with either $\geq 4$ tumours or those with two or three tumours at least one of which is >3.0 cm in diameter. The combination of chemoembolization with ethanol injection was performed in 186 patients.

### Treatment methods

Preoperative planning including ultrasound examination and evaluation of all imaging findings was performed to identify the tumours and to determine the access route. The procedure was performed according to an institutional protocol and under the supervision of experienced physicians who had performed this treatment more than 200 times. The precise techniques of ethanol injection are described elsewhere (12). Briefly, all procedures were performed percutaneously under ultrasound guidance. Artificial pleural effusion or artificial ascites method is much less frequently used in ethanol injection compared with radiofrequency ablation, because the procedure is necessary to be repeated several times. Since 1990, we have used two or three needles to inject ethanol into several sites in one procedure (12). Ethanol injection was performed twice per week. The procedure was repeated until ethanol appeared to have been injected throughout the tumour. To judge a timing to stop repetition of injecting ethanol and to order a CT scan, we considered total volume of injected ethanol and change of echogenicity. The general guideline for the necessary volume of injected ethanol was calculated according to the following numerical expression, $V = (4/3)\ \pi\ (r + 0.5)^3$, where V (in millilitres) is the volume of ethanol and r (in centimetres) is the radius of the tumour; 0.5 is added to provide a safety margin, which is based on the concept that some surrounding liver parenchyma all around the tumour as well as the tumour itself must be ablated (5).

A CT scan was then performed 1–3 days after the procedure to evaluate technique effectiveness (14). Complete ablation was defined as hypoattenuation of the entire tumour. When the presence of unablated tumour portions was suspected, a few more procedures were performed. We did not predefine the number of procedures in a treatment. The ethanol injection treatment was generally continued until CT demonstrated the entire tumour necrosis.

### Follow-up

Follow-up investigations consisted of CT, ultrasonography and measurement of serum α-fetoprotein (AFP), des-γ-carboxy-prothrombin (DCP) (since April 1993) levels and lectin-reactive AFP (AFP-L3) (since July 1997) every 4 months. Local tumour progression was defined as appearance of viable tumour touching the original tumour (14) and distant recurrence as emergence of tumour(s) separate from the primary site. Ethanol injection was used for recurrence if the patient still met the indication criteria. If multiple recurrences were not treatable with ethanol injection, chemoembolization was generally performed.

### Statistical analyses

This study is a report of a consecutive case series. All ethanol injection treatments performed on primary HCC patients at the Department of Gastroenterology, University of Tokyo between 1985 and 2005 were included. Data are presented as mean ± SD for quantitative variables, and as absolute frequencies for qualitative variables.

A 'procedure' was defined as a single intervention episode that consisted of one or more ablations performed on tumours, and a 'treatment' as the completed effort to ablate tumours. A treatment consisted of several procedures (14). 'Technique effectiveness' rate was defined as the percentage of successfully eradicated macroscopic tumours as evidenced at CT scan after the last procedure (14). In cases in which there was Lipiodol deposit inside the tumour because of the combination of chemoembolization with ethanol injection, we judged that the tumour had been successfully eradicated if it was surrounded with completely non-enhanced tissue in final CT.

Overall survival was calculated in the 685 primary HCC patients. Survival curves were generated using the Kaplan–Meier method. In addition to overall survival, subgroup analyses were performed with clinical characteristics including tumour size, tumour number and Child–Pugh class. Recurrence was evaluated in 591 patients in whom ethanol injection was performed with curative intent. All tumours were treated by ethanol injection in those patients. The remaining 94 patients were excluded from the recurrence analysis because some small tumours had been left untreated by ethanol injection on account of detection failure by ultrasonography. Recurrence rates were calculated using the Gaynor method (15). All time estimates were made from the date of the first ethanol injection. The follow-up was finalized at either death or the last visit to the outpatient clinic before December 31 2010. Transplanted patients were censored from this study at the date of transplantation.

The prognostic relevance of baseline variables (Table 1), the combination of chemoembolization,

**Table 1.** Baseline characteristics of the 685 Patients undergoing percutaneous ethanol injection for primary hepatocellular carcinoma

| Variable | |
|---|---|
| Age (years) | 64.0 ± 8.9 |
| Males, *n* (%) | 502 (73.3) |
| Viral infection* | |
| HBs-Ag positive, *n/N* (%) | 64/685 (9.3) |
| Anti-HCV positive, *n/N* (%) | 570/673 (84.7) |
| Both positive, *n/N* (%) | 11/673 (1.6) |
| Both negative, *n/N* (%) | 52/673 (7.7) |
| Alcohol consumption >80 g/day, *n* (%) | 143 (20.9) |
| Ascites, *n* (%) | 122 (17.9) |
| Encephalopathy, *n* (%) | 44 (6.5) |
| Albumin (g/dl) | 3.55 ± 0.50 |
| Total bilirubin (mg/dl) | 0.96 ± 0.536 |
| Prothrombin time (%) | 71.6 ± 15.9 |
| Platelet count ($\times 10^4$/mm$^3$) | 10.3 ± 4.6 |
| AST (IU/L) | 80.6 ± 48.2 |
| ALT (IU/L) | 79.2 ± 61.9 |
| Child–Pugh class, *n* (%) | |
| A | 425 (62.1) |
| B | 228 (33.3) |
| C | 32 (4.6) |
| Tumour size (cm) | 2.83 ± 1.47 |
| Tumour number | 2.0 ± 1.7 |
| Serum AFP (ng/ml), *n* (%) | |
| ≤ 100 | 525 (76.6) |
| 101–400 | 95 (13.9) |
| >400 | 65 (9.5) |
| Serum DCP (mA U/ml), *n* (%)† | |
| ≤ 100 | 428 (82.8) |
| 101–400 | 49 (9.5) |
| >400 | 40 (7.7) |
| Serum AFP-L3 (%), *n* (%)‡ | |
| ≤ 15 | 193 (86.2) |
| 15.1–40 | 16 (7.1) |
| >40 | 15 (6.7) |

*Anti-HCV was not tested in 12 patients.

†Serum DCP level was not measured in 168 patients.

‡Serum AFP-L3 level was not measured in 461 patients.

HBs-Ag, hepatitis B surface antigen; HCV, hepatitis C virus; AFP, α-fetoprotein; DCP, des-gamma-carboxy-prothrombin; AFP-L3, lectin-reactive α-fetoprotein.

Data are expressed as mean ± standard deviation.

HCC recurrence and the number of ethanol injection sessions to survival was analysed by univariate and multivariate models. The prognostic relevance of baseline variables (Table 1), the combination of chemoembolization and the number of ethanol injection sessions to local tumour progression and distant recurrence was also analysed by univariate and multivariate models. In multivariate analysis, we evaluated models including Child–Pugh class and excluding its components to avoid multicollinearity. Serum DCP and AFP-L3 levels were excluded from the multivariate model because of absence of data from 168 and 461 patients respectively. Some continuous variables in which log-linearity could not be assumed were transformed into categorical variables. Variables with a $P$ value <0.05 determined by univariate comparison were subjected to multivariate analysis. A stepwise variable selection was performed with Akaike Information Criteria in multivariate analysis. Results were expressed as hazard ratios with corresponding 95% confidence intervals (CI), with $P$ values from the Wald test. All significance tests were two-tailed, and differences with a $P$ value <0.05 were considered statistically significant.

Complications were defined according to the guidelines of the Society of Interventional Radiology (16).

## Results

### Antitumour effect

We performed 2147 ethanol injection treatments, comprising 13 526 procedures. Thus, procedure number per treatment was 6.3 ± 2.6. The total volume of injected ethanol per treatment was 40.9 ± 16.3 ml. Many patients received iterative ethanol injection treatments for recurrence. A total of 108 patients underwent ethanol injection treatment once, 118 patients twice, 196 patients 3 times, 153 patients 4 times, 71 patients 5 times, 28 patients 6 times, 8 patients 7 times and 3 patients 8 times.

Technique effectiveness rate was 98.2% (2108/2147 treatments). It was similar between the initial ethanol injection treatments and the other ethanol injection treatments for recurrence ($P = 0.397$). Complete ablation of the tumour was achieved in 675 (98.5%) of the 685 initial treatments and in 1433 (98.0%) of the 1462 other treatments. However, technique effectiveness rate significantly differed with tumour size ($P = 0.002$). No apparent viable portions remained in 758 (99.0%) of 766 treatments for tumours ≤ 2.0 cm in diameter, in 704 (98.4%) of 717 treatments for tumours 2.1–3.0 cm, in 570 (97.9%) of 582 treatments for tumours 3.1–5.0 cm and in 76 (92.7%) of 82 treatments for tumours >5.0 cm.

### Survival

Table 1 shows clinical characteristics of the 685 patients. A total of 136 patients (19.9%) were older than 75 years. In all, 180 patients had tumours ≤ 2.0 cm in diameter, 274 had tumours 2.1–3.0 cm, 192 had tumours 3.1–5.0 cm and 39 had tumours >5.0 cm. A total of 367 patients had one tumour, 238 patients had 2 or 3 tumours and 80 had 4 or more tumours.

As of December 2010 (with a median follow-up of 51.6 months), 70 patients (10.2%) remained alive, 52 (7.6%) were lost to follow-up and 563 (82.2%) had died. Of the 685 patients, two were transplanted. The number of patients who survived longer than 5, 10 and 20 years after the first ethanol injection treatment was 305, 97 and 3 respectively. The cause of death was HCC

in 297 patients (52.8%), liver failure in 129 (22.9%), upper gastrointestinal bleeding in 30 (5.3%), complications related to the procedure in 2 (0.4%), liver-unrelated diseases in 84 (14.9%) and undetermined in 21 (3.7%).

The 1-, 3-, 5-, 10-, 15- and 20-year survival rates of all 685 patients were 91.0% (95% CI = 88.9–93.2%), 67.6% (95% CI = 64.1–71.3%), 49.0% (95% CI = 45.3–53.0%), 17.9% (95% CI = 15.0–21.2%), 8.6% (95% CI = 6.4–11.7%) and 7.2% (95% CI = 4.5–11.5%) respectively (Fig. 2; Table 2). Survival rates significantly differed with tumour number ($P = 0.0001$), tumour size ($P = 0.0001$) and Child–Pugh class ($P = 0.0001$). In patients with 1–3 tumours, all $\leq 3$ cm, and in Child–Pugh class A or B, the 5-year survival rate was 59.5% (95% CI: 54.7–64.7%).

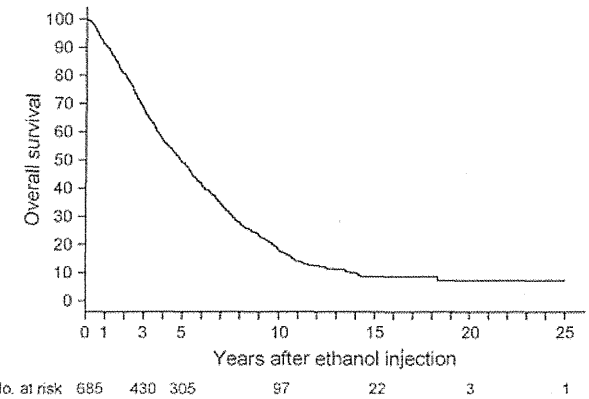


**Fig. 2.** Overall survival in 685 primary hepatocellular carcinoma patients who underwent ethanol injection.

Univariate analysis indicated that 13 of the 22 variables were relevant to survival. In multivariate analysis, a model that contained age, antibody to hepatitis C virus (anti-HCV), Child–Pugh class, tumour size, tumour number and serum AFP level was selected (Table 3).

Survival rates significantly differed with the time period in which the first ethanol injection was performed ($P < 0.0001$; Fig. 3). In 109 patients who underwent ethanol injection between 1985 and 1991, the 5-year survival rate was 30.3% (95% CI = 22.7–40.5%), whereas it was 51.2% (95% CI = 46.8–55.9%) in 476 patients between 1992 and 1998, and 61.1% (95% CI = 51.3–72.8%) in 100 patients between 1999 and 2005.

### Recurrence

Recurrence developed in 449 patients. Local tumour progression alone was found in 61 patients, local tumour progression with distant recurrence in 44 and distant recurrence alone in 344. Of these 344 patients, eight had recurrence in extrahepatic sites: five had lymph node metastasis, one had lung metastasis, one had bone metastasis and the remainder had both lymph node and lung metastasis. Of the 449 patients, the first recurrence was treated by iterative ethanol injection in

**Table 2.** Survival of patients undergoing ethanol injection, based on tumour number, tumour size and Child–Pugh class

| Grading | *n* | Survival (%) 3-Year | 5-Year | 10-Year | 15-Year | 20-Yar | Median (years) | *P* value |
|---|---|---|---|---|---|---|---|---|
| Overall survival | 685 | 67.6 | 49.0 | 17.9 | 8.6 | 7.2 | 4.9 | – |
| Tumour number | | | | | | | | |
| Solitary | 367 | 72.0 | 56.5 | 24.6 | 12.1 | 9.7 | 5.8 | 0.0001 |
| 2–3 | 232 | 71.5 | 46.3 | 12.9 | 5.9 | – | 4.7 | |
| ≥4 | 86 | 37.6 | 23.8 | 2.5 | 1.3 | – | 2.6 | |
| Tumour size | | | | | | | | |
| ≤2.0 cm | 240 | 83.6 | 63.8 | 27.6 | 12.3 | 6.1 | 6.9 | 0.0001 |
| 2.1–3.0 cm | 221 | 68.0 | 47.9 | 15.0 | 10.7 | 10.7 | 4.8 | |
| >3.0 cm | 224 | 50.2 | 34.4 | 10.1 | 3.5 | 3.5 | 3.1 | |
| Child–Pugh class | | | | | | | | |
| A | 425 | 77.3 | 58.7 | 24.4 | 12.5 | 10.4 | 6.2 | 0.0001 |
| B | 228 | 53.9 | 35.5 | 8.1 | 3.0 | – | 3.5 | |
| C | 32 | 37.5 | 18.8 | 3.1 | – | – | 1.9 | |
| Combination of tumour number, tumour size, and Child–Pugh class | | | | | | | | |
| Solitary, ≤3 cm | 275 | 77.5 | 62.2 | 28.8 | 14.5 | 10.8 | 6.8 | – |
| Solitary, ≤3 cm, Child–Pugh A | 185 | 84.9 | 69.2 | 36.7 | 20.2 | 15.1 | 7.6 | – |
| 1–3 tumours, ≤3 cm | 419 | 78.6 | 58.0 | 23.5 | 12.2 | 9.1 | 6.1 | – |
| 1–3 tumours, ≤3 cm, Child–Pugh A/B | 402 | 80.5 | 59.5 | 24.3 | 12.8 | 9.6 | 6.2 | – |
| Satisfied the indication criteria of surgical resection proposed in the BCLC protocol* | 121 | 86.3 | 72.8 | 31.1 | 14.8 | – | 7.2 | – |

*Child–Pugh class A with a normal level of bilirubin, no significant portal hypertension and a single HCC.
BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma.

**Table 3.** Multivariate analysis of variables relevant to survival, local tumour progression and distant recurrence

| Variable | Multivariate analysis Hazard ratio (95% CI) | *P* value |
|---|---|---|
| Survival | | |
| Age (per year) | 1.03 (1.02–1.04) | <0.0001 |
| Anti-HCV-positive | 0.81 (0.69–0.94) | 0.006 |
| Child–Pugh class | | |
| A | 1 | |
| B | 2.01 (1.66–2.44) | <0.0001 |
| C | 3.11 (2.08–4.65) | <0.0001 |
| Tumour size (cm) | | |
| ≤2.0 | 1 | |
| 2.1–3.0 | 1.26 (1.00–1.58) | 0.051 |
| 3.1–5.0 | 1.51 (1.18–1.93) | 0.001 |
| >5.0 | 2.31 (1.61–3.31) | <0.0001 |
| Tumour number | | |
| solitary | 1 | |
| 2–3 | 1.10 (0.90–1.35) | 0.34 |
| ≥4 | 2.11 (1.59–2.78) | <0.0001 |
| Serum AFP (ng/dl) | | |
| ≤100 | 1 | |
| 101–400 | 1.47 (1.14–1.90) | 0.003 |
| >400 | 2.16 (1.57–2.97) | <0.0001 |
| Local tumour progression | | |
| Tumour size (cm) | | |
| ≤2.0 | 1 | |
| 2.1–3.0 | 1.47 (1.15–1.88) | 0.002 |
| 3.1–5.0 vs. ≤2.0 | 1.30 (0.97–1.75) | 0.08 |
| >5.0 vs. ≤2.0 | 2.81 (1.64-4.82) | 0.0002 |
| Distant recurrence | | |
| Tumour size (cm) | | |
| ≤2.0 | 1 | |
| 2.1–3.0 | 1.42 (1.11–1.82) | 0.006 |
| 3.1–5.0 | 1.28 (0.95–1.72) | 0.10 |
| >5.0 | 2.48 (1.43–4.28) | 0.001 |
| Tumour number | | |
| solitary | 1 | |
| 2–3 | 1.47 (1.16–1.85) | 0.001 |
| ≥4 | 2.12 (1.36–3.28) | 0.0008 |

AFP, α-fetoprotein; CI, confidence interval; HCV, hepatitis C virus.

399 (88.8%), chemoembolization in 44 (9.8%), systemic chemotherapy in three (0.7%) and best supportive care in three (0.7%).

The 1-, 3-, 5-, 10-, 15- and 20-year rates of local tumour progression with or without distant recurrence were 7.9% (95% CI = 5.7–10.0%), 15.6% (95% CI = 12.6–18.6%), 18.2% (95% CI = 15.0–21.4%), 18.4% (95% CI = 15.2–21.6%), 18.4% (95% CI = 15.2–21.6%) and 18.4% (95% CI = 15.2–21.6%) respectively (Fig. 4). Univariate analysis demonstrated that three variables were relevant to local tumour progression, whereas multivariate analysis indicated that only tumour size was significantly related to local tumour progression (Table 3).

The 1-, 3-, 5-, 10-, 15- and 20-year rates of distant recurrence without local tumour progression were 17.1% (95% CI = 14.0–20.1%), 42.6% (95% CI = 38.6–46.7%), 53.5% (95% CI = 49.4–57.7%), 60.4% (95% CI = 56.3–64.5%), 60.8% (95% CI = 56.7–64.9%) and 60.8% (95% CI = 56.7–64.9%) respectively. Univariate analysis demonstrated that five variables were relevant to distant recurrence, whereas multivariate analysis indicated that tumour size and tumour number were significantly related to distant recurrence without local recurrence (Table 3).

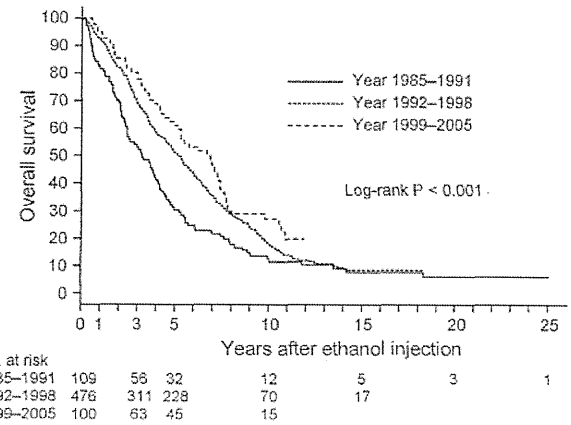


**Fig. 3.** Survival according to the time period in which the first ethanol injection was performed (1985–1991 vs. 1992–1998 vs. 1999–2005)

### Complications

Table 4 shows complications encountered. The incidence rates per treatment and per procedure were 2.1% (45 of 2147) and 0.33% (45 of 13 526) respectively. A patient died of multiple organ dysfunction syndrome caused by procedure-related hemoperitoneum. The tumour was not on the surface but inside the liver. The patient did not have marked bleeding tendency. The other developed myocardial infarction, resulting in death during the procedure. The treatment mortality rate was 0.06%.

## Discussion

This study describes a 20-year experience with ethanol injection at a high-volume centre. We performed 2147 ethanol injection treatments on the 685 primary HCC patients, showing that ethanol injection has a high antitumour effect. Tumours were judged to have been completely ablated by final CT imaging in 98.2% of the treatments. The complete response rate may be higher in this study than others (17, 18), probably because we did not predefine the number of procedures in a treatment. We generally repeated the procedure until CT demonstrated complete tumour necrosis. Many other studies limited the procedure number of ethanol injection. Complete tumour ablation has been reported to relate to improved survival (19).

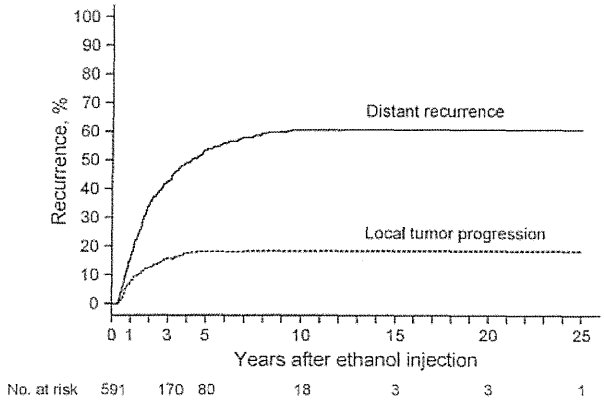


**Fig. 4.** Local tumour progression or distant recurrence in patients who underwent ethanol injection.

**Table 4.** Complications in 2147 treatments of ethanol injection for hepatocellular carcinoma

| Complication | Number |
|---|---|
| Neoplastic seeding | 9 |
| Hemoperitoneum | 9 |
| Hemobilia | 6 |
| Liver abscess | 6 |
| Symptomatic pleural effusion | 3 |
| Massive hepatic infarction | 3 |
| Biliary cast | 2 |
| Hemothorax | 2 |
| Abnormal decrease in blood coagulation factor VIII | 2 |
| Biloma | 1 |
| Biliary bronchial fistula | 1 |
| Myocardial infarction | 1 |

This study showed that ethanol injection could achieve long-term survival over 20 years. Ninety-seven patients survived for more than 10 years and three for more than 20 years. Both tumour factors and liver function were relevant to survival. In addition, age was among the prognostic factors. In this study, 19.9% were older than75 years, which may have resulted in the higher percentage (14.9%) of liver-unrelated deaths compared with other studies. Ant-HCV positivity was a good prognostic factor in this study.

Survival in ethanol injection appears to have improved with times. This is probably because of advances in imaging techniques, such as ultrasound and CT, more refined skills and greater experience in ablation and innovations in the treatment of underlying liver diseases.

Hepatocellular carcinoma frequently recurred after ethanol injection. Most recurrences were, however, not local tumour progression but distant recurrence. Frequent recurrence is not specific to ethanol injection. After hepatic resection, the tumour recurrence rate exceeds 70% at 5 years (20, 21). In this study, periodic follow-up detected most recurrence at limited stage. Ethanol injection was performed again for first recurrence in 88.8% of the cases. In hepatic resection, the rate of repeat resection for first recurrence has been reported to range from 10.4 to 30.6% (21, 22). As ethanol injection is less invasive than hepatic resection, iterative ethanol injection can be performed for recurrence more easily.

Ethanol injection was a safe procedure, although many patients in this study were at risk for surgical treatment because of advanced cirrhosis or other comorbidities. Only 121 (17.7%) of the 685 patients satisfied the indication criteria of surgical resection proposed in the BCLC (Barcelona Clinic Liver Cancer) protocol (23) and were, thus, considered good candidates for surgical resection. Other investigators also reported low complication rates of 0–3.2% (6–8, 24). For hepatic resection, morbidity rates have been reported to be 38–47% even in recent studies (25–27).

Radiofrequency ablation has steadily replaced ethanol injection (11). At our institution, radiofrequency ablation is currently the first option for percutaneous ablation (28). Several randomized controlled trials including ours (12, 18, 29, 30) demonstrated more reliable local antitumour effect and higher survival. Our 10-year outcome of radiofrequency ablation (28) appears superior to this 20-year outcome of ethanol injection. In addition, radiofrequency ablation requires fewer treatment sessions and shorter hospitalization.

A meta-analysis showed, however, that ethanol injection did not differ from radiofrequency ablation for tumours $\leq 2$ cm in diameter (31). A recent randomized controlled trial also demonstrated similar 5-year survival between the two ablations (32). Ethanol injection is at least more feasible and cheaper than radiofrequency ablation.

Surgical resection has been considered the treatment of first choice for HCC. Our first option for resectable tumours was also surgery. However, most patients who came to our department declined surgical resection. Thus, some patients in this study underwent ethanol injection not because of unresectable tumour but because of refusal of surgery. Those who preferred surgery would have gone directly to the surgical department, which has extensive experience in hepatic resection (27).

It is not easy to compare outcomes between ethanol injection and surgical resection. Indications are different between the two treatments. Furthermore, indications for each treatment are different from institution to institution. Thus, a case adjudged to be treatable by ethanol injection or surgical resection at an institution may not be given the same treatment at another. The best-known indication criteria may be those proposed in the BCLC protocol (23), which states that surgical resection should be restricted to patients with performance status 0, Child–Pugh class A, single HCC, normal portal pressure and normal serum bilirubin level. In patients satisfying

those criteria, the 5-year survival rate is expected to be >70% (20). In this study, 5-year survival rate of the patients satisfied the criteria was 72.8%, which appears satisfactory when compared with outcomes following surgical resection. Furthermore, in patients with solitary HCC, $\leq 3$ cm in diameter, and Child–Pugh A, 5- and 10-year survival rates were 69.2% and 36.7% respectively. In patients treated by surgical resection, 5- and 10-year survival rates were 34.4–70.0% and 10.5–52.0% respectively (22, 33–39). Although this is an observational study with no control, survivals following ethanol injection appear comparable to those reported following surgical resection.

A randomized controlled trial showed no significant difference in survival between ethanol injection and surgical resection (40). Several non-randomized controlled trials also reported similar overall survival between the two treatments (5–7, 40–43), whereas others reported higher survival with resection (44). Further studies are necessary to resolve this issue of comparing ablation with resection.

We made strenuous efforts to standardize the procedure of ethanol injection because many physicians performed ethanol injection at our institution. In addition to proficient practice of ethanol injection, detailed preoperative planning, cautious postoperative evaluation of therapeutic effect and careful follow-up are vital to achieve satisfactory outcomes.

Source population in this study may represent selection bias, as we performed ethanol injection on most patients who were hospitalized at our department; however, many patients with unfavourable tumour conditions for ethanol injection might not have been referred to us. Therefore, caution is required when extrapolating our findings to the general population of HCC patients.

A second limitation is that study population cannot be clearly defined. This study was based on daily clinical practice over a 20-year period. Indication criteria of ethanol injection changed over time, mainly because of the introduction of the other ablations: microwave ablation and radiofrequency ablation. Furthermore, various treatments besides percutaneous ablations were available for HCC, such as surgical resection and chemoembolization, with frequently overlapping indications.

One further limitation is the fact that this was a single-centre study. To extrapolate the findings in this study to patients at other institutions, consideration should be given to differences in the indications, methods, expertise, performance of available ultrasound and CT equipment and others. Treatment outcome may be influenced by the physicians' expertise and the institution's volume of care. We performed over 2000 ethanol injection treatments, which may represent a much greater number of treatments than those in most other institutions.

In conclusion, our 20-year experience shows that ethanol injection was potentially curative, resulting in long-term survival over 20 years. Findings in this study may suggest that other ablation therapies, such as radiofrequency ablation, will achieve similar or even better long-term results in HCC.

## Acknowledgements

The work had no specific funding.

*Financial disclosures*: There are no financial disclosures from any authors.

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J Gastroenterol (2012) 47:1152–1159
DOI 10.1007/s00535-012-0574-3

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

# Systemic combination therapy of intravenous continuous 5-fluorouracil and subcutaneous pegylated interferon alfa-2a for advanced hepatocellular carcinoma

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Received: 15 November 2011 / Accepted: 27 February 2012 / Published online: 22 March 2012


**Abstract**
*Background* In Japan, sorafenib is now the first-line therapy for individuals with advanced hepatocellular carcinoma (HCC), but no other treatment is available for such patients. The aim of this study was to assess the efficacy and safety of combination therapy with systemic continuous intravenous infusion of 5-fluorouracil (5-FU) and subcutaneous peginterferon alfa-2a, which was used before sorafenib was introduced to Japan.
*Methods* Two hundred and twenty-three HCC patients, who were not amenable to curative surgery, percutaneous ablation, or transarterial chemoembolization (TACE), and for whom intraarterial chemotherapy was not indicated because of the presence of extrahepatic metastasis or stenosis of the common hepatic artery, received peginterferon alfa-2a (90 μg subcutaneously on days 1, 8, 15, and 22) and 5-FU (500 mg/day intravenously given continuously on days 1–5 and 8–12). We assessed their response to treatment and survival, and treatment safety.
*Results* The response rate was 9.4 % (including six patients with complete response) and the disease-control rate was 32.7 %. The median time to progression was 2.0 months. The overall median survival time was 6.5 months (Child–Pugh class A: 9.2 months vs. Child–Pugh class B: 2.8 months). In a multivariate analysis, Eastern Cooperative Oncology Group (ECOG) performance status >0, Child–Pugh class B, and the presence of macroscopic vascular invasion were independent predictors of poor prognosis. The major grade 3–4 adverse events were leucopenia (13.9 %) and thrombocytopenia (5.8 %). No treatment-related deaths occurred.
*Conclusions* This combination therapy was well tolerated and showed promising efficacy. Further studies are needed to establish the usefulness of this treatment.

**Keywords** Hepatocellular carcinoma · Systemic chemotherapy · Survival analysis · Time to progression

**Abbreviations**

| | |
|---|---|
| AIC | Akaike information criterion |
| ALT | Alanine aminotransferase |
| AST | Aspartate aminotransferase |
| CR | Complete response |
| CT | Computed tomography |
| DCP | Des-gamma-carboxy prothrombin |
| ECOG | Eastern Cooperative Oncology Group |
| HBV | Hepatitis B virus |
| HCC | Hepatocellular carcinoma |
| HCV | Hepatitis C virus |
| MRI | Magnetic resonance imaging |
| MST | Median survival time |
| NA | Not assessable |
| PD | Progressive disease |
| PR | Partial response |

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| | |
|---|---|
| RECIST | Response to treatment in solid tumors |
| SD | Stable disease |
| TACE | Transcatheter arterial chemoembolization |
| TTP | Time to progression |
| 5-FU | 5-Fluorouracil |

## Introduction

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death, with a particularly high incidence in Asian countries, including Japan [1, 2]. HCC usually develops in a liver already suffering from chronic disease, most notably due to hepatitis B virus (HBV) or hepatitis C virus (HCV) infection [3]. In the past, HCC was diagnosed often only at a very advanced stage, which was associated with a very poor prognosis [4]. Close surveillance of designated high-risk patients, using advanced diagnostic modalities, has now facilitated HCC detection at a much earlier stage. Together with the considerable advances in HCC treatment, such as surgical resection, percutaneous ablation, transcatheter arterial chemoembolization (TACE), and liver transplantation, the survival time of HCC patients has been much prolonged in recent years [5–10].

However, the potentially curative treatment modalities described above are not indicated for patients with advanced HCC with extrahepatic metastasis or macroscopic vascular invasion , and their prognosis remains poor. In two recent large randomized controlled trials, sorafenib, a multi-kinase inhibitor, significantly prolonged survival in patients with advanced HCC, even when the primary lesion was associated with vascular invasion or extrahepatic metastases, and this agent is now widely regarded as the standard treatment for such patients [11, 12]. However, even with sorafenib, the median survival time (MST) of such patients is rather short, ranging from 6.5 to 10.7 months. Thus, the development of new drugs or new regimens that include cytotoxic and molecular-targeted agents still remains necessary.

Previously, we reported the efficacy of therapy using a combination of intrahepatic arterial 5-fluorouracil (5-FU) and subcutaneous interferon alfa for patients with advanced HCC with portal venous invasion [13]. Because most intraarterially administered 5-FU is taken up by the liver during the first pass, this combination chemotherapy would not be effective against extrahepatic metastasis. Nevertheless, the mechanism underlying the chemotherapy with intraarterial 5-FU would function if 5-FU could reach extrahepatic lesions via systemic administration. Therefore, we expected that a combination of systemic intravenous 5-FU and subcutaneous interferon would be effective against extrahepatic metastasis of HCC. We report the efficacy and safety of this treatment for advanced HCC, which we performed before sorafenib was introduced to Japan.

## Patients, materials, and methods

### Patients

The present study was conducted as a retrospective cohort study. We analyzed 223 consecutive patients who received combination therapy comprised of continuous intravenous infusion of 5-FU and subcutaneous pegylated interferon-alfa for advanced HCC at Kyoundo Hospital from January 1, 2004, to May 31, 2009, when sorafenib was licensed in Japan. The study population consisted of patients with advanced HCC who were not amenable to curative surgery, percutaneous ablation, or TACE, and for whom intraarterial chemotherapy was not indicated because of the presence of extrahepatic metastasis or stenosis of the common hepatic artery. Patients with a previous history of treatment, including systemic chemotherapy, were included. The eligibility criteria also included an Eastern Cooperative Oncology Group (ECOG) performance status score of 2 or less [14], Child–Pugh liver function class A or B, adequate hematologic function (white blood cell count, $\geq$3000/μL; hemoglobin, $\geq$8.5 g/dL; platelet count >30000/μL; and prothrombin time international normalized ratio, $\leq$2.3), adequate hepatic function (albumin, $\geq$2.8 g/dL; total bilirubin, $\leq$3 mg/dL; and alanine aminotransferase [ALT] and aspartate aminotransferase [AST], $\leq$5 times the upper limit of the normal range), and adequate renal function (serum creatinine, $\leq$1.5 times the upper limit of the normal range). Patients were required to have at least one measurable target lesion according to the response to treatment in solid tumors (RECIST) guidelines ver. 1.0 [15]. All patients provided written informed consent before treatment. The treatment protocol was approved by the ethics committee of the institution.

### Diagnosis of HCC

Intrahepatic lesions, vascular invasion, and extrahepatic metastasis of HCC were diagnosed with contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI), considering hyperattenuation in the arterial phase with washout in the late phase as the definitive sign of HCC [16, 17]. Ultrasound-guided tumor biopsy was also performed when radiological findings were atypical. Bone scintigraphy was added when bone metastasis was suspected because of symptoms but was not confirmed on CT or MRI.

### Treatment

One cycle of this treatment consisted of 4 weeks (days 1–28). Peginterferon alfa-2a (90 μg) was administered subcutaneously on days 1, 8, 15, and 22, and 5-FU (500 mg/day) was systemically administered via continuous intravenous infusion, using a portable infusion pump, on days 1–5 and 8–12. Treatment was continued until disease progression, unacceptable toxicity, or patient refusal occurred. This protocol had no treatment interval, and the next cycle started on the day after day 28 of the previous cycle. The first one or two treatment cycles were provided during hospitalization and 5-FU was administered through a peripheral intravenous catheter. Patients who could be expected to survive for a relatively long period underwent implantation of an indwelling central intravenous catheter and were treated on an outpatient basis thereafter. Indwelling central intravenous catheters were inserted by ultrasound-guided subclavian vein puncture and the catheter tip was placed into the superior vena cava using a guidewire under fluoroscopic guidance. When adverse events caused by 5-FU became clinically important, the dose of 5-FU was reduced by 50 %. As prevention and treatment for stomatitis, sodium gualenate hydrate and sodium bicarbonate were used as a gargle. Dexamethasone ointment was also used for stomatitis. Antidiarrheal agents such as loperamide hydrochloride were used for diarrhea.

### Response and toxicity assessment

To assess the response to treatment, contrast-enhanced CT or MRI was performed at the end of the first and second cycles and every two cycles thereafter. In principle, treatment responses were evaluated according to the RECIST guidelines ver.1.0 [15]. The best overall response was adopted in the analysis. Complete response (CR) was defined as the disappearance of both intrahepatic lesions and extrahepatic metastasis. CR was confirmed by repeat assessments performed 4 weeks or more after the criteria for response were first met. Patients who had not completed the first cycle were regarded as having progressive disease (PD) if radiological disease progression was confirmed at the time, and as "not assessable (NA)" if imaging was not performed at the time. Toxicity was evaluated using the National Cancer Institute Common Toxicity Criteria version 3.0. During hospitalization, patients were interviewed about their symptoms and underwent a daily physical examination. Blood tests were performed every week. When treated as outpatients, they were required to visit the outpatient department at least once every 2 weeks.

### Statistical analysis

We included in the analysis those patients who could not complete the first cycle. The categorical variables were compared by $\chi^2$tests, whereas continuous variables were compared with an unpaired Student's *t*-test (parametric) or Mann–Whitney *U*-test (nonparametric). A $P$ value of $<0.05$ was considered statistically significant. Overall survival and time to progression (TTP) were calculated using the Kaplan–Meier method. Patients were censored at the time of the last visit, when lost to follow up, or at the end of the study period. Follow up ended on June 30, 2010. The clinical data at baseline were assessed as predictors of survival using univariate and multivariate Cox proportional hazard regression analysis. The following variables were included in this analysis: age, sex, ECOG performance status, hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (HCVAb), Child–Pugh classification, platelet count, Barcelona-Clinic Liver Cancer (BCLC) staging classification [18], presence of viable intrahepatic lesions, macroscopic vascular invasion, extrahepatic metastasis, and a history of previous treatment. Stepwise variable selection with the Akaike information criterion (AIC) was used to find the best model in multivariate analysis. All analytical procedures were performed with S-plus Ver. 7.0 (Insightful, Seattle, WA, USA).

## Results

### Patients

A total of 223 patients, 176 male and 47 female, with an average age of 64.3 years, received this treatment. Patient characteristics are listed in Table 1. Child–Pugh classification was A in 166 patients (74.4 %) and B in 57 (25.6 %). Macroscopic vascular invasion was present in 103 patients (46.2 %). Extrahepatic metastasis was present in 166 (74.4 %) patients. Those patients without extrahepatic metastasis who were treated with this regimen had contraindications to intraarterial chemotherapy because of stenosis of the common hepatic artery, mainly due to repeated TACE. Two hundred and ten (94.2 %) patients had previously received some other treatment. The median number of cycles of the combination treatment was two (range 1–13). Four patients did not complete the first cycle because of deterioration of performance status, unacceptable toxicity, or patient refusal.

### Response to treatment

Six patients had CR (2.7 %), 15 (6.7 %) had a partial response (PR), 52 (23.3 %) had stable disease (SD), and