Evaluation of the effect of fluorescein sodium-guided under yellow 560nm surgical microscope filter in surgery for high grade gliomas: Preliminary results in a series of 27 patients

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Summary

Objective: The importance of a complete resection of high-grade gliomas (HGGs) has been highlighted in scientific literature, in order to limit tumor recurrence and above all to improve disease-free survival rates. Several fluorescent biomarkers have been tested to improve intraoperative identification of residual tumor. The fluorescein sodium (FS) are now starting to play an important role in glioma surgery. Under the YELLOW 560nm surgical microscope filter, low-dose FS (5mg/kg) as a fluorescent dye helps in visualization of tumor tissue. Our study provides preliminary results in investigating the safety and efficacy of this technique in patients with HGGs. *Subject and method:* A prospective, uncontrolled study of 27 patients with HGGs underwent FS-guided resection under YELLOW 560nm surgical microscope filter. We have analyzed the clinical features, size, tumor location, extent of tumor resection, pathologic of HGGs, patient status (Karnofsky Performance Scale) and FS side effects. *Result:* 7/27 patients (25.9%) were glioma grade III and 20/27 patients (74.1%) were glioma multiforme (GBM). With YELLOW 560nm surgical microscope filter (PENTERO 900 surgical microscope), FS-guided gross total resection (GTR) was achieved in 74.1% and subtotal resection (STR) in 11.1%. No side effects of FS were reported. *Conclusion:* FS-guided resection of HGGs under the YELLOW 560nm surgical microscope filter was effective and safe.

Keywords: High-grade glioma (HGG), glioblastoma multiforme (GBM), extent of resection, flourescein sodium.

1. Background

High-grade gliomas (HGGs), which account for about 80% of all primary malignant cerebral neoplasms, are aggressive tumors that require multimodal management. According to the classification of the World Health Organization (WHO), HGGs include grade III and grade IV tumors. Currently, standard management in this type of neoplasm is based on maximal tumor resection followed by radio and chemotherapy. Newly diagnosed GBM patients with favorable Karnofsky performance scale (KPS) > 70% and undergo the standard of care including surgical resection, chemotherapy and radiation, have a survival mean of approximately 15 months, with only 10% of patients living more than 5 years [5]. Nonetheless, the maximum extent of surgical resection is associated with a longer recurrence-free period and overall survival of patients with glioblastomas and other intracranial malignancies. There is strong evidence supporting the extent of resection (EOR) as a significant predictor of better survival in patients

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with HGGs [5], [7]. Such a surgical strategy becomes a balance of an aggressive tumor removal while producing no new or further permanent neurological deficit [6]. Currently, several measures are used to help the surgeon perform the procedure as precisely as possible. In addition to commonly used white light microscopes, newer devices include neuronavigation, electrophysiological cerebral mapping, and intraoperative magnetic resonance imaging (iMRI) systems. Some of the recent advances involve intraoperative implementation of fluorescent markers that may also be a significant support for the neuro-oncological surgeon [2], [10].

Over the past decade, the use of a photosensitive drug or dye that enhances tumor visualization by fluorescence, has been also proposed during removal of HHGs. In particular, 5-aminolevulinic acid (5-ALA), a natural biochemical precursor of hemoglobin that provokes the synthesis and accumulation of fluorescent porphyrins in different tumors, has been shown to increase the rate of complete resection and it has been recently approved also in the USA by FDA as an optical imaging agent in HGGs [2], [10]. The sodium salt of fluorescein (FS) represents an alternative to 5-ALA. FS presents a major blue excitation wavelength peak ranging from 460 to 500nm and a major green emission peak fluorescent radiation in the region ranging from 540 to 690nm. The microscope with a specialized filter of light at 560 nm (Yellow 560) that can detect FS at low doses [3]. The feasibility of fluorescein-guided removal of HGGs has been suggested by the results of several studies.

In Vietnam, the use of FS has been applied since the end of 2019 at some Hospitals such as University of Medicine and Pharmacy in Ho Chi Minh City, Bach Mai, etc. At 108 Military Central Hospital, the use of FS in the surgery of HGGs has been routinely applied since 2020. Our aim is to demonstrate the effectiveness and safety of fluorescence-guided surgery in HGG surgery.

2. Subject and method

2.1. Subject

Patient selection

Criteria for patient selection based on the criteria of the FUOGLIO trial performed by Acerbi et al (2011) [1], including: 1. Age over 18 years old; 2, suspection of HGG (astrocytoma III or IV grade according WHO classification) on the basis of contrast enhanced magnetic resonance imaging (MRI) findings; 3) tumor location that allowed a complete surgical resection of the enhanced area (i.e., no eloquent areas or near functional brain regions, according to Shinoda's classification, 2003 [12]) and 4, tumor size > 1cm and ≤ 5cm.

Exclusion criteria were as follows: 1) Severe heart, liver or kidney disease; 2) Recent acute ischemic stroke; 3) Prior history of adverse reaction to FS or severe reactions to other contrast agents; 4) Women during the first trimester of pregnancy; 5) Specific neural tumor locations such as corpus callosum, basal ganglia, brain stem, posterior cranial fossa; 6) Preoperative KPS (Karnofsky Performance Status) score of 60 or less; 7) History of non-neural malignant tumors.

Surgical protocol

Our surgical protocol of fluorescein-guided technique has been described in a previous report of the preliminary results of the FLUOGLIO trial [1]. Briefly, after intubation and before skin incision, patients received 5mg/kg of a 20% solution of sodium fluorescein (Monico Spa, Italy), administered intravenously. A dedicated surgical microscope (PENTERO 900 with fluorescence kit, YELLOW 560, Carl Zeiss) was used during the surgical procedure. Neuronavigation (StealthStation® S7® System, Medtronic, Louisville, USA) was allowed for surgical planning, initial tumor localization, and orientation during tumor removal, but not for judgment regarding extent of resection. During resection, the microscope could be switched alternatively from fluorescent to white-light illumination by pressing the specific button on the microscope handgrip. Tumor resection was completed in an inside-out fashion, usually by aspiration, until all the fluorescent tissue exposed was removed.

Postoperatively, all patients were admitted to the ICU for postoperative care. Postoperative MR images were obtained generally within 72 hours of surgery. During the whole hospital stay, the patient was closely monitored for side effects related to the use of FS such as clinical symptoms, liver and kidney function test index, etc.

2.2. Method

A prospective, descriptive, not controlled study was performed to collect data on 27 consecutive adult patients (aged 19 to 77 years) with histopathological diagnosed HGGs who underwent FS-guided resection from May 2020 to June 2021. All patients had first surgery and had not received preoperative radiotherapy or chemotherapy. Surgery was followed by radiotherapy with concomitant and adjuvant temozolomide in all patients.

Preoperative neurological status evaluation was performed at the admission to hospital. A second evaluation was conducted during the postoperative course, on discharge. The KPS was used to record the patients' general physical status both pre- and postoperatively one month.

All patients underwent preoperative MRI studies with a 1.5-T or 3.0-T with and without the use of a contrast agent. The preoperative MRI studies were performed within preoperative 7-10 days, and the postoperative studies within the first 72 hours after surgery. The preoperative MRI data was used for intraoperative neuronavigation system. The preoperative tumor volume and the postoperative residual tumor volume were calculated by an independent neuroradiologist. Postoperatively, the extent of tumor resection was identified by contrast enhanced T1 weighted MRI. Three categories were distinguished: No residual tumor tissue (gross total resection - GTR); minimal residual tumor tissue (subtotal resection - STR) and partial resection (PR). GTR was defined as resection where no residual enhanced tumor is visible, STR was defined as nearly total (> 95%). Postoperative tumor volumes after surgery were calculated using medical image viewer software (OsiriX for Mac) on enhanced residual tissue (in T1 weighted MRI).

The contrast of intraoperative FS is divided into 3 levels based on three color degrees of staining were observed, according to Shinoda (2003). That were deep yellow (stained tissue in yellow-green), light yellow and negatively stained. Normal brain parenchyma remained unstained. It was the basis for the surgeon to assess whether the use of FS was found "helpful" or not. "Helpful" meant that the tumor was clearly visible under fluorescent and distinctly different from surrounding brain parenchyma [7].

Histopathological analyses were performed with standard procedures. The classification was conducted on the basis of the current WHO classification of tumors of the Central Nervous System.

2.3. Data analysis

All statistical analyses were performed with SPSS, version 20.0 (SPSS Inc, Chicago, IL, USA).

3. Result

3.1. Demographic data and presenting characteristic of the patients

The study included 27 cases (16 men, 11 women), the mean age was 51.56 ± 13.69 years (range: 19 - 77). The mean tumor size was 3.4 ± 1.5cm. The clinical features, MRI images, histopathological results are shown in Table 1. The mean preoperative KPS score was 87.41 (range: 70-100). At neuropathological analysis, 20 patients (74.1%) were diagnosed with glioblastoma multiforme (GBM) while 7 patients (25.9%) were diagnosed with anaplastic astrocytoma grade III, according to the WHO classification (6 patients with astrocytoma, 1 patient with oligodendroglioma).

Table 1. Demographic data and presenting characteristic of the patients

Characteristic		Number of patients (%)
Sex	Male	16 (59.3)
	Female	11 (40.7)
Tumor location	Frontal	6 (22.2)
	Temporal	7 (25.9)
	Parietal	2 (7.4)
	Fronto-temporal	3 (11.1)
	Fronto-parietal	3 (11.1)
	Temporo-parietal	2 (7.4)
	Occipital	2 (7.4)
	Parieto-occipital	2 (7.4)
Average tumor size (cm)		3.4 ± 1.5
Preoperative KPS (70-100)		87.41 ± 5.0

Grading	Grade III	7 (25.9)
	Grade IV	20 (74.1)

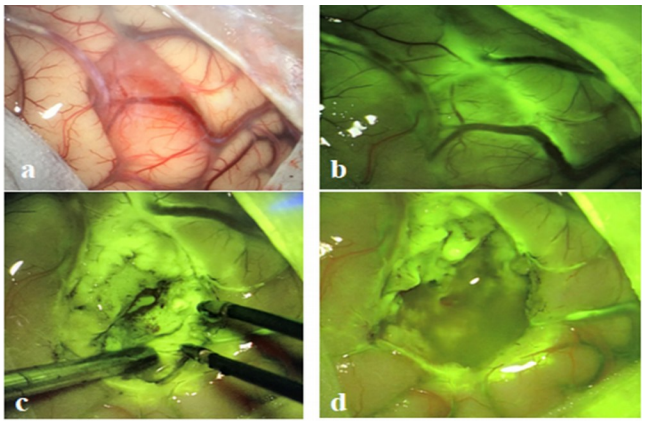

Figure 1. Intraoperative view of a temporal GBM tumor under the PENTERO 900 microscope

The image of tumor under normal white xenon-light illumination (a) and at the beginning of tumor removal under the YELLOW 560nm filter (b). During tumor removal, there was a clear delineation of the tumor area which shows significant FS enhancement revealing the boundary between the bright yellow tumor and the surrounding brain (c) and at the end of the removal (d). Source: Study patients, male, 48 years, ID 21480327.

3.2. Surgical results

The mean surgical time was 180.19 minute (range: 120 - 300 minute). The rate of complete and nearly complete resection of the tumor was 85.2%. The median length of postoperative hospital stay was 11.19 days (range: 7-20 days). The mean KPS score at hospital discharge was 87.78 ± 8.0. No adverse effects associated with the administration of FS were observed.

The surgical results are shown in Table 2. GTR was achieved in 74.1% (n = 19) of patients. A STR (> 95%) was achieved in 11.1% (n = 3) of them, while a PR (< 95%) was obtained in 14.8% (n = 5) of patients. Globally, a > 95% extent of resection was achieved in 85.2% (n = 22) of patients who underwent fluorescence-guided surgery. Post-operative neurological status was better for 18 patients, unchanged for 5 and worse for 4 patients. Overall, four serious complications emerged: One postoperative progressive cerebral edema, two postoperative hemorrhage, and one intracranial infection. Three of these patients were transient hemiparesis after surgery, who were completely recovery within postoperative one month later. There was no perioperative mortality.

Table 2. Surgical results

Variables		Number of patients (%)
The helpfulness of FS	Helpful	25 (92.6)
	Not-helpful	2 (7.1)
Extent of resection (EOR)	Gross total resection (GTR)	19 (74.1)
	Subtotal resection (STR)	3 (11.1)
	Partial resection (PR)	5 (14.8)
Post-operative neurological status	Better	18 (66.7)
	Unchanged	5 (18.5)
	Worse	4 (14.8)
Complications	Progressive cerebral edema	1 (3.7)
	Postoperative hemorrhage	2 (7.4)
	Intracranial infection	1 (3.7)
	Transient hemiparesis	3 (11.1)
	Death	0
	Adverse reactions to FS	0

Post-operative one month KPS (Mean ± SD)	87.78 ± 8.0

3.3. Intraoperative fluorescence characteristics

FS was administered intravenously at a constant dose of 5mg/kg following the induction of anesthesia. The mean intraoperative dose of FS was 280.56mg. All of the GBM tumors effectively stained yellow with FS during the surgical procedure under the Yellow 560 filter. According to surgeon's evaluations, FS was "helpful" in 25/27 cases (strong contrast enhanced).

No adverse effects associated with the administration of FS were observed. Yellow staining of sclera, skin, and urine disappeared within approximately 24 hours after the surgical procedure. No abnormal changes had been observed in routine blood or urine examinations, nor in liver and kidney function tests.

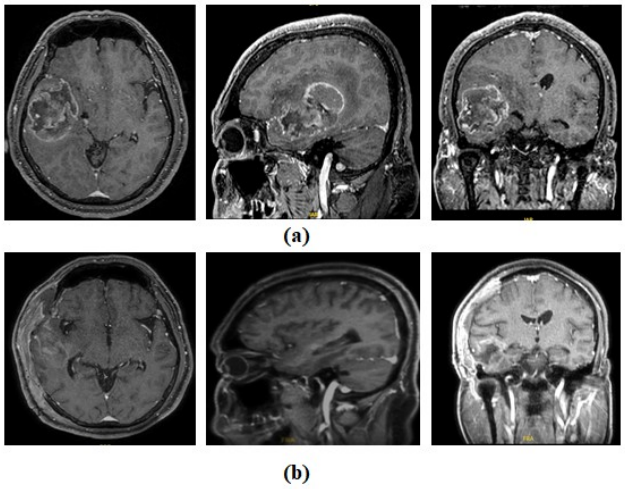

Figure 2. Preoperative (a) and postoperative 48-hours MRI images (b)

Source: Study patients, male, 48 years, ID 21480327

4. Discussion

For HGGs, the role of surgical radical resection of the tumor in improving symptom-free survival has been confirmed in many studies. Lacroix M et al studied 416 cases of GBM undergoing surgery and showed that patients with > 98% EOR had better overall survival [5]. Therefore, improving EOR of HGGs has become a major requirement in modern neurosurgery. Currently, there have been several intraoperative modalities to improve EOR [3], [10]. FS is a fluorophore, that has been used in medical applications for more than six decades. It has been routinely used in ophthalmology for retinal angiography. Since 1948, Moore et al. have addressed the role of FS in neuroblastoma by which it was used to distinguish fluorescence-enhanced neoplastic tumors from normal brain parenchyma. This dye penetrates in those areas of the brain where the blood-brain barrier is damaged, allowing real time enhancement of the areas enlightened by gadolinium in MRI. The first application of FS is in diagnostic biopsy; however, over time it has been applied as a guiding tool in surgery for HGGs. The fluorescence of FS can be grossly perceived to the naked eye, when this agent is injected at high doses (20mg/kg). However, when using a recent developed microscope integrated YELLOW 560 module (Carl Zeiss Meditec, Oberkochen, Germany), low dose of FS (5mg/kg) to detect an optimal fluorescence [3], [10].

Shinoda et al designed a topographical GBM staging system in which points are assigned based on tumor location, tumor size, and eloquence of adjacent brain tissue based on MR images (*Table 3*). This staging system is important in planning the ablation strategy of GBMs. According to this staging system, most authors adopted the following surgical policy for GBM resection: Stage I, the tumor should be resected as radically as possible with the aim of GTR; Stage II, risky resection should be avoided when the tumor extends to the eloquent area, Stage III, radical tumor resection should not be performed. Based on this staging system, all GBM diagnosed patients in our study were in Stage I, which in principle could be performed radical resection. In the present study, the rate of GTR was 74.1%; particularly, the rate of GTR and STR for the GBM patients was 93.6%. The mean post-operative KPS was 87.78 ± 8.0 and 23/27 patients had improved or unchanged postoperative neurological status. In 2011, Acerbi et al conducted a phase II (FLUOGLIO) trial with a 2-stage Simon minimax design to

evaluate the safety and efficacy of FS-guided HGG resection through a dedicated filter on the surgical microscope. The results showed a promising outcome in which the rate of GTR ranged from 75% to 100%, including tumors residing at functional regions [1].

Table 3. Determination of topographical stage of GBM (Shinoda, 2001) [7]

Stage Feature		Points Assigned*
Group for GBM location†	A	1
	B	2
	C	3
Size of GBM	size ≤ 5cm	0
	Size > 5cm	1
Eloquence of adjacent brain tissue	Noneloquent	0
	Eloquent	1

*Total points = (group) + (size) + (eloquence); that is, (1, 2, or 3) + (0 or 1) + (0 or 1). Stage I = 1 or 2 points; Stage II = 3 points; Stage III = 4 or 5 points.

† Group A, patients with a tumor located in the right hemisphere or left occipital lobe, including those with a tumor extending to the putamen and/or globus pallidus and those with a tumor just touching the corpus callosum and/or lateral ventricular wall; Group B, patients with a tumor located in the left frontal, parietal, or temporal lobe, including those with a tumor extending to the putamen and/or globus pallidus and those with a tumor just touching the corpus callosum and/or lateral ventricular wall; Group C, patients with a tumor located in or extending to the thalamus, caudate nucleus, and/or internal capsule, including those with a tumor apparently invading the fornix and/or corpus callosum and extending to the contralateral hemisphere.

In 2008, Koc et al. conducted an independent study to evaluate the role of FS in supporting HGG resection through a dedicated filter on the surgical microscope and they concluded that the use of FS in HGG resection was safe and convenient and could improve EOR [4]. Analogous results were also obtained for studies by Catapano et al. published in 2017. They concerned 23 patients operated on for HGG using a neurosurgical microscope fitted with a YELLOW 560 filter. The results were compared with a control group of 25 people operated on using standard methods. The authors showed an increase in the percentage of patients who achieved GTR (82.6% vs 52%) [6]. In a recent study, Katsevman et al conducted a comparative study between 2 groups: Fluorescein group (57 patients) and non-fluorescein group (132 patients) during surgery. The author found that, the intraoperatively hemorrhage value, and operation time were significantly less in fluorescein than in non-fluorescein group. Patients undergoing resection with sodium fluorescein, compared to the non-fluorescein treated group, had increased rates of gross- or near-total resection (73% vs 53%, respectively, $p<0.05$) as well as improved median survival (78 weeks vs 60 weeks, respectively; $p<0.360$) [9].

The introduction of light filters coupled with surgical microscopes brought about a real breakthrough, allowing optimization of the intensity of light with a small dose of dye. Naked eye guidance requires doses of 15 - 20mg/kg, while the use of filters allows it to be reduced to even 3-5mg/kg [3], [10]. Fluorescein in the body is mainly metabolized to fluorescein glucuronide, which has a half-life of 264 min. It is also a safe dye for patients with a very small list of potential side effects [2], [10]. To excite a dye to glow, it must be illuminated with light in the wavelength range from 460 to 500nm. The emitted fluorescence is in the wavelength range from 540 to 690nm. However, the mechanism of action is significantly different from that known from 5-ALA, where dyes showed affinity for cancer cells. In the case of fluorescein, under normal conditions, dye remains in the vessel. Fluorescein fluorescence within the tissue is only possible then under conditions of damage or disturbance of the blood-brain barrier (BBB) and penetration of the dye into the extravascular space. Therefore, it is primarily a non-specific marker of BBB function disorders, similar to gadolinium used as a contrast agent in imaging

studies [2]. Catapano et al. showed a close correlation between areas indicated in neuronavigation using FS and scans in the T1-weight magnetic resonance imaging (MRI) sequence obtained using gadolinium. It is estimated that the sensitivity of primary glioblastoma detection ranges from 79% to 94%, while the specificity ranges from 89.5% to even 100%, depending on the sample [6]. Several another studies have suggested that FS is a safe, effective, and convenient surgical adjunct to be used in the neurosurgical patient population to increase the extent of tumor resection. There has been concern, however, that FS is nonspecific, and may accumulate in any region where there is blood, edema, or surgical injury or trauma. This may suggest a tumor region that is in fact nononcological in nature, giving this adjunct in surgery a poor positive predictive value of tumor tissue. Studies have been undertaken to address such concerns. Neira et al demonstrated that intraoperative fluorescein staining correlated with histopathological alteration in both contrast-enhancing and non-contrast-enhancing regions, with a positive predictive value greater than 96% in non-contrast-enhancing regions, suggesting that FS can be used as a visual marker for glioma resection in both regions of GBM [8].

5. Conclusion

Based on the data collected in 27 patients (16 men, 11 women), the use of intravenous fluorescein during surgery for HGGs with a dedicated filter integrated into the PENTERO 900 surgical microscope able to allow a high rate of complete resection (the rate of GTR and STR was 85.2%). No side effect related to FS at the dose of 5mg/kg was reported.

The limitations of this study were the small number of patients, no control group, and no subgroup analysis. Larger-scale studies are now needed, to quantify the efficacy of fluorescein-guided surgery in improving the extent of resection as well as the progression-free and overall patient survival.

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