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Outcome comparisons of high-grade glioma resection with or without fluorescein sodium-guidance

Jian Hong*, Budong Chen, Xin Yao, Yushan Yang

Department of Neurosurgery, Tianjin HuanHu Hospital, Tianjin Key Laboratory of Cerebral Vascular and Neurodegenerative Diseases, Tianjin Neurosurgical Institute, Jinnan District, Tianjin, China

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ABSTRACT

Purpose: To compare surgical outcomes and adverse events between patients received microsurgical resection of high-grade gliomas with or without fluorescein sodium-guidance.
Patients: A retrospective study was conducted in our hospital between June 2016 and January 2017. Patients were divided into 2 groups: Fluorescein Group (42 patients) and Nonfluorescein Group (40 patients).
Measurements: Intraoperative (hemorrhage value and operation time) and postoperative (consistency between fluorescence-stained tissue and histopathological results, rate of resection) measurements were documented. Postoperative adverse events were recorded. Patients were followed up for 6 months to evaluate postsurgery glioma recurrence.
Main Results: Intraoperatively, hemorrhage value, and operation time were significantly less in Fluorescencein than in Nonfluorescencein group. The rate of glioma complete resection was significantly higher in Fluorescencein than in Nonfluorescencein group (85.7%vs 62.5%, $P=0.02$). The rate of glioma recurrence was significantly lower in Fluorescencein than in Nonfluorescencein group (11.9%vs 25.0%, $P=0.01$),

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* Correspondence to: Department of Neurosurgery, Tianjin HuanHu Hospital, Tianjin Key Laboratory of Cerebral Vascular and Neurodegenerative Diseases, Tianjin Neurosurgical Institute, No. 6 Jizhao Road, Jinnan District, Tianjin, China 300350.

E-mail address: hongjian80000@163.com (J. Hong).

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and no significant differences on adverse events were observed.

Conclusions: Intraoperative fluorescein sodium-guidance could facilitate the complete resection and significantly decrease the postoperative recurrence in patients with high-grade gliomas.

Introduction

High-grade gliomas refer to the WHO of III-IV grade malignant glioma originated from the neural epithelial tissue. They are characterized by abundant blood supply, rapid growth, low rate of complete resection, and high rate of postoperative recurrence. The median survival is about 1 year.[1] The high-grade glioma treatment effect is not ideal, because of its biological characteristics—no obvious boundary and infiltrating growth. It is difficult to determine the clear tumor boundary. The reported complete resection rate of grade glioma is only about 30%.[2]. Preoperative imaging examination, including magnetic resonance images (MRI), ultrasound and computed tomography, could only identify the tumor location, size, and unclear margins.[3-6] Furthermore, as brain tissue function, it is not allowed that neurosurgeons resect more brain tissue around glioma. As the consequences, higher rate of glioma recurrence has been a problem after neurosurgery.

In 1948, Moore *et al.* first reported that fluorescein sodium could be used intraoperatively to provide a real-time distinguishes of the tumor tissue from the surrounding normal structures.[7] Fluorescein sodium was not involved in the metabolism of the tumor cells. It enters the tumor tissue through the damaged blood-brain barrier and accumulates in the tumor mass.[8] The higher the grade of gliomas, the severer the damage to the blood-brain barrier, which results in more accumulation of fluorescein sodium and in the stronger fluorescence signals in tumor tissue.[9]

Recently, with the development of the fluorescence microscope, several studies have performed glioma resections with low-dose (3–10 mg/kg) fluorescein sodium.[10] This low-dose fluorescein sodium can be visualized by a microscope equipped with a yellow fluorescence emission filter, especially important for high-grade gliomas with ill-defined boundaries. Studies have shown that glioma resection guided by low-dose fluorescein sodium could achieve satisfactory postoperative outcomes.[10]

However, there were a few studies to comprehensively compare the fluorescence-guided with nonfluorescence-guided microsurgery resection of gliomas. More importantly, the fluorescence-guided technique was recently introduced into China, and it is necessary to explore and accumulate the clinical experiences.

In the current study, we comprehensively analyzed the data of patients with high-grade glioma, who received microsurgery resection with or without low-dose fluorescein sodium-guidance in our hospital. We compared the outcomes and adverse events between the 2 techniques.

Materials and methods

Study design and participants

The study was retrospective. The study protocol was approved by Ethics Committee of our hospital. Medical records were reviewed to select the patients who visited the Neurosurgery Department of our hospital between January 2016 and January 2017. The inclusion criteria for the selection were: (1) patients were first time to receive glioma surgery; (2) glioma was

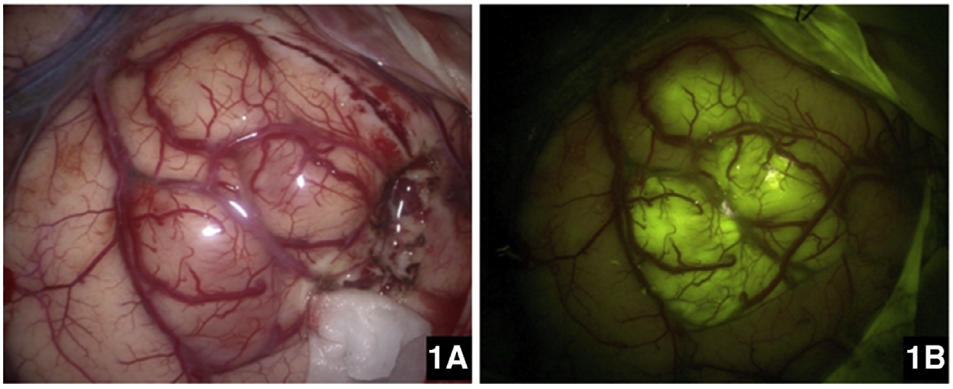

Fig. 1. Represented images of visualized glioma under the conventional white light microscope with no fluorescence-labeling (A) and the same glioma with fluorescent-labeling under the microscope equipped with a YELLOW 560 nm filter (B). Tumor margins are clearly identified in (B).

considered to be suitable for a complete surgical resection; (3) received microsurgical resection of tumor with or without fluorescein sodium-guidance; 4) postoperative pathological examination showed grade III or IV glioma.[11] Exclusion criteria were: (1) severe comorbidity, including heart, liver, or kidney disease; (2) recurrence of gliomas.

Study protocol and surgical procedure

Patients' age, gender, period from tumor diagnosis to surgery, weight, and height were recorded. The location and size of tumor in each patient was preoperatively identified with contrast-enhanced brain MRI.

For patients who received fluorescein sodium injection (as Fluorescein Group, 42 patients), a test dose of diluted 5mL of fluorescein sodium (1%, Alcon) was administrated intravenously 30 minutes before the general anesthesia. If there was no skin rash or signs for any allergic reaction, a single injection of 1.5-2.0 mg/kg fluorescein sodium was given intravenously to the patient. At least 90 minutes were passed before the dura mater was open in order to ensure that fluorescein sodium had sufficient time to accumulate in the brain tumor tissue. Patients who did not receive fluorescein sodium injection (as Nonfluorescein Group, 40 patients) went to operation without this step.

Craniotomy was performed and dura mater was open. A surgical microscope (OPMI, Pentero 900, Carl Zeiss, Germany) was used for the surgical procedure. For patients in the Fluorescein Group, the fluorescent dye could be visualized under the fluorescence microscope equipped with a YELLOW 560 nm filter. The tumor mass was identified (Fig 1) and removed with the standard microneurosurgical technique by alternating between YELLOW 560 nm fluorescence and white light illumination. At the final step of the procedure, particular attention was given to the tumor margins under the YELLOW 560 nm filter in order to ensure a total or subtotal removal of the tumor tissue. For patients in the Nonfluorescein Group, brain tumor was resection under the conventional white light microscope and ultrasound navigation. The resected glioma tissue was confirmed with the histopathological examination (Fig 2). The gliomas were graded based on the current WHO classification of tumors of the central nervous system.[11]

All the surgical operations were performed by a team of experienced neurosurgeons at our hospital. Patients were admitted into the neurointensive care unit postoperatively. Contrast-enhanced brain MRI was performed again to evaluate the residue tumor tissue (Fig 3). Radiochemotherapy was started as determined by the consultation with hospital oncologists.

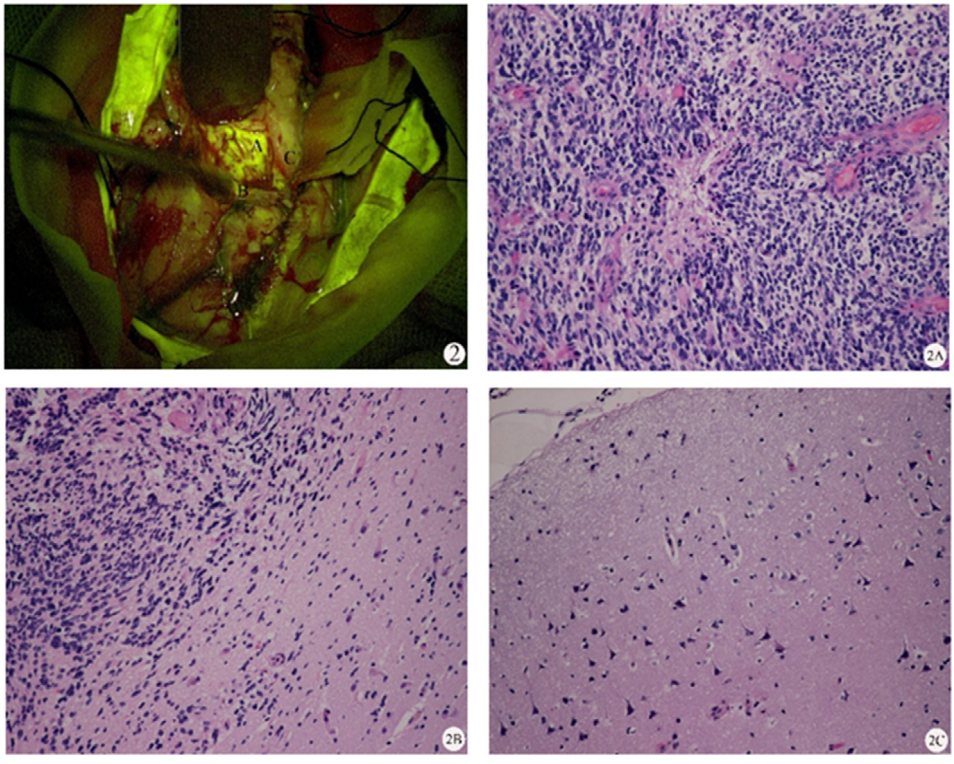

Fig. 2. A glioma specimen with different intensities of fluorescein staining in the upper left panel, and its corresponding pathological characteristics in A, B and C. A shows extensive palisade necrosis in tumor tissue with strong fluorescein accumulation. B shows the boundary between tumor cells and normal cells in tumor tissue with weak fluorescein accumulation. C shows normal neurons from normal neuro-tissue without fluorescein accumulation. (H&E staining with 200 magnification.)

Study outcomes

The outcomes of the study included intraoperative (volume of hemorrhage, duration of operation) and postoperative (consistence with pathological report, degree of resection) measurements. Volume of hemorrhage and duration of operation were obtained from the operation records. Consistence with pathological results was recorded when the glioma tissue was identified in the resected tumor mass. Complete resection was determined with postoperative brain MRI examination, that is, no any residue tumor tissue. Otherwise, any residue tumor tissue shown in postoperative brain MRI indicated a partial resection. Recurrence of the disease was considered when brain MRI showed new occupying lesions with hemorrhage, necrosis, or cystic formations.

We also calculated the sensitivity and specificity of the fluorescence sodium-guided technique. Sensitivity was calculated as the number of fluorescence staining samples and/or all samples confirmed to be high-grade glioma by postoperative histopathological examination. Specificity was calculated as the number of nonfluorescence samples and/or all samples confirmed not to be neoplastic by postoperative histopathological examination.

Adverse events after the operation, including electrolyte abnormality, upper gastrointestinal bleeding, delayed intracranial bleeding, intracranial infection, postoperative seizure, and multiple-organ failure, were documented. These adverse events were classified as mild, moderate, and severe based on. Patients were followed up for 6 months to evaluate tumor recurrence by repeating contrast-enhanced brain MRI examinations.

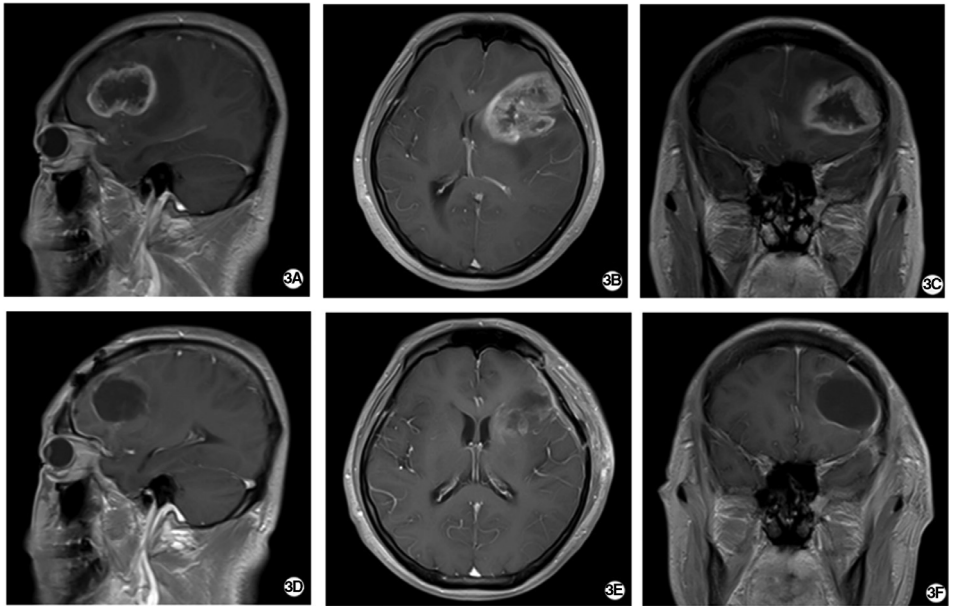

Fig. 3. Preoperatively -enhanced (A-C) MRI images of glioblastoma were located on the left forehead. Images taken 48hours after surgery on the same sections (D-F) shows that the tumor was completely resected. Images are shown on the sagittal plane (A and D), on the cross-section (B and E) and on coronal section (3C and 3F). All images were from a male patient (54 years old) with a gradeIV glioma.

Statistical analysis

Continuous data were presented as mean ± standard deviation if with a normal distribution, or as median and confidence interval if with a skewed distribution. Categorical data were presented as number and percentage. Continuous data between 2 groups were compared with student's t-test or Wilcoxon 2 sample test when appropriate. Categorical data between 2 groups were compared with chi square (Fisher exact test) or Wilcoxon rank sum test when appropriate. All statistical analyses were performed with SAS (SAS 9.3, SAS Institute). A $P < 0.05$ was considered statistically significant.

Results

A total 82 patients were identified, with 42 in the Fluorescein Group, in which Male/Female was 25/17, mean age 48 with range 25-73 years old, and with 40 in the Nonfluorescein Group, in which Male/Female was 28/22, mean age 26 with range 22-76 years old. All patients completed the surgical operation. The follow-up review with MRI for each patient was at 6-month postoperatively. There were no statistically significant differences in their baseline characteristics between 2 groups (Table 1).

Intraoperatively, hemorrhage value, and operation time were significantly less in Fluorescencein than in Nonfluorescencein group. Importantly, the rates of tumor complete resection was significantly higher in Fluorescencein than in Nonfluorescencein group (85.7%vs 62.5%, $P = 0.02$, respectively, Table 2). Moreover, the rate of glioma recurrence was significantly lower in Fluorescencein than in Nonfluorescencein group (11.9%vs 25.0%, $P = 0.01$, Table 2).

In Fluorescencein group, 87 samples with strong fluorescence staining were obtained during the surgical operation. 79 of them were confirmed to be gliomas by the postoperative

Table 1
Baseline characteristics in patients from both groups.

Characteristics	Fluorescein Group n = 42	Nonfluorescein Group n = 40	*P*
Age, year, M ± SD	49.1 ± 12.6	52.2 ± 12.3	0.20
Gender, n (%)			0.19
Male	25 (59.5)	18 (45.0)	
Disease duration, month, M ± SD	4.8 ± 2.4	5.2 ± 2.2	0.43
BMI, Kg/m^2, M ± SD	24.2 ± 6.2	23.8 ± 5.5	0.76
Tumor			
Size, cm^3, M ± SD	31.2 ± 14.8	30.1 ± 14.7	0.72
Location, n (%)			1.00
Parietal	5 (11.9)	5 (12.5)	
Frontal	8 (19.1)	7 (17.5)	
Functional	12 (28.6)	11 (27.5)	
Temporal	5 (11.9)	6 (15.0)	
Others	12 (28.6)	10 (27.5)	
Biggest diameter, n (%)			0.97
<4 cm	11 (26.2)	11 (27.5)	
4–6 cm	20 (47.6)	18 (45.0)	
>6 cm	11 (26.2)	11 (27.5)	
Pathological type, n (%)			1.00
Anaplastic oligodendrogioma	9 (21.4)	8 (20.0)	
Anaplastic ependymoma	6 (14.3)	5 (12.5)	
Anaplastic astrocytoma	8 (19.1)	7 (17.5)	
Glioblastoma	14 (33.3)	15 (37.5)	
Others	5 (11.9)	5 (12.5)	
WHO classification, n (%)			0.85
III	24 (57.1)	22 (55.0)	
IV	18 (42.9)	18 (45.0)	

M ± SD, mean ± standard deviation; BMI, body mass index.

histopathological examination. Eight resected samples without fluorescence were confirmed to be gliomas with the histopathological examination. Therefore, the sensitivity and specificity of fluorescein in identifying brain glioma were 90.8% and 83.3%, respectively.

The overall incidence of postoperative adverse events was higher in the Nonfluorescencein than that in the Fluorescencein group, though it did not reach a statistical significant difference (45.0% and 23.8%, respectively, $P = 0.06$). Detailed analyses did not show any statistically significant differences in each category of the adverse events between 2 groups (Table 3).

Discussion

In the current study, yellow fluorescence could be easily visualized under the fluorescence microscope in patients in the Fluorescencein Group. The fluorescence staining was strongest in the center of the tumor mass, and gradually faded when going to the edges of the tumor mass. The area of the fluorescence staining was consistent with the tumor mass identified by the contrast-enhanced brain MRI. Fluorescencein-guidance could facilitate a real-time delineation of gliomas from the surrounding normal tissue,[10] which increased the operation precision, shortened the operation time and, thus, reduced the bleeding during surgery. Most importantly, postoperative recurrence rate was significantly decreased in Fluorescencein Group, which was probably due to the increased rate of glioma complete resection under fluorescence signal guidance.

In the current study, the complete resection rate was 62.5% in the Nonfluorescencein Group, which was higher than previous reports performed under the conventional white light microscopy (30%-55%).[12] During our operation, we used intraoperative sonogram navigation to identify the boundaries of tumor mass. This technique was better than the one with only the microscopy, thus, improved the resection rate. However, the rates of tumor complete resection were significantly higher in patients in the Fluorescencein Group than in the Nonfluorescencein

Table 2
Intraoperative and postoperative comparisons between 2 group patients.

Variables, n (%)	Fluorescein Group n = 42	Nonfluorescein Group n = 40	*P*
Intraoperative			
Hemorrhage			<0.01
<100 mL	23 (54.8)	7 (17.5)	
100-400 mL	16 (38.1)	22 (55.0)	
>400 mL	3 (7.1)	11 (27.5)	
Duration of operation			0.03
<120 min	13 (31.0)	7 (17.5)	
120-240 min	25 (59.5)	22 (55.0)	
>240 min	4 (9.5)	11 (27.5)	
Tumor mass resection			0.02
Complete	36 (85.7)	25 (62.5)	
Partial	6 (14.3)	15 (37.5)	
Complication	3 (7.1)	4 (10)	> 0.05
Death	2 (4.8)	1 (2.5)	N/A
Postoperative			
Consistence with pathological result	39 (92.9)	32 (82.1)	0.18
At 6-month follow-up postsurgery			
Death	1 (2.5)	2 (5.1)	N/A
KPS	82	75	
Recurrence			
Yes	5 (11.9)	10 (25.0)	0.01

Table 3
Records of adverse events between 2 groups.

Adverse events, n (%)	Fluorescein Group n= 42	Nonfluorescein Group n = 40	*P*
Overall	10 (23.8)	18 (45.0)	0.06
Mild			
Electrolyte abnormality	1 (2.4)	5 (12.6)	0.11
Upper gastrointestinal bleeding	2 (4.8)	2 (5.0)	1.00
Delayed intracranial bleeding	1 (2.4)	2 (5.0)	0.61
Intracranial infection	2 (4.8)	2 (5.0)	1.00
Postoperative seizure	0 (0.0)	1 (2.6)	0.49
Moderate			
Electrolyte abnormality	1 (2.4)	0 (0.0)	1.00
Upper gastrointestinal bleeding	0 (0.0)	1 (2.6)	0.49
Delayed intracranial bleeding	0 (0.0)	1 (2.6)	0.49
Intracranial infection	1 (2.4)	0 (0.0)	1.00
Severe			
Upper gastrointestinal bleeding	0 (0.0)	1 (2.6)	0.49
Intracranial infection	1 (2.4)	2 (5.0)	0.61
Multiorgan failure	1 (2.4)	1 (2.6)	1.00

Group, suggesting that fluorescence staining was a better technique compared to ultrasound-guided technique.

Postoperative histopathological examination showed the fluorescence staining technique had the sensitivity and specificity of 90.8% and 83.3%, respectively, which were consistent with previous reports.[13,14] The detectable fluorescence signals in the study group could make it easier for the surgeons to identify the boundaries of the gliomas in order to have a successful complete dissection of the tumor mass with less injury to the surrounding structures. This was consistent with our outcome analysis, which showed less intraoperative hemorrhage, shorter surgical duration, and more frequent complete tumor resections in the study group when compared to the control group.

A previous study reported that fluorescence sodium should be given 20 minutes before the craniotomy.[15] In the current study, we administrated intravenous fluorescence sodium 30 minutes before the general anesthesia. It was approximately 90 minutes after the intravenous

fluorescence sodium injection till the dura matar was open. We had achieved satisfactory tumor staining by the protocol. The optimal period between intravenous fluorescence sodium injection and craniotomy requires further investigation.

The overall incidence of severe adverse events was low, which was consistent with previous studies.[10,12] The control group had a higher number of incidence for the adverse events than the study group, but the difference between 2 groups did not reach a statistical significance. These showed that fluorescein sodium-guided glioma resection was a safe procedure.

Over survival period after surgery has been an important indicator for patients received glioma resection. Both groups showed the same numbers of death in 6 months after surgery. Because the limitation of the study was that the follow-up period was shorter, we have not obtained an over survival in terms of longer period from the comparison of the 2 groups. We required each patient to come to our department for MRI examination to see if any glioma recurrence at 6 month after operation. We are planning to require each patient to receive a 12-month MRI examination additionally.

Conclusions

Compared to the traditional technique, application of fluorescein sodium injection and a microscope with a YELLOW 560 nm filter could achieve better intraoperative and postoperative outcomes, mainly on the increased complete resection of gliomas and the reduced rate of post-operation recurrence.

Declaration

Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent for publication

All patients gave their written information consent.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author' s contributions

JH and BC contributed to the study design; BC, XY and YY collected the data and performed the data analysis. All authors drafted the manuscript. JH revised the manuscript critically.

References

1. Stupp R, Brada M, van den Bent MJ, Tonn JC, Pentheroudakis G, Group EGW. High-grade glioma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2014;25(Suppl 3):iii93–ii101.

2. Lacroix M, Abi-Said D, Fourney DR, et al. A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. *J Neurosurg*. 2001;95:190–198.
3. Li P, Qian R, Niu C, Fu X. Impact of intraoperative MRI-guided resection on resection and survival in patient with gliomas: a meta-analysis. *Curr Med Res Opin*. 2017;33:621–630.
4. Mahboob S, McPhillips R, Qiu Z, et al. Intraoperative Ultrasound-Guided Resection of Gliomas: A Meta-Analysis and Review of the Literature. *World Neurosurg*. 2016;92:255–263.
5. Wirtz CR, Albert FK, Schwaderer M, et al. The benefit of neuronavigation for neurosurgery analyzed by its impact on glioblastoma surgery. *Neurol Res*. 2000;22:354–360.
6. Groshev A, Padalia D, Patel S, et al. Clinical outcomes from maximum-safe resection of primary and metastatic brain tumors using awake craniotomy. *Clin Neurol Neurosurg*. 2017;157:25–30.
7. Moore GE, Peyton WT, et al. The clinical use of fluorescein in neurosurgery; the localization of brain tumors. *J Neurosurg*. 1948;5:392–398.
8. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci*. 2006;7:41–53.
9. Nduom EK, Yang C, Merrill MJ, Zhuang Z, Lonser RR. Characterization of the blood-brain barrier of metastatic and primary malignant neoplasms. *J Neurosurg*. 2013;119:427–433.
10. Schebesch KM, Brawanski A, Hohenberger C, Hohne J. Fluorescein sodium-guided surgery of malignant brain tumors: history, current concepts, and future project. *Turk Neurosurg*. 2016;26:185–194.
11. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol*. 2016;131:803–820.
12. Schebesch KM, Proescholdt M, Hohne J, et al. Sodium fluorescein-guided resection under the YELLOW 560 nm surgical microscope filter in malignant brain tumor surgery–a feasibility study. *Acta Neurochir (Wien)*. 2013;155:693–699.
13. Acerbi F, Cavallo C, Broggi M, et al. Fluorescein-guided surgery for malignant gliomas: a review. *Neurosurg Rev*. 2014;37:547–557.
14. Martirosyan NL, Eschbacher JM, Kalani MY, et al. Prospective evaluation of the utility of intraoperative confocal laser endomicroscopy in patients with brain neoplasms using fluorescein sodium: experience with 74 cases. *Neurosurg Focus*. 2016;40:E11.
15. Okuda T, Yoshioka H, Kato A. Fluorescence-guided surgery for glioblastoma multiforme using high-dose fluorescein sodium with excitation and barrier filters. *J Clin Neurosci*. 2012;19:1719–1722.