Ultrarapid Evaluation of Meningioma Malignancy by Intraoperative Flow Cytometry

Go Matsuoka[1], Seiichiro Eguchi[1], Hidenori Anami[3], Tatsuya Ishikawa[1], Koji Yamaguchi[1], Masayuki Nitta[1], Yoshihiro Muragaki[1,2], Takakazu Kawamata[1]

BACKGROUND: **The prognosis for World Health Organization (WHO) grade II/III meningiomas is worse than for WHO grade I meningiomas. Histopathologic grade should ideally be identified during tumor resection, but current methods are time-consuming and have doubtful reliance. The aim of this study was to evaluate intraoperative flow cytometry (iFC) as a method for providing ultrarapid evaluation of meningioma malignancy.**

METHODS: **A total of 117 meningiomas were analyzed with iFC during surgery. For each, the malignancy index (MI) was calculated as the number of cells with a greater than normal DNA content as a proportion of the total number of cells. Each specimen was investigated histopathologically and was diagnostically graded according to the 2016 WHO grading system. MI results were compared with WHO grades of the meningiomas.**

RESULTS: **The automatic measurement of iFC took approximately 9 minutes on average. The difference in MI between grade I and grade II/III meningiomas was statistically significant ($P < 0.001$). Receiver operating characteristic analysis provided an optimal cutoff MI value of 8.0% for discrimination between grade I and grade II/III groups, with 64.7% sensitivity and 85.0% specificity for grade II/III meningiomas.**

CONCLUSIONS: **Our method of calculating MI with iFC appears to be technically feasible and reliable for ultrarapid evaluation of meningioma malignancy. MI with iFC could potentially enable determination of an optimal treatment strategy during surgery, such as extent of resection of the tumor and management of invaded normal brain or nerves.**

INTRODUCTION

The best treatment goal for meningiomas is gross total removal, which results in a longer recurrence-free period and improved overall survival.[1] However, even after complete resection, nonbenign World Health Organization (WHO) grade II/III meningiomas, which include atypical and anaplastic meningiomas, frequently recur,[2] with recurrence 8 times more likely than for WHO grade I meningiomas.[3] Furthermore, grade II meningiomas tend to grow more aggressively when they recur than at their initial presentation.[4] Radical resection during the initial surgery is therefore required, particularly for grade II/III meningiomas. However, it is unusual for the WHO grade of a meningioma to be established during the initial surgery, partially because of the difficulty and the time required for making such an assessment. A rapid and reliable intraoperative method for evaluating the malignancy of meningiomas could be of crucial importance. Identification of histopathologic malignancy during tumor resection could help the surgeon determine the optimal treatment strategy for the patient, such as the extent of resection of tumors and the management of invaded normal brain tissue or nerves, thus providing the best chance of long-term tumor control.

Several reports have described new intraoperative methods for determination of meningioma malignancy using flow cytometry and Ki-67 immunohistochemistry; however, most of them are time-consuming or demonstrate inadequate reliability of

Key words
- Intraoperative flow cytometry
- Ki-67 immunohistochemistry
- Malignancy index
- Meningioma
- MIB-1 index

Abbreviations and Acronyms
iFC: Intraoperative flow cytometry
MI: Malignancy index
ROC: Receiver operating characteristic
WHO: World Health Organization

From the [1]Department of Neurosurgery and [2]Faculty of Advanced Techno-Surgery, Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo; and [3]Department of Neurosurgery, Tokyo Metropolitan Health and Medical Treatment Corporation, Tokyo, Japan

To whom correspondence should be addressed: Go Matsuoka, M.D. [E-mail: matsuoka.go@twmu.ac.jp]

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malignancy evaluation.[5-14] Shioyama et al.[15] reported a method that used intraoperative flow cytometry (iFC) for rapid evaluation of the malignancy of a glioma, allowing an assessment within 10 minutes. The aim of the present study was to evaluate the feasibility and efficacy of applying iFC to establish the WHO histopathologic grade of intracranial meningiomas during resection using fresh material and a new technique in a shorter evaluation period than that of Shioyama et al.[15]

MATERIALS AND METHODS

Patients

The experimental protocol was approved by the ethics committee of our institution (3014-R), and the study was conducted in accordance with the principles of the Declaration of Helsinki. All patients provided written informed consent. Between November 2014 and March 2017, we resected meningiomas in 138 consecutive patients at our institution. Of these patients, 21 were excluded from the investigated prospective cohort because of technical errors or insufficient specimens for 1 or more of the studies. Thus, the analysis included 117 patients. All were Asian, and 82 (70%) were women. Median age at surgical treatment was 55.8 ± 13.6 years (interquartile range, 46–67 years).

The senior author (T.K.) removed the tumor and determined the extent of resection of each tumor. The diagnosis of meningioma and final WHO grade were confirmed histopathologically, and an immunohistochemistry evaluation of the monoclonal antibody Ki-67, a marker of proliferation, was performed. We reviewed the pathologic diagnosis according to the criteria listed in the WHO 2016 classification.[16] In addition, we prospectively collected information about each patient's clinical history, neuroimaging, and microsurgical dissection findings.

iFC Analysis

The technique of iFC was previously applied by Shioyama et al.[15] to cases of intracranial glioma. In this study, we adopted a similar method but with new-generation equipment, which resulted in improved automation and a shorter procedure time. The first author (G.M.) performed the iFC measurement for all cases. After the tumor was exposed, tumor tissue specimens, each with a volume of approximately 2 mm^3, were obtained using forceps. The specimens were placed in a test tube with a DNA staining reagent and set in the FCM-2200 Celltac Peak analyzer (Nihon Koden, Tokyo, Japan). Cell isolation initiated automatically. Staining, cell counting, and DNA analysis were also automatic, with the complete process taking 9 minutes. Human peripheral blood cells were used as a reference for the DNA histogram analysis and for defining the position of the diploid G_0/G_1 peak in the DNA histograms. The malignancy index (MI) was calculated as the ratio of the number of cells with a greater than normal DNA content to the total number of cells.[15]

Histopathologic Analysis

Three neuropathologists analyzed the intraoperative frozen and permanent tissue samples, reached a consensus opinion, and decided the final histopathologic diagnosis; all 3 were blinded to the calculated MI. The resected tumors were fixed and embedded in paraffin and stained with hematoxylin and eosin. Their proliferative activity was assessed by Ki-67 immunohistochemistry. When a differential diagnosis for lesions such as hemangiopericytoma and solitary fibrous tumors was needed, signal transducer and activator of transcription immunohistochemistry was additionally performed.

Statistical Analysis

JMP 13 software (SAS Institute Inc., Cary, North Carolina, USA) was used for statistical analysis. The relationship between intraoperative MI values and postoperative WHO grades was evaluated. The patients were divided into 2 groups according to WHO grade I and grade II/III. Grades II and III were grouped together because there were only 4 grade III cases, which was insufficient to allow significance in the statistical analysis. Differences between the 2 groups were assessed using χ^2 tests for categorical variables and one-way analysis of variance for continuous variables. $P < 0.05$ was considered significant. Logistic regression analysis was used to establish a statistical model of the relationship between WHO grades (as the dependent variable) and MI (as the explanatory variable). Receiver operating characteristic (ROC) analysis was applied, with grade II/III meningioma taken as positive and grade I meningioma taken as negative, and the area under the ROC curve was measured. The optimal cutoff value of MI for discriminating grade II/III meningiomas from grade I meningiomas was evaluated by the Youden index, and its sensitivity and specificity were calculated.

RESULTS

Meningiomas

Table 1 summarizes the demographic characteristics of the patients included in this study. Of 117 patients, 83 (71.0%) had grade I meningiomas, 30 (25.7%) had atypical meningiomas (grade II), and 4 (3.4%) had anaplastic meningiomas (grade III). There were 13 recurrent meningiomas (**Table 2**). Grade I tumors included 28 meningothelial, 26 fibrous, 19 transitional, 2 microcystic, 1 psammomatous, 4 angiomatous, and 3 unclassifiable meningiomas. The locations of the tumors were as follows: convexity ($n = 29$), falcine ($n = 18$), parasagittal ($n = 8$), skull base ($n = 58$), and intraventricular ($n = 4$). The iFC analysis was completed in 9 minutes.

Outcomes

There was a statistically significant difference in mean MI values for the grade I and grade II/III groups (6.08% ± 0.86% vs. 14.1% ± 1.35%; $P < 0.001$) and for the grade I and grade II groups (6.08% ± 0.86% vs. 12.5% ± 11.2%; $P < 0.001$). The ROC analysis indicated that an MI of 8.0% was the optimal cutoff value for distinguishing between the 2 groups (WHO grade I and grade II/III), with 64.7% sensitivity and 85.0% specificity (**Figure 1**).

Illustrative Case 1

A 33-year-old man presenting with headache was referred to our outpatient clinic. Magnetic resonance imaging of the head showed a tumor that extended along the cerebral falx and