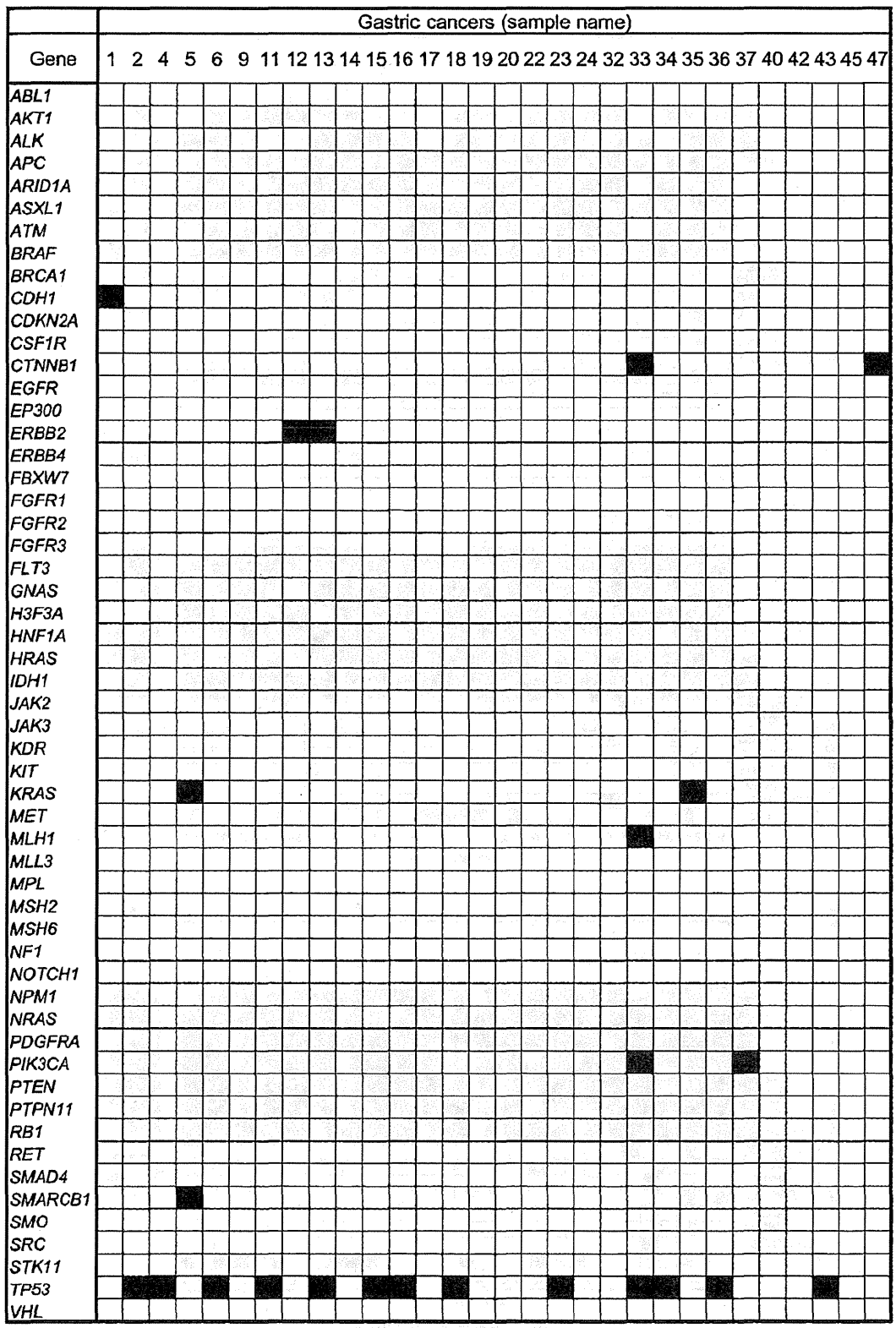

Fig. 2. Results of extensive mutation analysis of the 30 GCs. Mutations of the 55 known cancer-related genes were analyzed by Ion Torrent PGM sequencer. Among the 30 GCs, 19 had 24 somatic mutations of 8 different genes. *TP53* was mutated in 13 GCs (43%, 13 of the 30 GCs), and *CTNNB1*, *ERBB2*, *KRAS*, and *PIK3CA* were mutated in two GCs, respectively. The presence of a somatic mutation is shown by a filled square.

in tumor progression are silenced by aberrant DNA methylation in GCs with the CIMP.

In conclusion, integrated analysis of genetic and epigenetic alterations revealed that the CIMP was associated with mutations of oncogenes, including *ERBB2*, *CTNNB1*, *KRAS* and *PIK3CA*, in GCs.

Acknowledgements

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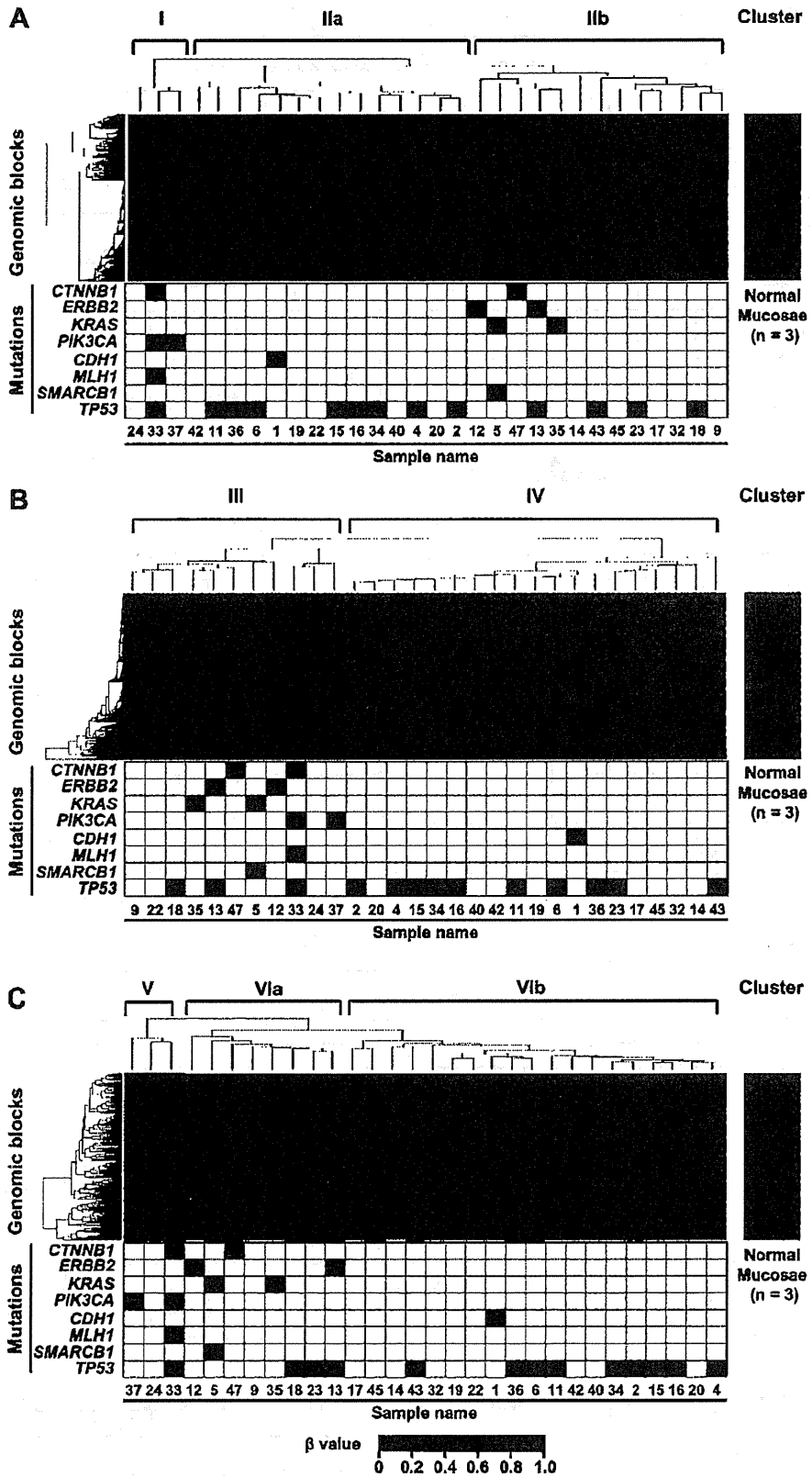

Fig. 3. The association between the DNA methylation profile and gene mutations. (A) Unsupervised hierarchical clustering analysis using DNA methylation profiles of 25,000 genomic blocks with CGIs. Clusters I ($n = 3$) and IIb ($n = 13$) contained GCs with a relatively large number of aberrantly methylated genes, and seven of the 16 GCs were shown to have mutations of oncogenes. (B) Unsupervised hierarchical clustering analysis using DNA methylation profiles of the 6877 blocks (genes) unmethylated in normal gastric mucosae. Cluster III ($n = 11$) contained GCs with a relatively large number of aberrantly methylated genes, and seven of the 11 GCs were shown to have mutations of oncogenes. (C) Unsupervised hierarchical clustering analysis using DNA methylation profiles of the 263 methylation-silenced genes. Cluster V ($n = 3$) contained GCs with the largest number of aberrantly methylated genes, and two of the three were shown to have *PIK3CA* mutations.

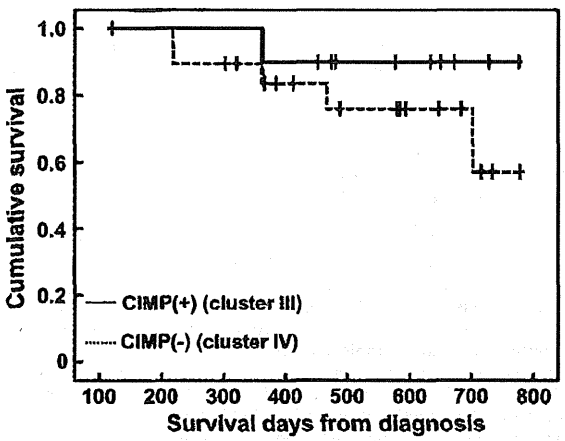

Fig. 4. The possible association between the CIMP and good prognosis. Kaplan-Meier curves were drawn using overall survival (OS). The CIMP status was determined based on the DNA methylation profile of the 6877 genes unmethylated in normal gastric mucosae. The prognosis of the CIMP(+) patients ($n = 11$) tended to be better than that of the CIMP(−) patients ($n = 19$) ($P = 0.285$).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.canlet.2012.11.022.

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Hepatology Research

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Original Article

Low-dose pegylated interferon-alpha-2a monotherapy in elderly and/or cirrhotic patients infected with hepatitis C virus genotype-2 or genotype-1 low level infection

Hideyuki Tamai, Kosaku Moribata, Yoshiyuki Mori, Naoki Shingaki, Hisanobu Deguchi, Kazuki Ueda, Izumi Inoue, Takao Maekita, Mikitaka Iguchi, Jun Kato and Masao Ichinose

Second Department of Internal Medicine, Wakayama Medical University, Wakayama, Japan

Aim: Elderly and/or cirrhotic patients with hepatitis C virus (HCV) are at high risk of adverse effects during interferon therapy. The aim of the present study was to evaluate the efficacy, safety and predictive factors for sustained virological response (SVR) of low-dose pegylated interferon-α-2a (PEG IFN-α-2a) monotherapy in elderly and/or cirrhotic patients with HCV genotype-2 or genotype-1 low level infection.

Methods: Sixty-four elderly (≥65 years) and/or cirrhotic patients with HCV genotype-2 or genotype-1 low level (<5 logIU/mL) infection underwent low-dose PEG IFN-α-2a (90 μg/week) monotherapy for 24 weeks. Sixty patients were available for efficacy assessment.

Results: SVR was achieved in 78.3%. SVR rates according to genotype-1 low, genotype-2 low and genotype-2 high viral load were 90.0%, 87.1% and 57.9%, respectively. The discontinuation rate was 12.5%. PEG IFN-α-2a was interrupted or discontinued in four patients because of severe thrombocytopenia (<25 000/mm^3). The baseline platelet counts of all these patients were less than 70 000/mm^3. On univariate analysis of factors contributing to SVR, significant differences were noted in viral load, platelet count, γ-glutamyltransferase, ferritin, α-fetoprotein level and rapid viral response (RVR). On multivariate analysis, RVR was the only independent factor ($P = 0.010$, odds ratio = 47.27). The positive and negative SVR-predictive values based on RVR were 95% and 82%, respectively.

Conclusion: Low-dose PEG IFN-α-2a monotherapy was effective and tolerable in elderly and/or cirrhotic patients with genotype-2 or genotype-1 low HCV level infection. However, a baseline platelet count of more than 70 000/mm^3 is needed for safety. RVR can predict SVR accurately.

Key words: cirrhosis, elderly patient, genotype 2, hepatitis C virus, low viral load, pegylated interferon-α-2a

INTRODUCTION

HEPATITIS C VIRUS (HCV) elimination in cirrhotic patients leads to not only improved histological findings[1] but also to reduced progression rates to decompensated cirrhosis and hepatocellular carcinoma and improved prognosis.[2,3] By extension, HCV clearance for elderly patients after interferon (IFN) therapy can significantly reduce the risk of hepatocellular carcinoma (HCC) development and achieve prolonged survival.[4,5] Thus, as the survival benefit of HCV clearance in elderly or cirrhotic patients is reasonably larger than that in younger or non-cirrhotic patients, when the prognosis is expected to be prolonged, antiviral therapy should be performed. Because patients with HCV genotype-2 or low viral load irrespective of genotype are more sensitive to IFN than patients with genotype-1 high viral load, these patients should be treated without delay.

Current standard therapy for patients with HCV is PEG IFN and ribavirin combination therapy. However, IFN-based therapy cannot be given safely to all HCV-infected patients due to severe adverse effects. In particular, dose reduction or discontinuation of therapy due to adverse effects is more frequent in elderly or cirrhotic patients.[6–8] Many elderly patients with HCV have other comorbid diseases and cytopenia due to progressive cirrhosis. Cytopenia during IFN therapy becomes worse in such patients, and the drug often has to be reduced in dosage, interrupted or discontinued.[9] To make matters

Correspondence: Dr Hideyuki Tamai, Second Department of Internal Medicine, Wakayama Medical University, 811-1 Kimiidera, Wakayama City, Wakayama 641-0012, Japan. Email: tamahide@wakayama-med.ac.jp

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worse, the sustained viral response (SVR) rates tend to be lower for elderly or cirrhotic patients than for younger and non-cirrhotic patients.[10]

In Japan, standard treatment for naive patients with HCV genotype-2 high viral load is pegylated (PEG) IFN-α plus ribavirin combination therapy for 24 weeks; for patients with low viral load, conventional IFN monotherapy for 24 weeks or PEG IFN-α-2a monotherapy for 24–48 weeks is standard.[11] In practice, a reduced dose can often be given to cirrhotic patients with cytopenia to reduce the risk of severe adverse effects.[11,12] If possible, IFN treatment should be tailored on the basis of safety and prediction of efficacy in high-risk patients.

Pegylated interferon-α-2a monotherapy is more effective than IFN monotherapy[13] and better tolerated than ribavirin combination therapy, although the SVR rate is lower for PEG IFN-α-2a monotherapy than for ribavirin combination therapy.[14] However, because elderly and/or cirrhotic patients have not been included in most randomized, clinical trials, there are not enough data on the safety and efficacy of the current recommended dose of IFN-based therapy for such patients.[15] Therefore, in the present study, low-dose, shorter duration PEG IFN-α-2a monotherapy, which can decrease drug toxicity, was performed to establish an adequate and safe treatment strategy for elderly and/or cirrhotic patients who are expected to be sensitive to IFN. The aim of the present study was to evaluate the efficacy, safety and predictive factors for SVR of low-dose PEG IFN-α-2a for 24 weeks in elderly and/or cirrhotic patients with HCV genotype-2 or genotype-1 low viral load.

METHODS

Patients

THIS WAS A prospective cohort study of low-dose PEG IFN-α-2a monotherapy for high-risk patients, such as elderly and/or cirrhotic patients. A total of 64 elderly patients and/or cirrhotic patients infected with HCV genotype-2 or genotype-1 low viral load who consented to participate in this study underwent low-dose PEG IFN-α-2a monotherapy from January 2004 to December 2010 in our hospital. Patients were enrolled if any of the following were present: (i) patients were 65 years of age or older; (ii) platelet count of less than 130 000/mm^3; and (iii) presence of liver cirrhosis. The exclusion criteria were: (i) hemoglobin (Hb) levels of less than 10 g/dL; (ii) platelet count of less than 50 000/mm^3; (iii) white blood cell (WBC) count of less than 1500/mm^3 (or granulocyte count <1000/mm^3); (iv) hepatic failure or cancer; (v) patients who used *sho-saikoto* (a Kampo medicine); (vi) intractable heart disease; and (vii) uncontrollable psychoneurotic disorders. All enrolled patients underwent abdominal ultrasonography and contrast-enhanced computed tomography for diagnosis of liver cirrhosis and HCC screening within 1 month before the start of therapy. Liver cirrhosis was diagnosed clinically by imaging and laboratory tests or liver histology. A liver biopsy was performed in all patients. Even if the fibrosis grade was underestimated on liver biopsy tissue examination, liver cirrhosis was diagnosed using the morphological appearance of cirrhosis with portal hypertension, such as portosystemic shunt or hypersplenism, on imaging. Steatosis was defined as positive if hepatorenal contrast was detected on ultrasonography. In the present study, the potential benefits and risks were explained to all patients before obtaining their written, informed consent. All study protocols were approved by the ethics committee of Wakayama Medical University. The study was performed according to the World Medical Association Declaration of Helsinki.

Treatment regimens

Half of the recommended dose of PEG IFN-α-2a (Pegasys; Roche, Basel, Switzerland) was used; 90 μg PEG IFN-α-2a was administrated s.c. once a week for 24 weeks without ribavirin. There were no dose reduction criteria in this study. PEG IFN-α-2a was interrupted based on the following criteria: (i) if the Hb fell below 8.5 g/dL; (ii) if the granulocyte count fell below 500/mm^3, or the platelet count fell below 25 000/mm^3; and (iii) if deemed necessary by the attending physician because of adverse events. The treatment could be restarted if cytopenia improved. If there was no improvement in hematological parameters or adverse events within 4 weeks, this therapy was discontinued. Although there are no guidelines on the use of granulocyte colony-stimulating factor (G-CSF) for IFN-induced granulocytopenia, G-CSF (Neutrogin 100 μg; Chugai Pharmaceutical, Tokyo, Japan) was used if severe granulocytopenia (<500/mm^3) developed. If PEG IFN-α-2a was repeatedly interrupted due to granulocytopenia, G-CSF was administrated 2–3 days before weekly PEG IFN-α-2a.[16] However, erythropoietin was not allowed as supplementary treatment because the Ministry of Health in Japan had not approved its use.

Laboratory tests and liver histology

Hepatitis C virus genotype was determined using the antibody serotyping method. HCV serotype-1 and -2

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correspond to genotype-1a/1b and -2a/2b, respectively. If HCV serotype could not be determined, genotype was examined. HCV RNA was measured using the quantitative and qualitative Amplicor HCV monitor ver. 2.0 test (Roche Diagnostics, Branchburg, NJ, USA) until March 2008. If HCV RNA was undetectable using quantitative reverse transcription polymerase chain reaction (RT–PCR), it was measured using qualitative RT–PCR. From April 2008, the amount of HCV RNA was measured using a COBAS TaqMan PCR assay (Roche Diagnostics). A HCV RNA level of more than 100 KIU/mL or 5 logIU/mL was defined as a high viral load, and less than 100 KIU/mL or 5 logIU/mL was defined as a low viral load. In addition to biochemical analyses including serum alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), total bilirubin, prothrombin time and albumin, levels of fibrosis markers (type IV collagen 7S and hyaluronic acid), ferritin and α-fetoprotein (AFP) were also measured within 1 month before the start of therapy. During therapy, blood cell counts were checked before treatment every week, and HCV RNA and biochemical analyses were measured every 4 weeks up to 24 weeks after the end of therapy. In all patients, within 3 months before the start of therapy, a core needle biopsy of the liver was done under ultrasound guidance using a 16-G core biopsy needle (Bard Monopty, Covington, GA, USA). The METAVIR scoring system[17] was used to analyze the histological findings and to classify patients based on activity (grades A0–A3) and fibrosis (stages F0–F4).

Assessment of effectiveness

During IFN therapy, rapid virological response (RVR) was defined as viral negativity using qualitative RT–PCR at week 4 from therapy initiation, corresponding to 1.7 logIU/mL by the TaqMan PCR assay. SVR was defined as follows: the HCV RNA was negative at the end of therapy and remained negative for 24 weeks after the end of therapy. No response was defined as detectable HCV RNA at week 24 from treatment initiation or at the end of treatment. Relapse was defined as negative at the end of therapy but positive 24 weeks after the end of therapy.

Assessment of safety and tolerability

Patients were assessed for safety and tolerability during treatment by their attending physicians who monitored adverse events and laboratory abnormalities, such as blood cell counts, every week up to week 24 and monthly thereafter. Adverse events were graded as mild (not requiring interruption or discontinuation), moderate (requiring interruption) or severe (requiring discontinuation), according to World Health Organization recommendations. The incidence and reasons for therapy discontinuation were analyzed.

Statistical analysis

Therapeutic effectiveness was determined using an intention-to-treat analysis that included patients who did not complete the scheduled course of therapy. Predictive factors for SVR were analyzed using a per protocol analysis that excluded patients who discontinued because of adverse events. The Mann–Whitney *U*-test was used to analyze continuous variables. Fisher's exact test or the χ^2-test was used to analyze categorical variables. Multivariate analysis was performed using a logistic regression model with the stepwise method. The criteria for selecting factors for multivariate analysis was $P < 0.05$. Each optimal cut-off value for continuous variables of SVR-predicting factors was decided by the Youden Index method on the basis of the receiver–operator curve (ROC). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy for SVR of RVR were calculated. Values of $P < 0.05$ were considered significant. The statistical software used was SPSS ver. 20.0J for Windows (SPSS, Tokyo, Japan).

RESULTS

Baseline background factors

THE PATIENTS' BASELINE characteristics are summarized in Table 1. There were 41 male and 23 female patients. Their median age was 68 years (range, 35–79); 45 (70.3%) patients were aged 65 years or older. Overall, 59 patients had cirrhosis; of the 45 elderly patients, 40 had cirrhosis. While 22 patients had a past history of treatment for HCC, 27 had hypertension, 15 had diabetes mellitus, 10 had diabetes mellitus and hypertension, four had thyroid dysfunction, three had chronic renal failure and three had a past history of cerebral stroke. A total of 32 patients had HCV genotype-2 low viral load, 22 had genotype-2 high viral load and 10 had genotype-1 low viral load.

Therapeutic effectiveness

Efficacy 24 weeks after the end of treatment could be assessed in 60 patients. SVR was achieved in 78.3% (47/60), relapse occurred in 16.7% (10/60) and there was no response in 5.0% (3/60). The mean PEG IFN-α-2a adherence (mean ± standard deviation) was

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Table 1 Patients' baseline characteristics

Age, years (range)	68 (35–79)
<65/≥65 years (%)	19/45 (29.7/70.3)
Cirrhosis/elderly/cirrhosis and elderly	59/45/40
Sex, male/female	41/23
Bodyweight (kg)	60.4 (35.0–92.6)
Body mass index (kg/m^2)	22.4 (18.0–30.0)
Prior interferon therapy (%)	4 (6.3)
History of HCC treatment (%)	22 (34.4)
Genotype (1/2)	10/54
HCV viral load (L/H)	41/23
Genotype and viral load (1L/2L/2H)	10/32/22
White blood cells (/mm^3)	3950 (1700–7300)
Hemoglobin (g/dL)	13.0 (10.5–16.2)
Platelets (×10^4/mm^3)	9.4 (5.5–23.8)
ALT (IU/L)	45 (11–233)
GGT (IU/L)	45 (15–641)
Type IV collagen 7S (ng/mL)	8.0 (3.1–19.1)
Hyaluronic acid (ng/mL)	235.5 (29.0–1960.7)
Prothrombin time (%)	80.9 (54.2–108.0)
Albumin (g/dL)	3.7 (2.6–4.7)
Total bilirubin (mg/dL)	1.1 (0.5–2.5)
Ferritin (ng/mL)	131 (22–1034)
AFP (ng/mL)	13.7 (0.4–610.9)
Activity grade (A1/A2/A3)	22/31/11
Fibrosis stage (F1/F2/F3/F4)	0/12/30/22
Steatosis (%)	12 (18.8)
Prior splenectomy (%)	4 (6.3)

Values are expressed as medians (range) or number of patients (percent).
AFP, α-fetoprotein; ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; H, high; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; L, low.

95.2% ± 18.0% in the SVR group and 95.2% ± 14.0% in the non-SVR group. There was no significant difference in PEG IFN adherence between the SVR group and the non-SVR group ($P = 0.402$). The SVR rates in the group less than 65 years old, the group aged 65–69 years old and the group aged 70 years and older were 78.9% (15/19), 78.3% (18/23) and 77.8% (14/18), respectively ($P = 0.996$). The SVR rates were 90.0% (9/10) for patients with genotype-1 low viral load, 87.1% (27/31) for patients with genotype-2 low viral load and 57.9% (11/19) for patients with genotype-2 high viral load. Relapse occurred in 10.0% (1/10) of genotype-1 low viral load, 9.7% (3/31) of genotype-2 low viral load and 31.6% (6/19) of genotype-2 high viral load. No response was seen in 0.0% (0/10) of genotype-1 low viral load, 3.2% (1/31) of genotype-2 low viral load and 10.5% (2/19) in genotype-2 high viral load. Therapeutic effectiveness according to genotype and viral load is shown in Figure 1.

Safety and tolerability

Mild adverse events occurred in seven patients; these were fatigue ($n = 4$), retinopathy with mild bleeding ($n = 2$) and psoriasis ($n = 1$). Moderate adverse events occurred in five patients. Those were thrombocytopenia ($n = 3$), acute pyelitis ($n = 1$) and granulocytopenia ($n = 1$). Therapy was discontinued in eight (12.5%) of the 64 patients. The discontinuation rates in the group less than 65 years old, the group 65–69 years old and the group 70 years and older were 10.5% (2/19), 4.3% (1/23) and 22.7% (5/22), respectively ($P = 0.168$). The reasons for therapy discontinuation due to adverse events were severe fatigue ($n = 1$), femoral neck fracture due to fall ($n = 1$), cerebral contusion due to fall ($n = 1$), thrombocytopenia ($n = 1$), severe dermatitis ($n = 2$), bacterial pneumonia ($n = 1$) and rupture of esophageal varices ($n = 1$). The ages of the two patients injured due to falls were 78 and 79 years, respectively; in both cases, the falls were accidental, and neither patient had anemia nor cerebral ischemia.

Pegylated interferon-α-2a was interrupted or discontinued in four patients because of severe thrombocytopenia (<25 000/mm^3). The baseline platelet counts of these patients were all less than 70 000/mm^3; they accounted for 40.0% (4/10) of patients with platelet counts of less than 70 000/mm^3.

Contributing factors for SVR and prediction of SVR

On univariate analysis of factors contributing to SVR, significant differences were noted in the platelet count,

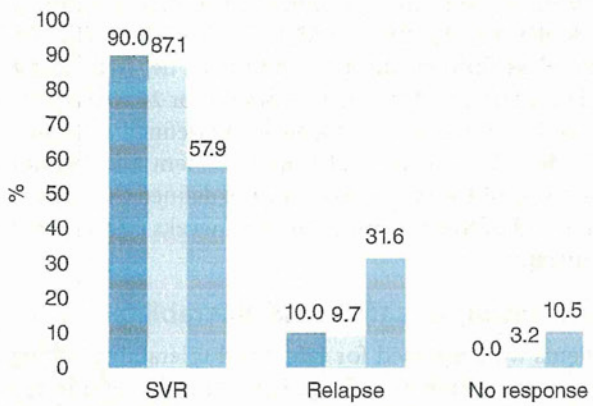

Figure 1 Therapeutic effectiveness according to genotype and viral load. SVR, sustained virological response; G1L, genotype-1 low viral load; G2L, genotype-2 low viral load; G2H, genotype-2 high viral load. ■, G1L; ■, G2L; ■, G2H.

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Table 2 Comparison of factors between patients with and without SVR

Factors	SVR ($n = 44$)	Non-SVR ($n = 12$)	*P*-value
Age (years)	68	67	0.696
Sex, male/female	29/15	6/6	0.335
Bodyweight (kg)	61.0	58.0	0.516
Body mass index (kg/m^2)	22.5	22.5	0.960
White blood cells (/mm^3)	4160	3640	0.088
Hemoglobin (g/dL)	13.2	13.2	0.689
Platelets ($\times 10^4$/mm^3)	10.6	7.1	0.001
ALT (IU/L)	44	45	0.834
GGT (IU/L)	41	65	0.007
Ferritin (ng/mL)	111.9	251.9	0.021
AFP (ng/mL)	12	43.6	0.025
Type IV collagen 7S (ng/mL)	7.6	8.8	0.159
Hyaluronic acid (ng/mL)	212.6	327.0	0.168
Activity grade (A1/A2,3)	15/29	5/7	0.627
Fibrosis stage (F1,2/F3,4)	8/36	4/8	0.263
HCV RNA (logIU/mL)	4.4	5.7	0.001
Genotype (1/2)	9/35	1/11	0.671
History of HCC treatment (%)	13 (29.5)	6 (50.0)	0.185
Prior interferon therapy (%)	2 (4.5)	2 (16.7)	0.198
Steatosis (%)	9 (20.5)	3 (25.0)	0.707
RVR (positive/negative)	42/2	2/10	<0.001

Values are expressed as medians or number of patients (percent).
AFP, α-fetoprotein; ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; RVR, rapid virological response; SVR, sustained virological response.

GGT, ferritin, AFP level, HCV RNA load and RVR (Table 2). Cut-off values of continuous data for multivariate analysis were determined by ROC analysis as follows: HCV RNA, 5 logIU/mL; platelets, 8×10^4/mm^3; GGT, 45 IU/mL; AFP, 15 ng/mL; and ferritin, 170 ng/mL. RVR was the only independent factor on multivariate analysis (Table 3). The sensitivity, specificity, PPV, NPV and accuracy for SVR of RVR are summarized according to baseline viral load in Table 4. RVR was an accurate predictor for SVR regardless of viral load.

DISCUSSION

IN THE PRESENT study, there were some IFN-related adverse events. First, femoral neck fracture and cerebral contusion due to falls, characteristic adverse events in very elderly patients, occurred in two patients whose ages were 78 and 79 years, respectively. In our study of low-dose PEG IFN-α-2b plus ribavirin combination therapy for such high-risk patients infected with HCV genotype-1b high viral load, spinal compression frac-

Table 3 Independent factors contributing to SVR on multivariate analysis

Factors	*P*-value	Odds ratio	95% CI
HCV RNA (<5 logIU/mL)	0.289	7.02	0.19–257.73
Platelets (>8×10^4/mm^3)	0.082	53.83	0.60–4828.20
GGT (<45 IU/mL)	0.634	2.12	0.10–46.15
Ferritin (<170 ng/mL)	0.222	11.11	0.23–527.42
AFP (<15 ng/mL)	0.324	5.65	0.18–176.70
RVR	0.010	47.27	2.49–896.31

The cut-off value for each factor was determined by receiver–operator curve analysis.
AFP, α-fetoprotein; GGT, γ-glutamyltransferase; HCV, hepatitis C virus; RVR, rapid virological response; SVR, sustained virological response.

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Table 4 Sensitivity, specificity, PPV, NPV and accuracy for SVR of RVR according to viral load

Patients	Sensitivity	Specificity	PPV	NPV	Accuracy
All	95%	82%	95%	82%	92%
Low viral load	97%	75%	97%	75%	94%
High viral load	91%	86%	91%	86%	89%

NPV, negative predictive value; PPV, positive predictive value; RVR, rapid virological response; SVR, sustained virological response.

tures due to falls also occurred in two cases.[18] Therefore, elderly patients must be thoroughly warned about falls prior to IFN treatment. Other adverse events that should be monitored in elderly and/or cirrhotic patients are ruptured esophageal varices, bacterial infections and thrombocytopenia. To prevent bleeding from varices during IFN treatment, upper gastrointestinal endoscopy is performed before treatment in cirrhotic patients, and if gastroesophageal varices that have a high risk of rupture are detected, they should be treated before IFN treatment. Furthermore, Roomer *et al.*[19] reported that older patients and patients with poorly controlled diabetes mellitus have a greater risk of developing infections during HCV treatment. In the present study, acute pyelitis and bacterial pneumonia occurred in patients with diabetes mellitus and required the discontinuation of PEG IFN in the patient who had bacterial pneumonia. With respect to thrombocytopenia during treatment, the baseline platelet counts of all patients whose PEG IFN treatments were interrupted or discontinued because of severe thrombocytopenia (<25 000/mm^3) were all less than 70 000/mm^3; they accounted for 40% of the patients with platelet counts of less than 70 000/mm^3. Even this low-dose PEG IFN regimen should be avoided in patients with thrombocytopenia of less than 70 000/mm^3 for safety. Frequent monitoring for adverse events and caution with respect to unexpected fever are needed even with the low-dose regimen.

Because IFN treatment is associated with adverse effects, if SVR can be predicted even on treatment using a reduced dose and/or a shorter duration, reducing the dose or shortening the duration is desirable for safety and cost. In the present study, for high-risk patients, half of the recommended dose of PEG IFN-α-2a without ribavirin for 24 weeks was sufficiently effective (SVR rate ~90%) for patients with a low viral load irrespective of genotype and for selected patients with genotype-2 high viral load who achieved RVR. RVR is known to be the most useful predictor of SVR with standard PEG IFN plus ribavirin therapy for patients with HCV genotype 2/3 who are sensitive to IFN.[20] Using RVR, patients who can be treated for a shorter duration can be selected. With respect to PEG IFN-α-2a monotherapy, Etoh *et al.*[21] reported that PEG IFN-α-2a monotherapy using the recommended dose for 24 weeks or less may be sufficient to treat selected patients with HCV genotype 2, especially those with low viral load achieving RVR. In a small randomized trial, Iwasaki *et al.*[22] demonstrated that 24-week treatment with the recommended dose of PEG IFN-α-2a alone is clinically sufficient in patients with HCV genotype 2 and pretreatment viral load below 1000 KIU/mL who achieve RVR. Excluding patients with high titers of genotype 1 HCV, Jeong *et al.*[23] performed a prospective controlled trial to compare the efficacy of an 8-week and a 24-week course of PEG IFN-α-2a monotherapy using the recommended dose for patients negative for HCV RNA at 2 weeks after therapy initiation. Their results suggested that patients who achieved an ultra-RVR (HCV RNA negativity at week 2 from the start of therapy) can receive an 8-week course of PEG IFN-α-2a monotherapy. Thus, even for PEG IFN-α-2a monotherapy, RVR or ultra-RVR will become useful to monitor treatment efficacy and guide decisions on treatment duration. However, there are no data about whether RVR can select the patients who can be treated with a reduced dose. The present results indicated that RVR can predict SVR accurately irrespective of viral load even on low-dose PEG IFN-α-2a monotherapy for high-risk patients with genotype-2 or genotype-1 low viral load. However, the present study population was small and non-randomized. Further randomized trials in patients who achieve RVR are needed to determine the optimal dose and duration.

In conclusion, low-dose PEG IFN-α-2a monotherapy for 24 weeks was an effective and tolerable regimen for elderly and/or cirrhotic patients infected with HCV genotype-2 or genotype-1 low viral load. This low-dose regimen without ribavirin appears to be optimal in efficacy and cost for high-risk patients with low viral load irrespective of genotype. Furthermore, as an SVR of approximately 60% can be expected even for patients with genotype-2 high viral load, this regimen should

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also be attempted first for naïve, high-risk patients with HCV genotype-2 high viral load. RVR is also an accurate predictor for SVR with this regimen, and it can be used to select patients who can be treated with a reduced dose. This low-dose regimen may be sufficient to treat selected patients with genotype-2 high viral load achieving RVR. However, even with this regimen, a baseline platelet count of more than 70 000/mm^3 is needed for safety, and elderly/cirrhotic patients should be warned about and carefully monitored for severe adverse events such as severe cytopenia, infections and falls.

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Original Article

Usefulness of a continuous suction mouthpiece during percutaneous endoscopic gastrostomy: A single-center, prospective, randomized study

Takao Maekita,[1] Jun Kato,[1] Yukihiko Nakatani,[2] Shotaro Enomoto,[1] Takashi Kayama,[2] Masahiro Tsuji,[2] Tsuyoshi Nakaya,[2] Yosuke Muraki,[1] Hisanobu Deguchi,[1] Kazuki Ueda,[1] Izumi Inoue,[1] Mikitaka Iguchi,[1] Hideyuki Tamai[1] and Masao Ichinose[1]

[1]*Department of Gastroenterology, School of Medicine, Wakayama Medical University and* [2]*Department of Internal Medicine, Nakaya Hospital, Wakayama, Japan*

Background: No mouthpiece has been designed to control salivary flow during endoscopic procedures. A new continuous suction mouthpiece (CSM) was developed, and its usefulness for percutaneous endoscopic gastrostomy (PEG) was evaluated.

Patients and Methods: Seventy-two patients who were scheduled to undergo PEG or the exchange of a gastrostomy button or tube were assigned to one of two groups: the group using the CSM and the group using the conventional mouthpiece. Aspiration pneumonia, procedure duration, extent of salivary flow, frequency of saliva suction, and number of choking episodes during the procedures were evaluated and compared between the two groups.

Results: The same number of patients was randomly allocated to each group. There were no significant differences between the two groups in sex, age, procedure type, duration of procedure, depth of sedation, and indication for the procedure. The grade of salivary flow was significantly lower in patients with the CSM than in patients with the conventional mouthpiece ($P < 0.001$). Significantly fewer suctions and choking episodes were observed in patients with the CSM than in patients with the conventional mouthpiece ($P = 0.013$, and $P = 0.015$, respectively). Aspiration pneumonia and other significant adverse events were not observed in either group.

Conclusions: CSM reduced the number of episodes associated with salivary flow in PEG-related procedures. The device is expected to reduce complications such as aspiration not only in PEG but in other upper endoscopic procedures.

Key words: aspiration, mouthpiece, percutaneous endoscopic gastrostomy, salivary flow

INTRODUCTION

PERCUTANEOUS ENDOSCOPIC GASTROSTOMY (PEG) is a procedure that was developed to provide direct access to the stomach with endoscopy in 1980,[1] and it has been increasingly carried out for patients who suffer from dysphagia and/or need long-term enteral nutrition. As PEG is relatively safe and easy to carry out, it has become the preferred option for creating a gastrostomy.

However, there is a non-negligible risk of adverse events in association with PEG. In particular, aspiration is the most problematic, because PEG is carried out with the patient in the supine position, it takes a long time, and it is likely to be carried out in elderly patients with dysphagia. Moreover, sedation during the procedure may increase the risk. The reported rate of early phase complications in PEG is approximately 2.3%.[2]

One of the most important factors correlated with aspiration is salivary flow induced by the introduction/extraction of the endoscope into the oral cavity. In this context, control of salivary flow during PEG is important for the prevention of aspiration. However, few attempts have been made to control salivary flow, perhaps due to its difficulty. Currently, an endoscopist or an assistant must check the accumulation of saliva and suction it using a catheter in case the patient undergoing the procedure cannot discharge saliva from the mouth.

During the upper endoscopy procedure, a hard plastic mouthpiece is used to protect the endoscope from being bitten and for smooth insertion of the endoscope without

Corresponding: Takao Maekita, Department of Gastroenterology, School of Medicine, Wakayama Medical University, 811-1 Kimiidera, Wakayama City, Wakayama 641-0012, Japan. Email: maekita@wakayama-med.ac.jp

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hindrance from the tongue. A mouthpiece having the function of suctioning saliva might be useful for preventing aspiration during endoscopic procedures. However, no mouthpiece has been designed for this purpose.

With this background, a new continuous suction mouthpiece (CSM) was developed to prevent adverse events in association with salivary flow during endoscopy. The mouthpiece was made by hand by retrofitting a commercially available mouthpiece. The aim of the present study was to evaluate the usefulness and the ability of the newly developed CSM for the prevention of aspiration during PEG by examining salivary flow and the incidence of aspiration-related events during the procedure.

METHODS

Equipment

AFTER CUTTING THE junction part of a non-toxic polyvinyl chloride (PVC) suction tube (NIPRO SUCTION CATHETER® 14-Fr; NIPRO, Osaka, Japan), the tube was bent double, and each part was connected with two movable short bands made of non-toxic PVC suction tube (NIPRO SUCTION CATHETER® 16-Fr; NIPRO). The three parts divided by the short bands were made into: a 2–5-cm-diameter loop intra-oral part for suction; a binding loop part to fit mouthpieces of various sizes; and an extra-oral part having two ends, both of which were linked to the Y-shaped connector (ARAM, Osaka, Japan) (Fig. 1A). The Y-shaped connector could be connected to the main suction tube leading to the suction unit (Shin-Ei Industries, Tokyo, Japan).

Smooth, 2.7-mm-diameter holes were made spirally in six locations in the body of the intra-oral loop, at equally spaced intervals. The holes were arranged in such a way as to prevent direct suction of the oral mucosa. The size of the intra-oral loop can be changed from 2 to 5 cm by sliding movable short bands to match the physique of each patient. Finally, the MB-142 mouthpiece (Olympus, Tokyo, Japan) was inserted into the binding loop part. The finished product of the CSM is shown in Figure 1B,C.

For the upper endoscopic procedure, the patient was placed on his or her left side and asked to bite down on the mouthpiece with the intra-oral loop with holes placed inside the left cheek (Fig. 2). Suctioning was continuously carried out at low pressure (10 kPa) through the unification tube attached to the Y-shaped connector during the endoscopic procedure. For the control subjects, the MB-142 mouthpiece was used as it was.

Patients and study design

This was a single-center, prospective, randomized, controlled study. Patients with brain disease, neurological disease, dementia, temporomandibular joint disorder, disuse syndrome, or a psychiatric disorder who were scheduled to undergo PEG or the exchange of a gastrostomy button or tube with endoscopy at Nakaya Hospital (Wakayama, Japan) from March 2011 to December 2011 were recruited. Patients were excluded if they had a history of respiratory problems that could increase the risk of complications associated with aspiration pneumonia and salivary flow. Eligible patients were randomly assigned to each of the following groups: the group using the CSM and the group using the conventional mouthpiece for the procedure. Randomization was carried out using the sealed envelope technique. During the procedures, salivary flow and complications associated with aspiration were evaluated and compared between the two groups. By its nature, this study could not be blinded.

This study was approved by the ethics committee of Nakaya Hospital. Written, informed consent was obtained from each patient or the next of kin. We have registered this study into University hospital Medical Information Network (UMIN) as UMIN000008575. The CSM was developed by our institute without any financial or equipment support from companies.

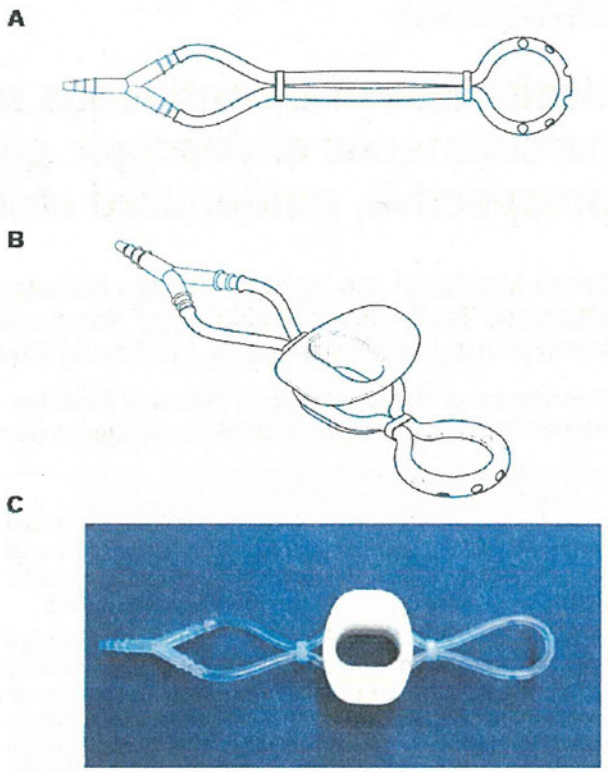

Figure 1 Continuous suction mouthpiece (CSM). (A) CSM without the mouthpiece. (B) CSM with the mouthpiece. (C) Photograph of the CSM.

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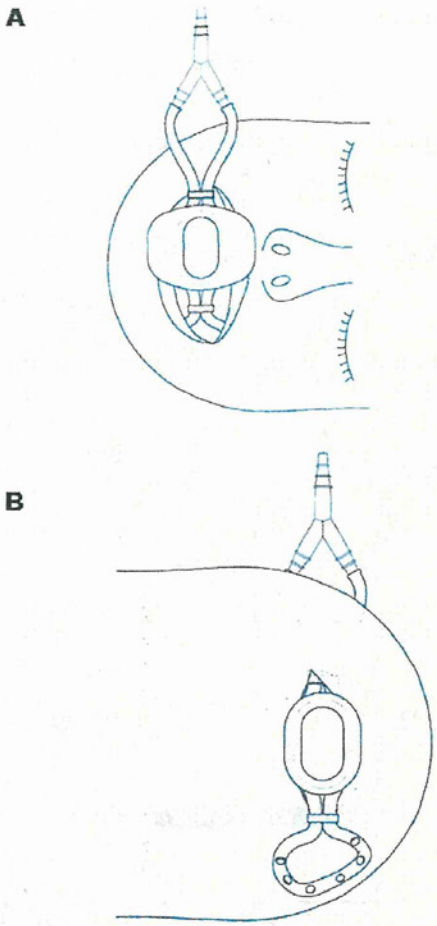

Figure 2 Schema for using the continuous suction mouthpiece (CSM). (A) View from outside of the body while using the CSM. Solid lines show the extra-oral part and broken lines show the intra-oral part. (B) View from inside of the body while using the CSM. Solid lines show the visible part and broken lines show the invisible part.

Procedures for PEG and exchange of a gastrostomy button or tube

Percutaneous endoscopic gastrostomy was carried out using a percutaneous endoscopic gastrostomy kit with a Funada-style fixture (Create Medic Co., Yokohama, Japan), whereas for the exchange of a gastrostomy button or tube, the new catheter (Kangaroo Button™ II; Nippon Sherwood Co., Tokyo, Japan) was placed after pulling the old button or tube out of the gastrocutaneous fistula. At each procedure, a conventional gastrointestinal videoscope (GIF-XP260N; Olympus) was orally inserted into the stomach to observe the upper gastrointestinal tract and to identify the site of insertion of the tube by transillumination and palpation of the abdominal wall. The endoscope was left in the stomach throughout the procedure. The procedures for all patients were carried out by one endoscopist and one assistant doctor.

Premedication with anticholinergic agents or glucagon was not used. Lidocaine 8% was sprayed into the posterior pharynx before insertion of the endoscope to reduce the gag reflex in all patients. Then, midazolam (1–5 mg) was given i.v. for sedation. During insertion of the endoscope, the patient was placed on his or her left side and, after the scope reached the inside of the stomach, the patient was shifted to the supine position, with only the patient's face facing to the left. Adequate monitoring of vital signs and oxygen saturation was carried out throughout the procedure.

Outcome assessment and evaluation

Primary outcome was occurrence of aspiration pneumonia. Secondary outcomes were extent of salivary flow, frequency of saliva suction, and the number of choking episodes during the procedure. Adverse events during and after the procedure were also examined. In addition, the oral cavity was meticulously examined after the procedure to determine whether blood blisters or any suction tube fragments were present in the oral cavity.

The duration of procedures using the CSM included the time required for biting down on the mouthpiece with the intra-oral loop placed inside the left cheek. The level of sedation was defined as follows: non, no use of sedatives; mild, conscious sedation; moderate, between conscious sedation and deep sedation; and deep, deep sedation. The extent of salivary flow was defined as follows: grade 1, no flow of saliva out of the mouth; grade 2, flow extending to the cheek; grade 3, flow extending to the ear; and grade 4, flow extending to the hair or clothing. When a rumbling sound was heard in the oropharyngeal region, the assistant suctioned the saliva during the procedure using the suction catheter (NIPRO SUCTION CATHETER® 14-Fr; NIPRO). Choking episodes were counted each time they occurred during the procedure, whereas consecutive coughs or chokes were counted as one choking episode.

Statistical analysis

Data were expressed as medians with ranges. Data were analyzed using the unpaired Mann–Whitney *U*-test and Fisher's exact test. The level of statistical significance was $P < 0.05$. All analyses were carried out using the SPSS 11.0 software package (SPSS Inc., Chicago, IL, USA).

RESULTS

A TOTAL OF 72 subjects (22 men and 50 women, median age 80.5 years [range 49–98]) were recruited during the study period, and all patients were considered

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Table 1 Characteristics of patients assigned to the CSM group or to the conventional (MB-142) mouthpiece group

	CSM	MB-142	*P*-value
Sex, male/female	14/22	8/28	0.125
Age, years median (range)	80 (49–92)	81 (58–98)	0.310
PEG procedure, placement/exchanging	4:32	4:32	1.000
Duration of procedure, minutes median (range)	8.5 (5–20)	8 (4–33)	0.258
Sedation, non/mild/moderate/deep	1/4/6/25	1/2/14/19	0.166
Underlying disease, brain/neurological/dementia/temporomandibular joint disorder/disuse syndrome/psychiatric disorder	23/2/6/1/4/0	24/1/6/0/4/1	0.798

CSM, continuous suction mouthpiece; MB-142, MB-142 mouthpiece (Olympus, Tokyo, Japan); PEG, percutaneous endoscopic gastrostomy.

eligible. Of the 72 patients enrolled, 66 (92%) could not express his or her will to participate in this study. In such cases, the next of the kin provided the consent. Of the patients, 36 were assigned to the CSM group, and the remaining 36 were allocated to the conventional mouthpiece group. The patients' characteristics are summarized in Table 1. There were no significant differences between the two groups in sex, age, procedure type, duration of procedure, depth of sedation, and indication for the procedure.

Aspiration pneumonia was not observed in any of the participating patients. The grade of salivary flow was significantly lower in patients with the CSM than in patients with the conventional mouthpiece ($P < 0.001$) (Fig. 3). Significantly fewer suctions and choking episodes were observed in patients with the CSM than in patients with the conventional mouthpiece ($P = 0.013$, and $P = 0.015$, respectively) (Figs 4,5). Complete obstruction of the holes, blood blisters or fragments of the PVC suction tubes were not observed in patients with the CSM. No other significant adverse events were observed in any of the patients.

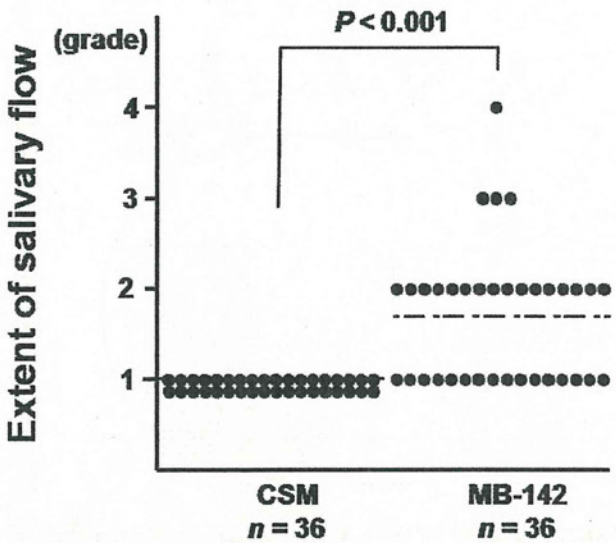

Figure 3 Extent of salivary flow. Grade of salivary flow is significantly lower in patients with the continuous suction mouthpiece (CSM) than in patients with the conventional mouthpiece (MB-142; Olympus, Tokyo, Japan) ($P < 0.001$). •, each value for each case; ——, mean value for CSM; ·–·–·, mean value for MB-142.

DISCUSSION

ALTHOUGH ENDOSCOPY-RELATED COMPLICATIONS associated with salivary flow can be quite troublesome, little attention has been paid to these complications. To the best of our knowledge, this is the first attempt to control salivary flow by continuous suctioning during endoscopic procedures. The present study showed that, during PEG procedures, salivary flow was less extended in patients with the CSM than in patients with the conventional mouthpiece. Moreover, significantly fewer suctioning and choking episodes were observed in patients with the CSM.

The present results suggest that the CSM has many advantages during endoscopic procedures. First, the CSM may decrease complications of aspiration during endoscopic procedures, because use of the CSM reduced the frequencies of saliva suction and choking episodes. It has been reported that there is considerable risk of aspiration during the PEG procedure in elderly patients and in patients with brain infarction, neurological disease, or dementia, because these patients cannot gag or choke adequately.[2] Therefore, although no aspiration pneumonia was observed in patients in either group in the present study, perhaps due to the small number of patients, use of the CSM in PEG procedures is recommended. Second, a nurse's time and effort can be saved during endoscopic procedures, because the frequency of suctioning is reduced. Third, the CSM could prevent exposure of the patient's body, clothing or operating bed to saliva. This advantage might reduce patient discomfort, as well as the time and cost required for clean-up. Fourth, continuous

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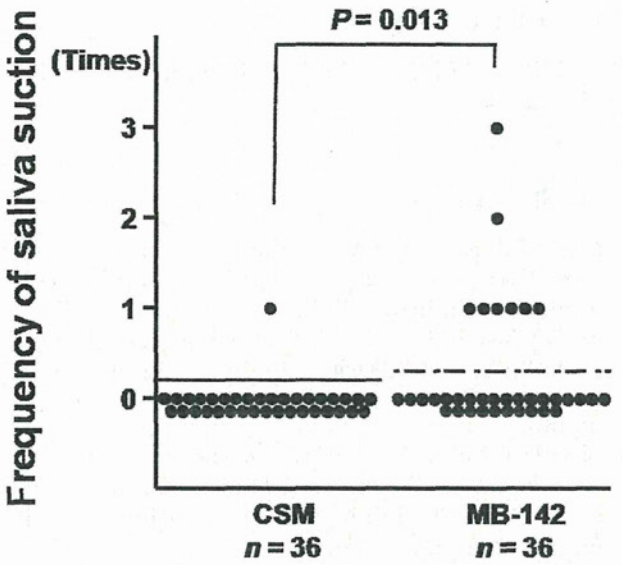

Figure 4 Frequency of saliva suction. Significantly fewer suctions were observed in patients with the continuous suction mouthpiece (CSM) than in patients with the conventional mouthpiece (MB-142; Olympus, Tokyo, Japan) ($P = 0.013$). •, each value for each case; ——, mean value for CSM; ·–·–·, mean value for MB-142.

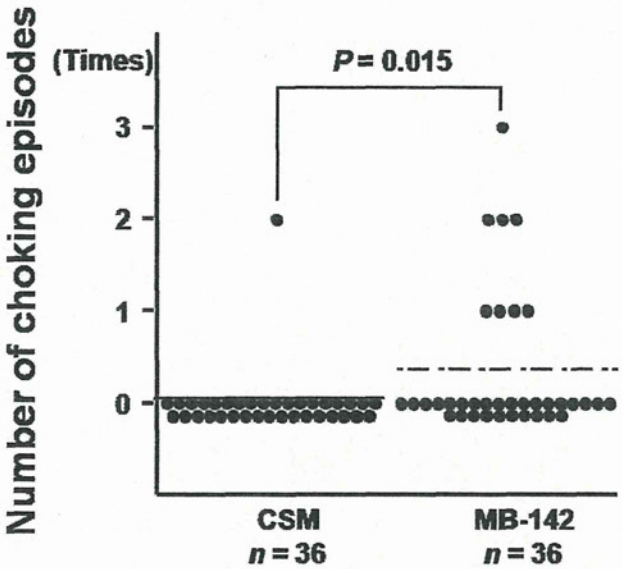

Figure 5 Number of choking episodes. Significantly fewer choking episodes were observed in patients with the continuous suction mouthpiece (CSM) than in patients with the conventional mouthpiece (MB-142; Olympus, Tokyo, Japan) ($P = 0.015$). •, each value for each case; ——, mean value for CSM; ·–·–·, mean value for MB-142.

suction creates airflow in the oral cavity and may reduce discomfort in the oral cavity caused by the endoscopic procedure. Finally, ease in preparing the equipment with no particular materials and low cost would be advantageous.

To reduce salivary secretion during endoscopic procedures, a strategy using anticholinergic agents could be an alternative. However, there are drawbacks in using these agents. First, because the effect of anticholinergics is not maintained during prolonged examinations or procedures, the effect is limited during long-term endoscopic procedures. Next, there are many patients in whom anticholinergics cannot be used due to heart disease, glaucoma, or prostate enlargement. In particular, patients who undergo PEG are rarely allowed to receive anticholinergics, because they are likely to be elderly subjects with serious underlying diseases. In contrast, the CSM can be used for all patients because it is associated with no serious adverse effects. In addition, it works throughout the endoscopic procedure. Thus, the improved mouthpiece would be superior to anticholinergics in terms of controlling salivary secretion during endoscopic procedures.

During the course of constructing the mouthpiece, the detailed specifications, including loop size and the number, size, and locations of the holes were determined through a trial and error process. The prototype device with a single hole caused blood blisters on the mucosal membrane inside the cheek by focused suction pressure. Therefore, devices with multiple holes and a unique loop shape were made so as to not adhere to the mucosal membrane. However, in terms of the number and location of the holes, suction pressure, and loop size, there may be room for further improvement.

In this context, development of equipment in which the suctioning function is integrated into the body of the mouthpiece could be expected, although an external suctioning part was used in the present study. Integration of the suctioning function in the body would completely prevent adherence of a tube to the oral mucosa. In addition, concerns regarding increased saliva production associated with tube placement can be eliminated.

The CSM may also be effective in endoscopic procedures other than PEG. Recently, many kinds of upper endoscopic procedures taking a long time have become common, such as endoscopic submucosal dissection, peroral double-balloon enteroscopy, endoscopic retrograde cholangiopancreatography, endoscopic ultrasonography, endoscopic hemostasis, and endoscopic procedures for gastroesophageal varices. Because these procedures are also associated with an increased risk of aspiration,[3,4] all patients who undergo these procedures may be candidates for the CSM. The usefulness of this item in various procedures should be evaluated in the future.

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The present study had several limitations. First, neither the endoscopist nor the assistant was blinded as to which mouthpiece was used. Because the shapes of the mouthpieces were different, blinding was not possible. Second, the frequency of aspiration pneumonia, the primary outcome of this study, could not be evaluated due to the small number of patients, because the reported rate of the aspiration pneumonia with conventional methods in PEG was only approximately 2.3%.[2] Finally, there are problems with the time and cost of the CSM. However, construction of one CSM costs no more than 1 US dollar, in addition to the cost of the MB-142 mouthpiece.

CONCLUSION

THE CSM REDUCED the extent of salivary flow and the frequencies of suction and choking episodes during PEG-related procedures. This type of simple and inexpensive device is expected to reduce complications such as aspiration not only in PEG but also in other upper endoscopic procedures.

CONFLICT OF INTERESTS

AUTHORS DECLARE NO conflict of interests for this article.

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ORIGINAL ARTICLE

Ultrasonogram of hepatocellular carcinoma is associated with outcome after radiofrequency ablation

Kosaku Moribata, Hideyuki Tamai, Naoki Shingaki, Yoshiyuki Mori, Tatsuya Shiraki, Shotaro Enomoto, Hisanobu Deguchi, Kazuki Ueda, Izumi Inoue, Takao Maekita, Mikitaka Iguchi, Masao Ichinose

Kosaku Moribata, Hideyuki Tamai, Naoki Shingaki, Yoshiyuki Mori, Tatsuya Shiraki, Shotaro Enomoto, Hisanobu Deguchi, Kazuki Ueda, Izumi Inoue, Takao Maekita, Mikitaka Iguchi, Masao Ichinose, Second Department of Internal Medicine, Wakayama Medical University, Wakayama 641-0012, Japan

Author contributions: Moribata K and Tamai H designed and proposed the research; all authors approved the analysis and participated in drafting the article; Moribata K, Tamai H, Shingaki N, Mori Y, Shiraki T and Enomoto S treated the patients; Moribata K, Tamai H, Shingaki N, Mori Y, Shiraki T, Enomoto S, Deguchi H, Ueda K, Inoue I, Maekita T and Iguchi M collected the clinical data; Moribata K, Tamai H and Ichinose M performed the statistical analysis; Moribata K and Tamai H wrote the manuscript.

Correspondence to: Hideyuki Tamai, MD, PhD, Second Department of Internal Medicine, Wakayama Medical University, 811-1 Kimiidera Wakayama City, Wakayama 641-0012, Japan. tamahide@wakayama-med.ac.jp
Telephone: +81-73-4472300 Fax:+81-73-4453616

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Abstract

AIM: To investigate the association between B-mode ultrasound classification of small hepatocellular carcinoma (HCC) and outcome after radiofrequency ablation (RFA).

METHODS: Ninety-seven cases of HCC treated using RFA between April 2001 and March 2006 were reviewed. Ultrasound images were classified as follows: type 1, with halo (n = 29); and type 2, without halo (n = 68). Type 2 was further categorized into three subgroups: type 2a, homogenous hyperechoic (n = 9); type 2b, hypoechoic with smooth margins (n = 43); and type 2c (n = 16), hypoechoic with irregular or unclear margins. Patients with type 2a HCC were excluded from analysis due to the small number of cases.

RESULTS: Two year recurrence rates for type 2b, type 1 and type 2c were 26%, 42% and 69%, respectively, with significant differences between type 2b and type 2c ($P < 0.01$), and between type 1 and type 2c ($P < 0.05$). Five year survival rates were 89%, 43% and 65%, respectively. Survival was significantly longer for type 2b than for other types (type 1 *vs* type 2b, $P < 0.01$; type 2b *vs* type 2c, $P < 0.05$). On univariate analysis, factors contributing to recurrence were number of tumors, tumor stage, serum level of lens culinaris agglutinin-reactive alpha-fetoprotein and ultrasound classification ($P < 0.05$). Factors contributing to survival were tumor stage and ultrasound classification ($P < 0.05$). Multivariate analysis identified ultrasound classification as the only factor independently associated with both recurrence and survival ($P < 0.05$).

CONCLUSION: B-mode ultrasound classification of small HCC is a predictive factor for outcome after RFA.

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Key words: B-mode ultrasound; Hepatocellular carcinoma; Radiofrequency ablation; Recurrence; Prognosis

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INTRODUCTION

Treatment strategies for hepatocellular carcinoma (HCC) are decided on the basis of tumor size and number, liver function and performance status[1,2]. Percutaneous local treatments that are less invasive than resection are performed for small HCCs that are unsuitable for resection, with the indications of ≤ 3 lesions, each with diameter ≤ 3 cm, in accordance with the Japanese guidelines[2] and the practice guidelines of the American Association for the Study of Liver Diseases[1].

Percutaneous radiofrequency ablation (RFA) is a well established local treatment for unresectable small HCC[3,4]. RFA is a curative treatment and achieves not only superior local control of the disease, but also better prognosis compared to percutaneous ethanol injection therapy (PEIT)[5,6]. Accordingly, RFA is now recommended over PEIT for the treatment of small HCC. Recently, RFA has also been adopted for patients with resectable early HCC, defined as single tumors > 2 cm in diameter or up to 3 nodules < 3 cm in diameter, with performance status 0 and Child-Pugh class A or B[7].

However, rapid aggressive recurrence with vascular invasion[8-10], intrahepatic dissemination[11,12], seeding or metastasis[13,14] has been reported after RFA. In particular, the risk of seeding is high in patients with poorly differentiated HCC[15]. Furthermore, the prognosis following RFA for poorly differentiated HCC is reportedly unfavorable[16,17]. A large proportion of patients with poorly differentiated HCC show microscopic vascular invasion and intrahepatic metastasis, even when the tumor is small[18]. As a result, curative treatment cannot be achieved using RFA alone and the procedure may thus cause dissemination or metastasis. Clinical diagnosis of poorly differentiated HCC with high-grade malignancy is therefore crucial when determining treatment strategies for small HCC.

Small HCCs show various images on B-mode ultrasound. However, the correlation between B-mode ultrasound image and prognosis has not been elucidated. We have previously reported that classification on B-mode ultrasonography of small hypervascular HCC correlated with histological differentiation and serum level of lens culinaris agglutinin-reactive alpha-feto protein (AFP-L3), an indicator of poor prognosis[19]. In particular, the presence of irregular or unclear margins was very important in screening for small, poorly differentiated HCC. The aim of this study was to determine whether B-mode ultrasound classification is associated with recurrence and survival after RFA.

MATERIALS AND METHODS

Patients

Our prospective database of 97 patients with initial hypervascular HCC (≤ 3 tumors, all ≤ 3 cm in diameter) who had undergone RFA between April 2001 and March 2006 was reviewed. Diagnosis of hypervascular HCC was based on the findings of tumor staining during the arterial phase of contrast-enhanced computed tomography (CT), dynamic magnetic resonance imaging (MRI) or contrast ultrasonography. If any of these diagnostic imaging techniques showed tumor stain in the arterial phase that was washed out in the equilibrium phase, imaging diagnosis was considered definitive. In all patients, tumor stage (tumor-node-metastasis classification as described by the Liver Cancer Study of Japan), etiology of hepatitis, Child-Pugh classification, levels of tumor markers (AFP, AFP-L3 and des-gamma-carboxy prothrombin), fibrosis stage and activity grade of the biopsied liver tissue using the new Inuyama classification[20] were evaluated before RFA. Eligibility criteria for RFA were as follows: (1) no vascular invasion on imaging diagnosis; (2) no severe ascites; (3) platelet count ≥ $5 \times 10^4/mm^3$; (4) prothrombin time ≥ 50%; (5) total bilirubin < 3 mg/dL; (6) no distant metastases; and (7) in principle, ≤ 3 tumors, all ≤ 3 cm in diameter. No exclusion criteria were set in terms of tumor location (i.e., near main vessels, adjacent organs). Furthermore, all patients with recurrent HCC underwent iterative RFA even when the above criteria for tumor size and number were not met, as long as complete ablation was considered achievable. Written informed consent was obtained from each enrolled patient and the protocol was approved by our institutional review board.

RFA technique

Percutaneous RFA using the Cool-tip RF system (Valleylab, Boulder, CO, United States) was performed under ultrasound guidance in all patients. Artificial pleural effusion or artificial ascites was produced using saline when necessary[21]. The impedance control mode was used with a 17-gauge, cooled-tip electrode with a 2 or 3 cm exposed tip. Ablation was started at 40 W for the 2 cm exposed tip and 60 W for the 3 cm exposed tip. Power was increased at a rate of 10 W/min. When a rapid increase in impedance occurred, output was automatically stopped and ablation was restarted after a short time at an output 10 W lower. Duration of a single ablation was 6 min for the 2 cm electrode and 12 min for the 3 cm electrode. After RF exposure, temperature of the needle tip was measured. When the temperature was below 65 ℃, additional ablation was performed. The electrode track was not treated by thermo-coagulation in any patients, to prevent seeding or hemorrhage.

Assessment of response and follow-up

Treatment response was assessed by contrast-enhanced CT or MRI at 1-3 d after the final session. Complete response was defined as no enhancement in the entire lesion with a safety margin on imaging. Additional ablation was performed until complete ablation was confirmed in each nodule. All patients were followed up on an outpatient basis every 3-4 mo using contrast-enhanced CT or MRI and measurement of tumor marker levels.

B-mode ultrasound imaging

We used either a SONOLINE Elegra™ Ultrasound Platform (Siemens Medical Systems, Erlangen, Germany)

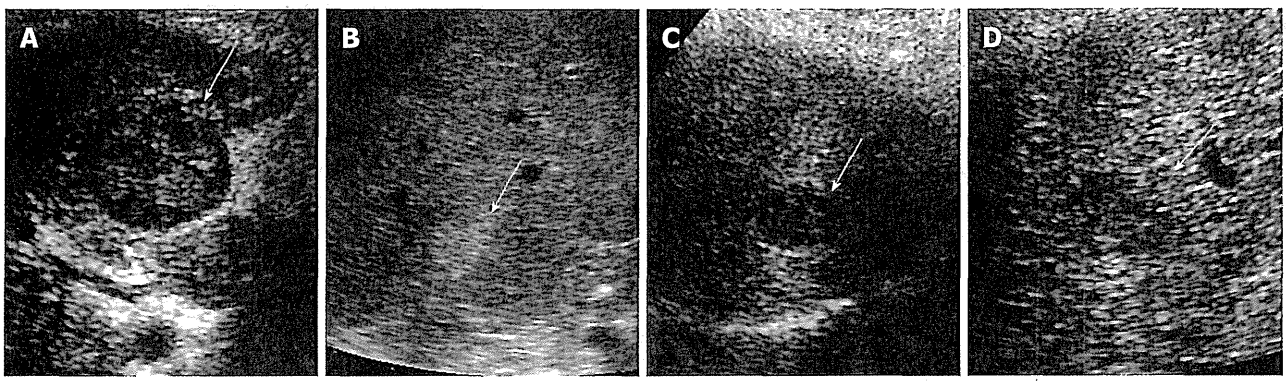

Figure 1 Classification of B-mode ultrasonographic images of small hepatocellular carcinoma. A-D: Hepatocellular carcinoma nodules < 3 cm in diameter were classified into two groups using B-mode ultrasonography: Type 1 with halo (A) and type 2 without halo. Type 2 was then further categorized into three subgroups: Type 2a, homogenous hyperechoic (B); Type 2b, hypoechoic with smooth margins (C); Type 2c, hypoechoic with irregular or unclear margins (D). Hepatocellular carcinoma nodules are indicated by arrows.

Table 1 Comparison of patient characteristics according to B-mode ultrasound-based classification

	Type 1 (n = 29)	Type 2b (n = 43)	Type 2c (n = 16)	P value
Age (yr)	66.4 ± 9.5	66.9 ± 8.7	69.3 ± 6.8	0.554
Gender (male/female)	23/6	25/18	8/8	0.085
HCV (positive/negative)	28/1	36/7	14/2	0.241
Number of tumors	1.2 ± 0.4	1.2 ± 0.5	1.6 ± 0.7	0.046
Size of tumor (mm)	22.5 ± 3.8	19.0 ± 5.2	23.1 ± 5.6	0.003
Child-Pugh classification (A/B)	16/13	31/12	9/7	0.272
Tumor stage (Ⅰ/Ⅱ/Ⅲ)	10/15/4	28/13/2	4/7/5	0.006
Activity grade (A0, 1/2, 3)	7/22	14/29	10/6	0.032
Fibrosis stage (F0-2/3, 4)	8/21	10/33	7/9	0.298
AFP (ng/mL)	124 ± 246	118 ± 274	207 ± 406	0.564
AFP-L3 (%)	7.3 ± 17.0	5.8 ± 16.5	17.5 ± 25.5	0.098
DCP (mAU/mL)	223 ± 489	210 ± 482	299 ± 486	0.817

Data are presented as mean ± SD or n/N. HCV: Hepatitis C virus; AFP: Alpha-fetoprotein; AFP-L3: Lens culinaris agglutinin-reactive alpha-fetoprotein; DCP: Des-gamma-carboxy prothrombin.

with a 3.5C40 convex probe or a SSA-770A ultrasound system (Toshiba Medical Systems, Tochigi, Japan) with a PVT-674BT ultrasound probe. Tissue harmonic imaging was performed in B-mode.

B-mode ultrasound classification

The B-mode ultrasound classification of small HCC we reported previously was used[19]. Nodules with a halo were regarded as type 1 and halo-free nodules were regarded as type 2. In addition, type 2 nodules were further classified based on the internal echo level and marginal features; uniform hyperechoic nodules were evaluated as type 2a, hypoechoic nodules with regular margins as type 2b and hypoechoic nodules with irregular or unclear margins as type 2c. B-mode ultrasound images were obtained within 1 mo before RFA. All recorded ultrasound images were analyzed by two skilled hepatologists (10 and 19 years of experience in abdominal ultrasonography) who were blinded to patient names. When a discrepancy existed in interpretation between the two hepatologists, a consensus was reached through discussion. If HCCs comprised two or three nodules, the largest nodule was selected and classified using our B-mode classification. B-mode ultrasound classified 29 cases as type 1, 9 as type 2a, 43 as type 2b, and 16 as type 2c. Given the small number of patients with type 2a HCC, these cases were excluded from analysis (Figure 1).

Statistical analysis

One factor analysis of variance and the Scheffe test were used to analyze continuous variables. Fisher's exact test or the χ^2 test were used to analyze categorical variables. Cumulative recurrence-free survival rates and cumulative survival rates according to B-mode ultrasound classification were constructed using Kaplan-Meier methods and compared using the log-rank test. Uni- and multivariate analyses using a Cox proportional hazard regression model were performed for factors contributing to tumor recurrence and survival. Results were expressed as hazard ratio with 95%CI. $P < 0.05$ was considered statistically significant for all analyses using SPSS Statistics Version 19 software (IBM, Tokyo, Japan).

RESULTS

The median follow-up interval was 1018 d. Two year recurrence rates for type 2b, type 1 and type 2c were 26%, 42% and 69%, respectively. Significant differences were seen between type 2b and type 2c ($P < 0.01$), and between type 1 and type 2c ($P < 0.05$). Five year survival rates were 89%, 43% and 65%, respectively. Survival was significantly longer for type 2b than for other groups (type 1 *vs* type 2b, $P < 0.01$; type 2b *vs* type 2c, $P < 0.05$).

Patient background variables at baseline according to B-mode ultrasound classification are compared in Table 1. Significant differences were evident among groups in terms of number of tumors, tumor size, tumor stage and activity grade of hepatitis. Mean tumor size was smaller in type 2b than in other types. Mean number of tumors was smaller in type 2b than in type 2c. High tumor stage was more frequent in type 2c than in other types. Severe activity grade of hepatitis was likewise more frequent