

## Cutoff Values for Dose-liver Function Parameters to Prevent Radiation-induced Liver Disease in Advanced Hepatoma

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### ABSTRACT

**AIM:** To retrospectively explore the cutoff values that predict radiation-induced liver disease (RILD) in hepatocellular carcinoma (HCC) patients with portal vein tumor thrombus (PVTT) in the main trunk and/or the first branch who had previously received single-photon-emission computed tomography (SPECT)-based three-dimensional conformal radiotherapy (3DCRT; SPECT-B-3DCRT) using Tc-99m-galactosyl human serum albumin (Tc-99m GSA).

**METHODS:** Seventy-five HCC patients with PVTT underwent SPECT-B-3DCRT (total dose of 45 Gy/18 fractions) in the stop-breathing position with an error of  $\leq 5$  mm. SPECT allowed the minimum possible irradiation of the functional liver (FL). Child-Pugh score deterioration (CPSD) by 2 (=RILD) or CPSD by 1 was scored within four months of completing SPECT-B-3DCRT. Receiver-operating characteristic (ROC) analysis of the outcomes RILD vs CPSD by 1 was conducted to determine the accuracy and cutoff values for  $FLV_{20Gy}$  (percentage of FL volume receiving  $\geq 20$  Gy),  $F_{20Gy}$  (GSA counts within the irradiated liver area receiving  $\geq 20$  Gy  $\times 100$ /GSA counts in the whole liver),  $F_{20Gy}/LHL15$  (liver radioactivity counts at 15 min/heart plus liver radioactivity counts at 15 min), and  $F_{20Gy}/total$  count ratio (liver radioactivity counts/liver plus total background radioactivity counts).

**RESULTS:** The cutoff values and accuracy of  $FLV_{20Gy}$ ,  $F_{20Gy}$ ,  $F_{20Gy}/LHL15$ , and  $F_{20Gy}/total$  count ratio were 26.4 and 0.826 ( $p=0.024$ ), 30.2 and 0.913 ( $p=0.004$ ), 37.7 and 0.913 ( $p=0.001$ ), and 43.0 and 0.957 ( $p<0.001$ ), respectively.

**CONCLUSION:** An  $F_{20Gy}/total$  count ratio of 43.0 is the most reliable cutoff value for preventing RILD.

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**Key words:** Functional image-guided radiotherapy; Hepatocellular carcinoma; Portal vein tumor thrombus; Dose-volumetric parameters; Dose-function parameters

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### INTRODUCTION

The prognosis of patients with hepatocellular carcinoma (HCC) associated with liver cirrhosis is unsatisfactory<sup>[1,2]</sup>, and it is still difficult to simultaneously eradicate the binary diseases of HCC and liver cirrhosis. Radiation treatment (RT) is not yet popular for HCC, particularly because it destroys the reserve capacity of the cirrhotic liver along the radiation beam, which can induce fatal radiation-induced liver disease (RILD)<sup>[3,4]</sup>. The severity of liver cirrhosis is considered one of the risk factors for RILD<sup>[3,4]</sup>.

Only two cutoff values are available for predicting RILD. Kim *et al* proposed using the total liver volume, with a cutoff value for  $TLV_{30Gy}$  (percentage of the total liver volume receiving  $\geq 30$  Gy) of 60% to predict RILD<sup>[5]</sup>. However, this value does not reflect liver function and is not applicable to giant HCC. Shirai *et al* defined the functional liver (FL) using Tc-99 m-galactosyl human serum albumin

(GSA)<sup>[6]</sup> and proposed a cutoff value for  $FLV_{20Gy}$  (percentage of the FL volume receiving  $\geq 20$  Gy) of 20% to predict RILD<sup>[7]</sup>. However, Shirai *et al* used analogue image data to evaluate the functional liver. To replace the dose–volume histogram, Marks *et al* proposed the idea of a dose–function histogram to merge the function of the organ bearing the tumor with the radiation dose distribution curve created by radiation treatment planning<sup>[8]</sup>. In planning radiation treatment for lung cancer, they used lung perfusion single-photon-emission computed tomography (SPECT) counts of Tc-99m macro-aggregated albumin as the lung function digital data to create the dose–function histogram<sup>[8]</sup>. Therefore, in this study, we attempted to create a cutoff value to predict RILD more exactly than  $FLV_{20Gy}$  using Tc-99m GSA counts as the liver function digital data<sup>[6]</sup>.

The purpose of this study was to retrospectively determine the cutoff values for predicting RILD in HCC patients with portal vein tumor thrombus (PVTT) at the main trunk and/or the first branch, associated with Child A or B liver cirrhosis, who had previously received SPECT-based three-dimensional conformal radiotherapy (SPECT-B-3DCRT)<sup>[9,10]</sup>, using the digital counts of Tc-99m GSA.

## METHODS

This retrospective clinical study was approved by the Ethics Committee of our institute and informed consent was obtained from all the patients.

The eligibility criteria were: (1) unresectable HCC with PVTT in the first branch and/or the main trunk; (2) unlimited HCC size; (3) the absence of extrahepatic metastasis; (4) ascites under medical control or no ascites; (5) a Child A or B score of 5-9; and (6) performance status of 0-2 on the Eastern Cooperative Oncology Group scale. HCC with PVTT was diagnosed according to the guidelines of the Liver Cancer Study Group of Japan<sup>[11]</sup>. Before RT, transcatheter arterial chemoembolization (TACE) with 3-10 mL of lipiodol with or without gelatin sponge particles was performed in each patient. As anticancer drugs, 40-60 mg of epirubicin hydrochloride and 10 mg of mitomycin C were mixed with the lipiodol. TACE using lipiodol (TACE-Lp) was applied to the whole tumor. TACE using lipiodol and gelatin sponge particles (TACE-Lp and GSP) was performed superselectively for tumors outside the main tumor and PVTT. In brief, the main tumor and PVTT were treated with TACE-Lp, and intrahepatic metastases were treated with TACE-Lp and GSP. TACE-Lp was expected to control any minute HCCs surrounding the main tumor and PVTT<sup>[12]</sup>.

### Retrospective analysis

One hundred two patients with HCC associated with PVTT at the main portal trunk and/or the first branch received SPECT-B-3DCRT plus TACE between March 2007 and February 2014. The data were analyzed in September 2014. Patient selection for the retrospective analysis is shown in figure 2. Three patients were excluded because they were unable to perform breath holding in the stop-breathing position to an accuracy of  $\leq 5$  mm; these patients underwent SPECT-B-3DCRT for PVTT alone, and not for the main tumor. Three more patients were excluded because of ruptured esophageal varices, obstructive icterus caused by invasion of the biliary tract, or a deterioration in performance status (PS) during RT, so they could not complete the total 45 Gy of the prescribed dose. Seven patients were not followed-up at our institute. Thus, 89 patients whose prognoses were identified (18 alive, 71 dead) were analyzed.

### SPECT analysis

GSA-SPECT<sup>[6]</sup> was performed with a double-headed camera (Infinia; GE Medical System, Waukesha WI USA). Tc-99m-GSA (185 MBq) was injected as an intravenous bolus via a cubital vein. Dynamic accumulation images of the heart and liver were obtained for the first 20 min after injection. Time-activity curves for the heart and liver were generated for regions of interest (ROIs) in the whole liver and heart. The receptor index was calculated by dividing the radioactivity in the liver ROI by the radioactivity in the liver plus heart ROIs 15 min after injection<sup>[13]</sup>. SPECT images were obtained from the upper margin to the lower margin of the liver. The total count ratio was calculated by dividing the radioactivity counts of the liver by the radioactivity counts of the liver plus the total background of the SPECT image. The SPECT data (90 steps, 360°) were obtained at 20 min with an image matrix of 128×128 and zoom of 1.28. Image reconstruction was conducted with the ordered subset expectation maximization (OSEM) method and Chang's attenuation correction<sup>[14]</sup>.

### Determination of functional liver volume (FLV)

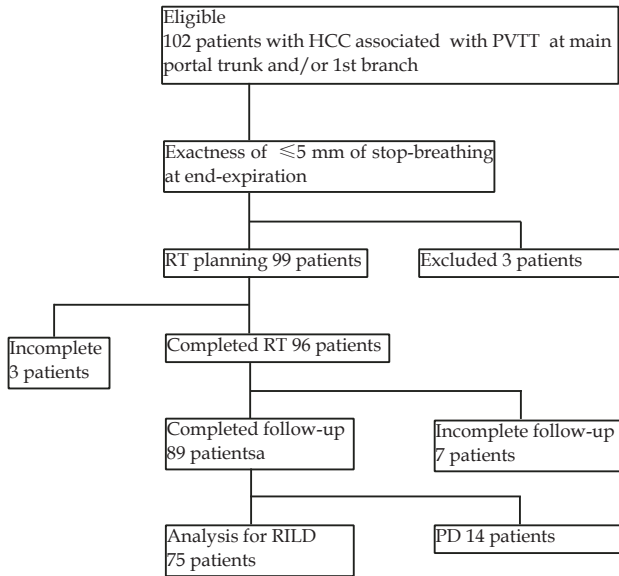
The FLV was distinguished from the normal liver volume (NLV) before radiation planning. For accuracy, Tc-99m-GSA SPECT<sup>[6]</sup>, dynamic contrast-enhanced computed tomography (CT) and simulation CT were performed no more than two weeks before SPECT-B-3DCRT. To allow the accurate fusion of the images, all images of the whole liver were obtained at 5 mm thickness under breath holding at end-expiration, in each modality. We contoured the whole liver, main tumor, PVTT, and other hepatic tumors (intrahepatic metastases) on the dynamic CT images of each patient. We defined NLV as the area remaining after subtracting the main tumor plus PVTT plus other hepatic tumors from the whole liver. Within the NLV, the areas that had the same radioisotope (RI) filling defects as the HCC were defined as the dysfunctional liver volume; the areas of hyperaccumulation relative to the HCC were defined as FLV<sup>[6]</sup>. The corresponding areas on the SPECT images were then outlined on the dynamic CT images, and these transferred areas were fused with the corresponding simulator CT images and outlined.

### Radiation planning and radiation therapy

Our three-dimensional conformal radiotherapy (3DCRT) method is summarized as follows. All patients underwent 3DCRT in the supine position with both arms raised above the head. We used no special apparatus for respiratory immobilization. To minimize the effects of respiration, the subjects practiced breath holding for 10-15 s at the time of end-expiration until the position could be maintained within 5 mm under X-ray fluoroscopic monitoring. RT was repeatedly delivered with a 10 MV linear accelerator during breath holding at end-expiration for 10-15 s at a time.

The simulation CT data were transferred to a 3D radiation treatment planning system (Pinnacle, ADAC Laboratories, Milpitas, CA). The subsequent CT treatment planning is shown in figure 1. The gross tumor volume (GTV) was defined as the main tumor plus PVTT. Because any tumor outside the GTV was expected to be controlled by TACE, the clinical target volume (CTV) was regarded as the same as the GTV. The planning target volume (PTV) included the CTV with a 10 mm margin: 5 mm to allow for respiratory-induced motion and 5 mm to allow for penumbra covering and variations in the daily setup.

The optimal 3DCRT beam directions (optimal angles of the gantry) were explored using the SPECT images for guidance, as proposed by Shirai *et al*<sup>[9,10]</sup>. The directions of the two high-dose beams were

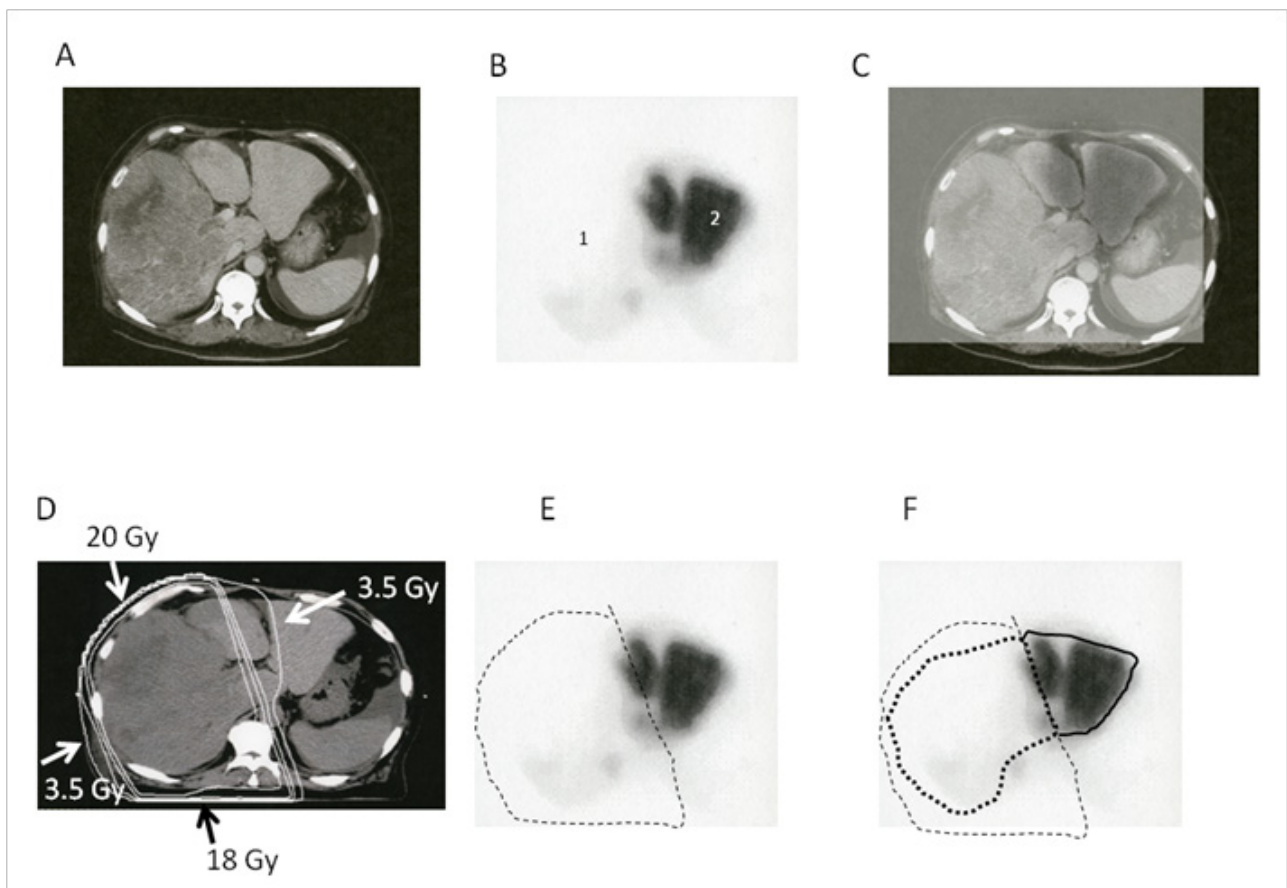


**Figure 2** Patient flow chart for the analysis of survival and radiation-induced liver disease. HCC: hepatocellular carcinoma, PVTT: portal vein tumor thrombus, RT: radiation therapy, RILD: radiation-induced liver disease, PD: progressive disease.

designed to primarily cover the main tumor and PVTT, and to irradiate the FLV as little as possible<sup>[9,10]</sup>. As shown in Figure 1-D, the doses of the high-dose beams were limited to 38 Gy/18 fractions/ four weeks to prevent adverse effects to the duodenum, spinal cord, and kidneys<sup>[15,16]</sup>. Two additional low-dose beams of 7 Gy/18 fractions/ four weeks were required to elevate the dose to the CTV, resulting in a total dose of 45 Gy to the isocenter<sup>[17]</sup>. These low-dose beams were designed to avoid irradiating organs at risk, such as the stomach, duodenum, and spinal cord. Doses of 3.5 Gy for each additional beam did not cause liver damage, even if the beams irradiated the FLV<sup>[9,10]</sup>. In terms of the kidney, the volume irradiated with 20 Gy or more was planned to be  $\leq 30\%$  of the total volume. Finally, the couch angle was adjusted to a maximum limit of 90°.

**Dose-Function Parameters**

Shirai *et al* proposed  $F_{LV20Gy} = 20\%$  as the cutoff value for SPECT-B-3DCRT with GSA causing RILD [Child-Pugh score deterioration (CPSD) by 2]<sup>[17]</sup>. However, it is difficult for the dose-volume parameters to accommodate the degree of liver function strength or to evaluate the severity of liver cirrhosis. Therefore, based on the report of Marks *et al*<sup>[8]</sup>, the concept of dose-function parameters was adopted to resolve the problem of the inhomogeneity of liver function strength. Percentage  $F_{20Gy}$  was defined with the following



**Figure 1:** A 71-year-old man with hepatocellular carcinoma (HCC). A: Contrast-enhanced computed tomography (CT) showed an HCC with a maximum size of 20 cm. B: Single-photon-emission computed tomography (SPECT) with Tc-99m-galactosyl human serum albumin (GSA) at the same level as A, shows a GSA deficit area (1) and a GSA high-accumulation area (2). C: Merged image of A and B indicates that the GSA deficit area corresponds to the main tumor located in the right lobe and the GSA accumulation area corresponds to the functional liver. D: Simulation CT after radiation treatment planning showing the isodose distribution curve for the total prescribed dose of 45 Gy to the planning target volume. E: 20 Gy isodose curve (thin dotted line) was merged with image B. F: Thick dotted area indicates the liver area irradiated with 20 Gy or more, and the solid line area indicates the liver outside the 20 Gy isodose curve.  $F_{20Gy}$  was calculated with the formula: [liver radioisotope counts within the 20 Gy isodose curve (thick dotted area) × 100 / [radioisotope counts in the whole liver area (thick dotted area plus solid area)].

formula: GSA counts within the liver area irradiated with  $\geq 20$  Gy  $\times 100$ /GSA counts in the whole liver (Figure 1). Figure 1D shows the dose–volume distribution image and figure 1E shows the fusion image of figure 1D plus the equivalent-level SPECT image, with the 20 Gy isodose curve shown as a dotted line. The receptor index (liver radioactivity counts at 15 min/heart plus liver radioactivity counts at 15 min, LHL15) is widely used to evaluate the severity of liver cirrhosis<sup>[13]</sup>, so  $F_{20\text{Gy}}/\text{LHL15}$  was defined as the correction indicator to evaluate both of liver function strength and liver cirrhosis. We also used the total count ratio (liver radioactivity counts/liver plus total background radioactivity counts on SPECT images) to evaluate the severity of liver cirrhosis.  $F_{20\text{Gy}}/\text{total count ratio}$  was also defined as the correction indicator to evaluate both liver function strength and liver cirrhosis.

### Follow-up evaluation

Laboratory tests for blood and liver function were performed weekly during SPECT-B-3DCRT and monthly for four months after the completion of SPECT-B-3DCRT, according to the definition of RILD by Pan *et al.*<sup>[18]</sup>. If changes in the levels of transaminases or other factors were detected, the tests were rechecked after an interval of 1–2 weeks.

All patients underwent follow-up CT one month after RT and every 3–4 months thereafter for as long as they survived. The local control rate of GTV was investigated based on the CT findings. Overall survival was investigated and the survival of patients with Child A or B cirrhosis was compared.

Sorafenib is administered for unresectable and TACE-unmanageable HCC<sup>[1,19]</sup>. In this study, TACE was first conducted for the main tumor and intrahepatic metastases outside the radiation field. SPECT-B-3DCRT was then administered for HCC with PVTT at the main trunk and/or the first branch of the portal vein. Thereafter, TACE was repeated for recurrent tumors. When tumor growth could not be controlled with repeated TACE and/or a distant metastasis was associated with Child A cirrhosis, sorafenib was administered and patient survival was analyzed, although the number of patients in this sample was limited.

### RILD and CPSD by 1

Classic and nonclassic RILD were assessed according to the endpoints of RILD described by Pan *et al.*<sup>[18]</sup>. Classic RILD involves anicteric hepatomegaly and ascites, as well as elevated alkaline phosphatase (more than twice the upper limit of the normal or baseline value). Nonclassic RILD involves elevated liver transaminase (more than five times the upper limit of normal, or 20 times the upper limit of normal in patients with baseline transaminase values more than five times the upper limit of the normal range) or a decline in liver function [defined as a worsening of the Child–Pugh score (CPSD) by 2 or more] within four months of the completion of RT.

Shirai *et al.* defined the cutoff value for  $\text{FLV}_{20\text{Gy}}$  that causes liver dysfunction with CPSD by 1 or greater to be 20%<sup>[7]</sup>. In this study, we investigated the cutoff values for the dose–volume parameters and dose–function parameters that differentiated CPSD by 1 from CPSD by 2 or greater (=RILD).

All patients were evaluated for evidence of RILD (CPSD by 2) or CPSD by 1 within four months of the completion of RT. This process excluded from the study all those patients who displayed progressive disease within four months of 3DCRT.

### Risk factors related to RILD

The clinical parameters, dose–volume parameters (DVPs), and dose–

function parameters (DFPs) were investigated as risk factors. The 13 clinical parameters tested were: sex, age, performance status (PS), PVTT, hepatitis virus type, alpha-fetoprotein (AFP), Child–Pugh class, the Cancer of the Liver Italian Program (CLIP) score<sup>[20]</sup>, previous treatment, GTV, FLV, NLV, and TLV. The three DVPs were: the percentage of TLV receiving  $\geq 20$  Gy ( $\text{TLV}_{20\text{Gy}}$ ), the percentage of NLV receiving  $\geq 20$  Gy ( $\text{NLV}_{20\text{Gy}}$ ), and the percentage of FLV receiving  $\geq 20$  Gy ( $\text{FLV}_{20\text{Gy}}$ ). The three DFPs were: (GSA count in the area irradiated with  $\geq 20$  Gy in the liver)/(GSA count in the whole liver)  $\times 100\%$  ( $F_{20\text{Gy}}$ ),  $F_{20\text{Gy}}/\text{LHL15}$ <sup>[13]</sup>, and  $F_{20\text{Gy}}/\text{total count ratio}$ .

### Statistical analysis

The binary variable RILD versus non-RILD was used as the dependent variable to analyze the characteristics of RILD using 13 clinical parameters, which were the independent variables.

Of these, sex (male vs female), age ( $\leq 60$  vs  $>60$  years), PS (0–1 vs 2), PVTT (major branch vs trunk), hepatitis B virus (no vs yes), serum AFP level ( $< 400$  vs  $\geq 400$  IU/mL), Child–Pugh class (A vs B), CLIP score ( $< 3$  vs  $\geq 3$ ), and previous treatment (TACE-Lp vs TACE-Lp and GSP) were used as binary variables, and GTV, FLV, NLV, and TLV were used as continuous variables in the univariate logistic regression analysis. Numerical data were expressed as means  $\pm$  SD and compared with Student's *t* test.

A receiver-operating characteristic (ROC) curve and Fisher's exact test were used to obtain the cutoff values for RILD (CPSD by 2) versus non-RILD (CPSD by 1) by analyzing the DVPs and DFPs:  $\text{TLV}_{20\text{Gy}}$ ,  $\text{NLV}_{20\text{Gy}}$ ,  $\text{FLV}_{20\text{Gy}}$ ,  $F_{20\text{Gy}}$ ,  $F_{20\text{Gy}}/\text{LHL15}$ , and  $F_{20\text{Gy}}/\text{total count ratio}$  (the independent variables). We calculated the DFPs based on the concept of DFP proposed by Marks *et al.*<sup>[8]</sup>

We calculated the cumulative local control rate and overall survival from the day of 3DCRT commencement until the date of tumor progression within the CTV and the date of death, respectively. We calculated the probability of local control and overall survival according to the Kaplan–Meier estimator. Statistical tests were two-sided and performed with SPSS version 11.0 (SPSS Inc., Chicago, IL). A *p* value of  $< 0.05$  was considered to indicate a significant difference.

## RESULTS

### Survival rates and local control rates

The median follow-up and the median overall survival were 10.3 months (range 1.8–78 months) and 10.9 months, respectively. The overall survival rates at one year, two years, and five years were 47.0%, 20.4%, and 11.2%, respectively (Figure 3). The overall local control rates of CTV at one year, two years, and five years were 77.2%, 73%, and 73%, respectively (Figure 4). The median overall survival times for patients with Child A ( $n=45$ ) and patients with Child B ( $n=44$ ) were 14.4 months and 10.0 months, respectively, which were significantly different ( $p=0.0289$ , log rank test).

Sorafenib was administered to five patients with progressive disease after repeated-TACE associated with Child A liver cirrhosis. All five patients were dead at the time of analysis and their median survival times after SPECT-B-3DCRT and after sorafenib were 25 months (19.5, 22.0, 25.0, 51.1, and 62.6 months) and 17.5 months (11.5, 14.0, 17.5, 24.3, and 36.4 months), respectively.

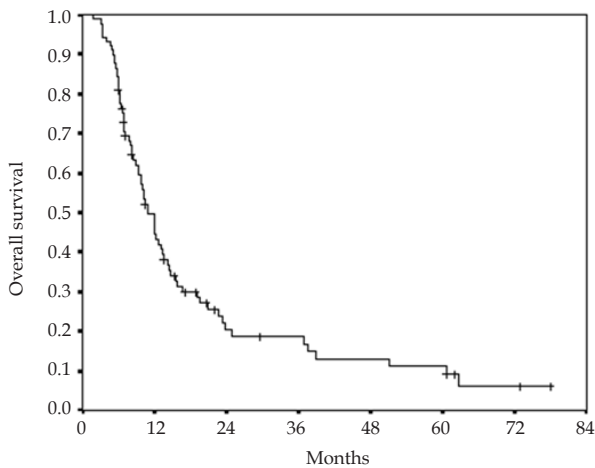
### Detection and analysis of RILD

Progressive HCC was observed in 14 of the 89 patients analyzed within four months of the completion of SPECT-B-3DCRT, so 75

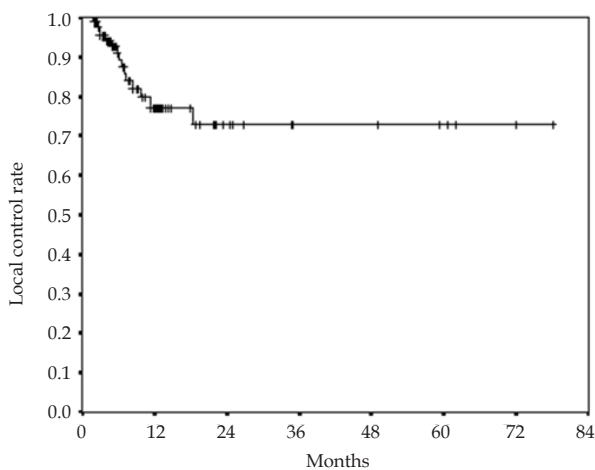
patients were analyzed for the presence or absence of RILD. The patient backgrounds and tumor characteristics are listed in Table 1. The clinical parameters for the radiation treatments (mean±SD) were: GTV, 448.7±600.6 cm<sup>3</sup> (range, 8.8-2927.8); FLV, 946.9±280.5 cm<sup>3</sup> (range, 223.2-1779.2); NLV, 1072.8±286.3 cm<sup>3</sup> (range, 516.0-1976.0); and TLV, 1624.6±687.7 cm<sup>3</sup> (range, 615.0-4002.0).

Of these 75 patients, no patient experienced CPSD by 3, indicating that there was no fatal RILD. CPSD by 1 occurred in 15 patients and CPSD by 2, corresponding to RILD, occurred in eight patients (three with albumin+ascites; two with albumin+encephalopathy; two with albumin+bilirubin; and one with albumin). The interval from the completion of RT to the occurrence of CPSD was 1-9 weeks (median, four weeks). The period of CPSD was 1-18 weeks (median, eight weeks). All patients recovered from CPSD.

The univariate analysis of the association between the clinical parameters and CPSD by 2 (RILD) is shown in Table 2. The relevant clinical parameters significantly associated with RILD were PS ( $p=0.008$ ), Child-Pugh class ( $p=0.043$ ), CLIP score ( $p=0.018$ ), and GTV ( $p=0.037$ ).



**Figure 3** Kaplan-Meier analysis of overall survival in 89 patients with unresectable hepatocellular carcinoma associated with portal vein tumor thrombus in the main trunk or the first branch after Tc-99m-galactosyl-human-serum-albumin-based three-dimensional radiotherapy.



**Figure 4** Kaplan-Meier analysis of the local control rate in 89 patients with unresectable hepatocellular carcinoma associated with portal vein tumor thrombus in the main trunk or the first branch after Tc-99m-galactosyl-human-serum-albumin-based three-dimensional radiotherapy.

**CPSD by 2 versus CPSD by 1**

To explore the appropriate cut-off values that differentiate the patients with CPSD by 2 from the patients with CPSD by 1, the values of the three dose-volume parameters (TLV<sub>20Gy</sub>, NLV<sub>20Gy</sub>, and FLV<sub>20Gy</sub>) and the three dose-function parameters (F<sub>20Gy</sub>, F<sub>20Gy</sub>/LHL15, F<sub>20Gy</sub>/total count ratio) were investigated, as shown in Figure 5. Because the maximum and minimum values for TLV<sub>20Gy</sub> occurred in the group with CPSD by 1, it was difficult to use TLV<sub>20Gy</sub> to differentiate the groups. However, because the maximum values of the other five parameters occurred in the group with CPSD by 2 and the minimum values occurred in the group with CPSD by 1, these parameters tended to differentiate the two groups. The values for F<sub>20Gy</sub>/LHL15 and F<sub>20Gy</sub>/total count ratio clearly differentiated the groups of CPSD by 1 and CPSD by 2 (Figure 5). In particular, all the values for F<sub>20Gy</sub>/total count ratio clearly differentiated the groups, except the maximum value in the group with CPSD by 1 (Figure 5).

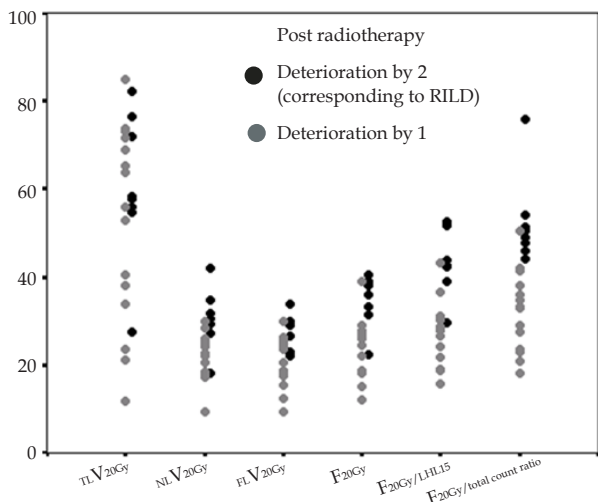
The values of LHL15 and the total count ratio were compared between the CPSD by 2 and the CPSD by 1 groups: LHL15, 0.793±0.079 vs 0.867±0.086, respectively,  $p=0.053$ ; total count ratio, 0.675±0.117 vs 0.745±0.080, respectively,  $p=0.105$ .

The values of the six parameters were compared between the CPSD by 2 and the CPSD by 1 groups: TLV<sub>20Gy</sub>, 60.6±16.9 vs 51.9±22.4, respectively,  $p=0.313$ ; NLV<sub>20Gy</sub>, 30.1±6.8 vs 22.0±5.3, respectively,  $p=0.013$ ; FLV<sub>20Gy</sub>, 27.0±4.3 vs 20.4±5.8, respectively,  $p=0.006$ ; F<sub>20Gy</sub>, 34.9±5.9 vs 23.8±6.4, respectively,  $p=0.001$ ; F<sub>20Gy</sub>/

**Table 1** Patient characteristics.

Characteristic	Value
Gender	
Male	63 (84.0)
Female	12 (16.0)
Age (years)	
Median	70
Range	42-87
≤ 60	20 (26.7)
> 60	55 (73.3)
Performance status	
0	23 (30.7)
1	36 (48.0)
2	16 (21.3)
PVTT	
Major branch	46 (61.3)
Trunk	29 (38.7)
Hepatitis virus type	
HBV	19 (25.3)
HCV	39 (52.0)
Both	4 (5.3)
Unknown	13 (17.4)
AFP (IU/mL)	
< 400	43 (57.3)
≥ 400	32 (42.7)
Child-Pugh class	
A	39 (52.0)
B	36 (48.0)
CLIP score	
1	14 (18.7)
2	18 (24.0)
3	24 (32.0)
4	14 (18.7)
5	5 (6.6)
Previous treatment	
TACE-Lp	23 (30.7)
TACE-Lp & GSP	52 (69.3)

Abbreviations: PVTT: portal vein tumor thrombus; HBV: hepatitis B virus; HCV: hepatitis C virus; AFP:  $\alpha$ -fetoprotein; CLIP: Cancer of the Liver Italian Program; TACE-Lp: transcatheter arterial chemoembolization with lipiodol; TACE-Lp & GSP: transcatheter arterial chemo-embolization with lipiodol plus gelatin sponge particles. Data are presented as numbers of patients, with percentages in parentheses.



**Figure 5** Relationships between the deterioration of the Child-Pugh score by 1 or 2 and the dose-volume and dose-function parameters. Black spots: deterioration of the Child-Pugh score by 2; gray spots: deterioration of the Child-Pugh score by 1; TLV<sub>20Gy</sub>: percentage of total liver volume receiving ≥20 Gy; NLV<sub>20Gy</sub>: percentage of the normal liver volume receiving ≥20 Gy; FLV<sub>20Gy</sub>: percentage of the functional liver volume receiving ≥20 Gy; F<sub>20Gy</sub>: percentage of the liver radioisotope (RI) counts in the area receiving ≥20 Gy divided by the total liver RI counts; LHL15: percentage of the whole liver counts divided by the whole liver and heart counts 15 min after the injection of Tc-99m-galactosyl human serum albumin; total count rate: percentage of RI counts in the whole liver divided by the RI counts in the whole SPECT images.

**Table 2** Univariate analysis of clinical parameters associated with risk of radiation induced liver disease (RILD).

Characteristic	RILD		p <sup>1</sup>
	No (n=67)	Yes (n=8)	
Gender (n)			0.463
Male	57	6	
Female	10	2	
Age (years)			0.91
≤60	18	2	
>60	49	6	
Performance status			0.008
0-1	56	3	
2	11	5	
PVTT			0.49
Major branch	42	4	
Truncus	25	4	
HBV			0.263
No	45	7	
Yes	22	1	
AFP (IU/mL)			0.755
<400	38	5	
≥400	29	3	
Child-Pugh class			0.043
A	38	1	
B	29	7	
CLIP score			0.018
<3	32	0	
≥3	35	8	
Previous treatment			0.659
TACE-Lp	20	3	
TACE-Lp & GSP	47	5	
GTV (cm <sup>3</sup> )	399.1±581.8	864.4±633.4	0.037
Functional liver volume (cm <sup>3</sup> )	966.4±285.1	783.6±176.2	0.081
Normal liver volume (cm <sup>3</sup> )	1095.2±291.7	885.5±138.5	0.05
Total liver volume (cm <sup>3</sup> )	1595.7±684.5	1867.2±712.5	0.294

RILD: radiation-induced liver disease; GTV: gross tumor volume; other abbreviations as in table 1. Data are presented as number of patients or mean±standard deviation. <sup>1</sup>Univariate logistic regression analysis.

LHL15, 44.2±7.8 vs 27.5±7.1, respectively, *p*<0.001; and F<sub>20Gy</sub>/total count ratio, 52.3±10.0 vs 32.3±8.8, respectively, *p*<0.001. F<sub>20Gy</sub>/LHL15 and F<sub>20Gy</sub>/total count ratio markedly differentiated the numerical data.

**ROC analysis**

Figure 6 shows the ROC analysis used to determine the cutoff values for the six parameters in predicting the differentiation of CPSD by 2 and CPSD by 1. The ROC analysis implied no significance on TLV<sub>20Gy</sub> [*p*=0.401, area under the curve (AUC)=0.608], so the cutoff value for TLV<sub>20Gy</sub> could not be calculated. However, the other five parameters showed significant differences (*p*=0.024 to *p*<0.001). The cutoff value and accuracy for NLV<sub>20Gy</sub> were 26.6 and 0.870, respectively; for FLV<sub>20Gy</sub> were 26.4 and 0.826, respectively; for F<sub>20Gy</sub> were 30.2 and 0.913, respectively; for F<sub>20Gy</sub>/LHL15 were 37.7 and 0.913, respectively; and for F<sub>20Gy</sub>/total count ratio were 43.0 and 0.957, respectively. Of these five parameters, the accuracy was highest for F<sub>20Gy</sub>/total count ratio. Furthermore, the AUCs for F<sub>20Gy</sub>/LHL15 and F<sub>20Gy</sub>/total count ratio were >0.9, so the cutoff values for F<sub>20Gy</sub>/LHL15 and F<sub>20Gy</sub>/total count ratio were reliably exactness.

**Adverse effects**

The adverse effects on the skin, upper gastrointestinal tract, and whole blood were analyzed based on the Common Terminology Criteria for Adverse Events v 3.0. Grade 1 skin effects were observed in 12 patients (12/75, 16.0%) and grade 1 gastrointestinal tract effects in nine patients (9/75, 12.0%). For the whole blood, grade 1, 2, and 3 effects on white blood cells were observed in 10 patients (10/75, 13.3%), 14 patients (14/75, 18.7%), and five patients (5/75, 6.7%), respectively, and grade 1, 2, and 3 effects on platelets were observed in 15 patients (15/75, 20.0%), seven patients (7/75, 9.3%), and six patients (6/75, 8.0%), respectively. All patients with grade 3 effects in whole blood had deteriorated from grade 2 before RT. All adverse events were transient and reversible.

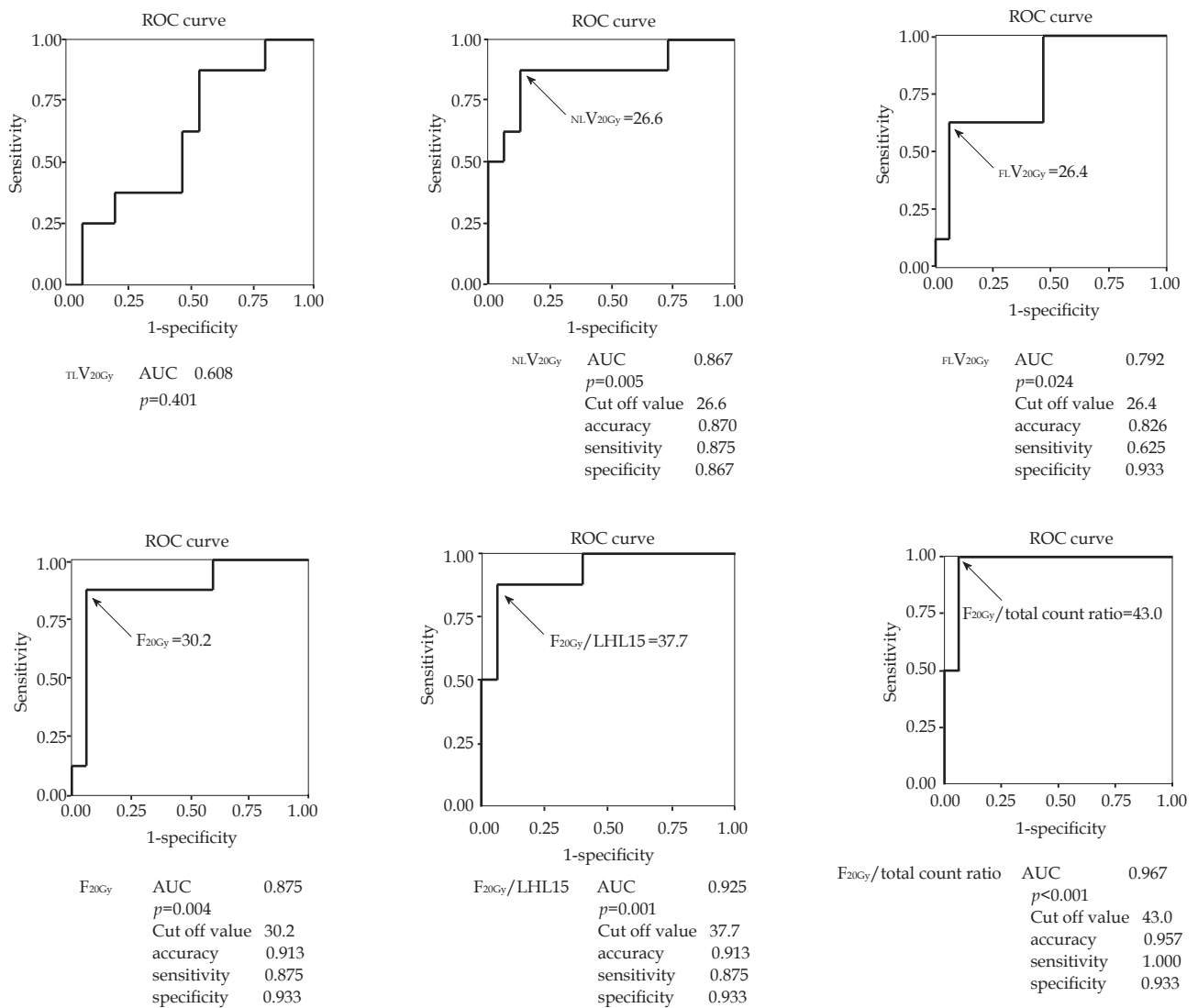
**DISCUSSION**

Sorafenib is currently recommended for advanced HCC<sup>[1,19]</sup>. In the present study, SPECT-B-3DCRT with TACE preceded the administration of sorafenib in five patients with HCC associated with PVTT at the main portal trunk and/or the first portal branch vein, leading to a median survival of 25.0 months. We believe that the prevalence of multidisciplinary treatments, including SPECT-B-3DCRT before sorafenib administration, has improved the survival of patients with advanced HCC.

However, RT for HCC is limited by the occurrence of RILD<sup>[3,4]</sup>. The clinical parameters associated with RILD in this study were PS, Child classification, CLIP, and GTV. Of these parameters, the Child classification has been repeatedly cited as a risk factor for RILD in previous reports<sup>[3,4]</sup>. It is imperative to know how to use these parameters in actual radiation planning to prevent RILD. Therefore, the cutoff values for the dose-volume parameters and functional volume parameters that would prevent RILD were investigated.

The first step in preventing RILD, before the cutoff value is determined, is to control the respiratory movement of the liver during irradiation<sup>[7]</sup>. We sought to achieve exactness in the stop-breathing position, within an error of ≤5 mm, and did so in 99 of 102 patients (97%) by breath holding for 10-15 s at the time of end-expiration. This is one of the most important procedures used to avoid unnecessary irradiation of the liver.

The second step was to determine the FL in the whole liver using



**Figure 6** Six receiver-operating characteristic (ROC) curves were created using six independent variables (TL.V<sub>20Gy</sub>, NL.V<sub>20Gy</sub>, FL.V<sub>20Gy</sub>, F<sub>20Gy</sub>, F<sub>20Gy</sub>/LHL15, F<sub>20Gy</sub>/total count ratio) to predict the deterioration in the Child-Pugh score by 2 (corresponding to radiation-induced liver disease) versus the deterioration in the Child-Pugh score by 1 after SPECT Tc-99m-galactosyl-human-serum-albumin-based three-dimensional radiotherapy. AUC: area under curve; TL.V<sub>20Gy</sub>: percentage of the total liver volume receiving ≥20 Gy; NL.V<sub>20Gy</sub>: percentage of the normal liver volume receiving ≥20 Gy; FL.V<sub>20Gy</sub>: percentage of the functional liver volume receiving ≥20 Gy; F<sub>20Gy</sub>: percentage of the liver radioisotope (RI) counts in the area receiving ≥20 Gy divided by the total liver RI counts; LHL15: percentage of the whole liver counts divided by the whole liver and heart counts 15 min after the injection of Tc-99m-galactosyl human serum albumin; total count rate: percentage of RI counts in the whole liver divided by the RI counts in the whole SPECT images.

GSA-SPECT and to create a dose–volume histogram (DVH)<sup>[21,22]</sup>. The functional DVH was originally created by Seppenwoolde *et al* and Cristian *et al* for RT of lung cancer using Tc-99m macro-aggregated albumin. We used it to create a functional DVH for RT of HCC using Tc-99m GSA. FL does not always correspond to the normal liver in patients with liver cirrhosis and/or HCC with PVTT<sup>[23]</sup>. GSA-SPECT usually shows no accumulation of radioactivity in the normal liver in the lobe with the tumor thrombus, so the normal liver is registered as dysfunctional liver<sup>[9,10]</sup>. We attempted to plan the radiation regimen to irradiate the HCC plus the PVTT and the dysfunctional liver to avoid irradiation of the FL area<sup>[9,10]</sup>. We thus successfully avoided fatal RILD<sup>[7,9-10]</sup>. However, we experienced transient RILD (CPSD by 2) in eight patients. This indicates the limitation of the functional DVH, which is based on an analogue image of the FL area. The FL area created by analogue images does not reflect the quantity of FL in the FL area.

The third step was to resolve the problem of the quantity of FL in the FL area. Based on the concept of the dose-function parameters of Marks *et al*<sup>[8]</sup>, we measured the radioactivity counts of GSA-SPECT and incorporated the quantities in both the ≥20 Gy-irradiated FL area and the whole liver. We then obtained the cutoff value for F<sub>20Gy</sub>, whose accuracy (0.913) in predicting RILD surpassed the accuracy (0.826) of FL.V<sub>20Gy</sub> in the ROC analysis. This increased accuracy implies a solution to the inhomogeneity of FL. However, the functional parameter F<sub>20Gy</sub> does not reflect the wide-ranging differences in the reserve capacity of the liver (severity of cirrhosis) in patients with Child A or B cirrhosis and Child-Pugh scores of 5-9.

The fourth step was to create a correction parameter to allow the incorporation of this wide range of different liver reserve capacities into F<sub>20Gy</sub>. LHL15 (= L15/L15+H15) is the most important parameter reflecting the liver reserve capacity<sup>[13]</sup>. Therefore, we introduced F<sub>20Gy</sub>/LHL15 as a correction parameter, leading to a cutoff value of 37.7,

with an accuracy of 0.913 and AUC of 0.925 (Figure 6). However, LHL15 is based on the radioactivity counts in the liver versus those in the heart, but not on the radioactivity counts in other abdominal organs or the background radioactivity.

Therefore, the fifth step was to create another correction parameter to incorporate the total radioactivity counts, including those in the heart, visceral organs, and background. We proposed the concept of the total count ratio, or the liver radioactivity count versus the total radioactivity count, and introduced the parameter  $F_{20Gy}/\text{total count ratio}$ . It is easy to obtain all the radioactivity counts when SPECT data acquisition for  $F_{20Gy}$  is used.  $F_{20Gy}/\text{total count ratio}$  produced a cutoff value of 43.0, with an accuracy of 0.957 and AUC of 0.967. Therefore, the cutoff value of 43.0 for  $F_{20Gy}/\text{total count ratio}$  is the most reliable cutoff of all the dose–function parameters examined in this study (Figure 6).

This study had several limitations. Although we used LHL15 and the total count ratio to represent the liver reserve capacity, radioactivity counts exist outside the SPECT scanning levels. Sugahara *et al.* reported that the index of liver counts/syringe counts for Tc-99m GSA immediately before intravenous injection was  $55.9 \pm 10.2\%$  in Child A patients and  $38.1 \pm 12.4\%$  in Child B patients, demonstrating the clear differentiation of liver reserve capacities<sup>[14]</sup>. Therefore, LHL15 and the total count ratio might be incorporated into this index. However, it was impossible to use syringe counts in this study because it was retrospective. We also did not identify the risk factors with a multivariate analysis. From a statistical point of view, previous reports failed to detect risk factors among the dose–function parameters because patients with RILD as an endpoint were too few to analyze with multivariate analysis and each dose–liver function parameter was not an independent variable but correlated with the other variables<sup>[3–5]</sup>. Therefore, instead of a multivariate analysis, we used a ROC analysis with individual monovariate analyses to assess each dose–liver function parameter, which allowed us to calculate the cutoff values with great exactness.

In conclusion,  $FLV_{20Gy}$  of 26.4%,  $F_{20Gy}$  of 30.2, and  $F_{20Gy}/LHL15$  of 37.7 are effective cutoff values for each dose–liver function parameter and above all, an  $F_{20Gy}/\text{total count ratio}$  of 43.0 is the most reliable cutoff value for preventing RILD when irradiation is performed in the stop-breathing position with an error of  $\leq 5$  mm.

## CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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