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Fluorescence In Situ Hybridization（FISH）Cut-off Value: Loss of Heterozygosity（LOH）on 1p and 19q in Oligodendroglial and Oligoastrocytic Tumors and Glioblastomas with an Oligodendroglial Component

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Abstract

Loss of heterozygosity（LOH）on 1p and 19q by fluorescence in situ hybridization（FISH）is increasingly performed to confirm histological diagnosis and to direct treatment for oligodendroglial and oligoastrocytic tumors and for glioblastomas that have oligodendroglial components（GBM-O）. However, each neuropathology laboratory sets own cut-off value for 1p and 19q LOH. The aim of this study was to determine the cut-off values for 1p and 19q LOH in our institution. We assessed overall survival（OS）in 35 cases, including oligodendrogliomas（OD: n=7）, oligoastrocytomas（OA: n=13）, anaplastic oligodendroglioma（AO: n=1）, anaplastic oligoastrocytomas（AOA: n=5）, and GBM-O（n=9）. The mean values for 1p and 19q LOH by FISH was 77.5% and 69.9%, respectively, in OD, 69.0% and 66.9%, respectively, in OA, 90.4% and 84.3%, respectively, in AO, 45.0% and 40.0%, respectively, in AOA, 55.9% and 46.0%, respectively, in GBM-O. The five-year overall survival of 35 cases was 60.7%. The group with greater than 60.0% LOH for 1p and 19q showed significantly longer overall survival than the group with less than 60.0% LOH for 1p or 19q（p=0.008）. Thus, 60% was a reliable cut-off value for 1p and 19q LOH by FISH.

Key words：**Oligodendroglial Tumor, Cut-off Value, Chromosome 1p and 19q, Loss of Heterozygosity, Fluorescence In Situ Hybridization**

Introduction

The incidences of loss of heterozygosity（LOH）for loci on 1p and 19q are particularly high in oligodendroglial tumors.[1)] Combined loss involving chromosomes 1p and 19q is statistically significantly associated with both chemosensitivity and longer recurrence-free survival after chemotherapy. Moreover, losses involving both chromosomes 1p and 19q are strongly associated with longer overall survival according to univariate and multivariate analyses.[2)] Fluorescence in situ hybridization（FISH）is the most common technique for detecting LOH at 1p and 19q.[3)] However, many neuropathology laboratories set their own cut-off values.[3),4),5)]

The present retrospective study was designed to identify a reliable cut-off value for 1p and 19q LOH in our institution. We examined histopathologically diagnosed oligodendroglial and oligoastrocytic tumors and glioblastomas with oligodendroglial components（GBM-O）and determined the 1p and 19q LOH FISH values that influenced overall survival after resection.

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Materials and Methods

Patients

We reviewed 35 cases of oligodendroglial and oligoastrocytic tumors and GBM-O, which had been surgically resected at the Department of Neurosurgery, Fukuoka University Hospital between 2000 and 2012. Recurrent cases were excluded. The age and sex of each patient was retrieved from the medical record. The follow-up period ranged from 0-146 months (median, 22 months) after surgery.

Pathological evaluation

Tissue sections were cut 4-μm thick and stained with hematoxylin and eosin (H&E). Oligodendroglial and oligoastrocytic tumors were classified as oligodendroglioma

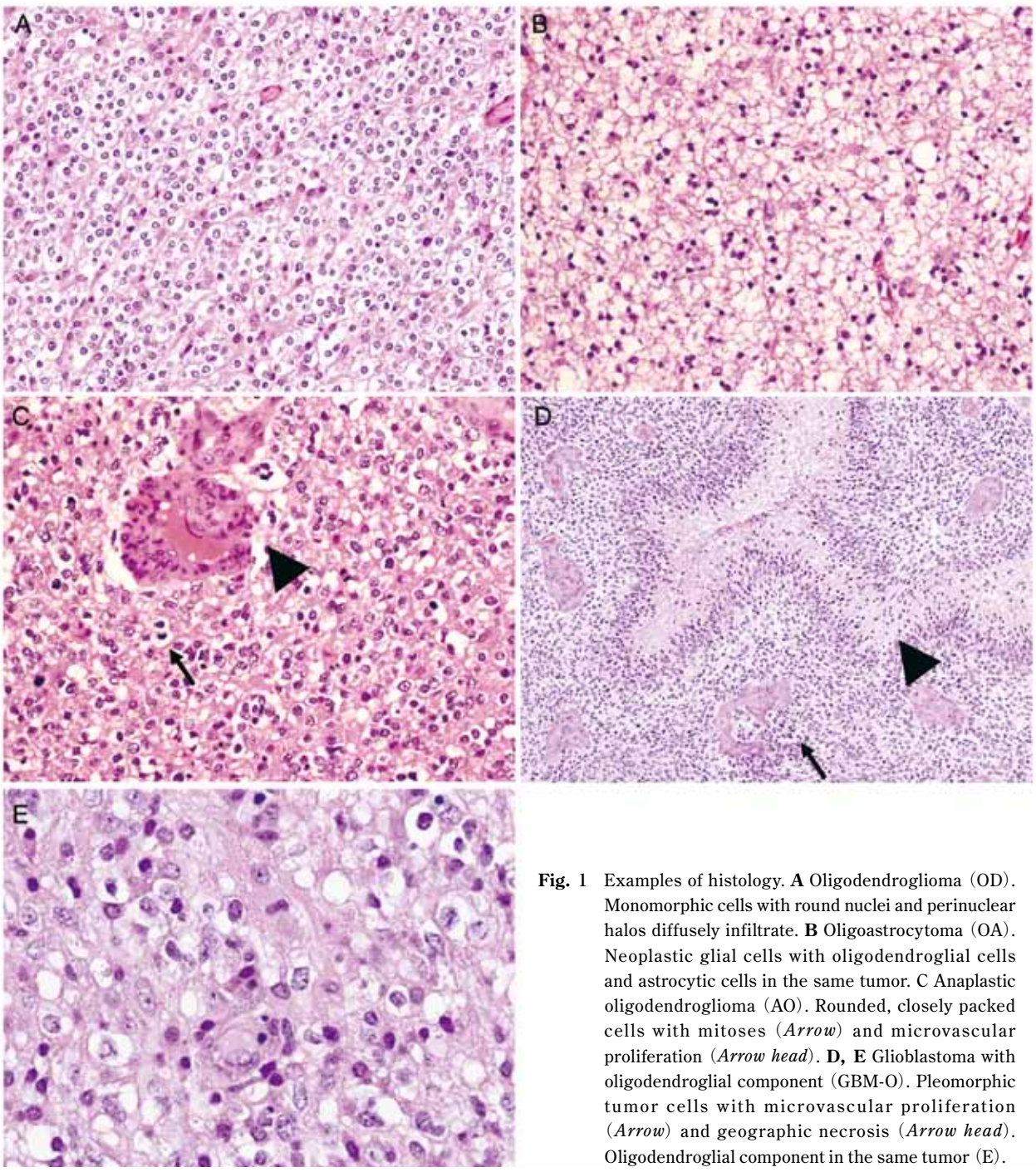

Fig. 1 Examples of histology. **A** Oligodendroglioma (OD). Monomorphic cells with round nuclei and perinuclear halos diffusely infiltrate. **B** Oligoastrocytoma (OA). Neoplastic glial cells with oligodendroglial cells and astrocytic cells in the same tumor. C Anaplastic oligodendroglioma (AO). Rounded, closely packed cells with mitoses (*Arrow*) and microvascular proliferation (*Arrow head*). **D, E** Glioblastoma with oligodendroglial component (GBM-O). Pleomorphic tumor cells with microvascular proliferation (*Arrow*) and geographic necrosis (*Arrow head*). Oligodendroglial component in the same tumor (E).

(OD) (Fig. 1A), oligoastrocytoma (OA) (Fig. 1B), anaplastic oligodendroglioma (AO) (Fig. 1C), or anaplastic oligoastrocytoma (AOA) according to the World Health Organization (WHO) classification of central nervous system tumors.[6] Glioblastomas with oligodendroglial components (GBM-O) (Fig. 1D, E) were also included.

FISH analysis for 1p and 19q LOH

Two-color FISH was performed with 1p36/1q25 and 19p13/19q13 dual -color probes (Vysis, Inc., Applied Biosystems, Downers Grove, IL, USA). Briefly, sections were deparaffinized and rehydrated with descending alcohol dilutions. This was followed by treatment with 2 × saline-sodium citrate (SSC) containing 0.3% Tween 20 (Sigma, St Louis, Mo) at 37℃ for 1 to 12 hours. Sections were then incubated in pretreatment solution (Histology FISH Accessory Kit; Dako, Carpinteria, Calif; 20 × dilution) at 95℃ for 10 minutes, and digested with pepsin solution (DAKO, 1 × dilution) at 37℃ for 25 minutes. After refixing in 10% buffered formalin at room temperature (RT) for three minutes, tissue sections were treated in 2 × SSC containing 0.3% Tween 20 at 43 ℃ for 10 minutes, dehydrated in ethanol, dried, and exposed to the two probes. The probes and tissue sections were denatured at 85℃ for five minutes in probe solution, which was included with the Kit (Abbott Japan), followed by hybridization at 37℃ for 20 hours in ThermoBrite (Abbott Japan). The tissue sections were washed in 2 × SSC containing 0.3% Tween 20 at 75℃ for two minutes and in 2 × SSC containing 0.1% Tween 20 at RT for five minutes. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI)/antifade (Vector Laboratories, Burlingame, Calif). Analyses were performed with a fluorescence microscope (Axio Imager Z1; Carl Zeiss Microimaging, Jena, Germany) and Isis analysis system (Metasystems, Altlussheim, Germany), which were equipped with filter sets with single- and dual-band excitors for Spectrum Green, Spectrum Orange, and DAPI (ultraviolet wavelength=360 nm). The signals of 100 – 200, non-overlapping intact tumor nuclei were assessed in each tumor case. A FISH result was considered positive if the ratio of target signal (red)/ reference signal (green) was within 1/2 for both chromosomes 1 and 19 (Fig. 2). Results were expressed as percentages.

Statistical analysis

Survival curves were plotted by the Kaplan-Meier method, and p values were calculated with the log-rank test. A p value of <0.05 denoted the presence of a statistically significant difference. All statistical evaluations were performed with SPSS II (SPSS Japan Inc., Tokyo, Japan) software.

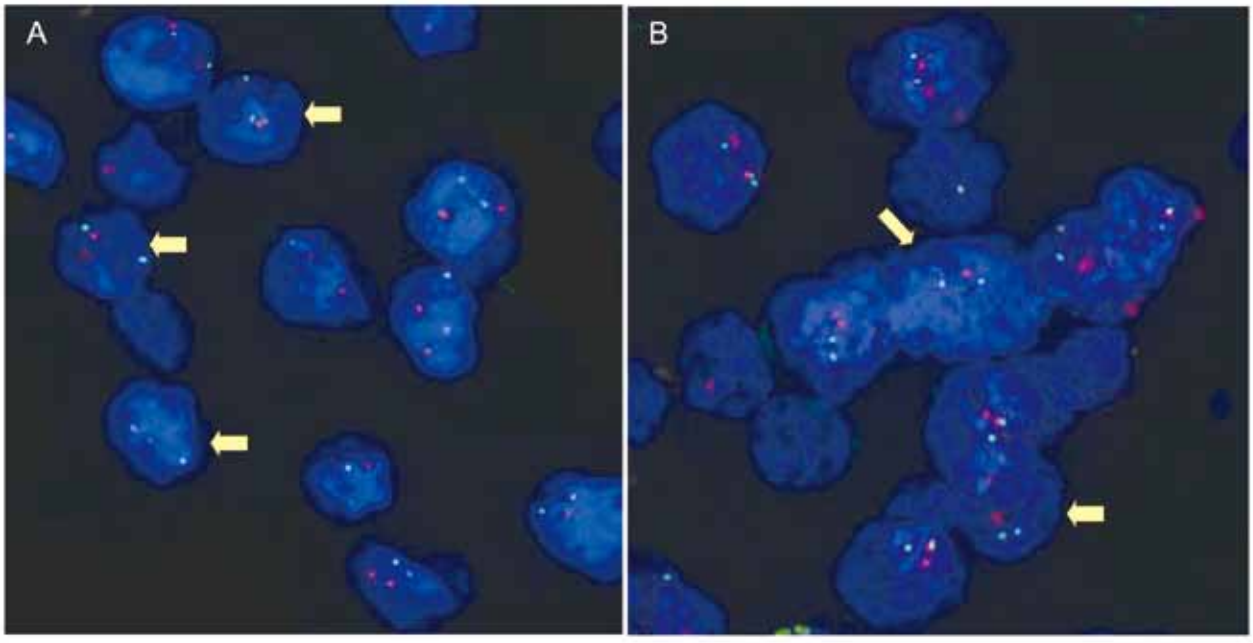

Fig. 2 Example of FISH analysis for 1p and 19q LOH. LOH was 35.5% for 1p (A) and 24.8% for 19q (B) in OA. *Arrow*, loss of heterozygosity (LOH).

Table 1 Clinicopathological characteristics of patients (n = 35).

Age	mean age was 48.7 years (range, 5-86)
Male/Female	19 (54.3%) / 16 (46.7%)
Oligodendroglioma (OD)	7 (20.0%)
Oligoastrocytoma (OA)	13 (37.1%)
anaplastic oligodendroglioma (AO)	1 (2.7%)
anaplastic oligoastrocytoma (AOA)	5 (14.3%)
Glioblastoma with oligodendroglial component (GBM-O)	9 (25.7%)

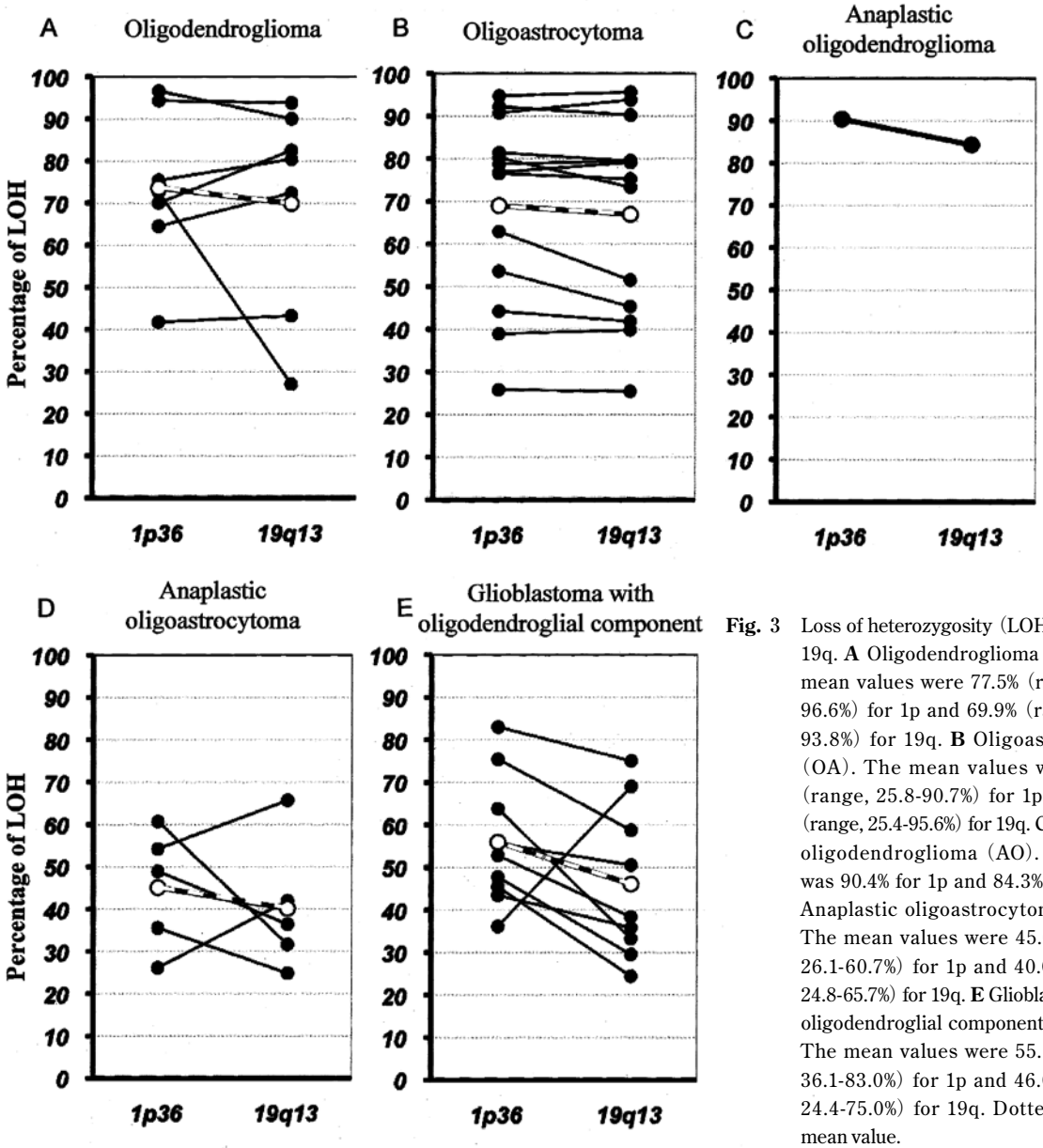

Fig. 3 Loss of heterozygosity (LOH) of 1p and 19q. **A** Oligodendroglioma (OD). The mean values were 77.5% (range, 41.7-96.6%) for 1p and 69.9% (range, 27.0-93.8%) for 19q. **B** Oligoastrocytoma (OA). The mean values were 69.0% (range, 25.8-90.7%) for 1p and 66.9% (range, 25.4-95.6%) for 19q. C Anaplastic oligodendroglioma (AO). The value was 90.4% for 1p and 84.3% for 19q. **D** Anaplastic oligoastrocytoma (AOA). The mean values were 45.0% (range, 26.1-60.7%) for 1p and 40.0% (range, 24.8-65.7%) for 19q. **E** Glioblastoma with oligodendroglial component (GBM-O). The mean values were 55.9% (range, 36.1-83.0%) for 1p and 46.0% (range, 24.4-75.0%) for 19q. Dotted line bar, mean value.

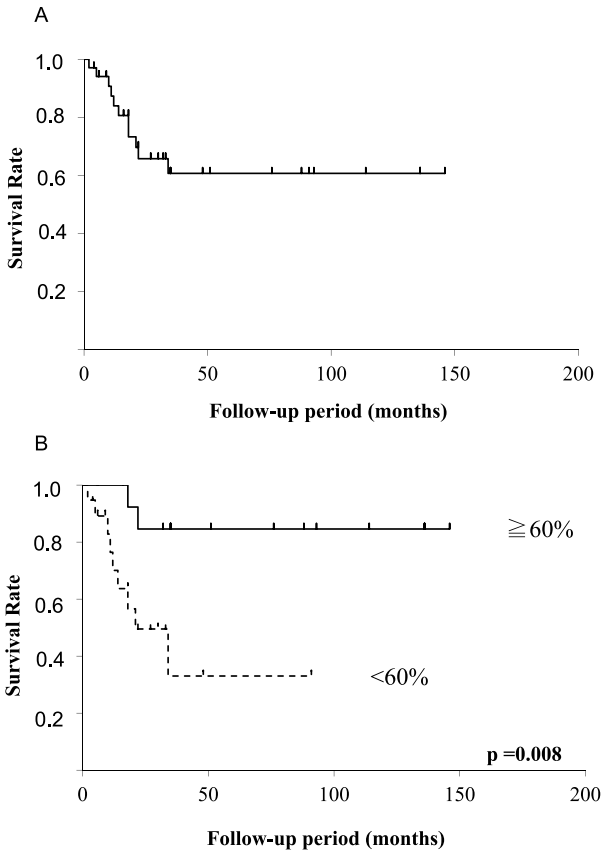

Fig. 4 **A** Kaplan-Meier survival curves for 35 cases. The five year overall survival of 35 cases was 60.7%. **B** Kaplan-Meier survival curves according to the cutoff value. The group with greater than 60.0% 1p and 19q LOH had significantly longer overall survival than the group with less than 60.0% 1p or 19q LOH (p=0.008).

Results

Clinicopathological characteristics

The clinicopathological characteristics of the 35 patients included in this study are presented in Table 1. The male/female ratio was 19/16. The mean age was 48.7 years (range, 5-86). Histological diagnoses were OD in seven cases, OA in 13 cases, AO in one case, AOA in five cases, and GBM-O in nine cases. The group with low grade glioma (OD, OA) had significantly longer overall survival than the group with high grade glioma (AO, AOA, GBM-O) by univariate analysis (p<0.001) (data are not shown).

FISH analysis for 1p and 19q LOH

An example of 1p and 19q LOH is shown in Fig. 2. The mean values of 1p and 19q LOH by FISH was 77.5% (range, 41.7-96.6%) and 69.9% (range, 27.0-93.8%), respectively, in OD, 69.0% (range, 25.8-90.7%) and 66.9% (range, 25.4-95.6%), respectively, in OA, 90.4% and 84.3%, respectively, in AO, 45.0% (range, 26.1-60.7%) and 40.0% (range, 24.8-65.7%), respectively, in AOA, 55.9% (range, 36.1-83.0%) and 46.0% (range, 24.4-75.0%), respectively, in GBM-O (Fig. 3). The five-year overall survival of 35 cases was 60.7%. The group with greater than 60.0% 1p and 19q LOH had significantly longer overall survival than the group with less than 60.0% 1p or 19q LOH (p=0.008) (Fig. 4).

Discussion

In oligodendroglial tumors, concurrent LOH of chromosomal arms 1p and 19q constitutes a hallmark alteration.[1), 6)] The incidence of 1p and 19q LOH is 85% in OD,[7), 8)] 65% in AO,[7)] 40% in OA,[7), 8)] and 3-25% in GBM-O.[5), 6)] Moreover, 1p and 19q LOH is a prognostic factor in OD [6), 9)] and AO [9), 10), 11)] and is associated with chemotherapy sensitivity.[9), 12), 13)] Classic oligodendroglial morphology shows diffuse infiltration of monomorphic cells with round nuclei and perinuclear halos and is highly associated with 1p and 19q LOH.[14)] In combination with classical histological and immunohistochemical data, 1p19q status provides pertinent information for discriminating between histological types of gliomas and identifying a subgroup of tumors that is associated with a better prognosis.[15)] Recently, the presence of LOH at 1p and 19q has been used to confirm histological diagnosis and for directing treatment.

Distinct genetic subsets of morphologically ambiguous gliomas are identifiable by FISH. Both histological grading and molecular analysis yield useful prognostic information.[16)] Moreover, FISH is the most common technique for detecting LOH at 1p and 19q.[3)] Many neuropathology laboratories select their own cut-off values.[3), 4), 5)] Cut-offs for overall target-to-control signal ratios currently range from 30–70%. In the present study, we analyzed the cut-off values that could influence overall survival after resection. Sixty percent was a reliable cut-off value for both 1p LOH and 19q LOH. The group with greater than 60.0% for both 1p and 19q LOH had significantly longer overall survival than the group with less than 60.0% for 1p or 19q LOH (p=0.008). The incidence of more than 60.0% 1p and 19q LOH was 71.4% in OD (5/7 cases), 46.1% in OA (6/13 cases) and 11.1%

in GBM-O (1/9 cases). The correlation between LOH and histological subtypes observed in our study was similar to those that were previously reported.[5),6),7),8)]

The occurrence of a balanced whole-arm translocation between chromosomes 1 and 19 results in the loss of 1p and 19q in oligodendroglioma.[17)] Robert et al. reported that 1p and 19q LOH were tightly associated, with 5.7% of cases showing alteration of either 1p or 19q by FISH in oligodendroglial neoplasms, including OD and OA.[18)] Similarly, there is the possibility that cases with partial LOH, not der (1;19) (q10;p10), exist in the group with less than 60.0% 1p or 19q LOH. Further clinicopathological studies are necessary to examine the possibility of partial LOH.

In conclusion, we examined 1p and 19q LOH by FISH in oligodendroglial, oligoastrocytic tumors, and GBM-O. Sixty percent was a reliable cut-off value that influenced overall survival after resection in our institution.

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