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CLINICAL INVESTIGATION

# Subclavian Vein Versus Arm Vein for Totally Implantable Central Venous Port for Patients with Head and Neck Cancer: A Retrospective Comparative Analysis

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**Abstract**
*Purpose* This study was designed to compare central venous ports (CVP) from two different routes of venous accesstthe subclavian vein and arm veiñin terms of safety for patients with head and neck cancer (HNC).
*Methods* Patients with HNC who underwent image-guided implantations of CVPs were retrospectively evaluated. All CVPs were implanted under local anesthesia. Primary outcome measurements were rates and types of adverse events (AEs). Secondary outcomes included technical success and rate and reason of CVP removal.
*Results* A total of 162 patients (subclavian port group, 47; arm port group, 115) were included in this study. Technical success was achieved in all patients. The median follow-up period was 94 (range, 1–891) days. Two patients in the subclavian port group experienced periprocedural complications. Postprocedural AEs were observed in 8.5 and 22.6% of the subclavian port and arm port group patients, respectively ($P = 0.044$). Phlebitis and system occlusions were observed only in the arm port group. The rate of infection was not significantly different between the two groups. The CVP was removed in 34 and 39.1% of the subclavian port and arm port patients, respectively.
*Conclusions* Both subclavian and arm CVPs are feasible in patients with HNC. AEs were more frequent in the arm port group; thus, the arm port is not recommended as the first choice for patients with HNC. However, further experience is needed to improve the placement technique and the maintenance of CVPs and a prospective analysis is warranted.

**Keywords** Central venous port · Venous access · Subclavian vein · Arm vein

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## Introduction

Multimodality treatment consisting of chemotherapy, radiotherapy, and surgery has improved head and neck cancer (HNC) mortality during the past decade [1–3]. This treatment approach also may improve organ preservation; however, episodes of acute and chronic treatment-related toxicity are frequent [1, 2, 4]. Thus, supportive treatments, such as nutrition support and venous access, have become increasingly important to accomplish antitumor therapies in advanced HNC. For this purpose, central venous access often is required for patients with HNC to enable repeat administration of chemotherapeutic agents, intravenous hydration, and parenteral nutrition. For long-term central venous access, central venous ports (CVPs) have been extensively used and image-guided placement of CVPs is now widely accepted as a safe and effective alternative to surgical placement [5, 6].

The insertion site of CVPs is controversial. In general, the subclavian approach is preferred because the long-term outcome is better; however, perioperative adverse events (AEs), such as pneumothorax and hemothorax, occur in 0.3–3.4% of patients [5–9]. Furthermore, patients with HNC are prone to infections because of oropharyngeal exudation, tracheostomy, and malnutrition during the treatment period [10], possibly resulting from infectious AEs related to CVP placement. Moreover, patients with HNC may have undergone radiation in the neck and upper

thorax adjacent to the insertion site of the CVP when the subclavian route is used. Only a few studies have dealt with the specific clinical outcomes of the different approaches for CVP insertion in patients with HNC [10]. At our hospital, patients with HNC have undergone CVP placements either via the subclavian vein through the chest wall or via the basilic or cephalic vein through the forearm.

The purpose of this study was to compare CVPs placed in two different sites—the chest and the arm—in terms of safety and efficacy in patients with HNC.

## Materials and Methods

### Patients

In this retrospective study, patients with HNC who underwent image-guided implantation of CVPs between 2006 and 2009 were included. Eligible patients were performed CVP placement via the subclavian vein and arm vein (i.e., basilic, median, cubital, or cephalic vein in antecubital fossa) and were followed up until CVP removal, death, or hospital discharge.

The study protocol was approved by the institutional review board. The procedure and its possible complications and benefits along with alternative procedures were explained to patients, and written informed consent was obtained. No additional consent was obtained for the retrospective analysis of the clinical records.

### Ports and Catheters

We used a port system consisting of a titanium reservoir with a silicone rubber septum for needle insertion (P-U Celsite Port, Toray Medical, Tokyo, Japan) and a 5-F hydrophilic heparinized polymer coated catheter (Anthron P-U Catheter, Toray Medical, Tokyo, Japan). We used two sizes of port systems for the chest and arm approaches: 8 and 2.5 g in weight and 0.5 and 0.2 ml in internal volume, respectively.

### Placement of CVP

All CVPs were implanted under local anesthesia in an angiographic suite using maximal sterile precautions by interventional radiologists. No routine prophylactic antibiotics were administered. We used 10% povidone-iodine for preprocedure cleansing of the patients' skin.

Arm ports were implanted in the forearm (Fig. 1A). The basilic or cephalic vein in the antecubital fossa was used. The CVP was inserted into the nondominant arm except in patients with small-caliber arm veins. Access vein was chosen with the use of tourniquet. After puncturing the vein

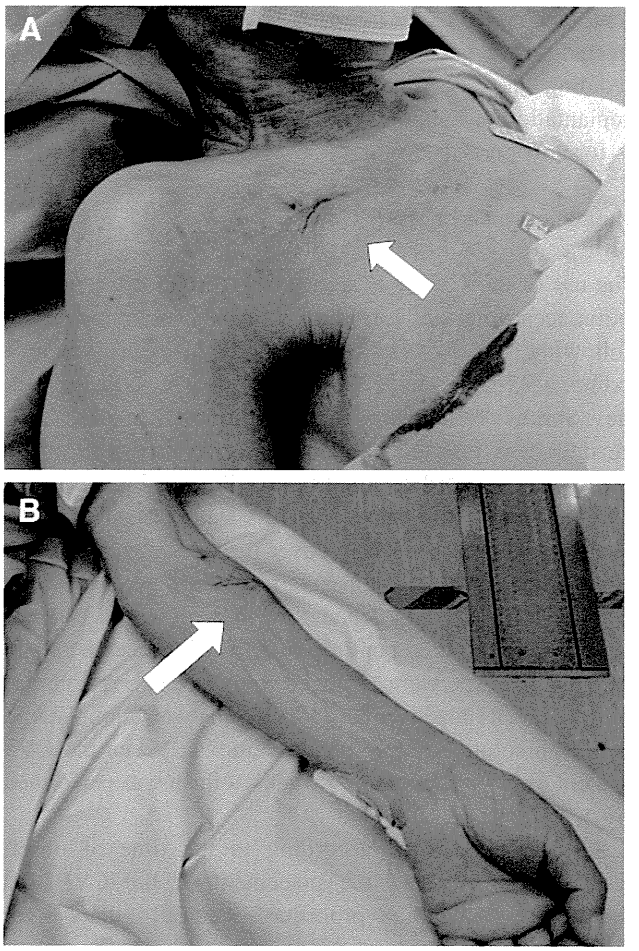


**Fig. 1** Insertion site of central venous port. **A** Subclavian ports were implanted in the anterior chest wall. **B** Arm ports were implanted in the forearm

with an 18-gauge elastic needle, a 0.035-in guidewire (Radifocus, Terumo, Tokyo, Japan) was advanced into the vena cava and a catheter was inserted. The tip of the catheter was positioned into the atriocaval junction using fluoroscopy. A subcutaneous pocket was created with a 3-cm incision in the region distal to the venipuncture site. A port was connected to the indwelling catheter through a subcutaneous tunnel and was implanted in the pocket. The wound was closed with 5–0 nylon sutures.

Chest ports were implanted via the right subclavian vein except in patients with occlusion or stenosis of that vein. Puncture of the subclavian vein was performed under real-time ultrasonographic guidance. A portable ultrasound (SonoSite iLook25, SonoSite, Seattle, WA) with a 10- to 5-MHz broadband linear array probe was used. The subclavian vein was punctured with an 18-gauge elastic needle through a needle attachment on the probe. After visual observation of the needle's penetration of the anterior wall of the subclavian vein and with confirmation of backflow of blood into a syringe, a catheter was advanced into the

vena cava with a guidewire. A subcutaneous pocket was created on the lateral side of the anterior chest wall while avoiding any possible radiation fields (Fig. 1B). The implantation of the port and the catheter was same process as for arm ports.

### Maintenance and Follow-Up

The use of the CVP was started 1 to 3 days after implantation according to the need of intravenous administration. Following the sterilization of the skin with povidone iodine, a 22-gauge noncoring Huber needle (Gripper needle, Smiths Healthcare Manufacturing S.A. de C.V., Mexico) was inserted percutaneously to access the port. Saline was injected to identify possible occlusion of the catheter or subcutaneous leakage. A semipermeable transparent dressing was used to cover the needle and attached to the skin with elastic adhesive tape. As a general rule, a needle was inserted on demand for intravenous access. For patients who required continuous infusion, a needle and an infusion line set were changed every week. A total of 10 ml of heparinized saline (100 IU/ml) was administered as a flush solution before needle removal. When the CVP was not used for more than 4 weeks, a flush was performed every 4 weeks.

AEs were recorded according to the time of onset: periprocedural (during the placement of the CVP and within 1 day after the procedure) and all others were considered postprocedural AEs.

### Study Outcomes

Primary outcome measurements for the comparison of the two CVP insertion sites included the rate and type of AEs. Periprocedural AEs were graded according to the Society of Interventional Radiology Classification System for Complication by Outcome (SIR Classification) [11]. Infectious AEs were divided into three categories in accordance with the Catheter-Related Infections published by the Centers for Disease Control and Prevention (CDC) [12], and the criteria for port-pocket infections described in previous reports [13]. They included port-pocket infection, bloodstream infection (BSI), and probable CVP-related infection. Port-pocket infection was diagnosed by the purulent discharge from the port-pocket or other suspicious symptoms, such as erythema, induration, or pain in the region of port-pocket. BSIs were defined as fever and blood cultures positive for microorganisms known to be associated with long-term venous access catheters (e.g., coagulase-negative staphylococci) [12]. Probable CVP-related infection was suspected when fever was observed without an obvious source of infection other than the CVP when blood cultures were negative or not performed. Other AEs were categorized according to the Common Terminology Criteria for Adverse Events (CTCAE) v3.0 and reported if applicable AEs of grade 2 or greater were encountered [14].

Secondary outcomes included the rate of technical success of CVP placement and the rate and reasons for CVP removal.

### Statistical Methods

Demographics and baseline variables were summarized with descriptive statistics. Categorical data were compared using the chi-squared test. Statistical significance was set at 0.05 for a two-sided test.

## Results

### Characteristics of Patients

A total of 162 patients were included in this study. The clinical characteristics of the patients are shown in Table 1. The majority of the tumors were seen in the oral cavity and

**Table 1** Characteristics of patients

| Characteristic | Subclavian ports | Arm ports | Total |
|---|---|---|---|
| Total patients | 47 | 115 | 162 |
| Age (year) | | | |
| Median | 65 | 64 | 64 |
| Range | 26–82 | 20–91 | 20–91 |
| Gender | | | |
| Male | 40 (85.1) | 81 (70.4) | 121 (74.7) |
| Female | 7 (14.9) | 34 (29.6) | 41 (25.3) |
| Primary tumor site | | | |
| Oral cavity | 15 (31.9) | 45 (39.1) | 60 (37.0) |
| Nasopharynx | 0 (0) | 10 (8.7) | 10 (6.2) |
| Oropharynx | 7 (14.9) | 23 (20) | 30 (18.5) |
| Hypopharynx | 6 (12.8) | 12 (10.4) | 18 (11.1) |
| Larynx | 12 (25.5) | 15 (13) | 27 (16.7) |
| Nasal cavity | 3 (6.4) | 4 (3.5) | 7 (4.8) |
| Unknown | 3 (6.4) | 2 (1.7) | 5 (3.1) |
| Other | 1 (2.1) | 4 (3.5) | 5 (3.1) |
| Tracheotomy | 18 (38.3) | 29 (25.2) | 47 (29) |
| Treatment | | | |
| Chemoradiotherapy | 43 (91.5) | 104 (90.4) | 147 (90.7) |
| Chemotherapy alone | 2 (4.3) | 3 (2.6) | 5 (3.1) |
| Radiotherapy alone | 0 (0) | 1 (0.9) | 1 (0.6) |
| Surgery | 9 (19.1) | 42 (36.5) | 51 (31.5) |
| Follow-up period, days | | | |
| Median | 110 | 89 | 94 |
| Range | 7–740 | 1–891 | 1–891 |

Data are shown as number of patients with percentages in parentheses

**Table 2** Procedure details

| Parameter | Subclavian ports | Arm ports | Total |
|---|---|---|---|
| Side | | | |
| Right | 45 (95.7) | 7 (6.1) | 52 (32.1) |
| Left | 2 (4.3) | 108 (93.9) | 110 (67.9) |
| Guiding image | | | |
| Ultrasound | 47 (100) | 0 (0) | 47 (29) |
| Fluoroscopy | 47 (100) | 115 (100) | 162 (100) |
| Venography | 0 (0) | 1 (0.9) | 1 (0.6) |

Data are shown as number of patients with percentages in parentheses

the pharynx in 118 patients (72.8%). A tracheostomy was performed on 47 (29%) patients. A total of 147 patients (90.7%) were treated with chemoradiotherapy.

## CVP placement

Procedural details are shown in Table 2. Technical success was achieved in all of the patients. The subclavian ports were implanted using both ultrasound and fluoroscopic guidance in all patients. The arm ports were implanted under fluoroscopic guidance. One patient underwent a venography because of the small diameter of the antecubital vein and access to an appropriate vein was needed for visualization.

## Safety

Table 3 presents AEs during and after CVP placement. One case of pneumothorax and one arterial puncture occurred in the subclavian port group. The patient with pneumothorax was treated with a chest drainage tube for 5 days. In the patient with the arterial puncture, hemostasis was achieved with manual compression of the puncture site. No periprocedural AEs were observed in the arm port group.

A total of 30 patients (18.5%) experienced postprocedural AEs: 4 in the subclavian port group and 26 in the arm port group. Overall, postprocedural AEs occurred more frequently in the arm port group than in the subclavian port group with a statistical significance ($P = 0.044$). Phlebitis and system occlusion were seen only in the arm port group. In patients with system occlusions, we injected a mixture of 60,000 IU urokinase and 5,000 IU heparin via a port and recanalization was achieved in one patient. In terms of the rates of infectious AEs, no significant difference was demonstrated between the two groups. Events per 1,000 catheter-days of each category of infection (i.e., bloodstream infection, port-pocket infection, and probable CVP-related infection) also demonstrated no statistically significant difference (Table 4). A total of four of 47 patients (8.5%) who underwent a tracheotomy experienced infectious AEs: 1 patient in the subclavian port group (2.0%) and three patients in the arm port group (2.6%) (Table 5).

## Removal of the CVP

We performed the removal of the CVP in 61 patients (37.7%). Elective removal after the completion of treatment was performed in 44 patients (27.2%). Emergency removal for AEs or system malfunctions, such as failed catheter recanalization, was needed in 17 patients (10.5%). Nine patients underwent bacteriological examination of the

**Table 3** Adverse events

| Adverse event | Subclavian ports | | Arm ports | | *P* value |
|---|---|---|---|---|---|
| | No. (%) | Median duration (days) | No. (%) | Median duration (days) | |
| Periprocedural | | | | | |
| Pneumothorax | 1 (2.1) | | 0 (0) | | 0.29 |
| Arterial puncture | 1 (2.1) | | 0 (0) | | 0.29 |
| Primary malposition | 0 (0) | | 0 (0) | | |
| Total | 2 (4.3) | | 0 (0) | | 0.83 |
| Postprocedural | | | | | |
| Infection** | 4 (8.5) | 49 | 9 (7.8) | 44.5 | 0.758 |
| Phlebitis | 0 (0) | | 11 (9.5) | 33 | 0.035[b]* |
| Fibrin sheath | 0 (0) | | 0 (0) | | |
| System occlusion | 0 (0) | | 5 (4.3) | 53 | 0.323 |
| Subcutaneous extravasation | 0 (0) | | 1 (0.9) | 34 | 1 |
| Venous thrombosis | 0 (0) | | 0 (0) | | |
| Catheter detachment | 0 (0) | | 0 (0) | | |
| Total | 4 (8.5) | 49 | 26 (22.6) | 66 | 0.044* |

*CVP* central venous port

* Details of infectious AEs are listed in Table 4

** $P < 0.05$ (Statistical comparison of the rate of AEs using unpaired *t* test)

**Table 4** Details of infectious AEs

| Adverse event | Subclavian ports | | Arm ports | |
|---|---|---|---|---|
| | No. of events (%) | Events/1,000 catheter-days | No. of events (%) | Events/1,000 catheter-days |
| Bloodstream infection | 1 (2.1) | 0.14 | 2 (1.7) | 0.12 |
| Port-pocket infection | 2 (4.3) | 0.27 | 5 (4.3) | 0.29 |
| Probable CVP-related infection | 1 (2.1) | 0.14 | 2 (1.7) | 0.12 |
| Total | 4 (8.5) | 0.55 | 9 (7.8) | 0.53 |

*CVP* central venous port

**Table 5** Removal details

| Parameter | Subclavian ports No. of patients (%) | Arm ports No. of patients (%) | Total No. of patients (%) |
|---|---|---|---|
| CVP removal | 16/47 (34) | 45/115 (39.1) | 61/162 (37.7) |
| Indication for removal | | | |
| No longer needed | 9 (19.1) | 23 (20) | 32 (19.8) |
| Catheter occlusion | 0 (0) | 4 (3.5) | 4 (2.5) |
| Bloodstream infection | 1 (2.1) | 2 (1.7) | 3 (1.9) |
| Port-pocket infection | 2 (4.3) | 5 (4.3) | 7 (4.3) |
| Probable CVP-related infection | 1 (2.1) | 2 (1.7) | 3 (1.9) |
| Other | 3 (6.4) | 9 (7.8) | 12 (7.4) |
| Catheter or port sent for culture | | | |
| Yes | 2 | 7 | 9 |
| No | 14 | 38 | 52 |
| Culture result | | | |
| No growth | 1 | 2 | 3 |
| ≥100 CFU | 1 | 5 | 6 |
| Median dwell time (days) | 112.5 | 63 | 101 |

*CVP* central venous port, *CFU* colony-forming units

removed port and catheter, and positive culture results were seen in six patients: methicillin-resistant *Staphylococcus aureus* in three patients, *Staphylococcus aureus* in two, *Candida albicans* in one, and *Escherichia coli* in one. Median time-to-removal was 134 (range, 8-807) days in elective patients and 49 (range, 6–227) days in emergency patients. Reimplantation was performed in three patients with an emergency removal.

## Discussion

In the present study, two different locations were used for CVPs in patients with HNC—the subclavian and the arm—which demonstrated different safety profiles. Overall, significantly more AEs were noted in patients with an arm port (22.6%) than in patients with a subclavian port (12.8%). Periprocedural AEs occurred only in the subclavian port group (4.3%). The number of postprocedural AEs was greater in the arm port group (22.6%) than in the subclavian port group (8.5%), and phlebitis was the most frequent AE (9.5%). Infectious AEs were observed in 8.5% of patients with subclavian ports and in 7.8% with arm ports.

For the placement of a central venous catheter (CVC), the subclavian vein has been a preferred route. Advantages of this route include the lower incidence of infection and the good stability of the system on the chest wall [15]. Drawbacks include potentially severe periprocedural AEs, such as pneumothorax, hemothorax, and compression of the catheter in the costo-clavicular space, which may lead to fracture of the catheter (pinch-off syndrome) [16]. The internal jugular vein is now widely accepted as a safe route when ultrasound guidance is used to avoid arterial puncture. Cephalic or forearm veins also are recognized as a lower risk site in regard to periprocedural AEs; however, a 5–10% failure rate was reported for the cephalic vein approach because of narrow vessel diameter, and thrombosis and phlebitis resulted more often than with other routes [7, 17, 18]. Femoral veins are not routinely used and are instead selected when other specific conditions, such as tumor involvement, stenosis, or thrombosis of superior vena cava exist. Preferred access route for the placement of CVP also has been subclavian vein. We chose the subclavian vein or the arm vein in our series, because the internal jugular vein is close to or involved with the tumor, lymph nodes, or radiotherapy field.

Recently, image guidance with fluoroscopy, venography, or ultrasound has been widely used for venous access, especially by interventional radiologists, to achieve a higher success rate and reduce periprocedural AEs. Real-time ultrasound-guided puncture is reported to be a reliable method to access the internal jugular and the subclavian veins. Lameris et al. first reported on the efficacy of ultrasound-guided puncture of the subclavian vein comparing with blind technique. In their series, technical success was achieved in all 31 ultrasound-guided procedures without puncture-related complications [19]. Sakamoto et al. reported on the results of a total of 500 patients with malignancies who underwent ultrasound-guided placement of CVPs via subclavian veins. This study demonstrated a small failure rate of 0.4% and a low periprocedural complication rate of 1.2% [20]. A randomized, controlled trial comparing three different routes for CVP by Biffi et al. demonstrated that ultrasound-guided access to the subclavian vein was associated with a very low (<1%) failure rate compared with 10.4% for internal jugular venous access and 15.7% for surgically exposed cephalic vein access [21]. However, they reported that the rates of periprocedural AEs were very low and did not differ among three routes: 0% in the subclavian and internal jugular veins, and 1.5% in the cephalic vein. In a report by Marcy et al. [18] of venography-guided arm port placement in 1,000 patients with cancer, technical failure was encountered in 6.3% of patients, and the periprocedural AE of symptomatic hematoma occurred in 0.9% of patients. Hata et al. [17] also reported that their outcome of arm-port insertion had no technical failures and no procedure-related AEs in 104 patients. Our results of technical success and periprocedural AEs in subclavian and arm ports were comparable to those in these previous reports. Placement of the CVP was well tolerated in each access route. However, given that periprocedural AEs can be almost completely avoided with arm vein access [22], the rare but severe periprocedural AEs associated with subclavian vein access should be taken into consideration when choosing the route for CVPs.

In our study, incidence of postprocedural AEs was significantly higher in the arm port group than in the subclavian port group. Phlebitis was the most frequently encountered AE in our study and seen only in patients with an arm port. Venous thrombosis may contribute to phlebitis; however, we did not encounter obvious thrombosis in our series. Previous studies on arm-port procedures demonstrated that the incidence of phlebitis was 0.7–8.2% [4, 15, 17, 21, 23, 24]. A possible reason for the higher rate of phlebitis (9.5%) in our study was the lack of preprocedural venography or ultrasound. Hata et al. demonstrated the importance of venography for selecting venous access [17]. The rate of phlebitis in their study was actually lower (5.8%) than in our study. Recently, guidelines regarding venous thromboembolism in patients with cancer were published, and an international consensus for prevention was established [25]. Although specific prophylaxis was not recommended for patients with CVCs, the importance of general thromboprophylaxis in hospitalized cancer patients was emphasized. In our study, no thromboprophylaxis was performed, thus the effect of prophylaxis on venous thrombosis and phlebitis was unclear.

Catheter-related infections were potentially life-threatening AEs. The mechanisms were comprised of several factors: infection of the exit site of the catheter, followed by migration of the pathogen along the external catheter surface; contamination of the catheter hub, resulting in the intraluminal colonization; and hematogenous seeding of the pathogen [26]. According to literature, the rate of catheter-related infection in long-term CVCs ranged from 0.6–52% [13, 27, 28]. Subcutaneous CVPs were associated with less frequent infections than external venous access devices, such as CVCs [13]. Huang et al. [29] reviewed the clinical features and outcomes of CVP-related infections in 896 CVPs in patients with cancer. A total of 42 patients (5%) encountered infectious AEs in their study. Of these, definite port-related bacteremia was seen in 28 patients, local infection in 5, and probable port-related bacteremia in 3. Johansson et al. [30] performed a direct comparison of CVPs with CVCs in patients with acute leukemia. The number of positive blood cultures per 100 central venous access device days was significantly higher in the CVC group (median 3.6 per 100 days) than in the CVP group (0.9 per 100 days), although this randomized trial was prematurely terminated due to extensive subcutaneous hematoma in the CVP group.

Incidence of infectious AEs varied depending on the access route. With CVCs, access from the femoral and internal jugular veins has been recognized to have a greater risk of infection than the access from the subclavian vein [26, 31, 32]. In the reports on CVP, subclavian access demonstrated infection rates of 1.4–2.4% [6, 24]. In arm ports, infection rates tended to be higher than with subclavian ports, ranging from 4.1–12% [4, 18, 22, 33], although direct comparison in a randomized trial demonstrated no significant difference between the two routes [21]. Marcy et al. also showed no significant difference in infection rates between the two routes in a retrospective study of HNC patients [10]. In our patients, no significant difference was shown in the rates of infectious AEs between the subclavian and arm port groups; however, the rates were higher than in previous studies. Possible explanations include the general high risk of infection in patients with HNC due to malnutrition or exudation from the nasal or oral cavities or the tracheostomy site, use of 10% povidone iodine instead of 2% chlorhexidine

gluconate, which is not available in our practice, inadequate maintenance of the site in our patients, which cannot be captured in the data due to the retrospective nature of this study, and prolonged hospitalization compared with other types of cancer due to the need for intensive supportive treatment during chemoradiotherapy.

Our study has several limitations. First, AEs were not fully evaluated because of the retrospective design. Consequently, types of AEs were limited in variety and the observation period was insufficient. Second, this was a single-center experience with a limited number of patients. Third, the results of the comparison between the two routes are not definitive and cannot be generalized, because this was not a randomized, controlled study. Moreover, the numbers of the patients in the two groups were not well balanced. Nevertheless, this study provides information on the safety of CVPs specific to patients with HNC, which had not been fully reported until now.

In conclusion, our results suggest that both subclavian and arm CVPs are feasible in patients with HNC. AEs were encountered more frequently in the arm port group with a statistical significance; thus, the arm CVPs are not recommended as the first choice for patients with HNC. However, more experience is needed to improve the placement technique and the maintenance of CVPs, and a prospective analysis is needed to establish definitive data on the safety and efficacy of CVPs in patients with HNC.

**Conflict of interest** The authors declare that they have no conflict of interest.

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# Significance of *IDH* mutations varies with tumor histology, grade, and genetics in Japanese glioma patients

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**Mutations in isocitrate dehydrogenase 1 (*IDH1*) and *IDH2* are found frequently in malignant gliomas and are likely involved in early gliomagenesis. To understand the prevalence of these mutations and their relationship to other genetic alterations and impact on prognosis for Japanese glioma patients, we analyzed 250 glioma cases. Mutations of *IDH1* and *IDH2* were found in 73 (29%) and 2 (1%) cases, respectively. All detected mutations were heterozygous, and most mutations were an Arg132His (G395A) substitution. *IDH* mutations were frequent in oligodendroglial tumors (37/52, 71%) and diffuse astrocytomas (17/29, 59%), and were less frequent in anaplastic astrocytomas (8/29, 28%) and glioblastomas (13/125, 10%). The pilocytic astrocytomas and gangliogliomas did not have either mutation. Notably, 28 of 30 oligodendroglial tumors harboring the 1p/19q co-deletion also had an *IDH* mutation, and these alterations were significantly correlated ($P < 0.001$). The association between *TP53* and *IDH* mutation was significant in diffuse astrocytomas ($P = 0.0018$). *MGMT* promoter methylation was significantly associated with *IDH* mutation in grade 2 ($P < 0.001$) and grade 3 ($P = 0.02$) gliomas. *IDH* mutation and 1p/19q co-deletion were independent favorable prognostic factors for patients with grade 3 gliomas. For patients with grade 3 gliomas and without 1p/19q co-deletion, *IDH* mutation was strongly associated with increased progression-free survival ($P < 0.0001$) and overall survival ($P < 0.0001$), but no such marked correlation was observed with grade 2 gliomas or glioblastomas. Therefore, *IDH* mutation would be most useful when assessing prognosis of patients with grade 3 glioma with intact 1p/19q; anaplastic astrocytomas account for most of these grade 3 gliomas. (*Cancer Sci* 2012; 103: 587–592)**

Gliomas are among the most common and formidable brain tumors.[1] Despite intensive treatment, most patients die within 2-10 years. Therefore, development of novel therapeutic strategies based on greater understanding of tumor characteristics is needed. Recently, a comprehensive sequence analysis of human GBM that included most human genes revealed frequent mutations in *IDH1*.[2] Subsequent analyses revealed that these mutations occur more frequently in low-grade glioma than in GBM, with a rate of *IDH1* mutation as high as 59–90%.[3–7] The *IDH* gene mutation is currently believed to occur in the early stage of gliomagenesis[4,6] and to play a critical role in tumor development.

The *IDH* genes encode redox enzymes; these enzymes convert isocitrate to alpha-ketoglutarate, use NAD(P)+ as a coenzyme, and function in energy metabolism. There are three *IDH* genes in humans, and only mutations in *IDH1* are frequently found in gliomas; the IDH1 enzyme resides in the cytosol and peroxisomes.[2–7] Mutations in *IDH2* are rare in gliomas; IDH2 localizes to mitochondria and functions in the Krebs (citric acid) cycle. To date, no *IDH3* mutation has been reported. Most *IDH1* mutations in gliomas are missense mutations at amino acid 132, which is in the catalytic domain and binds to substrate. Similarly, *IDH2* mutations in gliomas are substitutions at amino acid 172, which is functionally equivalent to amino acid 132 of IDH1. *IDH1* and *IDH2* mutations in the catalytic domain are also found in 8–23% of acute myeloid leukemias.[8,9] Mutations in *IDH* genes are rarely found in other tumors.[7,10]

In general, a tumor with an *IDH* mutation has either an *IDH1* or *IDH2* mutation, and the mutation is heterozygous with a wild-type allele.[2,4,6,7] This observation led to the notion that mutated *IDH1/IDH2* genes gain novel functions and are oncogenes, and that the wild-type *IDH* genes are not tumor suppressor genes. In fact, mutant IDH1 has novel enzymatic activity; it converts alpha-ketoglutarate to 2-HG, and accumulated 2-HG is presumed to contribute to tumorigenesis as an ''oncometabolite''.[9,11–13]

Malignant gliomas categorized as WHO grade 4[2,5,7,14,15] or grade 3[5,7,15–17] with an *IDH* mutation were reportedly associated with higher PFS[15–17] and OS[2,5,7,14–17] than those without an *IDH* mutation. However, for grade 2 gliomas, the relation between the presence of an *IDH* mutation and prognosis is controversial.[15,18–20]

The *IDH* mutations apparently have an important role in many aspects of glioma, including gliomagenesis, patient prognosis, and development of therapeutic strategies. However, information on *IDH* mutations in gliomas, such as prevalence, relation to other genetic alterations, and prognostic value, is still limited, particularly for Asian populations,[21,22] including Japanese patients.[5,17] Thus, to further clarify the significance of *IDH* mutations with regard to proper diagnosis and optimized treatment of malignant gliomas, we sought basic data on a large number of Japanese glioma patients for *IDH1* and *IDH2* mutations and other genetic and epigenetic alterations frequently found in gliomas, specifically 1p/19q LOH, *TP53* mutation, and *MGMT* promoter methylation.

## Materials and Methods

**Tumor specimens.** Tumor samples and paired blood samples were obtained following surgery. Of 250 gliomas, 168 tumors were collected at the University of Tokyo hospital (Tokyo,

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Japan) and 82 gliomas were collected at collaborating hospitals. The study was approved by the Ethics Committee of the University of Tokyo and all patients gave written informed consent. Histological diagnoses were made on formalin-fixed, paraffin-embedded tissues following the WHO classification[1] by a neuropathologist (J.S.) for samples from the University of Tokyo hospital and consensus diagnoses were made by four neuropathologists for samples from other hospitals as reported previously.[23] Genomic DNA was extracted for genetic analyses. Patients with the same grade (2, 3, or 4) glioma were treated similarly with surgical resection followed by radiotherapy and alkylating agent chemotherapy.

**Genetic analysis.** For *IDH* gene mutations, the genomic regions spanning the catalytic domain of *IDH1*, including codon 132, and of *IDH2*, including codon 172, were analyzed by direct sequencing using the Genetic Analyzer 310 (Applied Biosystems, Foster City, CA, USA). An aliquot of DNA was amplified by PCR using AmpliTaq Gold (Applied Biosystems) with annealing temperature at 55°C. The primers 5′-TGCCACCAACGACCAAGTCA and 5′-TGTGTTGAGATGGACGCCTATTTG were used for *IDH1* amplification and sequencing, as reported previously.[15] Amplification of *IDH2* was carried out using the primers 5′-CTCTGTCCTCACAGAGTTCAAGC and 5′-CCACTCCTTGACACCACTGCC, and the *IDH2* sequencing reactions were carried out using the primers 5′-AAGTCCCAATGGAACTATCCG and 5′-TCTGTGGCCTTGTACTGCAGAG.

Loss of heterozygosity on chromosomes 1p and 19q was determined using microsatellite analysis as described previously.[23] When tumors had no available paired blood DNA or when the LOH assay was ambiguous because of non-informative microsatellite markers, MLPA assay was carried out using the SALSA MLPA kit P088 (MRC Holland, Amsterdam, the Netherlands) following the manufacturer's instructions. *TP53* gene mutation was determined by direct sequencing following PCR-SSCP screening of exons 5–8 of *TP53*, as described previously.[24]

**Methylation-specific PCR.** Genomic DNA samples (250 ng each) were used for bisulfite reactions using the EZ DNA Methylation Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's protocol. DNA methylation status of the *MGMT* promoter was then determined by methylation-specific PCR as described by Esteller *et al.*[25]

**Statistical analysis.** Fisher's exact test was used to compare the genotype distributions. Overall survival was defined as the time between initial surgery and death or last follow-up. Progression-free survival was defined as the time between initial surgery and recurrence or last follow-up. Both OS and PFS were calculated according to the Kaplan–Meier method, and differences among patient subsets were evaluated using the log–rank test. Statistical calculations were carried out using JMP 9 (SAS Institute, Cary, NC, USA).

## Results

**Frequency and characteristics of *IDH* mutations in glioma samples from Japanese patients.** We analyzed 250 human glioma samples obtained following surgery; these tumors consisted of 125 GBM, 29 AA, 29 DA, 52 oligodendroglial tumors, 9 PA, and 6 GGL. Mutations of *IDH1* and *IDH2* were found in 73 (29%) and 2 (1%) tumors, respectively. All detected mutations were heterozygous, missense mutations. Among the 73 *IDH1* mutations, the G395A (R132H) substitution was the most frequent mutation (occurring in 70/73 cases, 96%), C394A (R132S) substitutions occurred in two cases, and a C394T (R132C) substitution occurred in one case. Of the two *IDH2* mutations, one was a G515A (R172K) substitution, and the other was an A514T (R172W) substitution. *IDH* mutations (*IDH1* or *IDH2*) were found in 13 (10%) of 125 GBM, 8 (28%) AA, 17 (59%) DA, and in 37 (71%) oligodendroglial tumors (Table 1). In the 52 oligodendroglial tumors, *IDH* mutations were found in 19/25 (76%) OG, 4/7 (57%) OA, 10/15 (67%) AOG, and 4/5 (80%) AOA. No mutation was detected in any case of PA or GGL. A higher rate of *IDH* mutation was found in secondary GBM (6/13, 46%) than primary GBM (6/109, 6%). In the three GBMO cases, there was only one *IDH1* mutation.

**Association between *IDH* mutation and 1p/19q co-deletion, *TP53* mutation, or *MGMT* promoter methylation.** The frequencies of 1p/19q co-deletion and *TP53* mutation and their relationship with *IDH* mutations are shown in Table 1. As expected, 1p/19q co-deletion was common in oligodendroglial tumors, especially those without an astrocytic component (OG 76%, AOG 67%), whereas *TP53* mutations were common in lower-grade astrocytomas (DA 45%); these genetic aberrations were never coincident. In OG, 1p/19q co-deletion was significantly correlated with *IDH* mutation ($P < 0.001$), and almost all oligodendroglial tumors with 1p/19q co-deletion had an *IDH* mutation (28/30, 93%).

The *TP53* mutation was more prevalent in DA (45%) than in AA (34%) or primary GBM (22%). However, when *IDH* mutation was present, *TP53* mutation was more frequent, and *TP53* mutations were found in 12/17 (71%) DA, 5/8 (63%) AA, and 3/6 (50%) primary GBMs that also had an *IDH* mutation. The rates of *IDH* mutation in astrocytic tumors with *TP53* mutation were higher than those with wild-type *TP53* (92% *vs* 31% in DA, 50% *vs* 16% in AA, and 13% *vs* 4% in primary GBM), and the association between *TP53* and *IDH* mutation was significant in DA ($P = 0.0018$), but not in AA or GBM. The majority of DA tumors with *TP53* mutation had *IDH* mutation (12/13, 92%); in contrast, only a few primary GBM tumors with *TP53* mutation also had an *IDH* mutation (3/23, 13%).

Of a total 250 gliomas, *MGMT* promoter methylation status was analyzed for 132 gliomas (grade 2, 3, and 4) resected at the University of Tokyo hospital. Methylation was evident in 37/69 GBM (54%), 5/18 AA (28%), 10/17 DA (59%), 8/10 AOG/AOA (80%), and 13/18 OG/OA (72%) (Table 1). The association between *IDH* mutation and *MGMT* methylation was significant in grade 2 ($P < 0.001$) and grade 3 gliomas ($P = 0.02$), but not in grade 4 gliomas ($P = 0.11$).

**Prognostic value of *IDH* mutation and other genetic alterations.** We evaluated the potential prognostic value of *IDH* mutation and other genetic alterations in WHO grade 2, 3, and 4 gliomas. For patients with grade 2 gliomas, univariate analysis showed that *IDH* mutation was not associated with OS ($P = 0.07$) or PFS ($P = 0.29$). Codeleted 1p/19q and wild-type *TP53* each slightly correlated with increased PFS ($P = 0.014$ and $P = 0.029$, respectively), but they were not correlated with OS, and neither of these genetic alterations showed significant association with prognosis in multivariate analysis (Table 2). *MGMT* promoter methylation was also not associated with prognosis. Similarly, we did not observe a significant association of *IDH* mutation with better prognosis for DA (OS, $P = 0.10$; PFS, $P = 0.58$).

In grade 3 gliomas, univariate analysis showed that the association between *IDH* mutation and prolonged survival (OS, $P = 0.0004$; PFS, $P < 0.0001$) was significant and that 1p/19q co-deletion was associated with prolonged survival (OS, $P = 0.028$; PFS, $P = 0.0025$), but that neither *TP53* mutation nor *MGMT* promoter status was associated with prognosis. Although *IDH* mutation and 1p/19q co-deletion were tightly associated with one another, the multivariate analysis further indicated that these alterations were independent indicators of a favorable prognosis (Table 2). *IDH* mutation was present in almost all tumors with the 1p/19q co-deletion.[26] Therefore, grade 3 gliomas were divided into three genetic subgroups: (i) 1p/19q codeleted tumors, most of which carry *IDH* mutation and show oligodendroglial phenotype; (ii) tumors without 1p/19q co-deletion and with mutant *IDH*; and (iii) tumors


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**Table 1. *IDH* mutation and common genetic and epigenetic alterations in gliomas from Japanese patients**

| Tumor pathology (WHO grade) *IDH1* or *IDH2* status | No. of patients | Frequency of *IDH1* or *IDH2* mutation | Median age, years | Male sex (%) | 1p/19q co-deletion | *TP53* mutation | Methylated *MGMT* promoter (%) |
|---|---|---|---|---|---|---|---|
| GBM primary (Gr. 4) | 109 | 6/109 (6%) | 62 | 61 | 1 | 23 (22%) | 27/57 (47) |
| Mutant | 6 | | 43 | 50 | 1 | 3 n.s. | 1/1 (100) |
| Wild-type | 103 | | 62 | 61 | 0 | 20 | 26/56 (46) |
| GBM secondary (Gr. 4) | 13 | 6/13 (46%) | 47 | 69 | 0 | 2 (15%) | 7/9 (78) |
| Mutant | 6 | | 52 | 50 | 0 | 2 n.s. | 4/5 (80) |
| Wild-type | 7 | | 43 | 86 | 0 | 0 | 3/4 (75) |
| GBMO (Gr.4) | 3 | 1/3 (33%) | 80 | 67 | 1 | 0 | 3/3 (100) |
| Mutant | 1 | | 62 | 100 | 1 | 0 | 1/1 (100) |
| Wild-type | 2 | | 80 | 50 | 0 | 0 | 2/2 (100) |
| Anaplastic astrocytoma (Gr. 3) | 29 | 8/29 (28%) | 57 | 55 | 1 | 10 (34%) | 5/18 (28) |
| Mutant | 8 | | 46 | 50 | 1 | 5 n.s. | 2/5 (40) |
| Wild-type | 22 | | 60 | 57 | 0 | 5 | 3/13 (23) |
| Anaplastic oligoastrocytoma (Gr. 3) | 5 | 4/5 (80%) | 43 | 40 | 0 | 4 (80%) | 2/3 (66) |
| Mutant | 4 | | 48 | 25 | 0 | 3 n.s. | 2/2 (100) |
| Wild-type | 1 | | 11 | 100 | 0 | 1 | 0/1 (0) |
| Anaplastic oligodendroglioma (Gr. 3) | 15 | 10/15 (67%) | 62 | 56 | 10 (67%) | 0 | 6/7 (86) |
| Mutant | 10 | | 49 | 43 | 9* | 0 | 5/5 (100) |
| Wild-type | 5 | | 66 | 100 | 1 | 0 | 1/2 (50) |
| Diffuse astrocytoma (Gr. 2) | 29 | 17/29 (59%) | 32 | 61 | 3 | 13 (45%) | 10/17 (59) |
| Mutant | 17 | | 33 | 59 | 2 | 12*** | 10/10 (100) |
| Wild-type | 12 | | 30 | 64 | 1 | 1 | 0/7 (0) |
| Oligoastrocytoma (Gr. 2) | 7 | 4/7 (57%) | 44 | 71 | 1 | 1 | 5/6 (83) |
| Mutant | 4 | | 37 | 100 | 1 | 1 | 3/3 (100) |
| Wild-type | 3 | | 53 | 33 | 0 | 0 | 2/3 (67) |
| Oligodendroglioma (Gr. 2) | 25 | 19/25 (76%) | 46 | 52 | 19 (76%) | 3 | 8/12 (66) |
| Mutant | 19 | | 47 | 53 | 18** | 1 | 7/10 (70) |
| Wild-type | 6 | | 34 | 50 | 1 | 2 | 1/2 (50) |
| Pilocytic astrocytoma (Gr. 1) | 9 | 0% | 12 | 56 | 0 | 0 | N/A |
| Mutant | 0 | | N/A | N/A | 0 | 0 | |
| Wild-type | 9 | | 12 | 56 | 0 | 0 | |
| Ganglioglioma (Gr. 1) | 6 | 0% | 22 | 67 | 0 | 0 | N/A |
| Mutant | 0 | | N/A | N/A | 0 | 0 | |
| Wild-type | 6 | | 22 | 67 | 0 | 0 | |

*$P$ = 0.0037; **$P$ = 0.0001; ***$P$ = 0.0018. The association with *IDH* mutation (Fisher's exact test). GBM, glioblastoma; GBMO, glioblastoma with oligodendroglioma component; N/A, not analyzed; n.s., not significant.

**Table 2. Prognostic value of common genetic alterations for overall survival (OS) and progression-free survival (PFS) in gliomas (multivariate analysis)**

| | PFS | | | OS | | |
|---|---|---|---|---|---|---|
| | *P*-value | Hazard ratio | 95% CI | *P*-value | Hazard ratio | 95% CI |
| Grade 2 glioma | | | | | | |
| *IDH* mutation | 0.4408 | 0.602 | 0.1678–2.1535 | 0.1573 | 0.329 | 0.0728–1.5270 |
| 1p/19q co-deletion | 0.3591 | 0.495 | 0.1083–2.2020 | 0.7988 | 1.237 | 0.2353–6.0194 |
| *TP53* mutation | 0.2904 | 2.036 | 0.5526–7.7157 | 0.4693 | 0.537 | 0.0685–2.6350 |
| Grade 3 glioma | | | | | | |
| *IDH* mutation | <0.0001† | 0.059 | 0.0086–0.2395 | 0.0403† | 0.319 | 0.0985–0.9519 |
| 1p/19q co-deletion | 0.0016† | 0.055 | 0.0025–0.3904 | 0.0170† | 0.184 | 0.0271–0.7567 |
| *TP53* mutation | 0.4144 | 0.646 | 0.2045–1.7994 | 0.0300† | 0.294 | 0.0786–0.8937 |
| Primary GBM | | | | | | |
| *IDH* mutation | 0.8456 | 0.898 | 0.2575–2.4255 | 0.8560 | 0.905 | 0.2609–2.4203 |
| *TP53* mutation | 0.1533 | 0.605 | 0.2792–1.1944 | 0.3089 | 0.705 | 0.3354–1.3613 |
| Methylated *MGMT* promoter | 0.0031† | 0.407 | 0.2216–0.7375 | 0.0058† | 0.429 | 0.2324–0.7820 |

†Significant value. Cox proportional hazard modeling for OS or PFS was applied for the major variable for prognostic factors. CI, confidence interval; GBM, glioblastoma.

without 1p/19q co-deletion and with wild-type *IDH*. Grade 3 gliomas were assessed with regard to the association between the genetic alterations and disease course (Fig. 1). In these genetic subgroups, grade 3 gliomas without 1p/19q co-deletion and with wild-type *IDH* were revealed to have markedly worse OS ($P < 0.0001$) (Fig. 1C) and PFS ($P < 0.0001$) (Fig. 1D), but

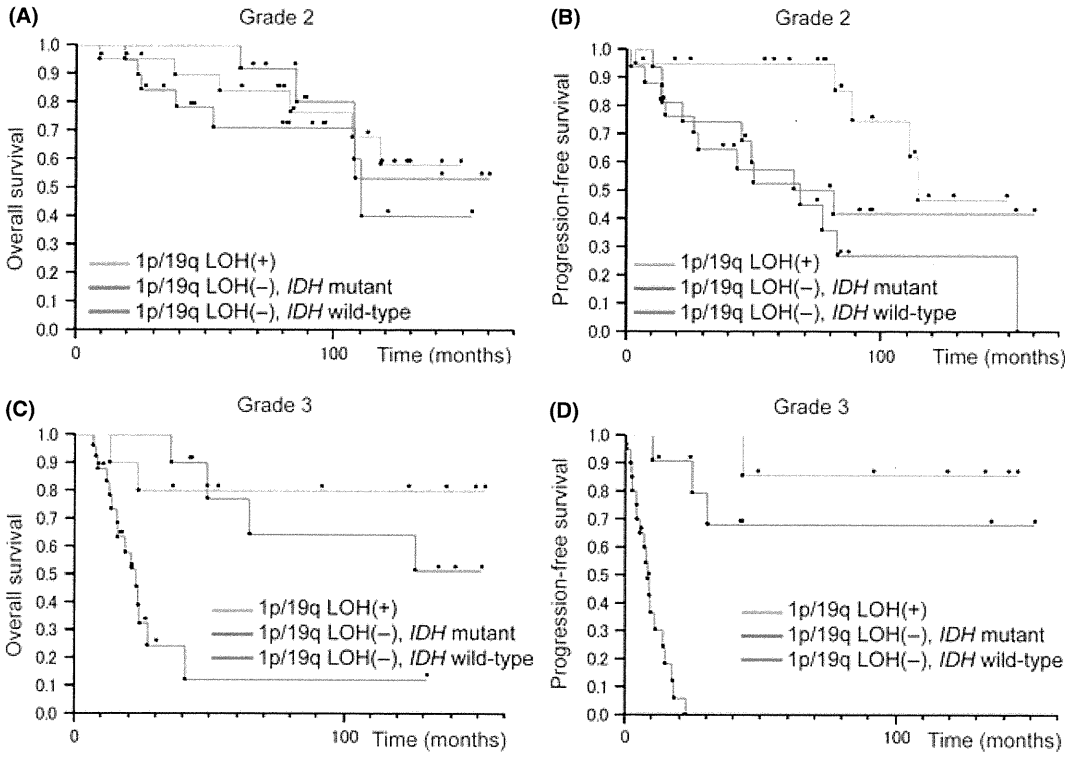


**Fig. 1.** Overall survival (OS) and progression-free survival (PFS) curves for patients with grade 2 and 3 gliomas with or without 1p/19q loss of heterozygosity (LOH) and/or isocitrate dehydrogenase (*IDH*) mutation. Overall survival (A) and PFS (B) in grade 2 gliomas; OS (C) and PFS (D) in grade 3 gliomas.

these lower survival rates were not observed for patients with grade 2 gliomas lacking 1p/19q co-deletion and *IDH* mutation (Fig. 1A,B). Grade 3 gliomas without 1p/19q co-deletion were predominantly AA (28 AAs, 5 AOAs, and 5 AOGs), and only 1 AA had the 1p/19q co-deletion (Table 1). The *IDH* mutation was significantly associated with increased OS ($P = 0.0064$) and PFS ($P = 0.0001$) for patients with AA based on the univariate analysis. *TP53* mutation was also correlated with increased PFS ($P = 0.013$), but *MGMT* promoter methylation showed no significant association with PFS or OS.

In primary GBM, our univariate analysis showed that neither *IDH* mutation, 1p/19q co-deletion, nor *TP53* mutation was associated with PFS or OS, but *MGMT* promoter methylation was significantly associated with increased OS ($P = 0.0043$) and PFS ($P = 0.0038$).

## Discussion

Here we report that *IDH* mutation, which was tightly associated with 1p/19q co-deletion and *MGMT* promoter methylation, was common in grade 2 gliomas and also, but to a lesser extent, in grade 3 gliomas. Moreover, we found that *IDH* mutation would be an especially useful genetic marker for evaluating the malignancy of grade 3 gliomas that do not have a 1p/19q co-deletion and that these gliomas were predominantly AA.

The frequencies and patterns of *IDH* mutation in our glioma samples from Japanese patients were largely comparable to those in previous reports.[2–7] *IDH* mutation was found predominantly in grade 2 glioma, such as DA, OA, and OG. *IDH* mutation frequencies were lower in higher-grade gliomas, and less than 10% of GBM had an *IDH* mutation; however, nearly half of secondary GBM, which developed from malignant transformation of lower-grade glioma, had an *IDH* mutation. These observations supported the notion that the *IDH* mutation has a crucial role in the development of the majority of grade 2 gliomas. In grade 3 gliomas, the oligodendroglial tumors had higher frequency of *IDH* mutation than astrocytic tumors (OG 76% > DA 59%, $P = 0.18$; AOG 67% > AA 28%, $P < 0.05$; Pearson's chi-square-test). No *IDH* mutation was detected in any grade 1 glioma, PA or GGL; this observation indicated that these tumors had a different genetic etiology from that of grade 2 and 3 infiltrative astrocytic and oligodendroglial tumors. This observation also supported the usefulness of *IDH* mutations along with BRAF alterations for differential diagnosis of PA.[27] However, our results differed from two previous reports that detected *IDH1* mutation (8–38%) in GGL.[5,28] Further studies are needed to clarify the biological and clinical significance of *IDH1* mutation in GGL.

As reported previously,[23] *TP53* mutation and co-deletion of chromosomes 1p and 19q were frequent alterations in grade 2 and grade 3 gliomas. The 1p/19q co-deletions were mostly found in oligodendroglial lineage gliomas, whereas *TP53* mutations were more frequent in gliomas derived from the astrocytic lineage. *IDH* mutation is currently believed to precede 1p/19q LOH and *TP53* mutation during the early stage of gliomagenesis,[4,6] and consistent with this hypothesis, most of our grade 2 gliomas that had 1p/19q co-deletion or *TP53* mutations also harbored an *IDH* mutation. In one study, all the gliomas with deletions of the entire 1p and 19q arms carried an *IDH1* or *IDH2* mutation;[26] however, we found a few exceptions in which there was 1p/19q LOH, but no *IDH* mutation. These apparent exceptions might have been artifacts due to our imperfect methods for detecting the extent of 1p/19q LOH, specifically microsatellite analysis or MLPA; these methods do not effectively differentiate partial chromosomal loss from typical entire 1p/19q hemizygous deletion, which is generally found in OG harboring *IDH* mutation. It would be better to carefully evaluate the extent of 1p/19q LOH in such exceptional cases. *TP53* mutation was also



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associated with *IDH* mutation in DA. However, there was no association between *IDH* and *TP53* mutation in AA or primary GBM. This observation suggested that *TP53* mutation promoted tumor growth independently of *IDH* mutation, especially in higher grade gliomas. Most gliomas with an *IDH* mutation had either 1p/19q LOH or *TP53* mutation, further supporting the hypothesis that combinations of *IDH* mutation and subsequent genetic alteration are common pathways leading to low-grade glioma. However, there were also a few other *IDH*-mutated gliomas that had neither 1p/19q co-deletion nor *TP53* mutation. In these gliomas, the kind of alterations subsequent to *IDH* mutation that caused progenitor cells to give rise to low-grade glioma remains to be elucidated.

Methylation at the *MGMT* promoter was associated with *IDH* mutation, especially for low-grade gliomas. Some IDH enzymes with a mutation in the catalytic domain acquire a novel enzymatic activity[9,11] that causes accumulation of 2-HG, and 2-HG is known to inhibit enzymes such as 5-methylcytosine hydroxylases and histone demethylases. As a result, *IDH* mutations bring about genome-wide hypermethylation, which might lead to tumor initiation.[12,29] Reportedly, the majority of low-grade gliomas have hypermethylated CpG islands throughout the genome; this phenomenon is called the glioma CpG island methylator phenotype, and these tumors frequently harbor *IDH* mutation.[30–32] Therefore, frequent *MGMT* promoter methylation in *IDH*-mutated low-grade glioma was possibly simply a reflection of hypermethylation of a plethora of genes resulting from the methylator phenotype. In contrast, the majority of GBM had *MGMT* promoter hypermethylation without also having *IDH* mutation, indicating that *MGMT* promoter methylation occurs independent of *IDH*-related hypermethylation in most GBM. Probably because of such a background, the prognostic values of *MGMT* promoter methylation for *IDH*-mutated and *IDH*-wild-type gliomas are not equal. In GBM, *MGMT* promoter methylation is a predictive factor for the efficacy of temozolomide, which is a common alkylating agent used in the chemotherapeutic treatment of malignant glioma.[33,34] However, the predictive value of *MGMT* promoter methylation for chemosensitivity in grade 2 and grade 3 glioma is controversial.[19,35]

The prognostic significance of *IDH* mutation differed among WHO tumor grades. Unlike previous reports, *IDH* mutation was not associated with the PFS or OS of our GBM patients; however, this finding might result from insufficient numbers of GBM patients with *IDH* mutation. A methylated *MGMT* promoter, which reflects the sensitivity of a tumor to temozolomide, was associated with favorable PFS and OS for patients with GBM, further emphasizing the importance of detecting *MGMT* promoter methylation status in GBM.

Among patients with grade 2 gliomas, *IDH* mutation was also not associated with prognosis. Wild-type *TP53* and 1p/19q co-deletion were each associated with prolonged PFS, probably because these two genetic alterations were mutually exclusive and tumors with wild-type *TP53* likely have a 1p/19q co-deletion, which is a recognized favorable prognostic factor. The prognostic value and predictability of temozolomide efficacy associated with *IDH* mutation in low-grade gliomas has been controversial. Consistent with our results, Kim *et al.*[20] showed that *IDH1* and *IDH2* mutations are not prognostic in low-grade gliomas, but that *TP53* mutation is a significant prognostic indicator of shorter survival and 1p/19q loss is prognostic of longer survival. However, Sanson *et al.*[15] reported a different result, specifically that *IDH1* mutation is associated with a better outcome in grade 2 gliomas. Dubbink *et al.*[18] showed that *IDH* mutation is associated with better outcomes for relapsed astrocytomas previously treated with radiotherapy, but there was no relationship between *IDH* mutation and temozolomide responsiveness. Houillier *et al.*[19] showed that *IDH1* or *IDH2* mutations predict better prognosis of glioma treated with temozolomide, but they did not appear to influence the course of untreated low-grade glioma. Thus, the prognostic value of *IDH* mutation is different from that of 1p/19q co-deletion, which is prognostic as well as predictive for responsiveness to temozolomide in low-grade gliomas. These inconsistent results on the association between *IDH* mutation and survival in cases of low-grade gliomas might be caused by the variable numbers of OG and DA included in these studies. Almost all oligodendroglial tumors with 1p/19q co-deletion also have an *IDH* mutation;[26] therefore, many cases of OG with favorable prognoses may affect and confound measurements of survival rate in the whole group of low-grade gliomas with *IDH* mutations. To avoid the confounding influence of OG, we also focused on DA with wild-type *IDH*; these tumors generally have neither 1p/19q LOH nor *TP53*. However, they had outcomes comparable to those of DA with *IDH* mutation. This finding indicated that DA with wild-type *IDH* was not more malignant than DA with an *IDH* mutation. This observation differed from the observation that AA with wild-type *IDH* had markedly worse outcomes than AA with an *IDH* mutation (OS, $P = 0.0064$; PFS, $P = 0.0001$).

In contrast with grade 2 and 4 gliomas, the prognostic significance of *IDH* mutation was evident for grade 3 gliomas, and this finding was consistent with previous reports.[15,17,36] Almost all gliomas with 1p/19q co-deletion have an *IDH* mutation,[26] and anaplastic oligodendroglial tumors often harbor 1p/19q co-deletion; therefore, monitoring of *IDH* mutation might have more clinical significance for patients with grade 3 gliomas with intact 1p/19q, and these tumors are predominantly AA. In fact, as the histopathological differential diagnosis of AA from GBM or DA is often subjective and diagnoses frequently differ between pathologists,[23] a pathological diagnosis of AA may not always indicate sameness between gliomas and similar prognosis. However, accurate determination of the pathological group of a tumor is clinically critical for planning adjuvant therapy, such as radiation and chemotherapy. Therefore, genetic analyses, which may reflect causative origins of tumors, are expected to reveal biological traits with less inter-observer variation, as is the case of 1p/19q co-deletion in oligodendroglial tumors. Because *IDH* mutations have a defined role in gliomagenesis and indicate, to some extent, the nature of the original tumor cell, monitoring *IDH* mutational status may allow for accurate assignment of diagnosed AA to low-grade gliomas that frequently harbor *IDH* mutation or to primary GBM that usually have intact *IDH*. Therefore, we believe that monitoring *IDH* mutation in combination with 1p/19q co-deletion, which genetically differentiates oligodendroglial and astrocytic tumors, could be a useful genetic marker of prognostic value, especially for grade 3 glioma patients.


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## Disclosure Statement

The authors have no conflicts of interest.

## Abbreviations

| | |
|---|---|
| 2-HG | 2-hydroxyglutarate |
| AA | anaplastic astrocytoma |
| AOA | anaplastic oligoastrocytoma |
| AOG | anaplastic oligodendroglioma |

| | |
|---|---|
| DA | diffuse astrocytoma |
| GBM | glioblastoma |
| GBMO | glioblastoma with oligodendroglioma component |
| GGL | ganglioglioma |
| IDH | isocitrate dehydrogenase |
| LOH | loss of heterozygosity |
| MLPA | multiplex ligation-dependent probe amplification |
| OA | oligoastrocytoma |
| OG | oligodendroglioma |
| OS | overall survival |
| PA | pilocytic astrocytoma |
| PFS | progression-free survival |

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# Reactivation of Hepatitis B Virus After Glioblastoma Treatment With Temozolomide

## —Case Report—

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## Abstract

**A 61-year-old man with glioblastoma and positive for hepatitis B surface antigen (HBsAg) developed acute hepatitis due to hepatitis B virus (HBV) reactivation after concomitant postoperative treatment with temozolomide (75 mg/m$^2$/day) and radiation therapy (60 Gy in 30 fractions). Corticosteroids were not used during chemo-radiation therapy, and grade 4 lymphocytopenia was observed. The levels of liver function tests (LFTs), including levels of aspartate aminotransferase and alanine aminotransferase, increased 5 weeks after the completion of chemo-radiation therapy, and reached the maximum levels of 1,549 IU/l (normal 13 to 33 IU/l) and 1,653 IU/l (normal 8 to 42 IU/l), respectively, after 2 weeks. At this point, serum HBV-deoxyribonucleic acid (DNA) level had increased to 630-fold over the baseline, and therapy with the antivirus agent entecavir (0.5 mg daily) was started. Over the next 2 weeks, the levels of LFTs and HBV-DNA improved. The present and previous cases suggest that grade 3/4 lymphocytopenia or grade 2 lymphocytopenia with corticosteroid use might have a significant effect on HBV reactivation. To avoid this complication, HBsAg-positive patients with glioblastoma should consult a hepatologist for initiating antivirus therapy before temozolomide treatment.**

Key words: hepatitis B virus, reactivation, glioblastoma, temozolomide, immunosuppression

## Introduction

The reactivation of hepatitis B virus (HBV) is a well-recognized complication of cytotoxic chemotherapy for malignant disease. HBV reactivation usually occurs in patients with hematological malignancies, but is also known in patients with solid tumors, including breast cancer, gastrointestinal cancer, and lung cancer.[1,2]

Temozolomide is an alkylating agent that exerts cytotoxic activity by inducing deoxyribonucleic acid (DNA) damage and apoptosis of tumor cells,[7] and is part of the standard postoperative chemotherapy for the treatment of glioblastoma.[9] Temozolomide carries the risk of HBV reactivation,[1,2] but few cases of temozolomide-induced HBV reactivation have been reported, so the incidence and associated risk factors, and the optimal management of glioblastoma patients with chronic HBV infection remain unclear.

We treated a patient with glioblastoma who was positive for hepatitis B surface antigen (HBsAg) and developed acute hepatitis due to HBV reactivation during temozolomide treatment, and discuss the management of patients with glioblastoma who have chronic HBV infection.

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## Case Report

A 61-year-old man presented with generalized convulsions. He had been informed of his HBV carrier status but had not received any treatment. On admission, he tested positive for HBsAg and hepatitis B envelope (HBe) antibody, and negative for HBe antigen, hepatitis C virus antibody, and human immunodeficiency virus. The blood HBV-DNA concentration was $10^3$ copies/ml. Magnetic resonance imaging of the brain showed a tumor in the bilateral frontal lobes involving the corpus callosum (Fig. 1A). The patient presented with slight disorientation and left hemiparesis. Partial tumor removal was achieved through a right frontal craniotomy, and the histological diagnosis was glioblastoma (Fig. 1B).

Betamethasone 8 mg was intravenously administered for 3 days following the operation. Ten days after resection, local radiation therapy (60 Gy in 30 fractions over 6 weeks) and temozolomide chemotherapy (75 mg/m$^2$/day) were initiated. Before chemotherapy and radiotherapy, the liver function tests (LFTs) were normal: aspartate aminotransferase (AST) was 26 IU/l (normal 13 to 33 IU/l), and alanine aminotransferase (ALT) was 28 IU/l (normal 8 to 42 IU/l). During chemo-radiation therapy, the lowest measured white blood cell count was 2900/$\mu$l, absolute neutrophil count was 2240/$\mu$l, and absolute lymphocyte

count was 190/μl. Four weeks after the completion of chemo-radiation therapy, the levels of LFTs started to increase, and one week later continued to deteriorate. AST increased to 685 IU/l and ALT increased to 744 IU/l. At this time we consulted with a hepatologist to determine the cause of the LFT changes. Abdominal computed tomography (CT) with contrast medium revealed a mass lesion in the liver (Fig. 2). Alpha fetoprotein (AFP) and protein induced by vitamin K or antagonists-II (PIVKA-II) were elevated to 257.6 ng/ml (normal <10.0 ng/ml) and 8,349 mAU/ml (normal <40 mAU/ml), respectively.

Our diagnosis was hepatocellular carcinoma (HCC) that had possibly developed before temozolomide treatment. Further, since the HCC was not obstructing the bile duct, we thought that the HCC was not the cause of the LFT changes. The patient's medication regimen at initial presentation consisted of valproic acid, propranolol, and famotidine. After the LFT changes, we stopped administration of famotidine but continued valproic acid and propranolol. The HBV-DNA level increased to $10^{5.8}$ copies/ml. On the basis of the laboratory data and radiological findings, we determined that temozolomide-induced HBV reactivation was the main cause of the LFT changes and acute hepatitis, although the possibility of drug-induced hepatitis or HCC-related LFT change was not completely excluded. Accordingly, we started treatment with the antivirus agent entecavir (0.5 mg daily). For 5 days after the start of entecavir treatment, AST and ALT continued to increase to the maximum levels of 1,549 IU/l and 1,653 IU/l, respectively, but thereafter improved markedly. Two weeks after the start of entecavir treatment, the LFTs returned to almost normal levels. The HBV-DNA level also decreased to $10^{1.8}$ copies/ml (Fig. 3).

After normalization of the LFTs, we started treatment with adjuvant temozolomide at 150 mg/m$^2$ daily for 5 days/28 days while continuing entecavir therapy. The second cycle used 200 mg/m$^2$ daily for 5 days/28 days, and no further elevation of LFTs and HBV-DNA level was observed, even though the lowest lymphocyte count was 110/μl (Fig. 3). Four weeks after the onset of LFT changes, the levels of AFP and PIVKA-II were 405.2 ng/ml and 10,195 mAU/ml, respectively, and continued to exacerbate his condition. Transarterial embolization was performed for the treatment of HCC. Three weeks later, the patient's AFP and PIVKA-II levels improved, decreasing to 166.4 ng/ml and 182 mAU/ml, respectively. However, after sec-

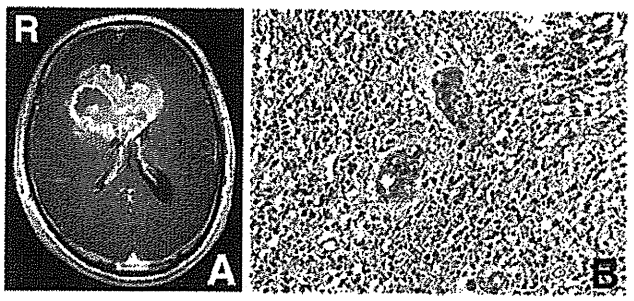


**Fig. 1 A: Preoperative $T_1$-weighted magnetic resonance image with contrast medium showing a tumor in the bilateral frontal lobes involving the corpus callosum. B: Photomicrograph of the tumor specimen showing glioblastoma with cellular anaplasia and prominent microvascular proliferation. Hematoxylin and eosin stain, original magnification ×200.**

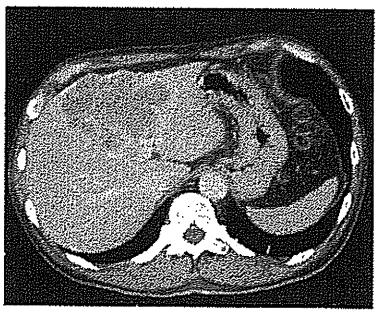

**Fig. 2 Abdominal computed tomography scan with late phase contrast enhancement showing a low density mass lesion of 9-cm diameter in the liver.**

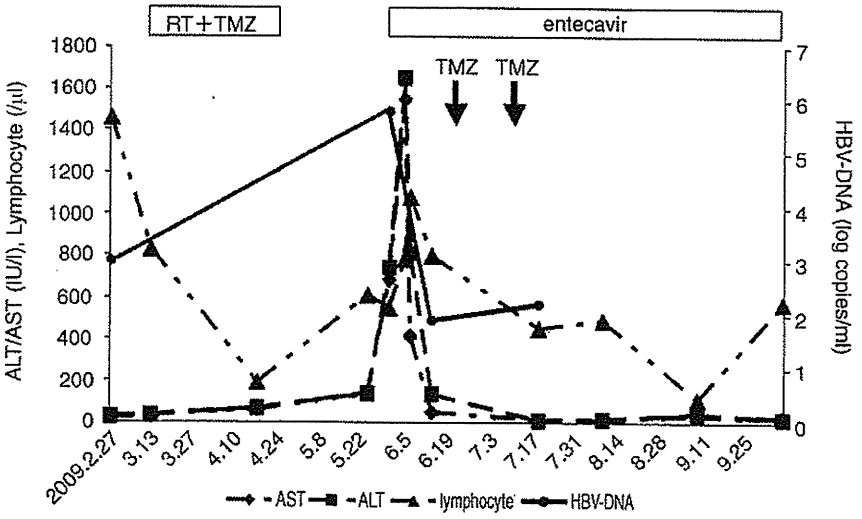


**Fig. 3 Time courses of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lymphocytes, and hepatitis B virus-deoxyribonucleic acid (HBV-DNA) levels. Four weeks after completion of concomitant temozolomide and radiation therapy (RT + TMZ), AST and ALT began to increase and continued to increase to a maximum of 1,549 IU/l and 1,653 IU/l, respectively. HBV-DNA level increased to $10^{5.8}$ copies/ml. After entecavir administration, ALT, AST, and HBV-DNA levels improved during the ensuing 2 weeks. After normalization of liver function, two cycles of adjuvant temozolomide (arrows) were initiated while continuing entecavir, without further elevation of AST and ALT even though the lowest lymphocyte count was 110/μl.**

ond transarterial embolization, he developed conscious disturbance, and was transferred to a nursing hospital 6 months after the completion of chemo-radiation therapy. Twelve months after the neurosurgical operation, he died of glioblastoma progression.

## Discussion

The reactivation of HBV by immunosuppressive agents is characterized by increased levels of serum HBV-DNA, abnormal LFTs, and clinical hepatitis of varying degrees of severity, which may result in death.[13] HBV reactivation occurs in 38–48% of HBsAg-positive patients with lymphoma or other hematological malignancies, who are undergoing conventional therapies, including cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP).[4] The risk factors for HBV reactivation include male sex, young age, steroid use, anthracycline use, high pre-chemotherapy HBV-DNA level, and diagnosis of lymphoma or breast cancer.[3,12,13] Two possible mechanisms may explain HBV reactivation during chemotherapy: immunosuppression enhances virus replication, leading to hepatic toxicity, or chemotherapy-induced T-cell depletion dampens the host response to viral antigens, which enables broader hepatocyte infection, and following the subsequent withdrawal of cytotoxic chemotherapy, a rebound immune response results in hepatocyte destruction.[12]

HBV reactivation-induced hepatitis has been defined as an increase in HBV-DNA level to 10-fold or more when compared with the baseline level, or as an absolute increase in HBV-DNA level to more than $1,000 \times 10^5$ genome equivalents/ml in the absence of other systemic infections.[11] In our patient, the HBV-DNA level increased by 630-fold over the baseline, when the LFTs were elevated, indicating HBV reactivation. However, we could not completely exclude the possibility of drug-induced hepatitis or HCC-related LFT elevation, because the patient had received medication (valproic acid, propranolol, and famotidine) during chemotherapy just before the LFT changes and had underlying HCC. However, the patient continued to receive valproic acid and propranolol even after the LFTs were elevated. Abdominal CT did not reveal bile duct stenosis due to HCC, and both the LFTs and HBV-DNA level improved shortly after entecavir treatment before HCC therapy. Therefore, we presume that the possibility of drug-induced hepatitis or HCC-related increase in LFTs is much lower than that of HBV reactivation, although famotidine-induced hepatitis remains much less likely. Famotidine is also known to induce agranulocytosis[5] and can cause immunosuppression. However, the lowest white blood cell count and absolute neutrophil count in our patient were 2900/μl and 2240/μl, respectively, so the possibility of famotidine-induced agranulocytosis leading to HBV reactivation was thought to be quite low.

HBV infection is one of the causative factors in the development of HCC, and the patient probably had HCC before temozolomide treatment. However, HCC was unlikely to be involved in the development of HBV reactivation after temozolomide treatment, because HCC was localized at the time of increases in LFTs and did not impair the patient's general condition, including the immune system.

Only two cases of HBV reactivation after temozolomide treatment for glioblastoma have been reported (Table 1).[1,2] A 65-year-old woman with glioblastoma presented with HBV reactivation on day 27 of cycle 3 of adjuvant temozolomide therapy and died 2 weeks after the onset.[2] She had a remote history of hepatitis B infection but did not undergo hepatitis examination before starting treatment. She did not receive steroid medication before the onset of HBV reactivation, and her lowest lymphocyte count was 450/μl. A 50-year-old HBsAg-positive man with glioblastoma presented with HBV reactivation 5 weeks after the completion of concomitant radiotherapy and temozolomide.[1] He was successfully treated with the antiviral agent lamivudine over the ensuing 7 weeks. He was treated with 4 mg of dexamethasone during radiation therapy and 2 mg just before the onset of HBV reactivation. His lowest lymphocyte count was 580/μl. Our patient developed the symptoms 4 weeks after completing concomitant radiotherapy and temozolomide, and was successfully treated with entecavir during the ensuing 2 weeks. He had no steroid exposure before the onset of HBV reactivation, and his lowest lymphocyte count was 190/μl. All these cases suggest that grade 3/4 lymphocytopenia or grade 2 lymphocytopenia with corticosteroid use might have a significant effect on the development of HBV reactivation. The guidelines issued in the Joint Report of the Intractable Liver Disease Study Group of Japan and the Japanese Study Group of the Standard Antiviral Therapy for Viral Hepatitis recommend that all patients should be screened for HBsAg, and anti-hepatitis B core and anti-HBs antibodies before chemotherapy is initiated. HBsAg-positive patients should be advised to consult a hepatologist for initiating antiviral therapy, such as entecavir before starting chemotherapy.[10]

HBV reactivation after chemotherapy with temozolomide may be a rare complication. However, temozolomide is associated with $CD4^+$ T-cell dysfunction and therefore may cause increased susceptibility to opportunistic infections by agents such as *Pneumocystis* pneumonia.[8] This characteristic immunosuppression may also induce HBV reactivation. In the Japanese population, 1.4% of individuals are positive for HBsAg,[6] so HBV reactivation during glioblastoma treatment with temozolomide may become a critical issue. To avoid this complication, patients with glioblastoma should be screened for hepatitis B, and HBsAg-positive patients should be referred to a hepatologist for initiating antivirus therapy before starting temozolomide treatment. Moreover, HBV reactivation should be included in the differential diagnosis of patients with elevated LFTs.

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**Table 1 Summary of three cases of hepatitis B virus (HBV) reactivation induced by temozolomide (TMZ)**

| Author (Year) | Age (yrs)/ Sex | HBV status | Diagnosis | Onset | Nadir | | | Steroid | Status of hepatitis |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | WBC (/μl) | Neutrophil (/μl) | Lymphocyte (/μl) | | |
| Grewal et al. [2007][2] | 65/F | remote history of HBV infection, not determined by laboratory test | GBM | day 27 of cycle 3 | N/A | 1250 | 450 | stop 1 week after operation | died of hepatitis 2 weeks after onset |
| Chheda et al. [2007][11] | 50/M | HBsAg (+) | GBM | 5 weeks after completion of TMZ concomitant with RT | 5300 | 3890 | 580 | dexamethasone 4 mg 5 weeks before onset and 2 mg at onset | successfully treated with lamivudine over the next 7 weeks |
| Present case | 61/M | HBsAg (+) | GBM | 4 weeks after completion of TMZ concomitant with RT | 2900 | 2240 | 190 | stop 3 days after operation | successfully treated with entecavir over the next 2 weeks |

F: female, GBM: glioblastoma, HBsAg: hepatitis B surface antigen, M: male, N/A: not available, RT: radiation therapy, WBC: white blood cell.

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ORIGINAL ARTICLE

# Pathological findings and prognostic factors in recurrent glioblastomas

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**Abstract** Glioblastomas, which are the most common primary intracranial tumor, are associated with the poorest survival time, which is typically 1–2 years. Age at initial diagnosis, Karnofsky performance score, and $O^6$-methylguanine DNA-methyltransferase (*MGMT*) promoter methylation status are the most well-documented predictors of survival in patients with newly diagnosed glioblastoma. Few studies have examined prognostic factors in patients with recurrent glioblastomas. At relapse, the pathological features of glioblastomas are affected by tumor regrowth and the influence of chemoradiotherapy during the initial treatment. Morphological transformations at recurrence include quantitative changes in tumor cells, such as the presence of giant cells and gemistocytic cell formation, radiation necrosis, and vascular structural changes. Therefore, we should carefully examine pathological findings at recurrence. In this report, we analyzed *MGMT* promoter status, the MIB-1 index, and the pathology of tumor samples at the first (primary tumor) and second (recurrent tumor) surgeries and clarified prognostic factors in patients with recurrent cases. In the multivariate analysis, we showed that MIB-1 indexes at the time of the second surgery ($p = 0.004$) persisted as a significant independent prognostic factor in survival of patients with recurrent glioblastoma.

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**Keywords** Recurrent glioblastoma · MIB-1 index · *MGMT* promoter methylation status · Prognostic factor

## Introduction

Temozolomide (TMZ) is the standard therapy for patients with glioblastomas [1]. A recent study showed an improvement in median survival time (MST) from 12.1 to 14.6 months with the addition of concurrent TMZ to the previous standard therapy of surgery and radiotherapy in patients with glioblastomas [1]. Age at diagnosis, Karnofsky performance score (KPS), extent of surgical resection, and *MGMT* promoter methylation status have been well-documented prognostic factors of survival in patients with newly diagnosed glioblastomas [2–6]. Only a few studies have reported prognostic factors in patients with recurrent glioblastomas. The initial histology of the glioblastoma, increased patient age, KPS <80, and corticosteroid use have been reported to be poor prognostic factors for survival in patients with recurrent gliomas [7]. However, the prognostic factors of recurrent glioblastomas remain unclear. Higher MIB-1 indexes of gliomas have been demonstrated to correlate well with poorer survival time [4, 8, 9]. However, MIB-1 indexes of glioblastomas at the first surgery do not predict overall survival or the response to adjuvant therapy as an independent risk factor [10], and the significance of MIB-1 indexes in glioblastomas remains unclear. Methylated $O^6$-methylguanine DNA-methyltransferase (*MGMT*) promoter status could serve as a good prognostic factor for glioblastomas [11]. Variations in *MGMT* promoter methylation can occur within the same tumor after treatment [12, 13]. However, the prognostic value of variations in *MGMT* promoter methylation before and after chemoradiotherapy has been estimated in only a

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few reports [14]. Giant cells, gemistocytic cell formation, and coagulation necrosis are often found in recurrent gliomas, and these findings suggest the presence of degenerative changes in tumor cells that are caused by hypoxia, irradiation, and chemotherapy [15]. Therefore, we also estimated the degenerative changes of tumor cells that are influenced by chemoradiotherapy in order to determine whether these degenerative changes may be a prognostic factor. Pathological features of glioblastomas at recurrence are affected by tumor regrowth and the influences of irradiation and chemotherapy during the initial treatment. Morphological transformations at recurrence include quantitative changes of tumor cells, radiation necrosis, and vascular structural changes [15–17]. Therefore, it is very difficult to estimate pathological findings at recurrence. In this study, we analyzed a number of prognostic factors, including MIB-1 indexes, methylation statuses of the *MGMT* promoter, and pathological findings in patients with recurrent glioblastomas.

## Materials and methods

### Patient and tissue collections

One hundred eighty-nine patients with glioblastoma were treated from 1996 to 2010 at our institute. Thirty-two patients (16.9%) were diagnosed initially with glioblastomas from 1996 to March 2010 and underwent second surgical resections for recurrence in the National Cancer Center Hospital. The recurrent surgical cases did not include any case of glioblastoma with oligodendroglial component (GBMO). Those patients underwent surgery twice or more during the treatment period of 1996–2010. They underwent initial surgeries, followed by chemoradiotherapy with nimustine hydrochloride (ACNU) or TMZ. Tumor samples were analyzed from primary and recurrent resected tumors; however, not all primary tumor samples resected in other hospitals were obtained. We only evaluated tumor samples with sufficient specimens for immunohistochemistry and DNA extraction. The MIB-1 index and *MGMT* promoter methylation status of the tumor samples from the first (primary tumor) and second (recurrent tumor) surgeries were determined. The presence of degenerative changes in the tumors, including pseudopalisading necrosis, coagulation necrosis, gemistocytic cells, and giant cells, was observed. The internal review board of the National Cancer Center approved this study. We defined the first progression-free survival (PFS) time as the time from the first operation to the first recurrence, and the second PFS was defined as the time from the second operation to the second recurrence. Detailed information on all 32 patients is listed in Table 1.

**Table 1** Characteristics of patients with recurrent glioblastoma

| Characteristic | Number of patients | Percent |
|---|---|---|
| Sex | | |
| Male | 20 | 62.5 |
| Female | 12 | 37.5 |
| Age (years) | | |
| Median | 57 | |
| Range | 19–71 | |
| Extent of removal at the first surgery | | |
| Total removal | 11 | 34.4 |
| Subtotal removal | 5 | 15.6 |
| Partial removal | 11 | 34.4 |
| Biopsy | 5 | 15.6 |
| Extent of removal at the second surgery | | |
| Total removal | 5 | 15.6 |
| Subtotal removal | 5 | 15.6 |
| Partial removal | 20 | 62.5 |
| Biopsy | 2 | 6.3 |
| MIB-1 index at the first surgery (%) | | |
| Median | 22.5 | |
| Range | 6.8–90.0 | |
| MIB-1 index at the second surgery (%) | | |
| Median | 13.2 | |
| Range | 0.6–85.7 | |
| *MGMT* promoter status at the first surgery | | |
| Methylated | 6 | 31.6 |
| Unmethylated | 13 | 68.4 |
| *MGMT* promoter status at the second surgery | | |
| Methylated | 5 | 21.7 |
| Unmethylated | 18 | 78.3 |
| Initial chemotherapy | | |
| ACNU | 20 | 62.5 |
| TMZ | 12 | 37.5 |
| First PFS (months) | | |
| Median | 6.2 | |
| Range | 2.7–47.1 | |
| Second PFS (months) | | |
| Median | 6.9 | |
| Range | 0.6–68.3 | |
| Overall survival (months) | | |
| Median | 19.6 | |
| Range | 7.8–72.2 | |

*ACNU* nimustine hydrochloride, *TMZ* temozolomide, *PFS* progression-free survival

### Histopathological analysis

Surgical specimens were fixed in 10% formalin and embedded in paraffin. Hematoxylin-and-eosin (H&E)-stained specimens were examined to determine histological tumor type. Degenerative changes, including pseudopalisading necrosis,