coagulation necrosis, gemistocytic cells, and giant cells, were also examined in H&E-stained specimens from the second operation. Multiple serial sections were subjected to immunohistochemical analyses in order to determine local staining. Furthermore, tissue sections were subjected to 15 min of microwave heating to activate antigens in a retrieval solution composed of 0.1 mol/L sodium citrate (pH 6). This was followed with immunostaining of the specimens with the streptavidin–biotin–peroxidase complex method (Vectastain; Vector Laboratories, Inc., Burlingame, CA, USA). Human monoclonal antibodies were used that recognize MIB-1 (Dako, Tokyo, Japan). Positive immunostaining was demonstrated with diaminobenzidine reactions, and slides were subsequently counterstained with hematoxylin, dehydrated, cleared, and mounted. Cell counting was performed with the aid of a light microscope (Olympus Corporation, Tokyo, Japan) at a magnification of 400×. At least 200 tumor cells were counted, and data consisted of the mean of the counts from three different locations within the specimen. MIB-1-stained cells were also counted and the percentage calculated within the observed field as the MIB-1 index.

Extraction of nucleic acids

Tumor samples were immediately frozen in liquid nitrogen and stored at −80°C. From each patient, a peripheral blood sample was drawn and stored at −80°C. Total DNA was extracted from either frozen tissue samples or paraffin-embedded specimens and each patient's blood with a DNeasy Blood & Tissue Kit (QIAGEN Sciences, Germantown, MD, USA), according to the manufacturer's protocol.

MGMT promoter methylation analysis

MGMT promoter methylation status was determined by methylation-specific polymerase chain reaction (PCR) (MSP). Tumor DNA was subjected to bisulfite treatment overnight at 50°C and then purified using an EZ DNA Methylation Kit (Zymo Research Corporation, Irvine, CA, USA), according to the manufacturer's protocol. Tumor DNA obtained from paraffin-embedded specimens was amplified with the use of the first PCR using the following primers: 5′-GGATATGTTGGGATAGTT-3′ and 5′-CCA AAAACCCCAAACCC-3′ and then subjected to MSP (two-step approach) [11, 18]. Thermocycling conditions consisted of 5 min at 95°C, 35 cycles of 45 s at 95°C, 30 s at 52°C, and 50 s at 72°C. Tumor DNA obtained from frozen tissues was directly subjected to MSP. Primer sequences used to amplify sequences from the methylated or unmethylated *MGMT* promoter were 5′-TTTCGACG TTCGTAGGTTTTCGC-3′ (M-MGMT-F) and 5′-GCAC TCTTCCGAAAACGAAACG-3′ (M-MGMT-R) or 5′-TT TGTGTTTTGATGTTTGTAGGTTTTTGT-3′ (U-MGMT-F) and 5′-AACTCCACACTCTTCCAAAAACAAAACA-3′ (U-MGMT-R). Thermocycling conditions for the methylated *MGMT* promoter consisted of 5 min at 95°C, 35 cycles of 45 s at 95°C, 30 s at 67°C, and 50 s at 72°C. Thermocycling conditions for the unmethylated *MGMT* promoter consisted of 5 min at 95°C, 35 cycles of 45 s at 95°C, 30 s at 64°C, and 50 s at 72°C. PCR products were separated on 2% agarose gels.

Statistical analysis

A multivariate analysis with the Cox model, which was used to assess truly independent prognostic factors, was performed only for variables for which p values <0.1 were obtained in the univariate analysis (JMP ver 8, Tokyo, Japan).

Results

Overall and progression-free survival of glioblastoma patients

Overall survival and PFS of 189 patients with newly diagnosed glioblastomas who were treated from 1996 to 2010 at our institute were 15.1 and 7.7 months, respectively (M:F = 121:68; median age 60.0 years). The MST of patients treated with initial chemoradiotherapy with ACNU ($n = 79$), TMZ ($n = 91$), and radiation only ($n = 19$) were 14.8, 16.2, and 6.6 months, respectively. There was no significant difference in MST after initial chemoradiotherapy with ACNU and TMZ ($p = 0.32$). The PFS of patients treated with initial chemoradiotherapy with TMZ is significantly longer than those treated with ACNU (10.0 and 5.3 months, $p < 0.01$). Patients who had undergone surgery twice or more ($n = 35$; MST 21.0 months) showed longer overall survival time than those who had undergone surgery only once ($n = 154$; MST 12.9 months; $p = 0.008$). The MST of patients in the total or subtotal resection group ($n = 69$; median age 62.0 years) and that of patients in the partial or biopsy group ($n = 120$; median age 60.0 years) who underwent a first surgery was 17.6 and 13.4 months, respectively ($p = 0.03$). Thirty-two patients with glioblastoma relapsed and underwent a second surgery at the first recurrence. Eight of 32 patients underwent a third operation at the second recurrence. Only one patient underwent a fourth operation at the third recurrence. The first median PFS was 6.2 months and the second 6.9 months. In the univariate analysis (Table 2), patients ≤50 years had a longer survival time than those who were 50 years old ($p = 0.05$). MST of patients ≤50 years was 31.2 months and of those who were 50 years was 16.6 months.

Table 2 Univariate analyses of overall survival time of patients with recurrent glioblastoma

Variable	Number of cases	MST (95% confident interval)	*p* value (log-rank)
Age			
≤50 years	12	31.2 (21.0–44.7)	0.05
>50 years	20	16.6 (12.7–19.6)	
Operation time			
Twice	24	17.1 (13.6–30.8)	0.09
≥3 times	8	33.3 (21.0–64.9)	
MIB-1 index at the first surgery			
≤30%	15	22.4 (13.2–55.5)	0.097
>30%	11	17.9 (12.3–23.7)	
MIB-1 index at the second surgery			
≤10%	13	42.9 (17.3–64.9)	0.005
>10%	19	16.9 (12.7–22.8)	
MGMT promoter status at the first surgery			
Methylated	6	30.6 (NA)	0.6
Unmethylated	13	19.6 (14.5–41.3)	
MGMT promoter status at the second surgery			
Methylated	5	16.9 (NA)	0.7
Unmethylated	18	19.6 (14.5–41.3)	

NA not applicable

Patients with three or more surgical resections tended to have longer survival times than those with two operations ($p = 0.09$). The MST of patients with three or more surgical resections was 33.3 months and of those with two operations was 17.1 months (Table 2). The MST of the patients in the total or subtotal resection (≥90% removal) group ($n = 16$; median age 60.0) and of patients in the partial (<90% removal) removal or biopsy ($n = 16$; median age 54.0) group who underwent a first surgery were 18.0 and 22.4 months, respectively ($p = 0.2$) in recurrent surgical cases. However, the overall survival time of patients in the total or subtotal resection group ($n = 10$; median age 57.0) and the partial or biopsy group ($n = 22$; median age 57.0) who underwent a second surgery were 42.9 and 18.0 months, respectively ($p = 0.03$). The MST after the second surgery for each group was 16.4 and 11.1 months, respectively ($p = 0.02$).

MIB-1 index at the second operation showed prognostic value in relapsed glioblastoma patients

The MIB-1 indexes of primary tumors were obtained from 26 patients and those of recurrent tumors from all patients. The median MIB-1 index of primary tumors was 22.5% (range 6.8–90.0%) and of recurrent tumors was 13.2% (range 0.6–85.7%). MIB-1 indexes were smaller for recurrent than for primary tumors ($p = 0.03$, Mann–Whitney U test) (Fig. 1).

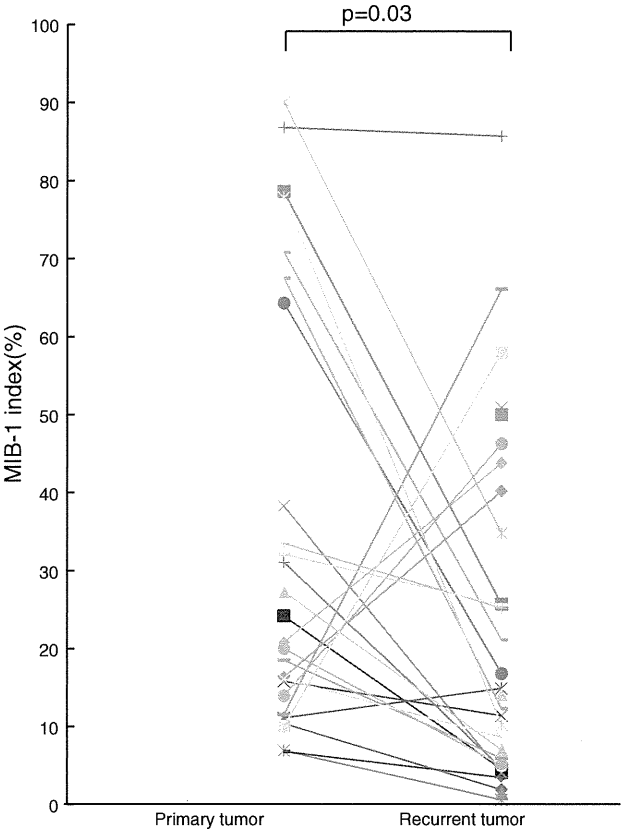
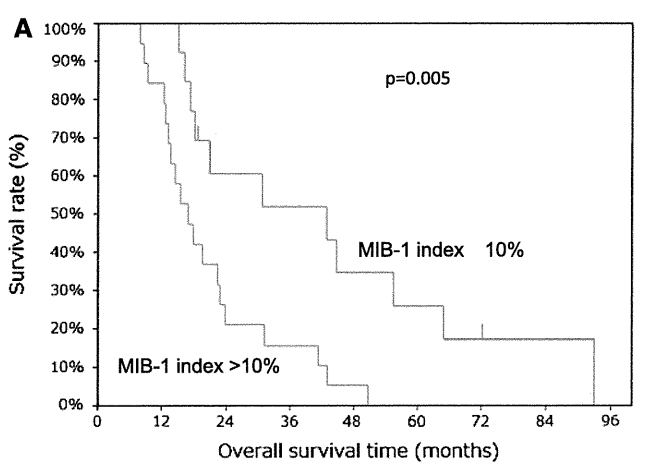


Fig. 1 Changes in MIB-1 indexes of primary and recurrent tumors. MIB-1 indexes were smaller in recurrent tumors than in primary tumors ($p = 0.03$, Mann–Whitney U test)

The MST of patients with MIB-1 indexes ≤30% was 22.4 months and of those with MIB-1 indexes >30% 17.9 months in primary tumors. Patients with MIB-1 indexes ≤30% in primary tumors tended to survive longer ($p = 0.097$) (Table 2). The MST of patients with MIB-1 indexes ≤10% was 42.9 months and of patients with indexes >10% 16.9 months in recurrent tumors. The MIB-1 indexes in recurrent tumors significantly correlated with MST ($p = 0.005$) (Table 2; Fig. 2a). The survival time of patients with MIB-1 indexes ≤10% after the second surgery was 21.6 months and of patients with indexes >10% was 6.0 months ($p = 0.0007$) (Table 3; Fig. 2b). The MIB-1 indexes in recurrent tumors significantly correlated with overall survival ($p = 0.004$), even in the multivariate analysis (Table 4). This analysis resulted in a hazard ratio (HR) of 5.252 [95% confidence interval (CI) 1.666–20.587).

Status of methylated *MGMT* promoter did not show prognostic value in relapsed glioblastoma patients

We obtained the *MGMT* promoter status in 19 cases with primary and 23 with recurrent tumors. The methylated *MGMT* promoter was found in six patients (31.6%) with primary and in five (21.7%) with recurrent tumors



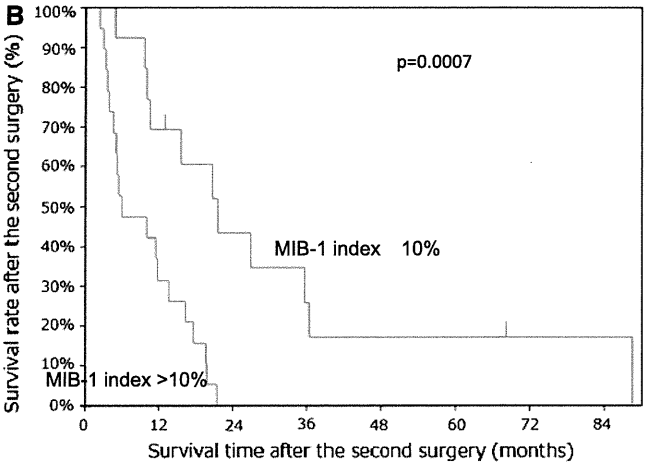


Fig. 2 **a** Kaplan–Meier survival curve comparing high and low MIB-1 indexes in recurrent tumors. The estimated overall survival rate of patients with MIB-1 indexes ≤10% was significantly higher than that of patients with indexes >10% ($p = 0.005$). **b** Kaplan–Meier survival curve after the second operation comparing high and low MIB-1 indexes in recurrent tumors. The estimated survival rate after the second operation of patients with MIB-1 indexes ≤10% was significantly higher than that of patients with indexes >10% ($p = 0.0007$)

Table 3 Univariate analyses of survival time after the second surgery of patients with recurrent glioblastoma

Variable	Number of cases	MST after second surgery (95% confident interval)	*p* value (log-rank)
MGMT promoter status at the second surgery			
Methylated	5	13.7 (NA)	0.8
Unmethylated	18	11.1 (5.0–19.9)	
MIB-1 index at the second surgery			
≤10%	13	21.6 (10.0–36.4)	0.0007
>10%	19	6.0 (3.9–13.7)	
Pseudopalisading necrosis			
Positive	8	10.9 (NA)	0.8
Negative	24	11.6 (6.0–19.7)	
Coagulation necrosis			
Positive	18	13.7 (5.4–19.7)	0.4
Negative	14	10.8 (3.7–35.7)	
Gemistocytic cell			
Positive	16	16.4 (9.7–21.6)	0.2
Negative	16	8.0 (3.9–13.7)	
Giant cell			
Positive	16	10.2 (4.7–16.4)	0.1
Negative	16	13.7 (5.0–21.6)	

NA not applicable

Table 4 Multivariate analyses of overall survival time of patients with recurrent glioblastoma

Variable	Number of cases	Hazard ratio (95% confident interval)	*p* value (Cox)
Age			
≤50 years	12	2.008 (0.811–5.506)	0.1
>50 years	20		
Operation time			
Twice	24	0.403 (0.119–1.146)	0.09
≥3 times	8		
MIB-1 index at the first surgery			
≤30%	15	1.073 (0.402–2.927)	0.9
>30%	11		
MIB-1 index at the second surgery			
≤10%	13	5.252 (1.666–20.587)	0.004
>10%	19		

(Table 1). The MST of patients with methylated or unmethylated *MGMT* promoters in primary tumors was 30.6 and 19.6 months ($p = 0.6$), respectively (Table 2). The MST of patients with methylated or unmethylated *MGMT* promoters in recurrent tumors was 16.9 and 19.6 months ($p = 0.7$), respectively (Table 2). No methylated *MGMT* promoter statuses in primary and recurrent tumors correlated with survival time (Table 2). Similarly, the methylated *MGMT* promoter in primary tumors did not correlate with the first PFS and in recurrent tumors did not correlate with the second PFS (Table 5).

Twenty patients underwent initial chemoradiotherapy with ACNU, and 12 underwent initial chemoradiotherapy with TMZ. Nine patients among 20 with initial chemoradiotherapy with ACNU were finally treated with TMZ. The MST of each of these groups was 23.3 and 14.0 months, respectively ($p = 0.02$). We then analyzed the correlation of survival time with initial chemotherapy regimen and *MGMT* promoter status in 18 patients whose *MGMT* promoter status was determined in both primary and recurrent tumors. The initial PFS of patients who received

Table 5 Univariate analyses of first and second progression-free survival (PFS) and mean survival time (MST) in 18 recurrent glioblastoma patients with *MGMT* promoter methylation status in both primary and recurrent tumors

Variable		Number of cases	First median PFS (95% confident interval)	*p* value (log-rank)	Second median PFS (95% confident interval)	*p* value (log-rank)	MST (95% confident interval)	*p* value (log-rank)
Initial treatment and the methylated *MGMT* promoter status in primary tumors								
Initial treatment	*MGMT* (primary)							
ACNU	Methylated	4	12.6 (9.1–18.3)	0.9	21.2 (2.1–NA)	0.3	49.3 (18.2–NA)	0.5
ACNU	Unmethylated	8	8.1 (3.3–26.3)		7.7 (1.6–13.6)		27.5 (15.1–64.9)	
TMZ	Methylated	1	5.2 (NA)	0.2	0.6 (NA)	0.03	9.2 (NA)	0.2
TMZ	Unmethylated	5	8.8 (2.7–22.9)		4.4 (1.0–16.4)		14.5 (7.8–41.3)	
Initial treatment and the methylated *MGMT* promoter status in recurrent tumors								
Initial treatment	*MGMT* (recurrent)							
ACNU	Methylated	3	18.1 (12.1–24.1)	0.8	13.4 (1.6–NA)	0.4	44.7 (15.5–NA)	0.6
ACNU	Unmethylated	9	9.1 (3.3–26.3)		6.6 (2.1–35.7)		31.2 (15.1–64.9)	
TMZ	Methylated	0						
TMZ	Unmethylated	6	7.6 (2.7–22.9)		3.8 (0.6–16.4)		13.4 (7.8–41.3)	
Changes of the methylated *MGMT* promoter status								
First surgery	Second surgery			0.9		0.3		0.5
Methylated	Methylated	1	3.3 (NA)		68.3 (NA)		72.2 (NA)	
Methylated	Unmethylated	4	10.9 (5.2–18.3)		4.4 (0.6–35.7)		30.6 (9.2–55.5)	
Unmethylated	Methylated	2	18.1 (12.1–24.1)		7.5 (1.6–13.4)		30.1 (15.5–44.7)	
Unmethylated	Unmethylated	11	6.4 (3.3–22.9)		5.0 (3.2–13.6)		19.6 (12.3–41.3)	

ACNU nimustine hydrochloride, *TMZ* Temozolomide, *NA* not applicable

chemoradiotherapy with ACNU and who had methylated ($n = 4$) and unmethylated ($n = 8$) MGMT promoters in primary tumors was 12.6 and 8.1 months, respectively ($p = 0.9$; Table 5). In contrast, the initial PFS of patients receiving chemoradiotherapy with TMZ and who had methylated ($n = 1$) and unmethylated ($n = 5$) *MGMT* promoters in primary tumors was 5.2 and 8.8 months, respectively ($p = 0.2$; Table 5). Three patients who initially underwent chemoradiotherapy with ACNU and who had methylated *MGMT* promoters, and five of nine patients who had unmethylated *MGMT* promoters in recurrent tumors were finally treated with TMZ. The MST of patients who underwent initial ACNU treatment followed by TMZ and who had methylated *MGMT* promoter status in recurrent tumors was longer than that of patients who had unmethylated *MGMT* promoter status (44.7 vs. 31.2 months, Table 5); however, this finding was not significant ($p = 0.6$). None of the patients who underwent the initial TMZ treatment received other alkylating agents. There was no significant difference in the PFS and MST of primary and recurrent tumors according to *MGMT* methylation status. Six patients showed changes in the methylated *MGMT* promoter in primary and recurrent tumors. Changes in the methylated *MGMT* promoter in primary and recurrent tumors did not correlate with the first and second PFS (Table 5).

Degenerative changes in tumor cells by chemoradiotherapy

We examined the degenerative changes in tumor cells, which included pseudopalisading necrosis, coagulation necrosis, gemistocytic cells, and giant cells, in H&E-stained specimens in recurrent tumors (Table 6). The MST of patients with pseudopalisading necrosis was 19.2 months and of patients without pseudopalisading necrosis 20.2 months ($p = 0.98$). The MST of patients with coagulation necrosis was 19.6 months and of patients without coagulation necrosis 25.4 months ($p = 0.16$). The MST of patients with gemistocytic cells was 22.4 months and of patients without gemistocytic cells 17.4 months ($p = 0.27$). The MST of patients with giant cells was 16.4 months and of patients without giant cells 22.4 months ($p = 0.08$). We found no correlations between morphological changes in tumor cells and survival time (Table 6).

Table 6 Univariate analyses of overall survival time of patients with recurrent glioblastoma with regard to degenerative changes of tumor at second surgery

Variable	Number of cases	MST (95% confident interval)	p value (log-rank)
Pseudopalisading necrosis			
Positive	8	19.2 (NA)	0.98
Negative	24	20.2 (16.2–31.2)	
Coagulation necrosis			
Positive	18	19.6 (13.6–23.7)	0.16
Negative	14	25.4 (15.5–44.7)	
Gemistocytic cell			
Positive	16	22.4 (16.2–50.8)	0.27
Negative	16	17.4 (13.2–30.8)	
Giant cell			
Positive	16	16.4 (13.2–30.8)	0.08
Negative	16	22.4 (16.9–44.7)	

MST mean survival time, *NA* not applicable

Discussion

We attempted to clarify the prognostic factors in recurrent glioblastomas. Clinically, patients ≤50 years had a longer survival time than patients who were >50 years old ($p = 0.05$). Glioblastoma patients who underwent surgery twice or more ($n = 35$; MST 21.0 months) showed increased overall survival compared with those who underwent only one surgery ($n = 154$; MST 12.9 months) at our institute from 1996 to 2010 ($p = 0.008$). Patients who had three or more surgical resections tended to survive longer than those who had two operations ($p = 0.09$). Whether surgical resections of recurring glioblastomas prolong survival of glioblastoma patients is unclear, but reoperations in recurrent patients have been reported to be beneficial for selected patients [19].

The extent of surgical resection in patients with newly diagnosed glioblastoma has been a well-documented prognostic factor for survival [20, 21]. In this study, we found a significant difference in survival time between the total and subtotal resection groups and the partial and biopsy groups of 189 newly diagnosed glioblastoma patients ($p = 0.03$). The extent of surgical resection during the first surgery had no correlation with the overall survival time in patients who underwent a second surgery in recurrent surgical cases; however, there was a significant difference between survival time and the extent of surgical resection during the second surgery. These data indicate that the extent of surgical resection is important, even in recurrent cases.

MIB-1 indexes that >30% in primary tumors tended to be poor prognostic factors, which were not significant. However, MIB-1 indexes in recurrent tumors had a definite correlation with overall survival time and survival time after the second surgery. Overall, survival of patients with MIB-1 indexes ≤10% at recurrence was 42.9 months and those with indexes >10% was 16.9 months, a significant difference. Schroder et al. [22] reported that MIB-1 indexes of glioblastomas at recurrence correlated with time to

recurrence. Kunishio et al. [23] reported that MIB-1 indexes of tumors with radiation necrosis after interstitial brachytherapy were 7.6 ± 5.5%, whereas that of primary tumors was significantly higher at 17.0 ± 11.2% ($p < 0.05$). These results were similar to ours. In contrast, Ralte et al. [24] reported that the difference in the MIB-1 indexes of initial (10.33 ± 7.98) and recurrent (13.8 ± 9.40) glioblastomas were not statistically significant ($p = 0.79$). Kodera et al. [25] reported that the MIB-1 indexes of recurrent glioblastomas after stereotactic radiosurgery were significantly lower than those before. In our study, MIB-1 indexes was also smaller in recurrent (13.2%) than in primary (22.5%) tumors ($p = 0.03$, Mann–Whitney U test). It has been postulated that MIB-1 indexes were smaller in recurrent than in primary tumors when tumor cells respond to initial chemoradiotherapy and degenerative changes of the tumor cells occur. Degenerative changes, such as giant cells, gemistocytic cell formation, and coagulation necrosis, are often found in recurrent gliomas [15], but these morphological changes did not correlate with survival time in our study.

It has been reported that the methylated *MGMT* promoter was found in 44.7–48.4% of newly diagnosed glioblastoma cases [11, 26, 27]. In our study, the methylated *MGMT* promoter was found in 31.6% of primary tumors. The first PFS was 6.2 months in patients treated with chemoradiotherapy with ACNU or TMZ, and the time was shorter than a previous report that found the first PFS to be 6.9 months with radiotherapy and TMZ treatment in newly diagnosed glioblastoma patients [1]. The rate of finding the methylated *MGMT* promoter seemed to be smaller in our series than in previous reports, which may be because our series could have included unfavorable cases with regrowth that did not respond to chemoradiotherapy and therefore needed a second surgery.

Brandes et al. estimated the correlation between *MGMT* promoter methylation status at first and relapse operation and survival time in 44 TMZ-treated paired tumors. They suggested that *MGMT* methylation status determined only at the first surgery appears to be of prognostic value. The MST of patients with methylated or unmethylated *MGMT* promoters in primary tumors was 30.6 and 19.6 months, respectively [14]. In contrast to their TMZ-treated series, in our series, 12 patients were initially treated with ACNU and six with TMZ. The *MGMT* promoter methylation status in primary tumors had no correlation with survival time and PFS. Brandes et al. also showed that overall survival and survival time after second surgery were not correlated with *MGMT* methylation status of recurrent tumors, even though most patients were treated with TMZ rechallenge and nitrosourea-based chemotherapy after the second surgery. The authors suggested that this may depend in part on the low activity of second-line therapies, especially those with alkylating agents, at the time of failure after chemoradiotherapy with TMZ [14].

Twenty patients in our study had initial chemoradiotherapy with ACNU and 12 patients had TMZ. The MST of each group was 23.3 and 14.0 months ($p = 0.02$), respectively. Nine patients among 20 patients with initial chemoradiotherapy with ACNU were finally treated with TMZ. The MST of patients with methylated *MGMT* promoter status in recurrent tumors treated with initial ACNU followed by TMZ tended to be longer than that with unmethylated *MGMT* (44.7 vs. 31.2 months, $p = 0.6$, Table 5). It is possibility that patients with recurrence who maintain the methylated *MGMT* status have sensitivity to TMZ even after the failure of initial ACNU. Nagane et al. [28] reported that protein expression of MGMT by Western blotting is an important prognostic factor for recurrent glioblastoma patients treated with TMZ after failure of initial ACNU-based chemotherapy. Methylated *MGMT* promoters were found in 31.6% of primary tumors and 21.7% of recurrent tumors. Six patients showed alterations in the methylation of the *MGMT* promoter between the primary and recurrent tumor. The *MGMT* promoter status of four of the five primary tumors with methylated *MGMT* promoters (80%) changed to unmethylated and the status of two of the 13 primary tumors with unmethylated *MGMT* promoters (15.4%) changed to methylated. Brandes et al. reported that *MGMT* status changed from methylated to unmethylated in eight of 13 (61.5%) tumors and from unmethylated to methylated in six of 25 (24%). Moreover, significant changes in *MGMT* methylation status occurred more frequently in *MGMT* methylated cases than unmethylated cases [14]. There are a number of potential explanations for these changes, including regional variation within the tumor, direct influence on methylation by treatment, selection of unmethylated cell populations by treatment, and further dedifferentiation of the tumor [12, 13].

In conclusion, we performed a multivariate analysis in relapsed glioblastoma patients and showed that only MIB-1 indexes in recurrent tumors persisted as significant independent prognostic factors in cases that had second surgeries. *MGMT* promoter status was frequently observed to change from methylated to unmethylated, but the *MGMT* promoter methylation status in primary and recurrent tumors had no correlation with survival time and PFS in recurrent surgical cases.

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CASE REPORT

Sentinel lymph node biopsy in breast cancer patients with previous breast augmentation surgery

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Abstract The number of breast augmentation surgeries (BAS) has increased. Therefore, the number of breast cancer patients with history of BAS has also increased. In this paper, we present two cases of sentinel lymph node biopsy (SLNB) in patients with previous BAS who were diagnosed with breast cancer. The patients were augmented using different approach; the first case was augmented through transaxillary incision, whereas the second case was augmented through periareolar incision. Lymphoscintigraphy (LPG) was performed on the patients 1 day prior to operation, enabling confirmation of lymphatic flow and SLN in both patients. SLNB was successfully performed in both cases. In one patient, SLNB was performed using indocyanine green (ICG) fluorescence and the Photodynamic Eye (PDE) system. Regardless of history of BAS, ICG and PDE system showed lymphatic flow and SLN in real time. LPG and ICG fluorescence were useful methods for SLN detection in patients with previous BAS, being able to confirm lymph flow before operation. Biopsy methods using LPG and PDE system were considered useful for difficult confirmation of lymph flow after breast augmentation. This is the first report of SLNB using ICG and PDE system for patients with previous BAS.

Keywords Sentinel lymph node biopsy · Augmentation · Lymphoscintigraphy · Fluorescence

Introduction

Sentinel lymph node biopsy (SLNB) technique has become the standard method for axillary staging in patients with early breast cancer. On the other hand, breast esthetic surgery is a recognized and accepted procedure. Among the procedures for breast esthetic surgery, the number of breast augmentation surgeries (BAS) has steadily increased in recent years. Therefore, the number of breast cancer patients with history of primary BAS has also steadily increased.

SLNB in patients with prior breast surgery is not recommended due to limited or insufficient data [1]. However, some authors have reported the possibility of SLNB after previous BAS [2–6].

We report two new cases showing that, after two different BAS procedures, SLNB can be performed successfully. This is the first report of SLNB performed successfully by fluorescence method using indocyanine green (ICG).

Case reports

Case 1

A 27-year-old woman with transaxillary augmented breast was referred to our hospital with a lump in the right breast.

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On physical examination, an elastic firm, mobile lump measuring 2 cm in diameter was palpable in the upper outer quadrant of the right breast. The lump was not adherent to skin. No lymphadenopathy was apparent.

Ultrasonography showed a lobulated, hypoechoic mass with unclear margins (Fig. 1). Magnetic resonance imaging (MRI) showed a clear shaped hypointense mass on T2 imaging. The mass was early blushing on dynamic study (Fig. 2). Blood examination including tumor marker was normal. There was no family history of breast cancer. Core-needle biopsy (CNB) was performed and revealed invasive ductal carcinoma. The patient underwent breast-conserving surgery and SLNB.

One day prior to operation, a dose of 74 mBq 99m-technetium-phytate (Daiichi RI Laboratory, Tokyo, Japan) was intradermally injected into the area overlying the tumor and periareolarly. LPG images were obtained immediately and 3 h after the injection. LPG showed lymphatic flow and hot spot (Fig. 3). Then, 5–10 min before surgery, blue dye was injected periareolarly. Two SLNs were found by gamma probe and by their blue tint during exploration. Lumpectomy with implant conservation was performed. The result of SLNB was negative for metastasis.

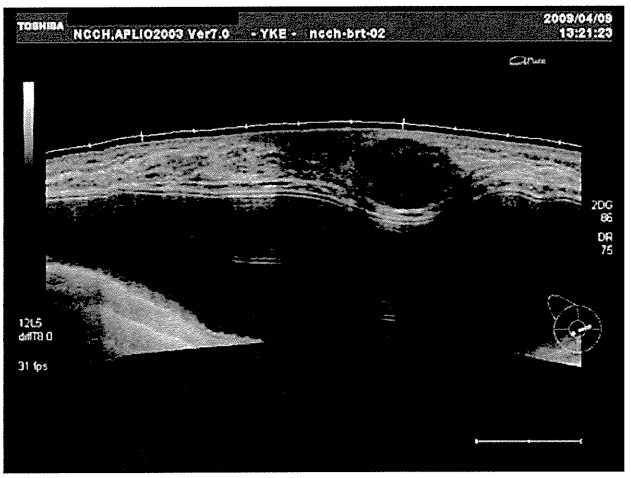

Fig. 1 Ultrasound of case 1. Implant was shown subglandular. A lobulated, hypoechoic mass with unclear margins was shown

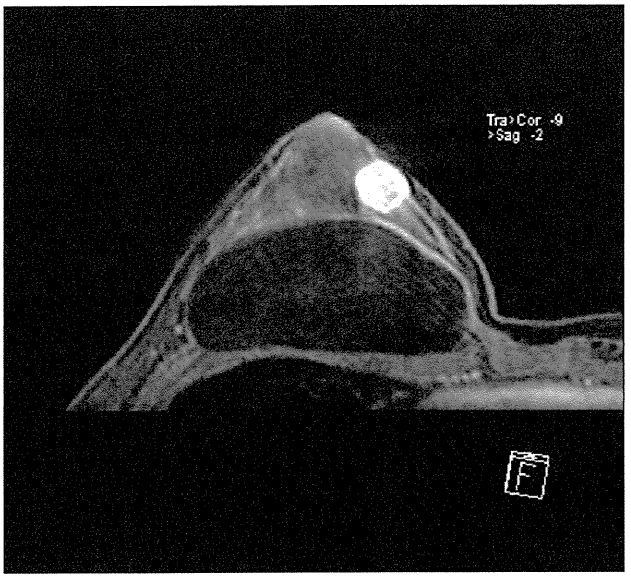

Fig. 2 Magnetic resonance imaging of case 1. A clear shaped tumor was observed on dynamic study. Implant was shown between the gland and pectoraris major

Case 2

A 48-year-old woman with a periareolarly augmented breast was referred to our hospital with a lump in the right

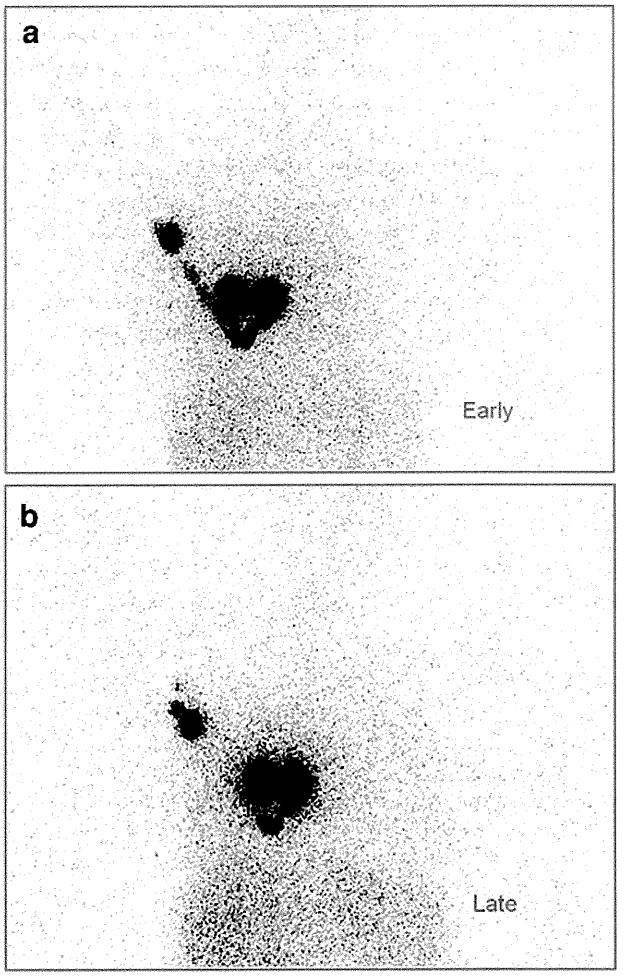

Fig. 3 Lymphoscintigraphy of case 1. **a** Lymphoscintigraphy showed lymphatic flow to sentinel lymph node in early phase. **b** Two hot spots were visualized in late phase

breast detected by MRI at regular check-up. Ultrasonography showed an irregular, hypoechoic mass with unclear margins (Fig. 4). MRI showed an unclear shaped mass on dynamic study (Fig. 5). CNB was performed and revealed invasive ductal carcinoma. The patient underwent mastectomy and SLNB.

LPG was performed by the same method as in case 1 and showed lymphatic flow and hot spot (Fig. 6). Then, 5–10 min before surgery, ICG was intradermally injected overlying the tumor and periareolarly. PDE system visualized lymphatic flow and SLN in real time. Four SLNs were detected by gamma probe and PDE system during exploration. One SLN was visualized by PDE system (Fig. 7). Mastectomy was performed with removal of the implant, which was subglandular. The result of SLNB was negative for metastasis.

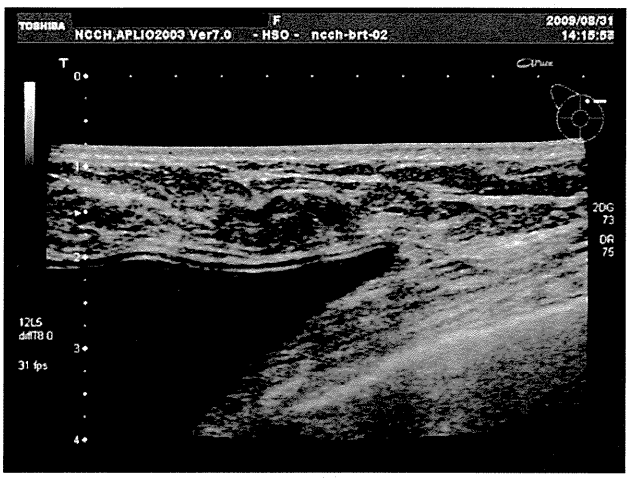

Fig. 4 Ultrasound of case 2. An irregular, hypoechoic mass with unclear margins was shown at right upper quadrant. Implant was shown subglandular

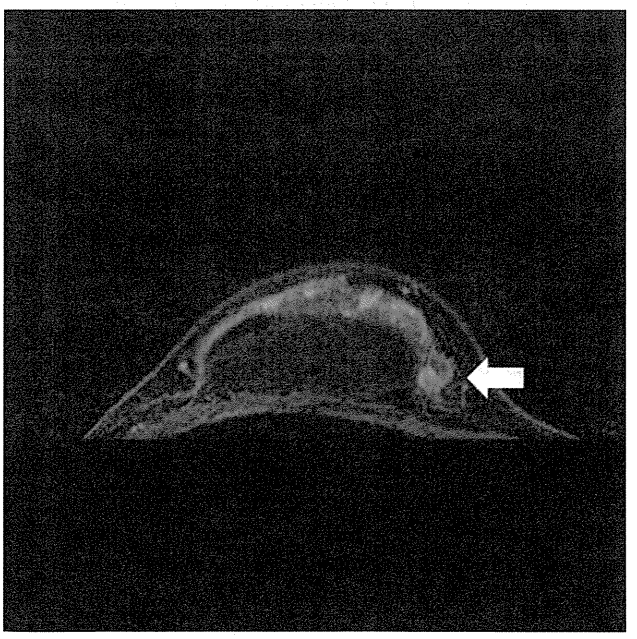

Fig. 5 Magnetic resonance imaging of case 2. An unclear shaped tumor was observed in a dynamic study (*arrow*). Implant was shown between the gland and pectoraris major

Discussion

SLNB has been indicated in oncologic surgery to avoid unnecessary total lymph node dissection. Treatment for breast cancer has become less invasive without affecting survival. Recently, SLNB has been demonstrated as the standard technique for treatment of early breast cancer.

The number of patients with breast cancer has been increasing. On the other hand, the number of breast esthetic surgeries has also been increasing. Breast augmentation and breast reduction are two of the most frequent

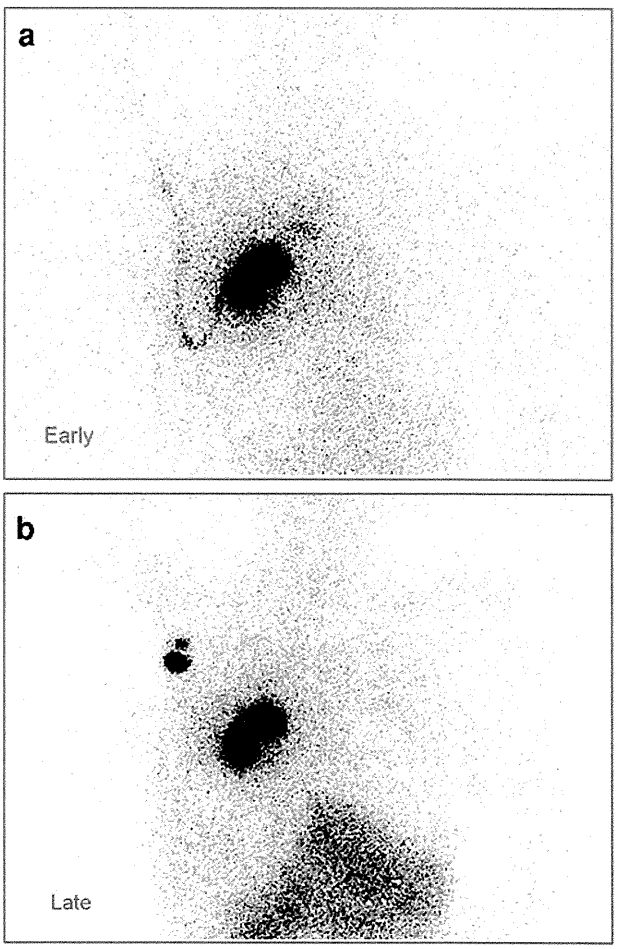

Fig. 6 Lymphoscintigraphy of case 2. **a** Lymphoscintigraphy showed lymphatic flow in early phase. **b** Two hot spots were visualized in late phase

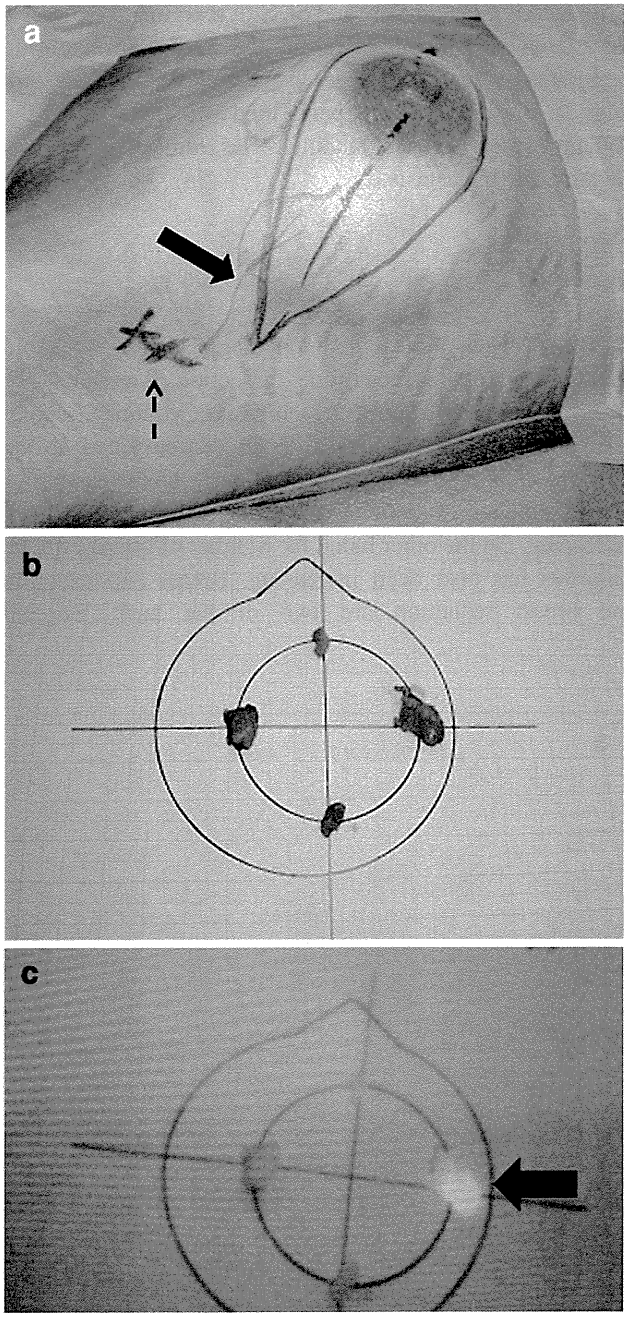

Fig. 7 Lymphatic route and sentinel lymph node of case 2. **a** Cutaneous labeling of lymphatic route detected by Photodynamic Eye system (*solid arrow*) and sentinel lymph node (*dashed arrow*). **b** Four sentinel lymph nodes were detected and removed. **c** One sentinel lymph node (*arrow*) was obtained with fluorescence and visualized using Photodynamic Eye system

procedures. BAS is a popular procedure to treat breast hypotrophy. It is estimated that, by 2007, about 300,000 cases had received breast augmentation [7]. Therefore, the number of breast cancer patients with previous BAS has been increasing steadily.

Due to interference with lymphatic drainage, SLNB with history of prior breast surgery is still controversial. The American Society of Clinical Oncology (ASCO) published a guideline in 2005 that SLNB was not recommended in patients with prior breast surgery or prior nononcologic breast surgery due to limited or insufficient data [1]. However, many reports of clinical experience of SLNB after excisional biopsy have been published. Therefore, the guideline concluded that excisional biopsy is not a contraindication to SLNB.

On the other hand, the feasibility of SLNB with previous BAS has not been evaluated, and clinical experience is insufficient at this time. Reported studies have suggested that SLNB for early breast cancer is possible after BAS. However, randomized study is still lacking and is needed for additional evaluation of SLNB after breast augmentation. The guideline mentioned that lymphatic drainage should be intact after breast esthetic surgery performed more than 6–12 months previously; therefore, SLNB after BAS cannot be considered an absolute contraindication. Also, it suggested that preoperative LPG could be performed for verification of integrity of the mammary and axillary lymphatics in patients with previous BAS.

SLNB technique is performed according to lymphatic drainage to SLN using radioisotope (RI), blue dye, and ICG. Among those methods, blue dye or ICG method is preformed to detect SLN only intraoperatively. However, when using RI method, injected RI is visualized on LPG before SLNB, besides detection of SLN by gamma probe intraoperatively. LPG is defined as external imaging and evaluation of lymphatic drainage and location of lymph nodes. Therefore, LPG has been recommended for validation of successful uptake and direction of lymph flow to SLN. RI method is more useful than other methods for SLNB after BAS.

In BAS, there are several approaches to implantation, such as axillary or periareolar, or inflamammary fold. All approaches may influence lymphatic flow to SLN. Especially, transaxillary breast augmentation runs the risk of false-negative or failure to identify SLN due to the possibility of lymph flow impairment during dissection and implantation.

Some authors have reported the possibility to use LPG and success of SLNB in patients with previous breast esthetic surgery. Sado et al. [8] and Munhoz et al. [9] reported that drainage pattern of patients with prior BAS is similar on LPG. Sado reported that SLN could be visualized in all patients. Gray et al. [2] and Jakub et al. [4] reported that all patients with previous BAS had SLN detected on LPG and SLNB performed. Fernandez et al. [6] reported that all patients with previous breast augmentation or reduction surgery had SLN detected on LPG and SLNB performed.

The anatomies of the axilla and the lymph nodes were described by Sappey and Rouviére [10, 11]. Borgstein

et al. [12] suggested that breasts share a common lymphatic drainage with the overlying skin, which converges to the subareolar plexus and drains through one or two lymph trunks toward the axillary chain. Suami et al. suggested that lymphatic drainage is organized into the superficial lymphatic system, internal mammary lymphatic system, and perforating lymphatic system. In human cadaver study, SLN drained almost the entire breast mainly through the superficial lymphatic system [13].

The difference in the superficial lymphatic system between after BAS and after excisional biopsy is the area of dissected skin. In excisional biopsy, skin incision is located on the tumor and skin must be dissected over the tumor and an additional region. As a result of skin dissection, superficial lymphatic system might be injured after excisional biopsy. On the other hand, in BAS, because the implant is placed under the mammary grand, skin incision is small and located on periareolar or axilla and skin need not be dissected much. Additionally, many breast cancer patients are diagnosed many years after performance of BAS. Therefore, superficial lymphatic system might be preserved or reconstructed during the many years after BAS.

Of our two cases, case 1 had received transaxillary and case 2 periareolar breast augmentation. LPG showed SLN and SLNB was successful in both patients with different augmentation procedure. Because these procedures dissected small part of the skin, most of the superficial lymphatic system was conserved. Therefore, the reason for successful LPG and SLNB was considered to be that the superficial lymphatic system was not impaired by BAS.

Case 2 underwent SLNB using ICG and PDE system. Because PDE system projects lymphatic flow synchronously, fluorescence method is useful for SLNB. Some authors have reported the possibility of SLNB using ICG and PDE imaging [14–16]. Hojo et al. [16] reported high identification rate using a combination of RI and fluorescence methods. In this case, the number of SLN obtained with fluorescence and visualized using PDE system was one of the four. However, in our institute, the number of SLN found using fluorescence is similar to when using indigocarmine blue, whereas the number of SLN found using RI is larger. Since there are pros and cons for each identification method, we used and recommend combined method using RI. This is the first report of SLNB performed by fluorescence method in a patient with BAS. In the patient with BAS, the flow of ICG was visualized in real time and the flow route was the same as that of radioisotope. Therefore, fluorescence method can be applied to the patient with BAS for SLNB.

Conflict of interest None.

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Original article

Sentinel lymph node biopsy using indigo carmine blue dye and the validity of '10% rule' and '4 nodes rule'

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ABSTRACT

This is the study which assessed sentinel lymph node biopsy (SNB) using indigo carmine blue dye and the validity of the '10% rule' and '4 nodes rule'. Patients (302) were performed SNB using the combined radioisotope (RI)/indigo carmine dye method. Excised SLNs were confirmed whether they were stained and numbered in order of RI count and the percentage of radioactivity as compared to the hottest node was calculated. The relationship between histological diagnosis, dyeing and RI count was assessed. All the patients were detected SLN. Positive nodes were identified in 84 (27.8%) patients and were identified up to the third degree of hottest. All the hottest positive nodes were stained by indigo carmine. From the results, removing the three most radioactive SLNs identified all cases of nodal metastasis without complications. These stopping rules were valid and useful under indigo carmine use too.

Introduction

Sentinel lymph node biopsy (SNB) has been established as the standard operative procedure for axillary staging in patients with early breast cancer.[1,2] SNB benefits patients without metastatic nodes by omitting unnecessary axillary lymph node dissection (ALND) with its concomitant morbidities. Since the introduction of SNB, the required number of nodes to excise to confirm metastasis has been discussed. To reduce the SNB false-negative rate, the number of excised nodes may be increased. However, if many nodes are excised at SNB, it practically approaches ALND and results in increased morbidity and decreased quality of life.

According to the study of SNB with the combined RI/dye method, the ideal number of excised nodes and the methods used to decide on this ideal number were analyzed. The two main procedures, '10% rule' and '4 nodes rule', used to determine the number of excised nodes[3] and the intensity of the radioisotope (RI) count[4] are analyzed. These procedures were studied under SNB using patent blue and isosulfan blue. However, instead of patent blue or isosulfan blue, indigo carmine blue dye has been used for SNB with a combination method in Japan. The aim of this study is to determine the ideal number of nodes for excision under RI and indigo carmine use.

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Materials and methods

Patients

This study is an analysis of 302 patients with clinical stage Tis–T3, node negative breast cancer who underwent SNB at the National Cancer Center Hospital, Tokyo, Japan from October 2008 to November 2009. Patients and tumors characteristics were shown at Table 1. All patients underwent SNB with a combination method using RI and indigo carmine blue dye. This procedure of SNB was approved from Ministry of Health, Labour and Welfare of Japan, and all patients provided written informed consent to be examined in this study.

Sentinel node biopsy

Technetium 99 m sulfur colloid (74 mBq) was injected subdermally into the periareolar area and the area around the primary tumor on the day before surgery. Lymphoscintigraphy was performed immediately after injection and after 3 h. At the time of surgery, 5 mL (20 mg) of indigo carmine blue dye (Daiichi-Sankyo, Tokyo, Japan) was injected subdermally into the periareolar area. SNB was performed by searching for the blue lymphatic stream and radioactivity using a gamma detecting probe (Neoprobe; Neoprobe Corp, Dublin, Ohio). All blue-stained and/or radioactive nodes were excised and regarded as sentinel lymph node (SLN). After removal

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Table 1
Clinicopathologic characteristics of the patients and tumors ($n = 302$).

	Number	%
Mean age, years (range)	56.4 (27–86)	
Menopausal status		
Premenopausal	109	36.1
Postmenopausal	193	63.9
Tumor laterality		
Right	166	55.0
Left	136	45.0
Tumor location		
Upper inner quadrant	90	29.8
Lower inner quadrant	40	13.2
Upper outer quadrant	126	41.7
Lower outer quadrant	42	13.9
Central	4	1.3
T stage		
Tis	39	12.9
T1	145	48.0
T2	109	36.1
T3	9	3.0
Tumor histology		
DCIS	39	12.9
Invasive ductal carcinoma	243	80.5
Invasive lobular carcinoma	16	5.3
Other	4	1.3

SD, standard deviation; and DCIS, ductal carcinoma in situ.

of the nodes, SLNs were confirmed whether they were stained by dye and were measured the radioactivity *ex vivo*. SLNs were numbered in order of RI count and the ratio of each node to the hottest node was calculated. All nodes with 10% or more of the *ex vivo* count of the hottest node were evaluated intraoperatively by frozen section. If metastases were identified in the SLNs, ALND was performed. In this study, patients with isolated tumor cells were considered to have SLN metastasis for which ALND was performed. ALND was omitted for patients without metastatic nodes.

Pathological examination

For frozen sections, the SLN was sectioned in the center. After the intraoperative frozen section, all nodes were submitted for permanent sectioning. The SLN was sectioned as close to 2–3 mm as possible, and processed with hematoxylin and eosin staining and immunohistochemistry using anti-cytokeratin antibodies (CAM 5.2 and AE1:AE3). Patients with metastases detected by either method were considered to be positive. If the metastatic lesion was between 0.2 and 2.0 mm in size, the node was defined as having micrometastasis. Macrometastasis was defined as a lymph node with metastatic lesions over 2.0 mm. Isolated tumor cells were defined as a lymph node with metastatic lesions less than 0.2 mm.

For comparison of categorical variables, the chi-square test was used. A p-value of 0.05 was considered statistically significant. All data were analyzed using SPSS software (SPSS Inc., Chicago, IL).

Results

In this study, SLN was successfully identified in all patients. More than one SLN was identified in 239/302 (82.5%) patients. The mean number of SLNs excised was 2.6 (range, 1–6). There were 84 patients with positive SLNs (27.8%) and 59 patients with only one positive SLN. The mean number of positive SLNs was 1.3 (range, 1–4). Total number of positive SLNs was 105. The number of positive node detected by RI and dye was 79 (75.2%). The rest was detected by RI or dye only and 24 (22.9%) were not stained and 2 (1.0%) were not detected by RI (Table 2). Table 3 shows the relationship between histological diagnosis and the order of RI count.

Table 2
The procedures of operation and the results of sentinel lymph node biopsy.

	Number	%
Surgery		
Total mastectomy	130	43.0
Lumpectomy	172	57.0
Total number of SLNs excised	782	
Mean number of SLNs excised (range)	2.6 (1–6)	
Number of positive SLNs	84	27.8
One positive SLN only	59	19.5
Total number of positive SLNs	105	
RI and dye	79	75.2
RI only	24	22.9
Dye only	2	1.0
Mean number of positive SLNs (range)	1.3 (1–4)	

SLN, sentinel lymph node; and RI, radioisotope.

Of the 105 total histological positive SLNs, 71 (67.6%) were the hottest node. All metastatic nodes were covered to the fifth degree of RI count. The most radioactive positive node of each patient was diagnosed up to the third hottest node (Fig. 1). Isolated tumor cells were only found in the hottest node. For each patient, the percentage of each node's RI count to the hottest node was calculated. When a RI count of 10% of hottest node is used as the cut-off, the proportion of positive patients captured by SNB was 94.1% and the false-negative rate was 5.9% (Fig. 2). All the hottest positive nodes were stained by indigo carmine. There was no complication associated with SNB.

Discussion

In the surgery for breast cancer patients, the theory of SLN has been established and since its introduction, SNB has allowed patients with negative biopsies to skip ALND and its associated morbidities. Though the procedure of SNB has been standardized by surgical oncologists, the ideal number of nodes to excise remains a question. In these stopping rules, patent blue or isosulfan blue was used. Instead of patent blue and isosulfan blue, indigo carmine has been used and can be used for SNB safety in Japan. This study investigated the ideal number of nodes to excise which satisfy a low false-negative rate under the use of indigo carmine blue dye.

Indigo carmine is the diagnostic dye and has been used for renal function test. Its molecular mass is 466.4 and near patent blue and isosulfan blue. Although Albo et al. and Montgomery et al. reported that anaphylactic reaction for isosulfan blue was appeared in 1.1–1.6% of patients,[8,9] there was no report of serious side effect with indigo carmine.

In this study, all positive nodes were captured up to the fifth rank in radioactivity, and the most radioactive positive node was captured up to the third rank in radioactivity. The optimal number of excised nodes was reported by some authors. McCarter et al. reported that 99.1% of patients were captured positive node up to the fourth site.[3] Almost all other studies reported that the only positive SLN is rarely identified beyond the fourth sampled node.[5] Since metastasis can be found in the 3–5th most radioactive SLN,

Table 3
Histological diagnosis of all sentinel lymph nodes in order of decreasing radioisotope count.

	1st	2nd	3rd	4th	5th
Isolated tumor cells ($n = 4$)	4	0	0	0	0
Micrometastasis ($n = 42$)	32	7	2	1	0
Macrometastasis ($n = 59$)	35	14	5	3	2
Total	71	21	7	4	2

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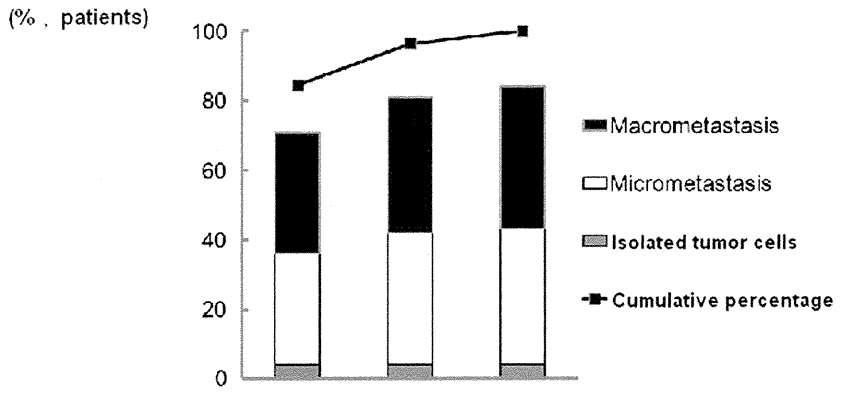

	1	2	3
Isolated tumor cells (n=4)	4	4	4
Micrometastasis (n=39)	32	38	39
Macrometastasis (n=41)	35	39	41
Total	71	81	84
Cumulative percentage (%)	84.5	96.4	100

Fig. 1. In patient with positive node ($n = 84$), the most radioactive positive node was within third rank of radioisotope count.

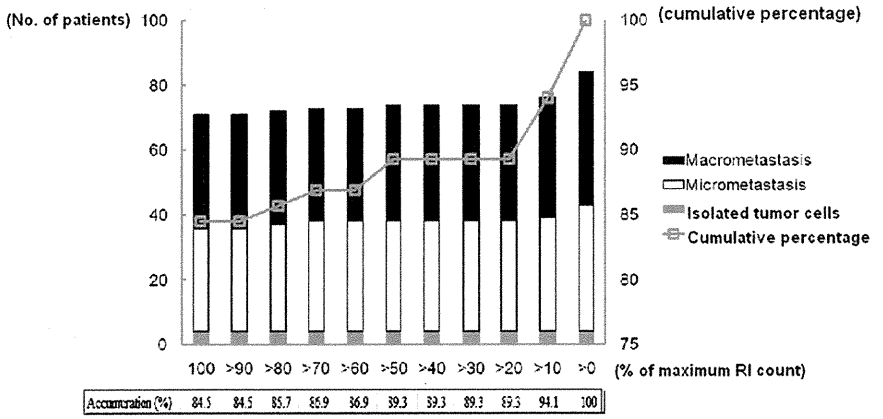

Fig. 2. Histological diagnosis according to percentage of the maximum radioisotope count.

and the TNM classification requires 6 or more lymph nodes to be excised for pN staging, the boundary between SNB and ALND can be defined at the number 6. Another guideline known as the '10% rule' was introduced in 2000 by Martin et al.[4] According to this rule, any node with 10% or more of the *ex vivo* RI count of the hottest node should be removed as a SLN. When the '10% rule' was used in this study, the false-negative rate was 5.9% and slightly high. The reported false-negative rates were 1.7% in Chung et al. [6] and 5.8% in Martin et al.[4] The accepted false-negative rate for SNB was 5%[7] and a record of less than 5% false-negatives at our institute since the introduction of SNB in 2000.

Although 22.9% of positive nodes were not stained but detected by RI only, all the hottest positive nodes were stained by indigo carmine. According to the current procedure of SNB, all the nodes that were stained and/or react to the gamma probe were excised by surgeon. Therefore the number of excised node by RI was apt to larger. And dye method is inferior to RI or combination method in detective rate, generally.[10,11] However, Narui et al. reported that a 4 node sampling method using only patent blue was reliable,[12] and in our study 2 nodes were found only through the dye method not due to accumulate RI. Metastatic disease can injure the lymphatic system and technical errors can occur during SNB. Recognition of the method's limitations and careful intraoperative palpitation are necessary.

In summary, the validity of these stopping rules in SNB under the use of indigo carmine blue dye was analyzed. Though the '10% rule' resulted in slightly high false-negative rate with the RI count method, a positive sentinel lymph node was identified in 100% of cases within the first 3 sentinel nodes when indigo carmine and RI was used. Under the use of indigo carmine, terminating SNB at 3 sampled nodes was the validity procedure to minimize the false-negative and complication rates.

Conflict of interest statement

None declared.

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Report

^{106}Ruthenium Plaque Therapy (RPT) for Retinoblastoma

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Summary

One hundred one ^{106}ruthenium plaque therapies were retrospectively analyzed that were performed in 90 eyes of 85 patients with retinoblastoma between 1998 and 2008.

Purpose: To evaluate the effectiveness of episcleral ^{106}ruthenium plaque therapy (RPT) in the management of retinoblastoma.

Methods and Materials: One hundred one RPTs were retrospectively analyzed that were performed in 90 eyes of 85 patients with retinoblastoma at National Cancer Center Hospital between 1998 and 2008. Each RPT had a corresponding tumor and 101 tumors were considered in the analysis of local control. Median follow-up length was 72.8 months. Median patient age at the RPT was 28 months. Median prescribed doses at reference depth and outer surface of the sclera were 47.4 Gy and 162.3 Gy, respectively.

Results: Local control rate (LCR) and ocular retention rate (ORR) at 2 years were 33.7% and 58.7%, respectively. Unilateral disease, International Classification of Retinoblastoma group C or more advanced at the first presentation or at the time of RPT, vitreous and/or subretinal seeding, tumor size greater than 5 disc diameter (DD), reference depth greater than 5 mm, dose rate at reference depth lower than 0.7 Gy/hour, dose at the reference depth lower than 35 Gy, and (biologically effective dose with an α/β ratio of 10 Gy) at the reference depth lower than 40 Gy_{10} were associated with unfavorable LCR. Two patients died of metastatic disease. Radiation complications included retinal detachment in 12 eyes (13.3%), proliferative retinopathy in 6 (6.7%), rubeosis iris in 2 (2.2%), and posterior subcapsular cataract in 23 (25.6%).

Conclusion: RPT is an effective eye-preserving treatment for retinoblastoma.

Introduction

Retinoblastoma is the most common intraocular malignancy of childhood that arises from neuroepithelial cells of the retina. The reported incidence of retinoblastoma is 1 in 16,653-22,166 live births in Japan (1).

For the management of children with retinoblastoma, mutilating enucleation and external beam radiation therapy (EBRT) are

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employed with a decreasing frequency, because of the facial disfigurement and increased incidence of the secondary malignancies after EBRT (2). Chemotherapy has been replacing EBRT as the modality for organ preservation (3, 4). Although chemotherapy can shrink the retinoblastoma lesion, local therapy is indispensable to attain local control. Episcleral plaque brachytherapy has emerged as a treatment option as a focal therapy in the primary or secondary treatment of retinoblastoma (3-5). Low-energy gamma-ray emitting ^{125}I plaque is most used around the world, which is inexpensive and can be customized to fit each tumor shape by arranging seed locations in the episcleral applicator (5-7). In contrast, the pure beta ray-emitting ^{106}ruthenium (^{106}Ru) plaque is used mainly in Europe (8, 9). Although ^{106}Ru plaque is very expensive and cannot treat tumors with a height greater than 5-6 mm because it emits purely beta rays (energy 3.54 MeV) (8-11), the thickness of the applicators is only 1 mm in contrast to 3 mm thickness of the I-125 applicators, which is greatly advantageous when an infant's very small eyes are dealt with. In Japan, National Cancer Center Hospital is the only institution performing episcleral brachytherapy using ^{106}Ru plaque applicators. This retrospective study analyzes the results of ^{106}Ru plaque therapy (RPT) in the management of retinoblastoma.

Methods and Materials

We retrospectively reviewed the clinical records of all patients undergoing RPTs for retinoblastoma between December 1998 and November 2008 in the National Cancer Center Hospital, Japan. One hundred one tumors of 90 eyes in 85 patients were treated by RPT during this period. In 10 eyes, multiple tumors were treated by simultaneous application of the plaques. Local status of the 101 tumors could be evaluated. All tumors were followed at least for 1 year. Patient and tumor characteristics at the initial presentation are listed in Table 1. Tumor stage is based on International Classification of Retinoblastoma (ICRB) (4, 12, 13). Only 31 (30.7%) of the 101 tumors presented with confined diseases of group A or B. Vitreous and subretinal tumor seedings were seen in 41.6% and 35.6%, respectively.

When RPT was the initial treatment, it was considered as the first-line treatment. When RPT followed after local and/or systemic therapies that had successfully reduced the tumor, it was considered as the second-line treatment. RPT was considered as salvage therapy, provided that it was employed to treat a refractory or relapsed tumor after the preceding therapies. In the current series, RPT was employed in only 4 tumors as the first-line therapy. The other 62 tumors underwent RPT as the second-line therapy and 35 as salvage therapy (Table 2). Some too-large tumors, apparently not suitable to be treated by RPT, underwent RPTs, because there was a strong wish of the parents to conserve

Table 1 Characteristics of patients and 101 tumors at the initial presentation

Characteristics	Number
Patients	85
Gender	
Male	52
Female	33
Age at the first brachytherapy	28 mo (range 7-240)
Laterality	
Bilateral	60
Unilateral	25
Family history	
Positive	9
ICRB	
Group A	2 (2.0%)
Group B	29 (28.7%)
Group C	15 (14.9%)
Group D	43 (42.6%)
Group E	7 (6.9%)
Unknown	5 (5.0%)
Tumor with vitreous seeding	42 (41.6%)
Tumor with subretinal seeding	36 (35.6%)
Median tumor size	5 DD (range 0.8-20)

Abbreviations: DD = disc diameter; ICRB = International Classification of Retinoblastoma.

Table 2 Tumor and treatment characteristics at the 101 first RPTs

Tumor characteristics	Number (%)
First-line therapy	4 (4.0)
Second-line therapy	62 (61.4)
Salvage therapy	35 (34.6)
ICRB at brachytherapy	
Group A	9 (8.9)
Group B	29 (28.7)
Group C	20 (19.8)
Group D	37 (36.6)
Group E	6 (5.9)
Tumor with subretinal seeding	28 (27.7)
Tumor with vitreous seeding	42 (41.6)
Response to preceding therapy	
Good	34 (33.7)
Stable	41 (40.6)
Poor	17 (16.8)
Unknown	5 (5.0)
Tumor size (DD)	
Median	5 DD (range 0.5-22)
Brachytherapy dose at outer surface of sclera	
Median	162.3 Gy (range: 61.3-950.0)
Brachytherapy dose at outer surface of sclera (BED_3)	
Median	854.9 Gy_3 (range 101.2-4317.0)
Dose rate at outer surface of sclera	
Median	7.5 Gy/h (range 4.5-10.3)
Brachytherapy reference depth	
Median	5 mm (range 3-9)
Dose rate at reference depth	
Median	0.83 Gy/h (range 0.11-2.22)
Brachytherapy dose at reference depth	
Median	47.4 Gy (range 24.3-86.1)
Brachytherapy dose at reference depth (BED_{10})	
Median	65.6 Gy_{10} (range 27.0-131.3)
Brachytherapy treatment time	
Median	53.3 h (range: 20.5-332.3)

Abbreviations: BED = biological effective dose; DD = disc diameter; ICRB = the International Classification of Retinoblastoma; RPT = ruthenium plaque brachytherapy.

the eyes of their children. For far more advanced disease in which tumor spread toward anterior structures of the eye or infiltrates into the optic disc, and if a massive hemorrhage was developed in retina or vitreous space with a loss of vision, enucleation was employed with or without systemic chemotherapy according to the pathological risk features. Systemic chemotherapy regimen mostly used in this cohort was 3-drug chemotherapy with carboplatin, etoposide, and vincristine.

Tumor response to the preceding therapies was defined as follows. The tumor whose stage attained down-grouping was classified as a good response, up-grouping as a poor response, and no group change as stable.

All episcleral ^{106}Ru plaque applicators (BEBIG Isotopen und Medizintechnik GmbH, Berlin, Germany) were inserted under general anesthesia. Before the operation, tumor location and height were assessed by slit lamp examinations with or without ultrasound and an appropriate plaque was selected. The plaques are hemispherically shaped with radii of 12 and 14 mm. CIA and CIB are used to treat anteriorly located tumor because they are semicircularly shaped concave in order to avoid cornea. COC are used to treat the tumor located in the posterior pole with a notch to avoid optic disc. CCA and CCB are round shaped and used to treat tumors which are away from cornea or optic disc. The diameters of A and B are 15.5 mm and 20 mm, respectively. To insert the plaques, extraocular muscles were separated temporarily. The selected plaques were sutured through the plaque eyelets to the sclera surface. The plaques were removed also under general anesthesia after the planned duration of radiation. The duration of radiation was calculated to administer prescription dose of 40 Gy to the reference depth. The reference depth was the height of tumor plus sclera thickness (1 mm) with a safety margin of 1 mm. Lateral tumor margin was set to 2-3 mm (10). Before July 2005, reliable ultrasound was not available to determine tumor height; therefore, the slit lamp was used to estimate it using its focus. Therefore before July 2005, only tumor width expressed by disc diameter (DD) and reference depths diagnosed approximately by slit lamp were available in the medical records. And for tumors with vitreous seeding, reference depth was set to 5-6 mm, which was regarded as the limit of the range of RPT. Hence, tumors with vitreous seeding without description of reference depth in medical record could be recalculated as having a reference depth of 5-6 mm. Before September 2006, the reference depth was 5 mm and thereafter it was set to 6 mm because of the dose tables provided by the manufacturer. Since May 2002, BEBIG has delivered its ^{106}Ru eye plaques with new protocols of radioactivity measurements in accordance with the National Institute of Standards and Technology calibration system. Therefore recalculations were performed for this study to correct the prescribed dose before the introduction of the new calibration system by using the conversion factor table provided by BEBIG (14). Because most of the conversion factors, which differ by applicator type and reference depth, were greater than 1.0, median dose at the reference depth became greater than 40 Gy after the recalculation (Table 2).

Because the biological effect of RPT could differ by dose rate and combined effect with EBRT must be considered, biologically effective dose (BED) was calculated according to the method of Dale (15) and is given by

$$\mathrm{BED} = \text{Total dose} \times 1 + \frac{2R}{\mu}\left(\frac{\beta}{\alpha}\right)\{1 - 1/\mu T[1 - \exp(-\mu T)]\}$$

where R indicates dose rate, T the treatment time, and μ the repair rate constant of sublethal damage. The value of μ was assumed as 0.46 hour^{-1} (corresponding to repair half time of 1.5 hours) (15). The α/β values used in this analysis were α/β = 10 Gy for tumor control and α/β = 3 Gy for late normal tissue morbidities. In 85 of 101 RPTs, the reference depth and prescribed dose could be obtained and BED_{10} (BED with an α/β ratio of 10 Gy) could be calculated. Because the outer surface of the sclera directly touches the plaque applicator (depth 0 mm), dose and BED_3 (BED with an α/β ratio of 3 Gy) of the outer surface of sclera could be calculated for 97 procedures whose applicator type and treatment time were known. For deriving total BED_3 of outer surface of sclera, BED_3 of EBRT, if any, before and after the RPT was added. In 16 eyes in which part of retina had overlapping multiple RPTs, BED_3 of outer surface of sclera of each RPT was added.

Ophthalmologic follow-up was performed with examinations under anesthesia every 1-2 months after the therapy until tumor control was achieved. Thereafter, examinations were performed every 2-6 months as needed.

The probabilities of local control rate (LCR), ocular retention rate (ORR), and overall survival (OS) were calculated using the Kaplan-Meyer method (16). For LCR, 101 tumors treated by 101 RPTs were taken into account. Local control was assessed by retinal diagram before and after the RPTs. Tumor persistent or regrowing within margins of the retina covered by the plaque applicator was considered as local failure. For the estimate of ORR, enucleation from disease progression or treatment-related complications and death from any causes were scored as an event and 90 eyes were subjects of the analysis. ORR was calculated from date of the last RPT to date of the events or to the last follow-up. The relationships between clinical and treatment variables and LCR were analyzed by the univariate and multivariate analyses. A P value of $<.05$ was considered statistically significant. The continuous variables were dichotomized to give the lowest P values in the log-rank test. The variables with P values $<.05$ were further analyzed in multivariate analysis by Cox proportional hazards test.

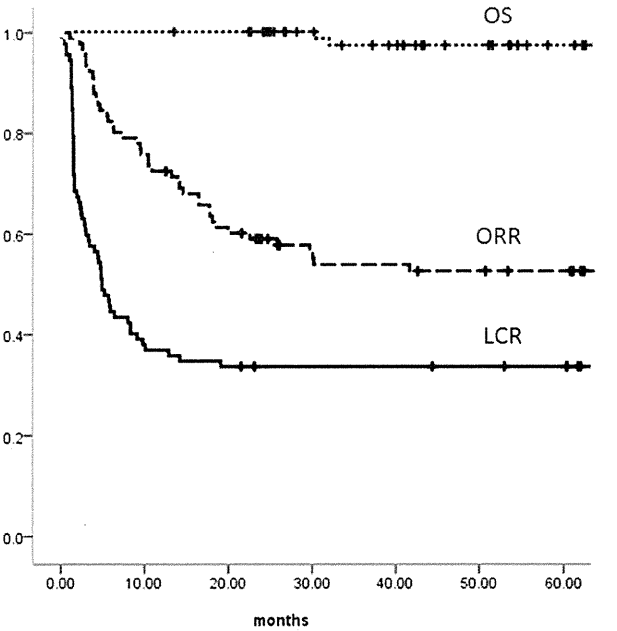

Fig. 1. Kaplan-Meyer curves of local control rate (LCR), ocular retention rate (ORR), and overall survival (OS).

Results

Tumor and treatment characteristics at the 101 RPTs were summarized in Table 2. Median patient follow-up length was 72.8 months (range 12.2-130). LCR of the 101 tumors treated by the 101 RPTs was 33.7% in 2 years with 31 tumors controlled (Fig. 1). All local failures were seen within 24 months after RPTs. The locally failed tumors were managed by various modalities including repeated RPT. Forty-two eyes (46.7%) were enucleated during the follow-up period and estimated 2 and 4 years ORR rates are 58.7% and 52.2%, respectively (Fig. 1).

Univariate analysis revealed clinical and treatment factors related with LCR (Table 3). Unilateral disease, ICRB group C or more at the presentation or at the time of RPT and vitreous seeding/subretinal seedings at the time of RPT, tumor size greater than 5 DD, dose at the reference depth lower than 35 Gy, BED_{10} for the reference depth lower than 40 Gy_{10}, reference depth greater than 5 mm, and dose rate at reference depth lower than 0.7 Gy/hour were associated with unfavorable LCR. Multivariate analysis revealed that ICRB group C or more at the initial presentation or at the time of RPT, and BED_{10} for the reference depth tumor lower than 40 Gy_{10} were statistically significant predictive factors for unfavorable LCR (Table 3). The tumors were classified into 2 groups according to the ICRB and BED_{10} for reference depth (BED_{10}). Group 1 was defined as ICRB A/B both at initial presentation and at RPT and BED_{10} for the reference depth $\geqq 40$ Gy_{10}. All other tumors were classified into group 2. There were 17 tumors in group 1 and 71 in group 2. Sixteen RPTs and 5 tumors lack the information of reference depth and initial ICRB, respectively. But if the tumor ICRB was not A/B at the time of RPT, it could be classified as group 2 even if neither reference depth nor initial ICRB were unknown. Therefore total number included in this grouping was above 85 but below 101. Two-year LCR were 64.7% and 25.4% in group 1 and group 2, respectively, with a statistical significant difference (Fig. 2). During the follow-up period, 2 patients died of brain metastasis with 3-year OS rate of 97.3% (Fig. 1).

As for morbidities, in 1 case, sclera ruptured during the operation, which required systemic chemotherapy but resulted in chemotherapy-refractory relapse and eventual enucleation. Twelve eyes (13.3%) developed retinal detachment, 6 eyes (6.7%) proliferative retinopathy, and 2 eyes (2.2%) rubeosis with abnormal neovascularization of iris. Both eyes with rubeosis eventually were enucleated because of glaucoma or disease progression. Twenty-three (25.6%) of 90 eyes developed posterior subcapsular cataract and 6 eyes required surgery for cataract. Median interval to cataract development after RPT was 35.0 months (range 0-87.33). Posterior subcapsular cataract development related only with whether or not EBRT was performed during the entire clinical course with cataract occurring in 28.1% of the patients undergoing EBRT at 3 years and 2.9% of those without EBRT ($P=.033$) (Fig. 3a). Thirty-four eyes (37.8%) had a retinal and vitreous hemorrhage after RPT. The incidence of retinal detachment, proliferative retinopathy, and rubeosis showed a correlation with radiation dose of the outer surface of sclera. $BED_3 \geqq 1200$ Gy_3 of the outer surface of sclera was significantly associated with a higher incidence either of retinal detachment, proliferative retinopathy or rubeosis ($P=.017$) (Fig. 3b).

There were 2 enucleations without tumor progression—1 of which developed after circulatory collapse of the retina after repeated selective ophthalmic arterial infusions (17) and transpupillary thermotherapy (18) for posterior pole of the retina. The other developed rubeosis iris caused by RPT as mentioned previously.

Two patients had a second malignancy after RPT. Both patients had hereditary retinoblastoma and 1 had family history of retinoblastoma. Both patients received EBRT and 1 had also received chemotherapy. One patient developed rhabdomyosarcoma in the nasal cavity within EBRT radiation field 27 months after the EBRT and 6 months after the RPT. The other had Ewing sarcoma in right mandible outside of EBRT fields 89 months after the EBRT and 76 months after RPT.

Discussion

In this study, we reported treatment results for RPTs for 101 retinoblastomas in 90 eyes of 85 patients in 10 years.

LCR of EBRT was reported to be 31%-64% (19, 20). Although small tumors could be controlled by 40-46 Gy of conventional fractionated EBRT, the control rate of greater tumors was unsatisfactory. Recently, 2 retrospective studies of RPT for retinoblastoma have been published (8, 9). Schueler et al (8) achieved excellent results of 92.9% LCR and eyes could be preserved in 88.6%. Abouzeid et al (9) also showed good results of 59%-73% eye preservation rate. Another radionuclide of ^{125}I also attained an excellent LCR ranging between 83% and 95% (6, 7). The prescribed dose of ^{125}I plaque brachytherapy was 40 Gy (6, 7) but those of RPT has not yet been standardized. In the study of Schueler et al (8) using the National Institute of Standards and Technology dosimetry standard, the dose at the apex ranged from 53-233 Gy and a mean dose extended up to 138 Gy with an estimated accuracy of no better than $\pm 35\%$. They concluded that the recommended dose should be 88 Gy at the tumor apex, although they mentioned the possibility of dose de-escalation (8). On the other hand, Abouzeid et al (9) prescribed 50 Gy at the tumor apex and found that the apical dose was not a predictive factor of local failure. They concluded that favorable tumor control could be achieved with a median dose at the tumor apex of 51.7 Gy. In this study, recalculated median dose at the tumor apex was 47.4 Gy (range 24.3-86.1 Gy) and comparable to that of Abouzeid et al (9). However, 2-year LCR of the current study was 33.7% and inferior to the other studies of RPT. The unfavorable LCR can be explained by the facts that 62.3% of the patients belonged to ICRB group C or more with unfavorable factors of vitreous seeding or subretinal seedings in the current study. In contrast, other studies included only the patients with tumors up to ICRB group C with a limited vitreous seedings. However, it has to be emphasized that as shown in Table 3, even with the presence of vitreous seedings about 20% of tumors could be controlled by RPT. Although tumor control rate of RPT with unfavorable factors were dismal, progressed tumors could be ultimately salvaged by enucleation without risking survival; therefore, it is meaningful to try to treat advanced tumors with a conservative approach including RPT especially for the patients whose contralateral eye had already been enucleated. As shown in Fig. 2, LCR for tumors without unfavorable factors were comparable to the other series (8, 9).

Factors that influenced LCR were disease laterality, ICRB, vitreous/subretinal seeding, tumor size, reference depth, dose, and dose rate at reference depth. It was in accordance with other reports that pointed out that vitreous seeding, subretinal seeding, and dose at the tumor apex were prognostic factors of local