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REVIEW

Fluorescein-assisted stereotactic needle biopsy of brain tumors: a single-center experience and systematic review

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Abstract

Over the last years, fluorescence-based technology has begun an emergent intraoperative method for diagnostic confirmation of brain tumor tissue in stereotactic needle biopsy. However, the actual level of evidence is quite low, especially about fluorescein sodium (FL) application. This method needs to be further validated and better analyzed about its impact in clinical practice. Retrospective analysis of 11 cases with contrast-enhancing brain tumors, underwent awake stereotactic needle biopsy with intraoperative FL assistance (group 1), was verified under the operative microscope filter. This group was matched with a control group of 18 patients (group 2). In addition, a systematic literature review was performed in PubMed/Medline database according to PRISMA statement. All studies concerning FL or 5-ALA application in stereotactic biopsy as intraoperative confirmation of brain tumor tissue were included. The primary endpoint was the evaluation of diagnostic accuracy. In group 1, all fluorescent specimens were diagnostic. The number of samplings was the useful minimum and non-use of intraoperative neuropathological examination allowed to significantly reduce procedure time (42.09 vs 69.72 min of group 2). No complications occurred, and the average hospitalization time after procedure was 1.09 days (vs 2.33 of group 2). Literature analysis supports the usefulness of photodiagnosis and its high diagnostic yield especially at the core of high-grade/contrast-enhancing tumors. FL assistance during stereotactic biopsy of contrast-enhancing brain tumors may give a real-time confirmation of tumor tissue, maximizing the diagnostic yield, and reducing time of procedure, morbidity, and hospitalization.

Keywords 5-ALA · Brain tumors · Fluorescein sodium · Fluorescence-assisted biopsy · High-grade glioma · Stereotactic needle biopsy

Introduction

Stereotactic needle biopsy is often considered a minimally invasive procedure, although complications even fatal are not uncommon. Intracranial hemorrhage may depend on several factors, including tumor vascularization and clinical features of the patient, but it is also related to the number of withdrawals performed to optimize the diagnostic yield of the procedure [27]. Non-definitive diagnosis, in fact, is about 2–10% of the reported case series [1, 2, 4, 9, 15, 20, 24, 27], and it is the main issue to overcome because of the need to repeat the biopsy. In the course of time, new intraoperative adjuncts are spreading to optimize the diagnostic yield and to reduce the related complications, limiting the use of intraoperative histology [4, 19, 23]. Fluorescence-based technology (5-ALA [28]and FL [16]) represents a very promising methodology in this field. However, the actual class of evidence is overall quite low, consisting in few observational cohort studies. In particular, FL application is very little reported and it needs to be further validated. Some technical and clinical aspects (like evaluation of operative times, execution in awake patient, and impact on morbidity and hospitalization) are not currently well analyzed.

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Materials and methods

The study includes 11 consecutive patients with contrast-enhancing brain tumors on MRI (Fig. 1a), not candidates for

surgical resection, underwent FL-assisted stereotactic needle biopsy at our Neurosurgical Unit from December 1, 2016 to September 30, 2017 (group 1). All cases were first discussed by the multidisciplinary neuro-oncological board at our center. Histories, preoperative MRI, intraoperative findings, post-operative CT scan, histologies, and clinical follow-up were retrospectively reviewed. All procedures were carried out in awake patient under local anesthetic. We performed a frameless pinless electromagnetic needle biopsy (StealthStation S7 AxiEM, Medtronic, Louisville, CO, USA) in two patients and a frame-based stereotactic needle biopsy with Leksell Stereotactic System (Elekta AB, Stockholm, Sweden) in nine patients. The choice between the two systems was made according to the location and size of the tumor and the operators' preference (frameless technique was preferred for both large and superficial tumors). Preoperative imaging was acquired with T1 pre- and post-gadolinium sequences MRI (GE Healthcare SIGNA Excite 1.5T, Little Chalfont, Buckingamshire, UK) no more than 48 h preoperatively for frameless procedures and with CT scan with contrast (GE Healthcare LightSpeed VCT, Little Chalfont, Buckingamshire, UK) immediately before frame-based procedures. Therapy post-procedure was decided according to histology and clinical characteristics of patients by the same multidisciplinary neuro-oncological team. All data are summarized in Table 1.

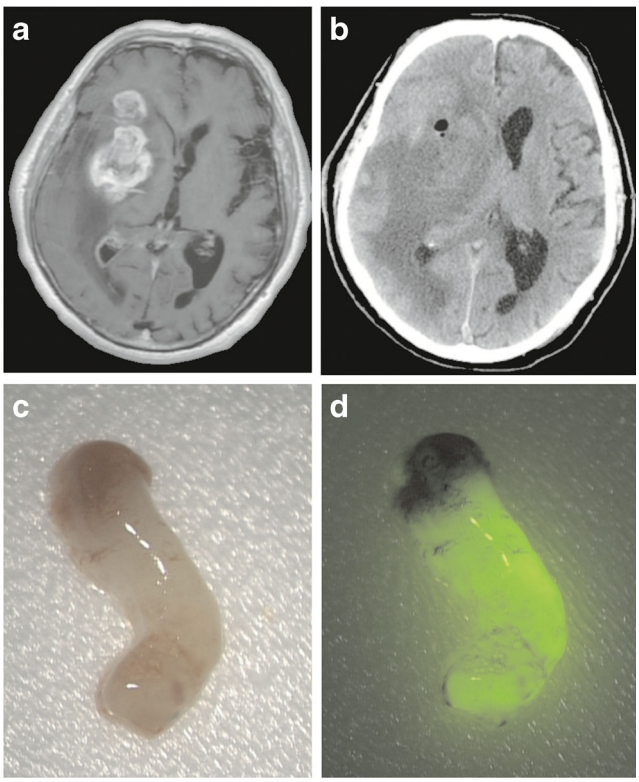

Fig. 1 Illustrative case of a patient with right fronto-temporal GBM on brain MRI (**a**), underwent FL-assisted stereotactic needle biopsy. Post-operative brain CT scan (**b**). Tumor sample under white light (**c**) and under YELLOW 560 filter (**d**) that shows intense fluorescence.

FL-assisted biopsy protocol

All patients with hepatic or renal dysfunction and hyper-reactivity against contrast agents were excluded. A low-dose (5 mg/kg) of fluorescein sodium 20% (Fluoresceina Sodica 20%, Monico SPA, Venezia/Mestre Italy) was administered intravenously in bolus 20 min before skin incision. The target point was systematically planned inside the contrast-enhancing area ("core-sampling" technique). Every sample was verified under the YELLOW 560 filter integrated into OPMI Pentero 900 (Carl Zeiss, Oberkochen Germany). If the first specimen was fluorescent, we did not plan any other collection if it was volumetrically adequate. Neuropathologist was blinded to fluorescence status. After procedure, all patients were transferred to our unit and they routinely underwent a brain CT scan (GE Healthcare LightSpeed VCT, Little Chalfont, Buckingamshire, UK) within 24 h. A formal consent for FL administration was not required because Technical-Scientific Advisory Committee of the Italian Medicine Agency (report number 38, May 18–20, 2015) approved FL use as tracer in neurosurgical oncology (determination 905/2015, Gazette no. 168, July 22, 2015).

Control group

Between 24 patients who underwent awake stereotactic needle biopsy at our center during the last 2 years, we excluded 6 cases (4 low-grade gliomas, 1 abscess, and 1 inflammatory disease). Therefore, we included a group of 18 patients with contrast-enhancing brain tumors on MRI, underwent procedure without FL assistance (group 2), using it similar to control group. It is matchable with group 1 for gender, age, histology, and surgical technique (Table 1). It includes 2 cases which underwent frameless pinless electromagnetic biopsy and 16 cases which underwent frame-based stereotactic needle biopsy. Post-operative management was the same of the group 1.

Statistical analysis

The Mann-Whitney *U* test was used to evaluate the differences between group 1 and group 2. The Spearman test was performed to evaluate correlations. All *P* values were based on two-tailed tests, with a level of significance of 0.05. All analyses were performed using software Kyplot 2.0 b15 (K. Yoshioka, Japan).

Table 1 Characteristics of the patients with contrast-enhancing brain tumors underwent awake stereotactic needle biopsy with FL assistance (group 1) and of the control group (group 2)

Characteristics	FL-assisted biopsy (group 1)	Control group (group 2)	*P* value
Number of patients	11	18	
Gender			
Female	4	8	0.69
Male	7	10	
Age (years)			
Mean	56.7	62.6	0.21
Range	40–77	47–79	
Standard deviation	12.51	9.63	
Histology			
Lymphoma	3	2	0.15
High-grade glioma (WHO III)	2	1	
High-grade glioma (WHO IV)	6	15	
Stereotactic technique			
Frame-based	9	16	0.62
Frameless	2	2	
Operation time (minutes)			
Mean	42.09	69.72	0.005
Range	30–60	30–145	
Standard deviation	11.09	34.91	
Intraoperative frozen section analysis	0 (0%)	4 (22.2%)	0.11
Number of sampling			
Mean	1.27	2.89	0.00002
Range	1–2	2–4	
Standard deviation	0.47	0.68	
Post-operative complications			
Minor bleeding	1 (9.1%)	4 (22.2%)	0.39
Hemorrhage	0 (0%)	1 (5.6%)	0.48
Hospitalization time after procedure (days)			
Mean	1.09	2.33	0.03
Range	1–2	1–11	
Standard deviation	0.3	2.45	

Literature review

Considering the few reports and the analogous results, literature review brought together both FL- and 5-ALA-based adjuncts to analyze more widely the feasibility of fluorescence technology in this field, taking rigorously into account the great difference between the two drugs from the biological point of view. Inclusion criteria were all studies concerning FL or 5-ALA application in stereotactic needle biopsy as intraoperative confirmation of brain tumor tissue. Exclusion criteria were studies including patients underwent biopsy techniques alternative to the stereotactic one, preclinical papers, and non-English articles. In cases in which different biopsy techniques were simultaneously considered in the same study, the subgroup of patients underwent stereotactic one was also eligible. When the same or partial patient population was used in more publications from the same Authors or same center, all studies were included in our qualitative analysis. The primary endpoint was the evaluation of diagnostic accuracy. The PRISMA Statement for Systematic Reviews and Meta-Analyses [13] was used. PubMed/Medline database was extensively searched with combinations of the terms "fluorescein and stereotactic biopsy," "5-ALA and stereotactic biopsy," "fluorescence and stereotactic biopsy," "(fluorescein[Title/abstract]) AND brain biopsy[Title/Abstract]," "(5-aminolevulinic acid[Title/abstract]) AND biopsy[Title/Abstract]," and "(fluorescence[Title/abstract]) AND brain biopsy[Title/Abstract]". Each article of interest was screened. Similar and related articles and reference list of each item were checked to not exclude any paper. Data were independently extracted from every report, and the Cochrane Risk of Bias Tool [7] was used to assess risk of bias in included studies.

Results

Group 1

There were no intraoperative and post-operative surgical complications (Fig. 1b), except one case (9.1%) of asymptomatic minor bleeding, that needed just a slightly longer clinical observation in our unit (2 days). Photosensitivity, side effects, or anaphylactic reactions related to additional use of FL did not occur. The awake procedure was well tolerated in all patients. The average time between FL injection and specimen collection was 38.2 min, and the average time of overall procedure was 42.09 min. No fluorescence was observed under white light (Fig. 1c) but only under the microscope filter (Fig. 1d). Samples were strongly fluorescent at the first withdrawal in 10/11 cases (90.9%). In the remaining case, a second sampling, near the previous one within the contrast-enhancing area, was necessary to observe fluorescence. Histology of this non-fluorescent specimen was in favor of non-specific gliosis. In other two cases, a second sampling was performed to obtain an adequate lesion volume. All fluorescent specimens were diagnostic for tumor tissue, and the mean number of sampling was 1.27. In the contrast-enhancing core of tumor, specificity and sensitivity were 100%. Intraoperative neuropathological examination was not performed in any case. The good post-operative course allowed the discharge the day after the procedure in almost all cases (Table 1).

Group 2

There were no intraoperative complications, and the awake procedure was well tolerated in all cases. The average number of sampling was 2.89 per patient. Intraoperative frozen section analysis was performed in four cases (22.2%). The average time of overall procedure was 69.72 min (vs 42.09 of group 1, $P = 0.005$), and it was significantly related to the choice of using intraoperative histology (Spearman's Rho 0.73, $P = 0.002$). In one case (5.6%), post-operative hemorrhage within surgical field occurred with a transient neurological deficit (Fig. 2a, b). In this case, just medical therapy was performed. During hospitalization, the patient was clinically and neuroradiologically observed by brain CT scans. Right hemiparesis progressively improved, and he was transferred to a rehabilitation center. In four asymptomatic cases (22.2%), a minor bleeding was observed on post-operative routine CT scan. In this group, the average hospitalization time after procedure was 2.33 days (vs 1.09 days of group 1, $P = 0.03$), and it was significantly related to the post-operative bleedings (Spearman's Rho 0.80, $P = 0.001$).

Literature review

The initial search obtained a total of 193 items. Records, after duplicates were removed, were 165. After screening for inclusion and exclusion criteria, just 12 studies were considered in the qualitative analysis (10 papers [6, 10, 14, 16, 21, 22, 25, 26, 28, 29] and 2 abstracts [11, 12]) (Fig. 3). Among the 12 considered records, we found 9 reports about 5-ALA-assistance [6, 10–12, 14, 25, 26, 28, 29] and only 3 about FL-assistance [16, 21, 22] during stereotactic needle biopsy (Table 2). Just two prospective studies were found [21, 25]. Case-control studies and concomitant awake procedures were not reported. The included studies were small but quite homogeneous in design. Main bias is represented by retrospective nature in all cases except two [21, 25]. In most papers, a detection bias, related to non-blindness of pathologist, was found [6, 10–12, 14, 22, 25, 28, 29]. The main finding was the high reported diagnostic accuracy both for FL and 5-ALA, but it significantly differed according to target and histologic entity and grading. Below, we resume the main features of each study briefly.

Yamaguchi et al. [28] in 2007 first reported the use of intraoperative 5-ALA fluorescence under a blue laser system during 6 cases (4 high-grade gliomas and 2 lymphomas) of frameless stereotactic needle biopsy in general anesthesia. Intraoperative rapid pathological examination was performed in all cases. Interestingly, with the increase of the level of protoporphyrin IX in the mitochondria, tumor samples were fluorescent also when rapid examination of frozen sections was non-diagnostic because of tissue breakdown.

Another pioneering use of 5-ALA fluorescence during stereotactic biopsy was reported by Hefti et al. [6] in 2008. They found strong fluorescence, under UV light of operative microscope, in the first sample at target in all cases of high-grade gliomas during 10 frameless procedures in general anesthesia. No further samples were considered if the first fragment showed intense fluorescence, and all histologies were conclusive.

Moriuchi et al. [14] reported in 2011 the 5-ALA assistance in two stereotactic needle biopsies (one frame-based and one frameless) of deep-seated brain tumors. The procedures were performed under general anesthesia, and the fluorescence status of the specimens was verified under UV light of operative microscope. This method allowed diagnostic confirmation avoiding further withdrawals in eloquent areas.

von Campe et al. [25] reported in 2012 their further experience [6] in this field. They obtained fluorescent specimens in 13 of 17 biopsies, performed with frameless technique and in general anesthesia. Fluorescent specimens were related to malignant or high-grade neoplasms. Non-fluorescent specimens were represented by WHO II astrocytomas and unspecific gliosis. No further samplings were taken, and no frozen section was obtained if strong positive fluorescence was visible

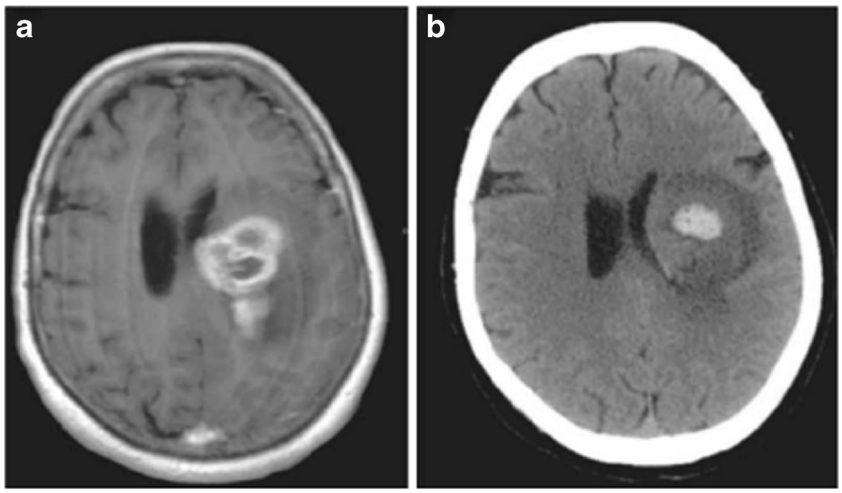

Fig. 2 Illustrative case of a patient of group 2 with left frontal GBM on brain MRI (**a**). Post-operative brain CT scan shows bleeding within biopsy areas (**b**).

under a 405–440-nm blue light source. This intraoperative adjunct allowed reduction of procedure time (30–45 min) for fluorescence positive cases.

Widhalm et al. [26] in 2012 reported a wide and prospective study about 5-ALA assistance. They performed 50 frameless biopsies under general anesthesia and with patient's head fixated in the head clamp. 5-ALA fluorescence was visible in 43/50 patients (strong in 39 and vague in 4 cases). Strong fluorescence was exclusively observed at the biopsy target (contrast-enhancing area on MRI in 39 cases, PET target in 8 cases, and chemical shift imaging in 3 cases) while it did not occur outside the target. Afterwards, the same group reported, in the form of abstract, the further experience with primary central nervous system lymphomas [12] and brain tumors in general [11]. This last case series amounts to 125 patients in which a final histopathological diagnosis was established in 121 cases (97%). A strong fluorescence was detected in 93/125 cases (78%) of which 92/93 (99%)

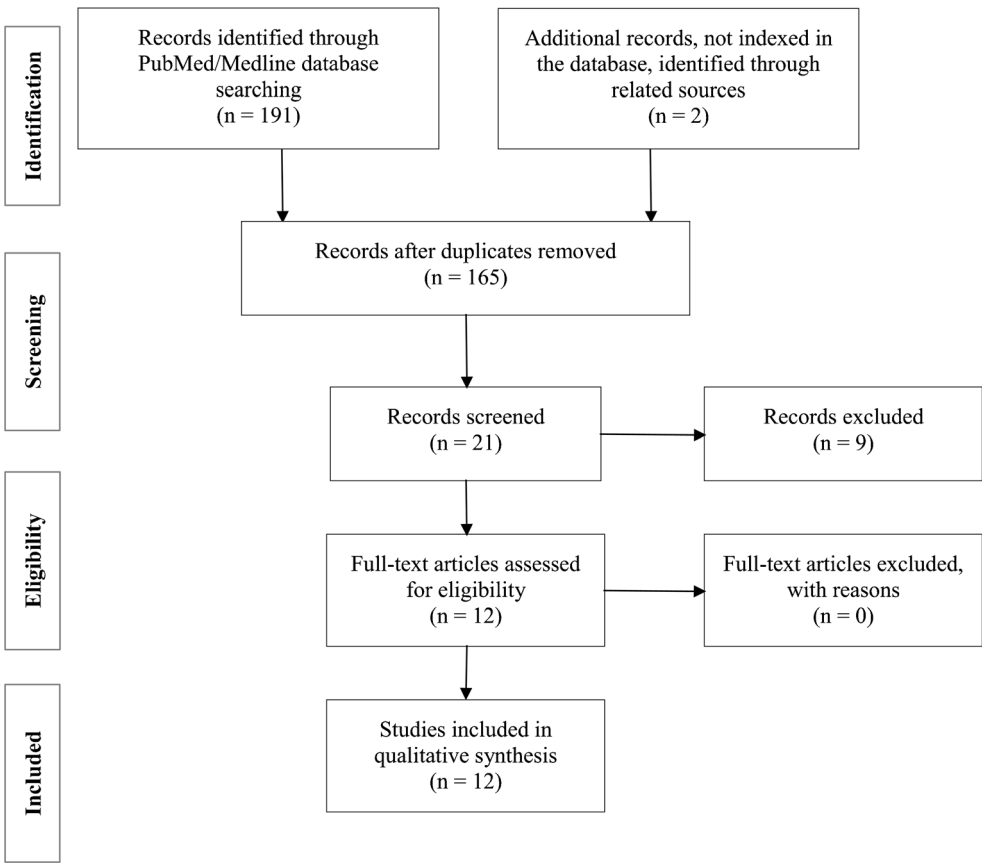

Fig. 3 PRISMA 2009 flow diagram about information through the different phases of systematic review

Table 2 Systematic literature review about the intraoperative fluorescence-based technology in stereotactic needle biopsy for brain tumors

Author, year	N. pts	Fluorescent tracer	Dose and administration timing	Histology	Surgical technique	Anesthesia	Study findings
Present study	11	FL under YELLOW 560 filter	e.v. 5 mg/Kg (20%); 20 min before skin incision	6 GBM, 2 anaplastic gliomas (WHO III), 3 lymphomas	Frame-based and frameless pinless electromagnetic stereotactic needle biopsy	Awake with local anesthetic	• 100% specificity and sensitivity of fluorescence within contrast-enhancing core of brain tumors • Reduction of samplings number, morbidity, time of procedure, and hospitalization compared to the control group
Thien, 2017 [21]	18	FL under YELLOW 560 filter or Olympus Inverted Microscope CKX41	e.v. 5 mg/Kg after anesthesia induction	5 GBM, 3 anaplastic gliomas (WHO III), 3 astrocytomas (WHO II), 4 lymphomas, 1 metastasis, 1 encephalitis, 1 tumefactive demyelination	Frameless stereotactic needle biopsy	General anesthesia	• comparison between FL and intraoperative histology • positive predictive value: 100% • negative predictive value: 25%
Millesi, 2017 [11]	125	5-ALA under modified operative microscope (Zeiss Pentero or NC4)	20 mg/kg; n/a	64 GBM, 14 astrocytomas (WHO III), 25 lymphomas, 22 others (n/a)	Frameless stereotactic needle biopsy	n/a	• Final histopathological diagnosis obtained in 97% • Strong fluorescence found in 78%, vague in 7% and none in 15% of cases • 99% of cases with strong fluorescence received diagnosis
Thien, 2016 [22]	4	FL under Fluoropen	e.v. 5 mg/Kg; n/a	GBM, diffuse astrocytoma (WHO II), lymphoma, low-grade glioma	Frameless stereotactic needle biopsy	General anesthesia	• Full concordance between Fluoropen and operative microscope filter • 80% of samples, within the contrast-enhancing part, were fluorescent • Specificity of 100% of fluorescence for tumor tissue • Non-contrast-enhancing tumor was non-fluorescent
Mischkulnig, 2015 [12]	19	5-ALA under modified operative microscope	n/a	Lymphomas	Stereotactic needle biopsy	n/a	• Strong fluorescence in 18/19 patients (99%) • in all cases with visible fluorescence, diagnostic tissue was found
Yamamoto, 2015 [29]	6	5-ALA	Oral 20 mg/kg; 4 h before anesthesia induction	Lymphomas	Frame-based and frameless stereotactic needle biopsy	General anesthesia	In some cases, alternative or none diagnosis was made on intraoperative frozen sections while definitive histology confirmed lymphoma in all cases of fluorescent samples
Rey-Dios, 2014 [16]	6	FL under YELLOW 560 filter	e.v. 3 mg/kg (10%); at anesthesia induction	GBM	Frameless stereotactic needle biopsy	General anesthesia	• All samplings within tumor core were fluorescent and diagnostic for tumor tissue (specificity of 100%)
Marbacher, 2014 [10]	82	5-ALA under 440-nm blue-violet light source	Oral 20 mg/kg; 3–5 h before anesthesia induction	52 HGG, 12 LGG, 3 gliomatosis cerebri, 1 ependymoma, 2 germinomas, 1 multiple myeloma, 6 lymphomas, 5 others (n/a)	Frameless stereotactic needle biopsy	n/a	• Fluorescence positivity in 44/52 cases (84.6%) of HGG • Fluorescence positivity in 3/6 cases (50%) of lymphomas • Fluorescence positivity in 3/12 cases (25%) of LGG
Widhalm, 2012 [26]	50	5-ALA under violet-blue	e.v. 20 mg/kg; 4 h before	25 GBM, 8 WHO III gliomas, 6 WHO II gliomas, 7 lymphomas, 4 metastasis	Frameless stereotactic needle biopsy	General anesthesia	• Strong fluorescence: 39 cases (32 HGG, 7 lymphomas)

Table 2 (continued)

Author, year	N. pts	Fluorescent tracer	Dose and administration timing	Histology	Surgical technique	Anesthesia	Study findings
		excitation light of operative microscope	anesthesia induction				• Vague fluorescence: 4 cases (1 HGG, 3 metastases) • None fluorescence: 7 cases (6 LGG, 1 metastasis) • Strong fluorescence only at biopsy target
von Campe, 2012 [25]	17	5-ALA under a 405–440-nm blue light source	Oral 20 mg/kg; 4 h before anesthesia induction	6 GBM, 2 astrocytomas (WHO III), 2 astrocytomas (WHO II), 4 lymphomas, 1 germinoma, 2 unspecific gliosis	Frameless stereotactic needle biopsy	General anesthesia	• Fluorescent specimens: malignant or high-grade neoplasms • Non-fluorescent specimens: WHO II astrocytomas and unspecific gliosis • No further samplings and no frozen section if strong positive fluorescence was visible • Reduction of procedure time (30–45 min) for fluorescence positive cases
Moriuchi, 2011 [14]	2	5-ALA under UV light	e.v. 20 mg/kg; 4 h before anesthesia induction	Lymphoma and diffuse astrocytoma (WHO II)	Frame-based and frameless stereotactic needle biopsy	General anesthesia	• Diagnostic confirmation avoiding further withdrawals in eloquent areas
Hefti, 2008 [6]	15	5-ALA under UV light	Oral 20 mg/kg; 5–6 h preopera-tively	HGG	Frameless stereotactic needle biopsy	General anesthesia	• Strong fluorescence with the first sample at target in all cases • No further samples were considered • Histologies were conclusive in all cases
Yamaguchi, 2007 [28]	6	5-ALA under blue laser system	Oral 20 mg/kg; 1 h before anesthesia induction	2 GBM, 2 astrocytoma (WHO III), 2 lymphomas	Frameless stereotactic needle biopsy	General anesthesia	• Confirmation of tumor tissue in biopsy samples also when intraoperative histology was non-diagnostic because of the tissue breakdown

received a final diagnosis. The non-diagnostic case that showed strong fluorescence (1/93) presented inflammatory changes in the specimen.

The largest case series in 5-ALA fluorescence-assisted stereotactic needle biopsy was reported by Marbacher et al. [10] in 2014. They performed 82 frameless biopsies of several entities of brain tumors. They observed a high fluorescence positivity rate only in HGG (84.6%). In lymphomas, it was positive for half (50%) and in LGG just 25%. No data were available about intraoperative histology use, planned target, surgical times, and procedure-related morbidity.

The last paper about 5-ALA in stereotactic biopsies was published by Yamamoto et al. [29] in 2015. This study included also further biopsy modalities; therefore, some results were mixed. The authors performed frame-based or frameless procedures in general anesthesia for 6 cases of lymphomas. They planned a first target at the central or near-central region of the enhanced mass and four or more samples around the first target and periphery of the tumor. Samples with positive fluorescence underwent intraoperative pathological examination. It is relevant to note that, in some cases, alternative or none diagnosis was made while definitive histology confirmed lymphoma in all cases.

The first paper about FL assistance in this field was reported by Rey-Dios et al. [16] in 2014. They used FL (3 mg/kg) fluorescence-assisted frameless stereotactic needle biopsy in 6 patients with glioblastoma. The patients were in general anesthesia and with the head fixated into three-point head holder. Fluorescence status was verified under filter integrated into operative microscope. Interestingly, they planned sampling within tumor core (enhancing and non-enhancing core) and margins to verify the different degree of fluorescence that was fluorescent only in enhancing core. All fluorescent samples were diagnostic for tumor tissue. Sensitivity and specificity were 79 and 100%, respectively. However, considering just results within enhancing core, sensitivity rate was also 100%.

The second paper about FL use was reported by Thien et al. [22] in 2016. They performed FL (5 mg/kg) fluorescence-assisted frameless stereotactic needle biopsy under general anesthesia in four patients with brain tumors (three contrast-enhancing and one non-contrast-enhancing on MRI). They verified fluorescence status through a new device (Fluoropen) that showed full concordance with operative microscope filter. Four of five samples (80%), taken from the contrast-enhancing part of the tumors, were fluorescent. All four specimens were diagnostic for tumor tissue. The contrast-enhancing but non-fluorescent sample was a lymphoma underwent high-dose dexamethasone preoperatively. Finally, the one non-contrast-enhancing tumor appeared non-fluorescent and histology was in favor of a low-grade glioma.

The same Singapore group [21]recently reported their further experience [22] with FL in the stereotactic biopsy of contrast-enhancing brain lesions in 18 patients. The most interesting aspect was the comparison between FL and intraoperative frozen section assessment to confirm pathological tissue samples. All fluorescent specimens (83%) were confirmed to be pathological on intraoperative histology (positive predictive value 100%). The non-fluorescent specimens (17%) were confirmed to be lesional in three of the four cases and non-diagnostic in the remaining case (negative predictive value 25%).

Discussion

Technical and clinical aspects

In our experience, FL adjunct was easy to integrate in practice and it resulted very helpful in the intraoperative assessment of the specimens. This tool modified our usual strategy because it systematically gave an immediate double check (macroscopic aspect and fluorescence status) without need of additional validations. In patients treated without FL assistance (group 2) in fact, we chose to make more samplings (mean 2.89) even in the cases of apparent pathological tissue at first withdrawal to not invalidate the procedure. Furthermore, the use of intraoperative frozen section analyses was more frequent and it lengthened a lot the procedure time (69.72 vs 42.09 min, $P = 0.005$). Of course, this last aspect may have a particular relevance on awake patient compliance. On the contrary, the fluorescence positivity in group 1 allowed us to speed up the procedure, to not consider intraoperative histology and to perform the useful minimum number of samples because of high specificity of dye. In general, fluorescence positivity, in fact, demonstrated to give information also when intraoperative neuropathological examination is non-diagnostic because of tissue breakdown [28]. Similarly, Hefti [6], Moriuchi [14], von Campe [25], and Thien [21] concluded the procedure with a very limited number of samples if fluorescence status was positive. In particular, Hefti [6] and von Campe [25] performed just one withdrawal with or without support of intraoperative histology, respectively. Anyway the impact of this choice on morbidity was not sufficiently stressed. Only Moriuchi [14] mentioned it for deep-seated tumors. In our practice, the group treated with FL did not undergo surgical complications and in all cases "a day procedure" was realizable, also with inevitable favorable cost-implications. If you consider the low cost of the drug (less than € 5 per vial), this last datum becomes particularly important. Though the rates of bleeding between the two groups were not statistically significant, in group 2, their higher occurrence (even if minor in most cases) needed a quite longer but significant observation period (2.33 vs 1.09 days, $P = 0.03$).

Finally, it is clear that photodiagnosis is only complementary to the traditional frameless or frame-based stereotactic

techniques and it cannot guide to the target. A correct preoperative planning remains paramount. However, intraoperative assessment of the specimens is equally a crucial point of the procedure that could take several intra- and post-operative implications. Therefore, in our opinion, the impact of this technology in clinical practice may be very relevant to reduce the rate of non-definitive diagnosis limiting, at same time, duration of the procedure, morbidity, and costs. Even the eventual choice of the awake method might take this into account.

Diagnostic accuracy

The patients' selection and the planning of the biopsy target (contrast-enhancing core of brain tumors) appear particularly important elements for a successful procedure. FL, in fact, is a marker of blood-brain barrier damage [5, 18] that showed strong correspondence with radiological contrast agents [3]. For this reason, its application for biopsies of contrast-enhancing brain tumors on MRI appears appropriate but the only indicated. In our study and in the others review, biopsies at the core of high-grade/contrast-enhancing tumors showed high rates of specificity and sensitivity (≈ 100%) of fluorescence for tumor tissue similar to 5-ALA. In relation to intraoperative histology, the positive predictive value of FL is 100% and the negative predictive value is 25% [21]. On the contrary, with FL, tumor non-enhancing core and margins may not show any fluorescence and biopsies carried out there may significantly reduce its sensitivity rate (from 100 to 79%) [16]. Histology of the periphery of the fluorescent area, in fact, may show a transition from low-grade to normal brain tissue with reactive gliosis that FL could not depict [3]. At tumor borders, also specificity rate could decrease with FL because of the possible false-positivity related to every cause of blood-brain barrier disturbance (e.g., perifocal edema, scar of a previous surgical, and/or radiant treatment [17]). Even so, specificity rate generally decreases much less than sensitivity rate (specificity 90.9–95% versus sensitivity 82.2–84.61% [3, 5]). Anyway, also 5-ALA fluorescence, even if tumor-specific, showed higher false-negative rates in low-grade/non-contrast-enhancing tumors [8, 28] and its adjunct may not add value [10]. However, it must be underlined that the reviewed papers complexively showed a weak evidence in the evaluation of diagnostic yield; 5-ALA accuracy was supported by some more and wider studies than FL. Anyway, the aggregation of information led to a slightly higher statistical power. The next step may be to conduct controlled prospective trials also to build a meta-analysis.

Finally, a singular aspect that would be interesting to consider is the late false-positive FL fluorescence, reported in high-grade glioma surgery [3, 17] as result of surgical brain-blood barrier damage. For ethical reasons, it was not verified in stereotactic neurosurgery. It should be considered that revisiting the same target or near-target area after a variable period of time (e.g., after intraoperative histology) may show a false-positive fluorescence of the tracer.

Limitations of the study

The main limits of the study are the small case series and studies design that do not offer a strong external validity. Especially, FL use as systematic alternative of intraoperative histology (gold standard) cannot be recommended until the clear benefit of FL had been proven in larger series.

Conclusions

FL assistance during stereotactic needle biopsy of contrast-enhancing brain tumors demonstrated to be able to give a real-time confirmation of tumor tissue and to reduce time of surgery, morbidity, and hospitalization. For this reason, it may help make stereotactic biopsy a low-risk procedure with high diagnostic yield and cost-effectiveness. In this setting, awake technique appears advisable. Anyway, further studies are needed to validate FL assistance in stereotactic biopsy.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical 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.

Informed consent Informed consent was obtained from all individual participants included in the study.

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