preparation of the determined radiation fields. A circular cone with a diameter of 5–6 cm and a bevel angle of 0 or 15 degrees was usually used. IORT encompassed the upper abdominal lymph node area, including the right and left cardia, left gastric artery, celiac artery, and upper para-aortic area. Maximum efforts were made to spare as much normal tissue as possible. The liver was mobilized superiorly and the stomach and the small intestine were mobilized inferiorly with the cone. However, the upper one-third of the head and body of the pancreas were exposed to radiation to the celiac axis. The absorbed doses in several planes or along various directions were measured and the beam profiles were plotted using a water phantom. A single-fraction dose of 20–30 (median 23 Gy) using high-energy electrons (9–12 MeV) was delivered. The energy of therapeutic electrons (9–12 MeV) was determined, depending on the depth, so as to cover the microscopic residual tumors.

Statistical analysis

The Mann-Whitney U-test and the chi-squared test were used to determine differences between the patient groups in the continuous numeric and nominal variables, respectively. Overall survival rates and regional control rates of the abdominal lymphatic system were estimated by the Kaplan-Meier method and compared using the log-rank test, with a *P*-value of less than 0.05 considered statistically significant. Multivariate analysis was performed using a proportional hazard model.

RESULTS

Median follow-up duration was 7.4 years (range, 0.1–16.6 years). Seven patients (6.0% of the total: 6 in the IORT group, 1 in the non-IORT group) were lost to follow-up before the end of the third year after treatment, which was determined as the final follow-up point. In addition, 9 patients (7.7% of the total: 7 in the IORT group, 2 in the non-IORT group) were lost to follow-up before the end of the fifth year after treatment.

The recurrence rate was 41.7% (30 patients) in the IORT group and 57.8% (26 patients) in the non-IORT group. The incidence of first recurrence in the upper abdominal lymph node area was lower in the IORT group (2.8%, 2 patients) than in the non-IORT group (13.3%, 6 patients). The incidence rates of other locoregional recurrence (including local recurrence and recurrence in the cervical lymph node area or mediastinal lymph node area) were not much different between the IORT group (18.1%, 13 patients) and the non-IORT group (20.0%, 9 patients). In the same way, the incidence rate of recurrence in the distant area was not much different between the IORT group (20.8%, 15 patients) and the non-IORT group (24.4%, 11 patients). When the control rates in the abdominal lymph node area (combining the upper abdominal lymph node area and the para-aortic lymph node area) were compared, both 3- and 5-year abdominal control rates were significantly higher in the IORT group than in the non-IORT group: 3- and 5-year control rates were 92.3% and 89.2%, respectively, in the IORT group, compared to 76.6% and 72.9%, respectively, in the non-IORT group ($P = 0.022$; Fig. 1).

Next, we compared the survival rates between the two patient groups. As shown in Figure 2, the respective 3- and 5-year overall survival rates were 57.6% and 52.8% in the IORT group and 48.8% and 34.7% in the non-IORT group, a non-significant difference ($P = 0.17$). We then grouped patients by pathological stage to perform subgroup analysis. Among patients with a pathological complete response (pCR) and pathological Stage 0–I tumor, the 5-year survival rate did not significantly differ between the IORT group (74.1%) and the non-IORT group (37.5%; $P = 0.18$). Similarly, there were no statistically significant differences

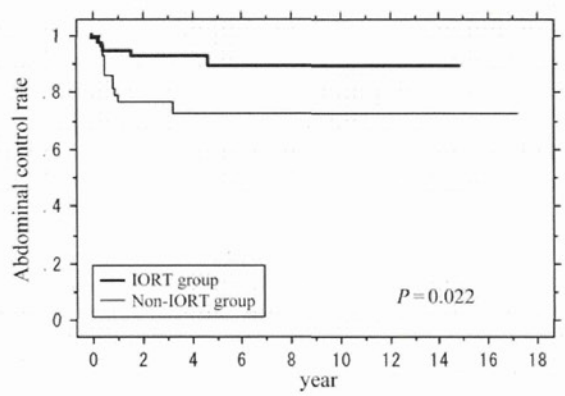

Fig. 1. Kaplan-Meier analysis of abdominal lymph node control rate in the intraoperative radiotherapy (IORT) group ($n = 72$) and non-IORT group ($n = 45$).

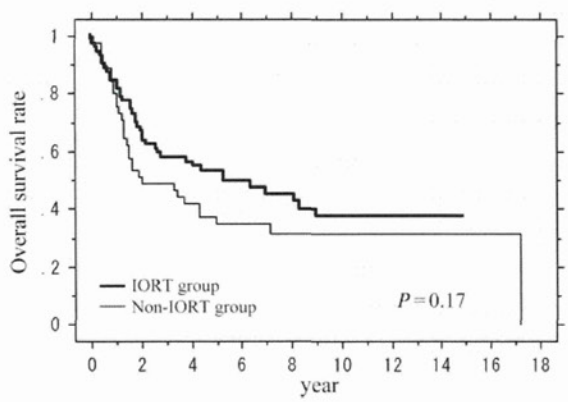

Fig. 2. Kaplan-Meier analysis of overall survival rate in IORT group ($n = 72$) and non-IORT group ($n = 45$).

in the survival rates between the two groups among pathological Stage II patients (56.1% in the IORT group, 54.2% in the non-IORT group; $P = 0.79$) or among pathological Stage III–IVA patients (33.3% in the IORT group, 11.8% in the non-IORT group; $P = 0.19$). We examined treatment outcomes in both groups among patients with and without lymph node metastases. The 5-year survival rate was 34.1% in the IORT group and 26.9% in the non-IORT group ($P = 0.65$) in patients with lymph node metastases, and it was 77.2% in the IORT group and 46.3% in the non-IORT group ($P = 0.088$) in patients without lymph node metastases. The differences in 5-year survival rates between the IORT and non-IORT groups were not significant, regardless of lymph node metastases. We also examined the contribution to the survival rate of using external radiotherapy to the upper abdominal area, as well as IORT. The 5-year survival rate was 0% in the group of 5 patients who received external radiotherapy to the upper abdominal area and 47.6% in the group of 112 patients who did not ($P = 0.0019$).

Furthermore, we assessed a subgroup of patients with the primary lesion located in the lower thoracic or abdominal part of the esophagus or measuring >6 cm in length, as patients in this subgroup tend to have lymph node metastasis in the upper abdominal area and thus are more likely to benefit from IORT targeted to this area. Among the 117 patients examined in this study, 75 patients belonged to this subgroup. Table 3 shows the number of patients in this subgroup who did or did not receive IORT, chemotherapy, and external radiotherapy. Univariate analysis examining the factors potentially associated with the overall survival rate (i.e. IORT, chemotherapy, external radiotherapy, age, and performance status (PS)) revealed that the 5-year overall survival rate was significantly higher in patients with PS 0 than in those with PS 1–4 ($P = 0.0029$). The 5-year overall survival rate was significantly different between patients who received IORT and those who did not receive it in this subgroup (61.7% versus 32.1%, respectively; $P = 0.033$; Fig. 3, Table 3). On the other hand, chemotherapy, external radiotherapy and patient age did not appear to influence the overall survival rate. We performed multivariate analysis using a proportional hazards model. The factors used were IORT, chemotherapy, external irradiation, age and performance status. In multivariate analysis, the 5-year overall survival rate was also significantly higher in those with PS 0 than in those with PS 1–4 ($P = 0.0019$) and in those who received IORT than in those who did not ($P = 0.026$; Table 3). In addition, in this subgroup of patients, the 5-year abdominal control rate was 88.8% in the IORT group and 62.7% in the non-IORT group. The difference between the two groups was significant ($P = 0.011$).

Table 3. Results of univariate analysis and multivariate analysis of factors influencing survival rate in 75 patients with a primary lesion in the lower thoracic or abdominal part of the esophagus or with a primary lesion >6 cm in length

Characteristics	Univariate analysis		Cox's multivariate regression analysis	
	5-year survival rate	*P*-value	HR (95% CI)	*P*-value
IORT		0.033	0.47 (0.25–0.92)	0.026
with IORT ($n = 45$)	61.7%			
without IORT ($n = 30$)	32.1%			
Chemotherapy		0.73	0.54 (0.27–1.01)	0.085
with chemotherapy ($n = 40$)	51.7%			
without chemotherapy ($n = 35$)	46.7%			
External radiotherapy		0.82	1.29 (0.65–2.58)	0.47
with external radiotherapy ($n = 43$)	52.8%			
without external radiotherapy ($n = 32$)	44.5%			
Age		0.26	1.58 (0.84–2.99)	0.16
≤60 years old ($n = 30$)	55.0%			
60 years old ($n = 45$)	45.4%			
Performance status		0.0029	0.36 (0.19–0.69)	0.0019
0 ($n = 44$)	63.3%			
1–4 ($n = 31$)	1.7%			

HR = hazard ratio, CI = 95% confidence interval.

In regard to perioperative complications (Table 4), the number of cases was higher, although not significantly so, in the IORT group compared to the non-IORT group (35 (48.6%) versus 22 (48.9%) of cases, respectively; $P = 0.98$). Typical complications included wound infection, pneumonia, anastomotic leaks and anastomotic stenosis, but incidence rates of these complications were not significantly different between the IORT and non-IORT groups. None of the 117 patients showed adverse events directly attributable to IORT, such as liver function disorder, pancreas function disorder or pancreatic necrosis.

DISCUSSION

Our approach to treating esophageal carcinoma, incorporating IORT immediately postoperatively, is thought to be a unique one. Although the efficacy of IORT, which delivers high-dose radiation directly to sites at a high risk of recurrence during curative surgery, has been theoretically accepted, detailed treatment outcomes have rarely been reported. We first examined the validity of IORT in the control of the abdominal lymphatic system, finding that the rate of first recurrence in the upper abdominal lymph node area was lower in the IORT group (2.8%) than in the non-IORT group (13.3%), suggesting better control in the former group. The control rate in the abdominal area was significantly higher in the IORT group than in the non-IORT group. On the other hand, the recurrence rates in the cervical area and mediastinal area were comparable between the two groups. Thus, it appears that performing IORT contributes to local control in the upper abdominal area. The overall survival rates, on the other hand, were not significantly different between the IORT and non-IORT groups, suggesting that the overall survival rate in patients with esophageal carcinoma cannot be effectively improved solely by adding IORT targeted to the upper abdominal area. This may be explained by the fact that the outcome of esophageal carcinoma treatment is influenced by complex factors involving surgical protocol, curability of surgical treatment, chemotherapy regimen and schedule, radiation field and dose used in external radiotherapy, and accuracy of preoperative diagnostic imaging.

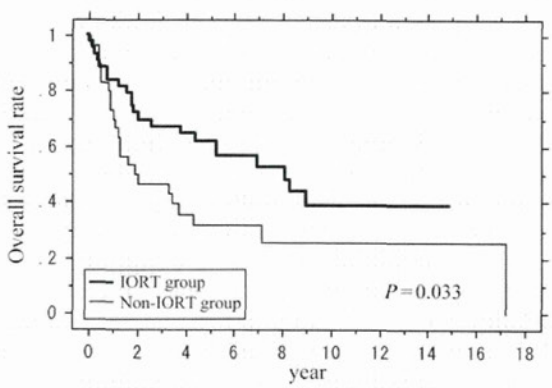

Fig. 3. Kaplan-Meier analysis of overall survival rate in the IORT group ($n = 45$) and non-IORT group ($n = 30$) within the subgroup of patients whose primary lesion was located in the lower thoracic or abdominal part of the esophagus or measured >6 cm in length.

We considered that external radiotherapy, as well as IORT, could influence disease control in the upper abdominal area. However, comparative analysis showed poorer treatment outcome in patients who received external radiotherapy than in those who did not. This poor outcome can be explained by the fact that external radiotherapy was performed mostly in patients at high risk of disease recurrence after surgery. The outcome may also have been affected by the small sample size used. Thus, our results remain inconclusive with regard to the effect of external radiotherapy targeting the upper abdominal area on treatment outcome.

Akiyama *et al.* demonstrated that the 5-year overall survival rate in patients with lower thoracic esophageal

Table 4. Complications

	IORT group $n = 72$	Non-IORT group $n = 45$	Total $n = 117$	*P*-value
No. of cases	35 (48.6%)	22 (48.9%)	57	0.13
Wound infection and abscess formation	5 (6.9%)	3 (6.7%)	8	0.75
Pneumonia, pleural effusion, and chylothorax	10 (13.8%)	7 (15.6%)	17	0.98
Anastomotic leak in the neck	9 (12.5%)	3 (6.7%)	12	0.48
Anastomotic leak in the upper abdomen	1 (1.4%)	0 (0%)	1	0.81
Anastomotic stenosis	6 (8.3%)	6 (13.3%)	12	0.58
Gastric necrosis	0 (0%)	1 (2.2%)	1	0.81
Recurrent nerve paralysis	6 (8.3%)	5 (11.1%)	11	0.86

carcinoma was 48.5% without abdominal lymph node metastasis and only 17.9% with it, and suggested that control in the abdominal area was an important factor for good outcome [16]. Also, interestingly, Eloubeidi *et al.* found that the survival rate was higher when tumor length was shorter, suggesting that tumor length is a prognostic factor and thus should be included in the factors determining TNM classification [27]. On the basis of these previous findings, we further examined a subgroup of patients whose primary lesions were located in the lower thoracic or abdominal part of the esophagus, or measured >6 cm in length (6cm is equivalent to about 1/3 or 1/4 of the full length of the thoracoabdominal esophagus): patients in this subgroup are considered to be at high risk for abdominal lymph node metastasis. Of our 117 patients with esophageal carcinoma, 75 (64.1%) belonged to this subgroup. Both univariate and multivariate analysis revealed that, within this subgroup, the survival rates were high in patients with PS 0 and in those who received IORT. This suggests that the control of the abdominal lymphatic system brought by performing IORT contributes to improving survival rate in this subgroup. In other words, lymphadenectomy alone is not sufficient in this subgroup, and this suggests that microscopic residual tumors are present in the treated area in these patients. We were concerned about recurrence in the abdominal area, and administered IORT to the upper abdominal area in addition to lymphadenectomy in high-risk patients for control of microscopic residual tumors. It is possible that this led to the improvement of therapeutic outcomes.

Our results agree with the study by Matsubara *et al.* in which they reported high incidence rates of metastasis in the para-aortic lymph node area, liver, and peritoneum in the patients with recurrence in the upper abdominal lymph node area [28]. Wu *et al.* also reported that, among patients with thoracic esophageal carcinoma, the survival rate was significantly poorer in those with perigastric lymph node metastasis than in those without it, suggesting the importance of control in this area [29]. Thus, it would seem worthwhile to offer IORT to patients with the above recurrence and metastasis profiles.

Lymph node recurrence is the key determinant of disease prognosis. Lanschot *et al.* studied the recurrence pattern after esophageal surgery in detail, finding that local control was crucial in improving the survival rate in one third of patients [30]. Further, Morita *et al.* assessed lymph node metastasis and hematogenous metastasis, and concluded that focus should be put on local control, as well as systemic management, in the treatment for esophageal carcinoma [31]. Taken together, both systemic management and the control of local lesions are essential in the treatment of esophageal carcinoma, and IORT can play an important role in local control. Esophageal carcinoma spreads via multiple routes, and local control and preventing tumor spread via lymphogenous and hematogenous routes should be carefully considered.

We did not find complications directly attributable to IORT. Also, despite our initial concerns, IORT itself and the accompanying longer operation times and longer duration of anesthesia did not increase the incidence of perioperative complications. A temporary elevation in the serum amylase level was reported in patients who underwent IORT targeted to the stomach, probably due to radiation to the pancreas [32], but this was absent in all patients in the present study. Thus, we believe that IORT is safe to perform during surgery for esophageal carcinoma.

In our study, external radiotherapy was adjunct to curative surgery, and postoperative radiotherapy was selectively performed according to surgical and histopathological findings. The significance of postoperative radiotherapy has been investigated in previous studies, wherein no improvement in survival outcome was observed [33, 34]. On the contrary, preoperative radiotherapy appears important to increase complete resectability. In Western countries, it has been reported that chemoradiotherapy, before curative surgery, significantly improved treatment outcomes compared with curative surgery alone in patients with resectable cancer [35]. However, in a separate report, preoperative chemoradiotherapy was shown to improve treatment outcome, but it also significantly increased postoperative deaths due to perioperative complications [36]. In our study, the incidence of perioperative complications in the IORT group was not significantly different to that in the non-IORT group, indicating the therapeutic benefit of IORT, a possible improvement in treatment outcome without compromising the safety of the treatment.

This study has certain limitations. First, the effects of selection bias are not negligible as this is a retrospective study. Second, the treatment protocol was not uniform among patients; for example, the use of chemotherapy and/or external radiotherapy was not in common, and thus the outcome of treatment in individual patients was influenced by the modalities used. The standardization of regimens for chemotherapy and external radiotherapy (field and dose of radiation) is necessary in the future.

CONCLUSION

In conclusion, albeit with some limitations, IORT targeted to the upper abdominal lymph node area is effective in obtaining good local control. It is also beneficial for treating patients with lower thoracic or abdominal esophageal carcinoma and those with a long primary lesion, as it can improve survival rates in these patients. IORT shows promise as an effective treatment option for such patients.

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RADIATION ONCOLOGY

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Patterns of failure after multimodal treatments for high-grade glioma: effectiveness of MIB-1 labeling index

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Abstract

Background: The purpose of the present study was to analyze the recurrence pattern of high-grade glioma treated with a multimodal treatment approach and to evaluate whether the MIB-1 labeling index (LI) could be a useful marker for predicting the pattern of failure in glioblastoma (GB).

Methods and materials: We evaluated histologically confirmed 131 patients with either anaplastic astrocytoma (AA) or GB. A median dose was 60 Gy. Concomitant and adjuvant chemotherapy were administered to 111 patients. MIB-1 LI was assessed by immunohistochemistry. Recurrence patterns were categorized according to the areas of recurrence as follows: central failure (recurrence in the 95% of 60 Gy); in-field (recurrence in the high-dose volume of 50 Gy; marginal (recurrence outside the high-dose volume) and distant (recurrence outside the RT field).

Results: The median follow-up durations were 13 months for all patients and 19 months for those remaining alive. Among AA patients, the 2-year progression-free and overall survival rates were 23.1% and 39.2%, respectively, while in GB patients, the rates were 13.3% and 27.6%, respectively. The median survival time was 20 months for AA patients and 15 months for GB patients. Among AA patients, recurrences were central in 68.7% of patients; in-field, 18.8%; and distant, 12.5%, while among GB patients, 69.0% of recurrences were central, 15.5% were in-field, 12.1% were marginal, and 3.4% were distant. The MIB-1 LI medians were 18.2% in AA and 29.8% in GB. Interestingly, in patients with GB, the MIB-1 LI had a strong effect on the pattern of failure ($P = 0.014$), while the extent of surgical removal ($P = 0.47$) and regimens of chemotherapy ($P = 0.57$) did not.

Conclusions: MIB-1 LI predominantly affected the pattern of failure in GB patients treated with a multimodal approach, and it might be a useful tool for the management of the disease.

Keywords: Pattern of failure, Glioblastoma, Radiotherapy, MIB-1 labeling index

Background

Glioblastoma (GB) is one of the most aggressive primary brain tumors. The standard treatment includes a multimodal approach with surgery, radiotherapy, and chemotherapy. Although concurrent chemoradiotherapy with temozolomide has been shown to have a survival benefit for GB [1], the overall outcome for GB has not improved significantly, and intracranial tumor recurrence or progression develops in most patients in less than 1 year.

"The nature of GB, with widespread tumor infiltration and lower radiosensitivity, has frustrated efforts to provide durable tumor control with radiotherapy." Although several investigators demonstrated the presence of widespread microscopic infiltration within the brain [2], local recurrence predominates with this disease, most often within 2 cm of the original tumor. Conformal radiotherapy that includes postoperative peritumoral edema in the target volume is currently being used in the Radiation

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Therapy Oncology Group (RTOG) trials (e.g., the RTOG 0525 and RTOG 8525 trials). Alternatively, treatment strategies based on contrast-enhancement of the tumor (using CT/MRI) plus 2-cm margins is currently employed in many European institutions, according to the European Organization for Research and Treatment of Cancer (EORTC) recommendations [3-6]. Therefore, consensus guidelines for clinical target volume (CTV) delineation remain controversial. On the contrary, with respect to radiation doses, although prescribed doses of 60 Gy are generally employed, attempts at dose escalation or altered fractionation of radiotherapy have been widely challenged [7,8]. Dose escalation to 90 Gy [7] or the addition of a stereotactic radiosurgery (SRS) boost [9] may reduce the failure rates in the high-dose region, although recurrence then tends to occur just outside the high-dose region.

Determination of proliferative activity using the monoclonal antibody MIB-1 labeling index (LI) has been investigated in malignant gliomas [10,11]. Although some studies indicated that MIB-1 LI was correlated with an increasing grade of malignancy, no study has evaluated whether MIB-1 LI might predict a pattern of failure in GB patients.

We evaluated the outcome of high-grade glioma treated with a multimodal approach, and investigated patterns of failure with regard to MIB-1 LI. The purpose of this study was to analyze the recurrence patterns and to evaluate whether MIB-1 LI could be useful as a marker to predict the pattern of failure in high-grade glioma.

Materials and methods

Patients

In this study, 142 consecutive patients with histologically proven high-grade glioma, i.e., anaplastic astrocytoma (AA) or GB, were retrospectively reviewed. These patients were treated with radiotherapy at Kobe University Hospital or Hyogo Cancer Center between 2000 and 2010. The retrospective review and the use of clinical data were approved by the institutional ethics board. Eleven patients were not included in the analysis: 5 patients who were treated with palliative therapy, 5 patients who discontinued the course of radiotherapy, and 1 patient who refused to enroll in the study. Therefore, a total of 131 patients were included in the analysis. Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Extent of surgical removal

The extent of surgical removal was assessed using surgical information and/or the comparison of preoperative and postoperative magnetic resonance imaging (MRI). The extent of surgical removal was categorized into 4 subgroups: gross total removal (GTR), subtotal removal (STR), partial removal (PR), and biopsy. GTR was defined as a resection of more than 99% of tumor volume; STR, a resection of 80–99% of tumor volume; PR, as a resection of 20–80% of tumor volume; and biopsy, a resection of less than 20% of tumor volume.

Methods of radiation therapy

Radiotherapy was started 2–4 weeks after surgery. Treatment planning computerized tomography (CT) scans obtained images at 2.5–5.0-mm slice intervals. Information obtained in the simulation CT scan was combined with any available MRI data, including post-contrast T1- and T2-weighted images or fluid-attenuated inversion recovery (FLAIR) images as fusion images. For the planning of radiotherapy, FOCUS (2000–2004) or Xio software (2005–2010) (CMS Co. Ltd., Japan) was used. The gross tumor volume (GTV) was carefully defined considering gadolinium-enhanced lesions in presurgical MRI and/or residual tumor lesions in post-surgical MRI. In our methods, the GTV was basically defined based on pre-surgical tumor extents. However, in case that range and portion of surgical cavities was beyond the pre-surgical tumor extents. The GTV were reconstructed by pre-surgical tumor extents combined with post-surgical imaging, because to set GTV from only presurgical images was considered to be insufficient. The CTV for the initial plan was delineated, including the area of peritumoral edema (high-intensity lesion on FLAIR images) plus the 1.5–2.0-cm margin, and the CTV-boost was defined based on the GTV with a 1.5–2.0-cm margin. For the setup margin, a 5-mm margin was applied to each CTV (CTV-initial, CTV-boost) to create PTVs (PTV-initial, PTV-boost). All patients were treated with conventional fractions of 1.8–2.0 Gy 5 times a week. A median total dose of 60 Gy (range: 54–71.2 Gy) was delivered in 27–39 fractions with concomitant and adjuvant chemotherapy. The prescribed dose was 40–50.4 Gy to the PTV-initial for both AA and GB, followed by 10–20.8 Gy to the PTV-boost. The 100% isodose line was defined at the isocenter, and the dose was prescribed to this point. All patients were treated with three-dimensional conformal radiation therapy consisting of 3–5 non-coplanar fields. The normal tissues delineated included the optic nerves, optic chiasm, brainstem, eye, and optic lens. The optic nerve and optic chiasm maximum doses were restricted to ≤50 Gy. The maximum dose to the brainstem was restricted to ≤54 Gy.

Chemotherapy

Regimens of chemotherapy shifted according to the study period. Between September 2000 and September 2006, 55 patients received a combination regimen that consisted of ACNU (nimustine), vincristine, and interferon with radiotherapy. After October 2006, 56 patients were scheduled

to receive temozolomide concurrent with radiotherapy at a dosage of 75 mg/m^2/day followed by adjuvant therapy at a dosage of 150–200 mg/m^2/day for 5 days every 28 days, unless the disease progressed or the patient experienced treatment-related toxicity or declining performance status necessitating the discontinuation of chemotherapy. In our policy, concomitant chemotherapy was considered to all patients with AA and with GB. However, 4 (15%) patients with AA could not receive chemotherapy because of their poor general conditions. Thus, 111 (84.7%) patients received chemotherapy.

Immunohistochemical analysis

Paraffin-embedded tissue blocks were sectioned (4-μm thick) onto slides and then deparaffinized. Sections were immunostained using the Vectastain ABC Elite Kit (Vector Laboratories, Burlingame, CA, USA) according to the manufacturer's instructions with anti-MIB-1 monoclonal antibody. The MIB-1 LI was calculated as the percentage of positively stained tumor cell nuclei among 1000 nuclei detected in areas with the greatest degree of immunostaining [11]. A median of the MIB-1 LI was used as reference to establish a cut off point [12].

Recurrence diagnosis methods

The diagnosis of tumor recurrence and disease progression was made using MRI. Positron emission tomography (PET) imaging was not routinely used. Recurrence was defined as follows: central failure if more than 95% of the recurrence volume was in the 95% isodose line of 60 Gy (boost volume); in-field if more than 95% of the recurrence volume was in the high-dose volume (50 Gy); marginal when less than 95% of recurrence volume was outside the high-dose volume; distant when recurrence was outside the RT field (<20% isodose line).

Assessment of the pattern of failure

There were 43 patients (8 AA patients and 35 GB patients) who showed no sign of recurrence. Among 88 patients who experienced recurrence, 14 patients' samples were not submitted for immunohistochemical evaluation because of insufficient samples. In total, data from 74 patients (16 AA patients and 58 GB patients) were evaluated for the pattern of failure in this study.

Follow-up evaluation and statistical analyses

In the follow-up evaluations, local and systemic tumor control was assessed at 1–3-month intervals using MRI. Progression-free survival (PFS) and overall survival (OS) rates were analyzed statistically in all patients. PFS was calculated from the first day of radiotherapy to the date of any recurrence or death, or censored the date of the last follow-up. OS was calculated from the first day of radiotherapy to the date of death, or censored the date of the last follow-up, and calculations were made using Kaplan–Meier estimates. Statistical significance was determined using the log-rank test. The chi-square test was used for the comparisons among cohorts in this analysis. Variables influencing OS and PFS were evaluated with multivariate Cox proportional hazards model with a 95% confidence interval. Differences were considered statistically significant at P values < 0.05. All statistical analyses were performed using StateView (version 5.0).

Results

Patients and treatments

Patient and treatment details are shown in Table 1. Twenty-six (20%) patients had AA, and 105 (80%)

Table 1 Patient characteristics and treatments (n = 131)

Characteristics	Anaplastic astrocytoma n = 26 (%)	Glioblastoma n = 105 (%)
Age		
Median	52.5 years	59 years
(range)	(18–75 years)	(16–77 years)
Sex		
Male	14 (54)	59 (56)
Female	12 (46)	46 (44)
Original tumor location		
Frontal lobe	11 (42)	36 (34)
Temporal lobe	5 (19)	35 (33)
Parietal lobe	3 (12)	20 (19)
Thalamus	4 (15)	3 (3)
Occipital	1 (4)	6 (6)
Cerebellum	2 (8)	2 (2)
Basal ganglia	0 (0)	2 (2)
Brainstem	0 (0)	1 (1)
MIB-1 labeling index		
Median (range)	18.2% (2–35)	29.8% (2–80)
Radiotherapy		
Median dose	60 Gy	60 Gy
(range)	(54–68.4 Gy)	(54–71.2 Gy)
Extent of surgical removal		
Gross total removal	3 (12)	18 (17)
Subtotal removal	5 (19)	15 (14)
Partial removal	11 (42)	59 (56)
Biopsy only	7 (27)	13 (12)
Chemotherapy		
ACNU (Nimustine)	11 (42)	44 (42)
Temozolomide	11 (42)	45 (43)
None	4 (15)	14 (13)
Unknown	0 (0)	2 (2)

Table 2 Patterns of recurrence after multimodal treatments (n = 74)

Recurrent sites	Anaplastic Astrocytoma	Glioblastoma
	n = 16 (%)	n = 58 (%)
Central	11 (68.7)	40 (69.0)
In-field	3 (18.8)	9 (15.5)
Marginal	0 (0)	7 (12.1)
Distant	2 (12.5)	2 (3.4)

patients had GB. There were no statistically significant differences between the 2 cohorts with regard to radiotherapy methods, the extent of surgical removal, or chemotherapy regimens.

Progression-free and overall survival

The median follow-up periods were 13 months for all patients and 19 months for those remaining alive. Among AA patients, the 2-year PFS and OS rates were 23.1% and 39.2%, respectively, while in GB patients, the rates were 13.3% and 27.6%, respectively (Figure 1). The median survival times (MSTs) were 20 months in patients with AA and 15 months in patients with GB. The cause of death among AA patients was identified as primary tumor deterioration in 17 patients (100%), while causes of death in GB cases were identified as primary tumor deterioration in 75 patients (93.8%), other diseases (pneumonia) in 4 patients (5%), and unknown in one patient (1.2%). Among 105 patients with GB, except for 16 patients who did not receive chemotherapy or unknown, 44 (42%) patients were treated with ACNU-based regimens during 2000 to September 2006, and 45 (43%) patients were treated with TMZ-based regimens in between October 2007 to thereafter (Table 1). Between ACNU-based and TMZ-based groups with GB, there were no significant difference in OS (P =0.86) and PFS (P =0.42), and in. In patients with AA, the tendency was similar, resulting in OS (P =0.93) and PFS (P =0.72).

Patterns of failure for AA or GB

Among the AA patients, 11 (68.7%) displayed recurrence in the central area, while 3 (18.8%) displayed recurrence in the in-field area and 2 (12.5%), in the distant area (Table 2). Among 5 patients who displayed recurrence outside of the central area, 1 (20%) patient also had a recurrent tumor in the central area. In contrast, among patients with GB, 40 (69.0%) displayed recurrence in the central area, while 9 (15.5%) displayed recurrence in the in-field area; 7 (12.1%), in the marginal area; and 2 (3.4%), in the distant area (Table 2). Among those 18 patients whose recurrence was outside of the central area, 6 (33%) patients also had a recurrent tumor in the central area. Representative cases of recurrent tumors observed in the central and distant areas are shown in Figure 2 and Figure 3, respectively.

Distribution of MIB-1 LI

MIB-1 LI scores were available in 101 patients (22 AA patients and 79 GB patients). The MIB-1 LI of AA was comparatively low, and the median MIB-1 LI was 18.2% (range: 2–35%). In contrast, the MIB-1 LI of GB was higher and more widely distributed. The median MIB-1 LI was 29.8% in GB (range: 2–80%) (Figure 4).

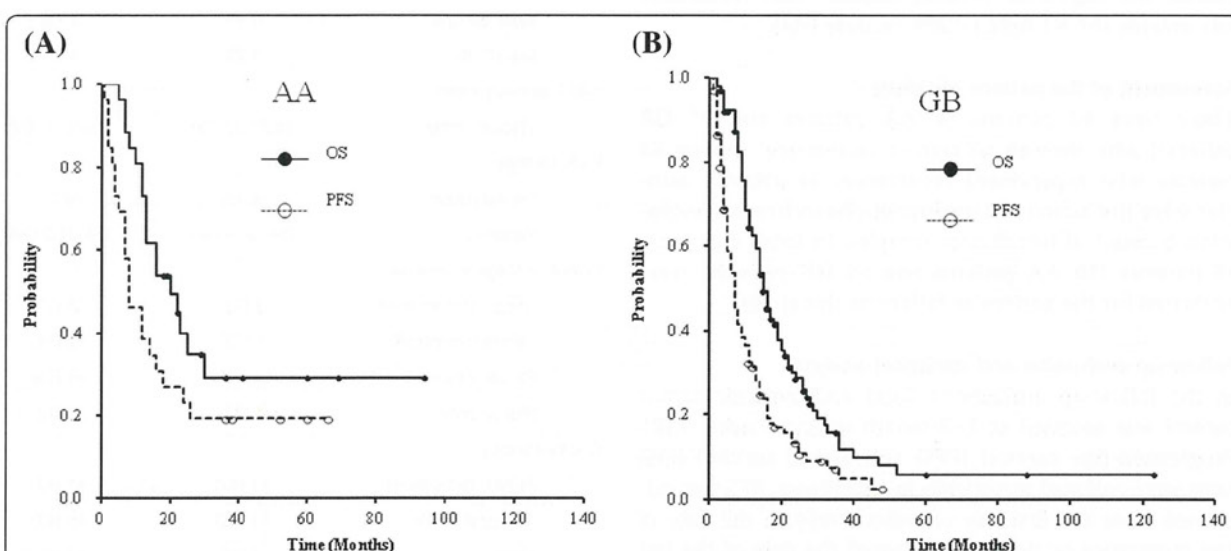

Figure 1 Kaplan-Meier curves of overall survival and progression-free survival for each histological grade. Kaplan-Meier curves of progression-free survival (PFS) and overall survival (OS) according to histology grade: (**A**) anaplastic astrocytoma (n = 26), (**B**) glioblastoma (n = 105).

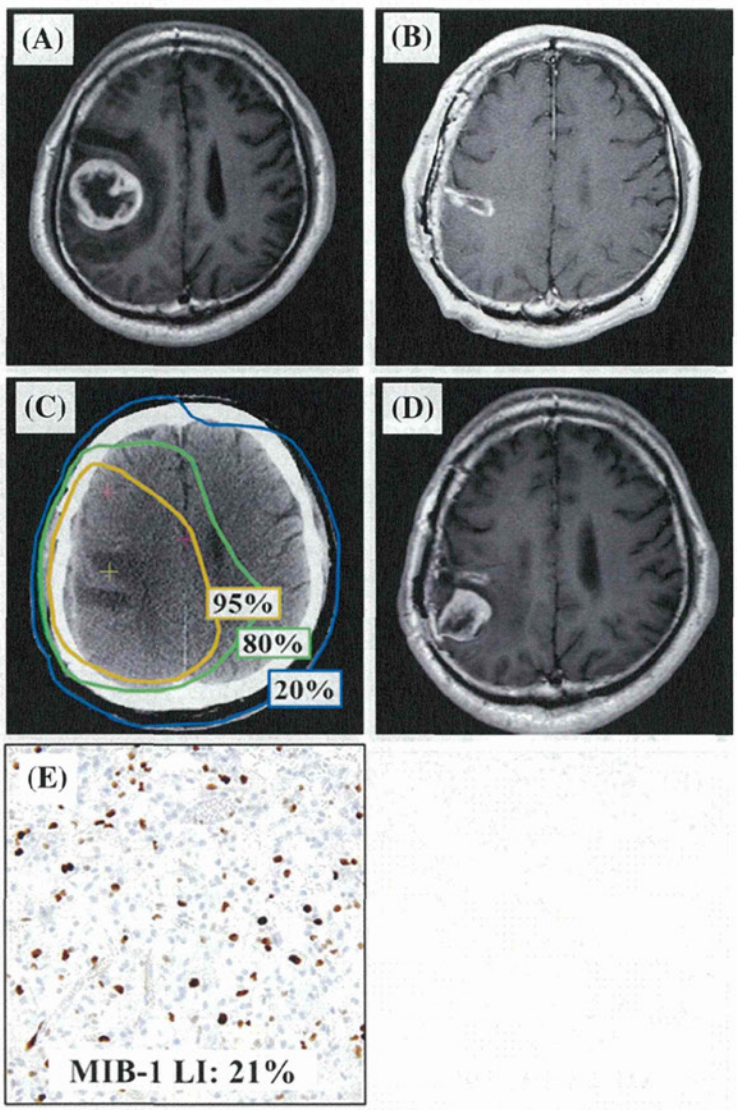

Figure 2 Representative images for the case of GB with lower MIB-1 LI. A 76-years female patient with GB showing a lower MIB-1 LI (21%) that recurred in the central region. (**A**) A postcontrast MR image (T1 weighted) before surgery. (**B**) A postcontrast MR image (T1 weighted) after a gross total removal. (**C**) Treatment planning CT image showing the 95% isodose curve (yellow), the 80% isodose curve (green), and the 20% isodose curve (blue). (**D**) A postcontrast MR image (T1 weighted) at 4 months after completing radiotherapy showing a recurrence tumor that developed in the central. (**E**) Immunohistochemical analyses (×200).

MIB-1 LI correlation with patterns of failure in GB

Of the 131 patients, 101 patients (16 AA patients and 58 GB patients) were available for evaluation of recurrence pattern and MIB-1 LI. Other 27 patients had not yet shown signs of tumor recurrence. Among the GB patients, recurrence occurred central area and others in 25 (83%) and 5 (17%) of patients with the MIB-1 LI <30%, and in 15 (54%) and 13 (46%) of patients with the MIB-1 LI ≥ 30%, respectively (P = 0.014) (Table 3) In contrast, among the AA patients, recurrence occurred central area and others (in-field, marginal, or distant) in 9 (69%) and 4 (31%) of patients with the MIB-1 LI < 30%, and in 2 (67%) and 1 (33%) of patients with the MIB-1 LI ≥ 30%, respectively (P = 0.931).

For patients with GB whose MIB-1 LI was < 30%, 25 (83%) developed a central recurrence, whereas 15 (54%) for those with MIB-1 LI ≥ 30% (P = 0.014). Among patients with the MIB-1 LI < 30% who recurred in the other sites, there were 2 (7%) patients in the in-field, 3 (10%) patients in the marginal, and none in the distant, whereas those with MIB-1 LI ≥ 30%, there were 7 (25%)

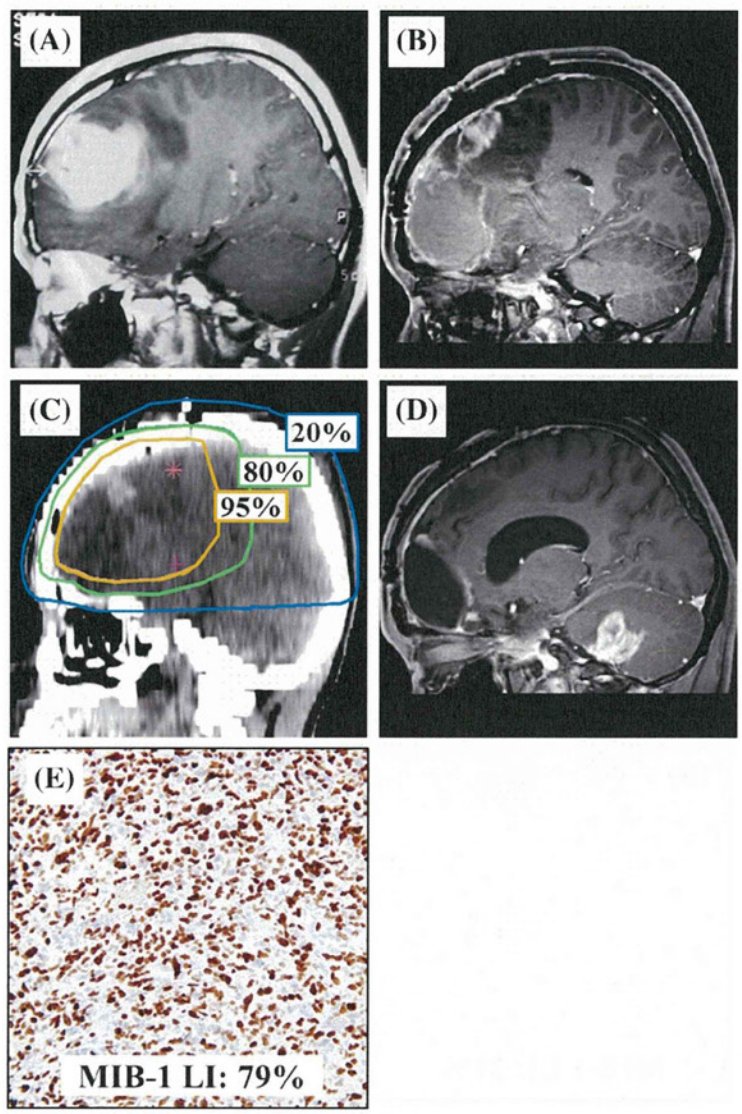

Figure 3 Representative images for the case of GB with higher MIB-1 LI. A 24-years male patient with GB showing a higher MIB-1 LI (79%) that recurred in the distant region. (**A**) A postcontrast MR image (T1 weighted) before surgery (**B**) A postcontrast MR image (T1 weighted) after a gross total removal. (**C**) Treatment planning CT image showing the 95% isodose curve (yellow), 80% isodose curve (green), and the 20% isodose curve (blue). (**D**) A postcontrast MR image (T1 weighted) at 11 months after completion of radiation showing a recurrence tumor that developed in the distant. (**E**) Immunohistochemical analyses (×200).

patients in the in-field, 4 (14%) patients in the marginal, and 2 (7%) patients in the distant, respectively.

In patients with GB, the MIB-1 LI was strongly affected by the pattern of failure ($P = 0.014$), while the extent of surgical removal ($P = 0.47$) or regimens of chemotherapy ($P = 0.57$) were not (Table 3). In contrast, in patients with AA, the MIB-1 ($P = 0.93$), extent of surgical removal ($P = 0.84$), and regimens of chemotherapy ($P = 0.61$) did not influence the pattern of failure.

Evaluation of prognostic factor for OS and PFS

Results of univariate and multivariate analysis of prognostic factors were shown (Table 4). In the multivariate analysis, age ($P = 0.04$), performance status ($P < 0.01$) and extent of resection ($P = 0.04$) were significant prognostic factors for OS. In case of PFS, performance status ($P = 0.01$) was the single significant prognostic factor. Om the contrary, MIB-1 LI was not significant factor for OS or PFS in both univariate and multivariate analyses.

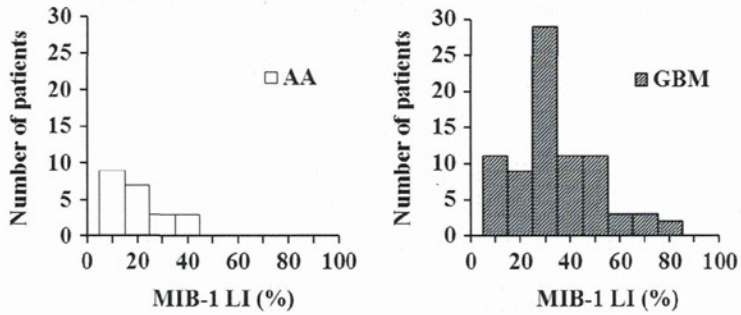

Figure 4 Distributions of MIB-1 LI. *Distributions of MIB-1 LI in patients with AA (n = 22) and in patients with GB (n = 79).*

Discussion

In the management of GB, the pattern of failure is one of the major concerns in relation to the CTV margins, optimal radiation dose, and identification of biomarkers. However, the factors affecting the pattern of failure in GB have not been described in detail. The present study is the first to report an association between the MIB-1 LI and the pattern of failure in patients with GB.

In prior studies, treatment factors, including CTV margins, radiation dose, and chemotherapy, were widely discussed as the most important aspects determining the pattern of failure of GB [3-5,7,13-15]. Chang et al. [3], in a study based on protocols following RTOG guidelines, conducted at the M.D. Anderson Cancer Center, reported similar patterns of failure in a series of 48 GB patients by comparing treatment plans based on residual tumor and resection cavity plus 2-cm margins. Liang et al. [4] studied 42 patients with malignant glioma irradiated with 60 Gy using conformal radiation techniques. All patients developed recurrences within 2 cm of the original tumor. Lee et al. [5] analyzed 36 patients with high-grade astrocytoma treated with 70 Gy and 80 Gy to a CT/MRI-localized PTV (tumor size: <2–3 cm) and found that 89% of the recurrences occurred with a central or in-field recurrence pattern. McDonald et al. [13] demonstrated that through the use of limited margins, 92% of the patients in their series had a PTV boost margin of 1 cm or less, only 5% of patients had a marginal failure, and 2% patients had a distant failure with temozolomide chemoradiation. Minniti et al. [14] demonstrated that the majority of patients treated with RT plus concomitant and adjuvant temozolomide have central recurrences, while distant new lesions may occur in more than 10% of patients. Milano et al. [15] demonstrated that central recurrence of glioblastoma treated with radiation and temozolomide predominates and persists over time, whereas new in-field, marginal, and distant recurrences commonly develop, particularly at later time points in patients with longer survival. Our results regarding the proportions of particular sites of recurrence were consistent with those reports (Table 5). Although the reports cited above included different CTV

Table 3 Factors may or may not affect the patterns of failure in patients with glioblastoma or anaplastic astrocytoma

Patients with glioblastoma									
	MIB-1 (n =58)			**Extent of surgical removal (n =58)**			**Chemotherapy (n =53)**		
Sites of recurrence	< 30	≧ 30	*P* value	> 80%	< 80%	*P* value	ACNU based	TMZ based	*P* value
	n = 30	n = 28		n = 20	n = 38		n = 23	n = 30	
Central	25	15	0.014	15	25	0.47	17	20	0.57
Others *	5	13		5	13		6	10	
Patients with anaplastic astrocytoma									
	MIB-1 (n =16)			**Extent of surgical removal (n =16)**			**Chemotherapy (n =13)**		
Sites of recurrence	< 30	≧ 30	*P* value	> 80%	< 80%	*P* value	ACNU based	TMZ based	*P* value
	n = 13	n = 3		n = 5	n = 11		n = 5	n = 8	
Central	9	2	0.93	3	8	0.61	4	6	0.84
Others *	4	1		2	3		1	2	

* Others = In-field + marginal + distant.
† > 80% = GTR or Sub-total, < 80% = Partial/Biopsy.

Table 4 Results of univariate and multivariate analyses for OS and PFS in patients with glioblastoma or anaplastic astrocytoma

Variable	Test for favorable status	OS	PFS
Univariate analysis		P value	P value
PS	0,1 (n = 60) vs. 2,3, and 4 (n = 60)	<0.01	<0.01
Age	< 50 (n = 37) vs. ≧ 50 (n = 94)	0.04	0.08
Extent of resection	GTR/STR (n = 41) vs. PR/biopsy (n = 90)	0.06	0.37
Histological grade	AA (n = 26) vs. GB (n = 105)	0.08	0.11
MIB-1 LI	< 30 (n = 61) vs. ≧ 30 (n = 43)	0.21	0.59
BED or Radiation dose	≤ 72 GyE (n = 77) vs. > 72 GyE (n = 54)	0.29	0.53
Gender	Male (n = 73) vs. Female (n = 58)	0.73	0.54
Chemotherapy (CT)	ACNU based (n = 55) vs. TMZ based (n = 56)	0.91	0.45
Multivariate analysis			
PS		<0.01	0.01
Age		0.04	0.07
Extent of resection		0.04	0.30
MIB-1 LI		0.07	0.12

margins, radiation doses, and regimens of chemotherapy, the patterns of failure of GB appear to be similar, suggesting that these treatment factors might not strongly affect the pattern of failure in GB.

Several clinical values of MIB-1 LI have been reported in human gliomas [10,11,17,18]. Johnnessen and Torp [10] evaluated the clinical usefulness of MIB-1 LI in grading for glioma, based on a review of 16 studies including a total of 915 patients. In addition, MIB-1 LI can be considered as an important proliferation parameter, and may be associated with clinical growth parameters independent of other prognostic factors in gliomas. Several cut-off studies have observed a significant correlation between MIB-1 LI and postoperative survival in astrocytic gliomas [17]. However, the majority of studies to date have failed to confirm such an association between the MIB-1 LI and survival in patients with GB [18]. In our series, values of the MIB-1 LI were widely distributed in both GB and AA cohorts. Median of the distribution in GB (cut off = 30) were first evaluated for correlation with patterns of failure, and other cut off points (cut off = 10, 20, 35, and 50) were also assessed. Among those cut off points, the cut off = 30 was found to be the only significant value as for the pattern of failure in GB. On the contrary, any cut-off point did not have useful values in AA. The discrepancy between GB and AA might be a matter of debate. It is at least speculated that, in our series, the distribution of the MIB-1 LI

Table 5 Comparison of published data with regard to patterns of failure in patients with glioblastoma

Author	Year	Number	Treatment	Dose (Gy)	Margin (cm)	Recurrence sites			
						Central	In-field	Marginal	Distant
Nakagawa [7]	1998	38	3DCRT + ACNU	60–80	0–2	90%†			5%
				90	0–2	46%††			8%
Lee [5]	1999	36	3DCRT	70–80	1.5	72%	17%	8%	3%
Chan [6]	2002	34	3DCRT	90	0.5	78%	13%	9%	
Chang [3]	2007	48	3DCRT ± chemo*	60	1	83%	6%	6%	4%
Brandes [16]	2009	95	3DCRT + TMZ	60	2–3	72%		6%	22%
Milano [15]	2010	54	3DCRT + TMZ	60	2–2.5	92%§		15%	13%
Minniti [14]	2010	105	3DCRT + TMZ	60	1–2	79%	6%	6%	14%
McDonald [13]	2011	41	(IMRT or 3DCRT) ± TMZ	60	0.8	78%	15%	5%	2%
This study	2012	58	3DCRT ± (ACNU or TMZ)	60	1.5–2	69%	16%	12%	3%

*21/48 patients received adjuvant or concurrent chemotherapy (carmustine, procarbazine, and temozolomide).
†5% were subependymal recurrences (did not apply to our classification method of recurrence sites).
††46% were subependymal recurrences (did not apply to our classification method of recurrence sites).
§Insufficient data to apply to our classification method of recurrence sites.

in AA was much smaller (2 - 35%) than that in GB (2 - 80%). Therefore, to set cut off might be difficult. Of course, other possibilities might be raised. This issue should be discussed more with accumulation of reports.

Notably, our results indicated that the MIB-1 LI affected the pattern of failure in patients with GB, while the extent of surgical removal or chemotherapy regimens did not (Table 3). Previously, Shibamoto et al. [19] demonstrated that extent of surgery was associated with the prognosis of GB in their analysis of 178 cases. However, there are no reports demonstrating that MIB-1 LI can provide clues for the recognition of the pattern of failure in patients with high-grade glioma. Thus, to our knowledge, this report is the first to show the clinical value of the MIB-1 LI in GB. On the contrary, in patients with AA, no association between MIB-1 and the pattern of failure was observed. Several reasons may explain this result. Because AA is less aggressive than GB, MIB-1 LI did not reflect disease behavior in AA. In addition, the number of patients with AA was quite small. Therefore, although our findings might be restricted to GB patients, they are valuable in elucidating novel aspects of GB. Because MIB-1 LI is known to be influenced by both the staining and counting methods, inter-laboratory and inter-observer variability need to be carefully determined [20]. Moreover, in addition to the grading system or prognostic significance, the cut-off value of MIB-1 LI for the pattern of failure in GB remains to be examined. Therefore, the present findings should be confirmed by larger-scale and multi-institutional studies with standardized evaluation methods for MIB-1 LI.

The MIB-1 LI had the impact in prediction of POF when sites of recurrence were stratified to the central versus others as shown in the Table 3. When the stratification was converted to the subgroups of the central or the in-field versus of the marginal or the distant, the value was not significant ($P = 0.23$). That might suggest that the MIB-1 with a cut-off = 30 was closely involved the in-field recurrence. Of course, other factors, such as definition of GTV (pre-surgical or post-surgical), margins for CTV, and total dose, should be standardized before proposing the association between the MIB-1 LI and the in-field recurrence, this result need to be clarified in the future study.

It is difficult to state distinct association between radiosensitivity and the MIB-1 LI in GB. As far as we investigated, there are no reports elucidating the direct link in GB. Possible explanation might be that, not only the MIB-1 LI, GB has been reported to contain several abnormalities of molecular parameters, including 1p19q deletion status [21,22], isocitrate dehydrogenase (IDH) genes mutation status [23,24], or methylation status of the O6-methylguanine-DNA methyltransferase gene MGMT promoter methylation status [25-27]. On the contrary, increased cell cycling and MIB-1 LI suggest more aggressive behavior of promote tumor progression and metastasis in variety of cancers [28,29]. Therefore, the MIB-1 with association with other molecular parameters might reflect tumor behavior and POF in patients with GB. Although the MIB-1 LI could be a useful tool to recognize disease characteristics including POF, further clinical and experimental studies are warranted.

From our result, it is possible to propose that patients with low MIB-1 LI have a benefit to receive increased dose of radiotherapy because more often central recurrence might occur. On the contrary, patients with the high MIB-1 LI might not have a benefit because prediction of sites of recurrence were difficult. Therefore, in those patients with the high MIB-1 LI, more aggressive chemotherapy seemed to be more important instead of a dose escalation of radiotherapy. Thus, the MIB-1 LI could be a useful tool to determine individual strategy in patients with GB with prediction of POF.

Among other biomarkers for GB, MGMT methylation seems to be the most intensively examined biomarker [14,16]. Brandes et al. [16] demonstrated that recurrence is correlated with MGMT methylation status in patients who received chemoradiation with temozolomide. Interestingly, Minniti et al. [14] also reported that patterns of recurrence were significantly different according to the methylation status of the MGMT promoter. Recurrences were central/in-field and distant in 64% and 31% of methylated patients versus 91% and 5.4% of unmethylated patients ($P = 0.01$), respectively. The present study did not include an evaluation of MGMT; however, the correlation between MIB-1 LI and MGMT or other markers and their combination should be examined in future studies. The combination efficacy between MIB-1 LI and MGMT could also affect the strategy for GB treatment.

The MIB-1 LI has been used as a marker of the proliferation rate of various intracranial and extracranial tumors. Okita and coworkers [30] reported that MIB-1 LI of the recurrent tumors identified as a significant independent prognostic factor in their multivariate analysis. Kim and coworkers [31] reported that the values of MIB-1 LI between primary and recurrent tumors were different, and that the MIB-1 LIs of the recurrent tumors were reduced in 75% of patients with GB. Their explanation to the result was that radiotherapy and/or chemotherapy might suppress proliferation, actively proliferating tumors, and they suggest that the MIB-1 LI may play a diagnostic role in recurrent GB. Thus, the prediction of POF by the MIB-1 LI will lead to evaluation and comparison between primary and recurrent tumors, and possibly might illustrate tumor characteristics of GB in the future.

Conclusions

The proportions of recurrence sites in our series of AA and GB were consistent with previous reports addressing

patterns of failure in high-grade gliomas. Our novel findings identified MIB-1 LI, but not treatment factors, as a biomarker that was significantly correlated with the pattern of failure in GB patients. Although the present findings need to be confirmed by larger-scale studies, this report provides important information to help elucidate the biological nature of GB.

Competing interest

The authors declare that they have no competing interests.

Authors' contributions

Conception and design: KU, RS, Provision of study materials or patients: TaS, HN, MN, ToS, KS, EK, Collection and assembly of data: KU, TaS, DM, OF, TS, Data analysis and interpretation: KU, TaS, DM, HN, KY, YO, NM, HA, RS. Manuscript writing: KU, RS. Final approval of manuscript: KU, TaS, DM, HN, KY, YO, NM, HA, MN, OF, ToS, KS, EK and RS. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Long-term outcome of hypofractionated radiotherapy to the whole breast of Japanese women after breast-conserving surgery

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Abstract
Background In Japan, there are still no reports of long-term outcome for hypofractionated radiotherapy to the whole breast after breast-conserving surgery (BCS). We report our institution's results from evaluation of the efficacy and safety of hypofractionated radiotherapy for Japanese women.
Methods Data in the medical records of 327 patients were retrospectively reviewed. The patients were treated with hypofractionated radiotherapy between January 2003 and December 2006 at the Kawasaki Medical School Hospital and were followed for more than 3 years. The median age was 54 years old (the age range was 28–80 years). The whole breast was irradiated with a total dose of 42.56 Gy/16 fx with boost irradiation to positive margins. Adjuvant therapy consisted of chemotherapy and/or hormone therapy and was administered to 300 patients, based on their stage or pathological findings.
Results Follow-up periods ranged from 21 to 92 months; the median follow-up period was 60 months. At 5-year follow-up, overall survival, cause-specific survival, relapse-free survival, and local control were 96.0, 97.5, 95.3, and 99.7% respectively. Grade 2 radiation pneumonitis occurred in five patients. Grade 2 radiation dermatitis occurred in 17 patients. Severe late complications were not observed.
Conclusions In our study, hypofractionated radiotherapy led to good results without severe toxicity. We believe hypofractionated radiotherapy after BCS is safe and efficient treatment for Japanese women.

Keywords Breast cancer · Breast-conserving surgery · Hypofractionated radiotherapy

Introduction

Breast-conserving therapy, consisting of breast-conserving surgery (BCS) followed by whole breast irradiation, is an established standard treatment for patients with early breast cancer [1–10]. In Japan, a fraction dose of 1.8–2 Gy and a total dose of 45–50 Gy are usually applied in the postoperative setting with or without additional boost irradiation of the excision site. In late years, alternative hypofractionated radiotherapy (e.g., 42.56 Gy/16 fx, 41.6 Gy/13 fx, 40 Gy/15 fx) has been reported to be feasible and acceptable [11–17] and is becoming popular as a convenient regimen. There are few reports of long-term results of hypofractionated radiotherapy for Japanese women. Morbidity of breast cancer is lower in Japan than in Europe and the United States. In addition, large breasts are more common in Europe and the United States than in Japan. Therefore, an original survey is needed to investigate the

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efficacy and safety of hypofractionated radiotherapy for Japanese women. We introduced hypofractionated radiotherapy after BCS as practical clinical treatment in 2003. We retrospectively investigate and describe the long-term results in this article.

Patients and methods

Patient selection

In our institution, since January 2003, all patients planned to receive postoperative radiotherapy after BCS were informed about the merits and demerits of two regimens, 50 Gy/25 fx (conventional regimen) and 42.56 Gy/16 fx (hypofractionated regimen), on the basis of published data [14]. The choice was entrusted to each patient, and more than 95% of patients selected the hypofractionated regimen, and gave written informed consent. Therefore, 910 patients were treated with the hypofractionated regimen from January 2003 to September 2010. Patient data in the medical records were retrospectively reviewed. Eligibility criteria for this analysis were:

1 treated with BCS followed by whole-breast hypofractionated radiotherapy consisting of 42.56 Gy in 16 fractions with or without boost irradiation to the tumor bed;
2 patients with early-stage breast cancer of pStage0-IIB; and
3 follow-up period of more than 3 years after the completion of radiotherapy.

To avoid statistical complexity, exclusion criteria were:

1 lost to follow-up within 3 years ($n = 16$);
2 died of other disease within 3 years without recurrence of breast cancer ($n = 3$);
3 simultaneous bilateral breast cancer ($n = 7$);
4 history of breast cancer ($n = 13$);
5 induction chemotherapy ($n = 5$); and
6 incomplete radiotherapy ($n = 2$).

As a result, 327 patients were included in this retrospective analysis. Patient characteristics are summarized in Table 1.

Table 1 Clinical, tumor, and treatment characteristics

Clinicopathologic characteristic	*n*
Age	28–80 (median 54)
<50/≥50	115/212
PS	
0-1/2/3≥	325/2/0
Surgery	
Bp or Bq only/SNB/Ax	29/117/181
Bp/Bq/Tm	185/142/0
Chemotherapy	
Yes/no	109/218
Hormone therapy	
Yes/no	261/66
pT stage	
is/1/2/3	29/206/91/1
pN stage	
0/1	261/66
pStage	
Stage 0	29
Stage I	164
Stage II A	107
Stage II B	27
Histology	
DCIS	28
IDC	268
Special types	29
Unclassified or NA	2
Margins	
Positive/negative	66/261
ER and/or PgR*	
Positive/negative/NA	253/38/36
HER2	
Positive/negative/NA	30/252/45

Ax axillary dissection; *Bp* wide excision; *Bq* quadrantectomy; *CR* complete response; *DCIS* ductal carcinoma in situ; *ER* estrogen receptor; *IDC* invasive ductal carcinoma; *NA* not available; *PgR* progesterone receptor; *SNB* sentinel node biopsy; *Tm* tumorectomy

* The definition of hormone-receptor positive is that one or both of ER or PgR receptor is positive

Surgery

All patients received BCS: wide excision (Bp) in 185 patients and quadrantectomy (Bq) in the other 142. No patient received tumorectomy (Tm). If cancer cells remained within 5 mm from the surgical margin, the specimen was defined as "stump-positive." In this study, 66 patients had positive margins at final pathological examination. 181 patients underwent level I/II axillary lymph nodes dissection. 117 patients underwent sentinel node biopsy (SNB) alone. Twenty-nine patients received no axillary surgery.

Radiotherapy

Techniques of radiotherapy, except for dose fractionation, were as same as for traditional methods. Patients were treated with external beam radiotherapy to the whole breast, using tangential fields with 4–6 MV photons. The field border was determined in the following way: the inferior border was located 1–2 cm below the

inframammary fold; the superior border, at the height of the suprasternal notch; the medial border, at the midsternal line; and the lateral border, at the mid-posterior axillary line. The anterior margin was located at least 2 cm from the surface of the breast and the posterior margin was maintained with a gantry-tilting technique to limit the maximum lung depth included in the field to 3 cm or less. A total dose of 42.56 Gy/16 fr was prescribed generally at the isocenter and, when necessary, wedge filters and/or the field-in-field technique were used to optimize dose homogeneity. The objective of optimization was to keep the minimum dose in the deep part of the breast no less than 95% and to limit the maximum dose within the breast to no more than 107% of the prescribed dose. For positive margins, additional boost irradiation of 10–13.3 Gy/4–5 fractions (using 4–11 MeV electrons) was administered to the excision site.

Adjuvant therapy

Any limitation of adjuvant therapy was not made in association with the hypofractionated radiotherapy. Adoption of adjuvant therapy depended on attending surgeon, and chemotherapy and/or hormone therapy were selected according to commonly-accepted criteria based on pathological stage or histological findings. 300 patients received adjuvant systemic therapy. Chemotherapy involved various combinations of the following drugs: cyclophosphamide, adriamycin, 5-fluorouracil, methotrexate, epirubicin, paclitaxel, docetaxel, tegaful-uracil, and doxifluridine. Chemotherapy was administered sequentially (not concurrently) with radiotherapy. Hormone therapy involved the following drugs: anastrozole, exemestane, tamoxifen, toremifene citrate, leuprorelin, goserelin, and letrozole. Hormone therapy was generally maintained from 2 to 5 years after the completion of radiotherapy.

Follow-up and patient evaluation

Patients were seen at least once a week during radiotherapy and at 1 month after completing radiotherapy. They were then seen every 3 months for 3 years. After 3 years, they were seen annually. At each visit, physicians recorded a history and performed a physical examination (mainly skin reaction and respiratory symptoms related to radiotherapy). A chest X-ray was taken before a radiotherapy session, and then at each follow-up visit during the first year. From the second year they had a routine chest X-ray semi-annually. When X-ray films or physical symptoms suggested radiation pneumonitis, more frequent X-ray tests or computed tomography (CT) were considered. Bilateral mammography, bone scan, and abdominal ultrasonography were performed annually. Radiation dermatitis and radiation pneumonitis were evaluated by use of the Radiation Therapy Oncology Group (RTOG)-acute radiation morbidity scale scoring criteria and the Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer (RTOG/EORTC) late radiation morbidity scoring scheme [18].

Statistical analysis

The survival time was calculated from the date of surgery. Survival curves and actuarial rates of recurrence were calculated using Kaplan–Meier method, and the significance of prognostic factors was assessed by log rank test [19]. Multivariate analysis was performed using stepwise Cox proportional hazard regression models [20]. A $P < 0.05$ between groups was considered significant. Stat View (version 5.0) software was used for all statistical analysis.

Results

Median follow-up period was 60 months. Of 327 patients, 14 patients died; 9 died of breast cancer and 5 died of other causes. Recurrence was observed in 15 patients. Of these patients, 14 developed distant metastases (bone, brain, lung, liver). Local recurrence in the ipsilateral breast was observed in one patient only, who was salvaged by mastectomy. Metachronous contralateral breast cancer occurred in four patients (one patient also had distant metastases).

Figure 1 shows overall survival (OS), cause-specific survival (CSS), relapse-free survival (RFS), and the local control (LC). Actuarial 5-year OS, CSS, RFS and LC were 96.0, 97.5, 95.3 and 99.7%, respectively. Figure 2 shows

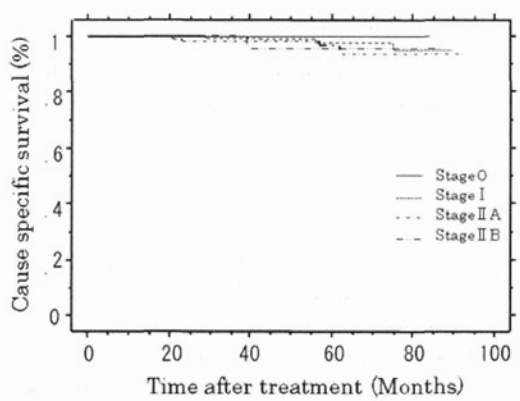

Fig. 1 Survival graphs showing overall survival, cause-specific survival, relapse-free survival, and local control ($n = 327$)