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Fluorescence guided resection and glioblastoma in 2015: a review

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Abstract:

High-grade gliomas represent a widely heterogeneous group of tumors, the most frequent of which is glioblastoma multiforme. Its annual incidence has risen over the last decades, particularly amongst elderly people. The actual standards of care allow for a 15-month median survival rate for WHO grade IV gliomas. As recurrence occurs in more than 85% of patients at the surgical margins, the initial resection extent is a cornerstone of disease control. Fluorescence guided resection (FGR) aims at increasing complete resections and, thus, local control. This technique uses 5-aminolevulinic acid (5-ALA), a natural intermediate substance in the heme-porphyrin biosynthesis pathway, and a protoporphyrin IX (PpIX) precursor. PpIX is fluorescent under blue light exposure. Recent studies reported a significant increase in complete resections using FGR, which were associated with prolonged progression free survival, fewer reinterventions, and delayed neurological deterioration. Here, we depict the principles of this surgical technique, its actual outcomes, and future developments.

Conflict of interest statement: None

Today's "State of Art"

Gliomas represent a wide and heterogeneous tumor type that account for half of the new primary brain tumor cases that are annually diagnosed in adults. Most of them, 80%, are high-grade (WHO grade III or IV) gliomas (HGG) [1]. The astrocytoma grade IV or glioblastoma multiforme (GBM) is the most common HGG type. The GBM annual incidence is greater than 5 per 100,000 of the population and leads to 3% of all cancer deaths for patients aged 35 to 64 years-old [2]. Over the past 30 years, coinciding with the introduction of the MRI, the GBM incidence has increased in Europe and USA, especially among individuals older than age 65 years [3, 4]. GBM is characterized by rapid proliferation, marked infiltration, and poor prognoses with a median survival rate of 15 months [5, 6]. The actual treatments for GBM include surgical resection, radiotherapy, chemotherapy, and more recently, antiangiogenic treatments [7, 8].

Regarding surgery, a landmark study by Lacroix et al reported that a larger extent of resection (EOR), reaching 98% of tumors, was correlated with increased median survival rates that were superior to 4 months [9]. More recently, Sanai et al noted a stepwise improvement in survival rates that correlated with EOR [10]. According to their study, the overall survival rate increase was observed at a 78% EOR and was still improving until a 100% EOR was observed. McGirt supported these data, confirming that complete resection of enhancing tumors increased the median survival rate, even for recurrent GBMs [11]. Stummer reviewed 3 prospective studies (the ALA-Glioma, BCNU wafer, and EORTC studies) and concluded that total resection appeared to improve survival and may enhance adjuvant therapy efficacies [12, 13]. Even in elderly people, open craniotomies have been reported to increase median survival times, up to 171 days (95% CI 146–278) versus 85 days (95% CI 55–157) when considering standard management that relies on biopsies ($p=0.035$) [14].

In 2008, The American Association of Neurological Surgeons and the Congress of Neurological Surgeons produced guidelines for the management of newly diagnosed GBMs [15]. The authors recommended maximum safe surgical resections of newly diagnosed GBM (type II scientific evidence), followed by 60 days of postoperative radiotherapy to the enhancing lesion (type I evidence) including a 2 cm margin that surrounds the lesion (type II evidence). The guidelines also recommended concurrent and postoperative temozolomide in newly diagnosed GBMs (type I evidence) as well as BCNUs (carmustine wafers) in those who undergo craniotomies (type II evidence). Stupp reported that, in newly diagnosed GBMs, after maximal tumor resection and post-operative concomitant radio-chemotherapy procedures, a median survival rate of 15 months was observed [7]. For recurrent GBMs, the median survival rate dropped to 3 months. Presently, the 5-year survival rate is less than 5% [16].

The previously mentioned treatments present several limits. For instance, total surgical resections may not be achievable without functional risks. Total resections of HGGs are hardly possible due to their invasiveness into the healthy surrounding parenchyma, which leads to an 85% local recurrence rate within 2.5 cm from the initial operative cavity [17]. It is now well established that glioma cells are not only able to destroy but also invade normal tissue around and away from the operative cavity. The concept of “total” removal that is based on the absence of a postoperative residual contrast enhancement is not satisfactory. Obviously, tumor cells remain among the T2 FLAIR hypersignal that surrounds the initial contrast enhancement [18]. Concerning radiotherapy and chemotherapy, these treatments are not selective and can affect normal functional cells in the short or long term, e.g., radiation-induced leucoencephalopathy. Therefore, some authors theorized that “There is no jacket that fits all GBMs” [19].

Further research is needed to improve the GBM patient survival rate. For neurosurgeons, maximizing the EOR is a cornerstone in GBM management and, in this context, fluorescence guided resection (FGR), using photodynamic diagnoses [20], may play an important role in improving patient healthcare. FGR is a surgical method that uses light induced photochemical reactions after the administration of a precursor (5-ALA) of a fluorescent agent (protoporphyrin IX, PpIX). Recent studies, including the Cochrane review [21], confirmed its efficiency, cost effectiveness, and low adverse event rate, making this technique suitable for daily practice and EOR optimization.

Photodynamic diagnoses with 5-Aminolevulinic acid: How it works

The biochemical aspects

Fluorescence guided resection is based on the tumor specific photosensitizing substance accumulation, which induces a selective fluorescence spectrum after specific wavelength light exposure. 5-Aminolevulinic acid (5-ALA) is the most commonly used molecule. 5-ALA is a natural prodrug that becomes protoporphyrin IX (PpIX) [22], which is a photoactive compound that absorbs blue light (375-440 nm). PpIX then emits red light in the visible spectrum, mainly at 635 nm. A correlation exists between higher cellular PpIX concentrations and greater fluorescence intensities [23, 24].

Normally, non-neoplastic brain PpIX accumulation is one of the lowest in all of the human organs [23]. However, HGG usually accumulates high PpIX levels. As Colditz reported, it may be related to a lower baseline heme requirement because tumor cells prefer glycolysis for obtaining energy rather than the heme-

regulated cytochrome oxidative phosphorylation pathway [22, 27]. Moreover, the PpIX concentration is correlated with increased cell proliferation [28] and others conditions, such as hypoglycemia [29] and acidosis [30], which are commonly found in the HGG tumor milieu. According to Kanako, the PpIX concentration in wet GBMs was 1004.6µg/g compared with 125.1 µg/g in the wet surrounding brain [31]. A previous study reported ratios ranging from 20:1 to 50:1 in HGG tissue compared with normal brain tissue [32]. Prominent downregulation of the ferrochelatase gene (FECH) mRNA expression was found in human glioblastoma tissues compared with normal brain tissues, suggesting that FECH is at least partly responsible for PpIX accumulation in glioblastoma cells [33]. Active efflux of PpIX from the cerebrospinal fluid via peptide transporter 2 (PEPT2) to the choroid plexus has been reported to play a role in protecting the brain from 5-ALA neurotoxicity [34, 35]. In strongly fluorescent GBMs, PEPT2 mRNA was downregulated, and this may be an additional mechanism for PpIX accumulation in GBM tissue [36]. Lastly, a small amount of 5-ALA is able to go through the intact blood brain barrier [37]. It was postulated that more 5-ALA can influx across disrupted blood brain barriers in the peritumoral area.

Treatment planning

Patient selection is a major step before undergoing FGR. In 2014, the Cochrane Review reported criteria that should be utilized before considering FGR use, such as patients with no persistent neurological deficit under corticosteroids, patients with a good performance status (Karnofsky Performance Scale > 60), patients that harbor a well defined tumor in a non-eloquent region, and patients that are amenable to a safe complete resection [21]. Surgical procedures are planned with T1-weighted 3D magnetization-prepared rapid gradient echo (MPRAGE) MR imaging with and without contrast enhancement as well as T2-weighted sequences to visualize edema [38, 39]. Well-defined tumors that are harbored with MRI give off a distinct ring-like pattern of contrast enhancement and a core area with reduced signal, which is suggestive of central necrosis [40]. Tumors that are invading functional neuron bundles, such as the midline, basal ganglions, cerebellum or brainstem regions should be excluded [40]. GBMs that are invading eloquent areas are usually not FGR candidates. However, Della Puppa et al reported 25 operated GBM cases in eloquent areas using MRI neuro-navigation and intra-operative monitoring [39]. This monitoring included: continuous electroencephalography, electrocorticography, electromyography, sensory motor evoked potentials, and cortical/subcortical stimulation [39]. Thanks to these preoperative recordings, a cortical functional map was obtained. Although 64% of their patients presented with neurological impairments during the first week of assessments, they reported only a 3% severe neurological morbidity rate at 3 months [39]. In the Feigl et al study, 15 patients that were harboring GBMs in the vicinity of the eloquent areas were operated on using FGR

combined with intra-operative monitoring [38]. They reported only 2 postoperative accentuated hemiparesis cases and one new homonymous hemianopia case [38].

FGR requires preoperative 5-ALA administration. The usual dose is 20 mg/kg [41], which is mixed in water and given orally three hours before anesthesia [42]. Higher systemic doses, ranging from 30, 50, and 60 mg/kg, have been tested in humans [43, 44] with no superiority. Fluorescence is supposed to be maximal 6 h after administration, then the plasma level decreases to zero at 48 h [45]. 5-ALA, at 20 mg/kg, presents minimal side effects, such as erythema (in cases of direct exposition to direct light for up to 24 h after administration), nausea, mild hypotension, and liver function test elevation [41]. Nevertheless, 5-ALA should not be delivered with other phototoxic substances (i.e., fluoroquinolones) in patients with inherited or acquired porphyrias and during pregnancy [46]. Patients that are suffering from chronic liver failure must avoid ALA according to several authors, such as Roberts et al and Eljamel et al [47, 48]. 5-ALA use in clinical practice has been authorized since 2007 in Europe (Gliolan[®] Medac, Germany) [41]. Neurosurgeons have to complete a certified fluorescence guided surgery course before they are authorized to use it clinically [41].

Others photosensitizers have been evaluated for performing FGR. Photofrin[®] (Axcan Pharma, Quebec, Canada) is a hematoporphyrin derivative that is an exogenous first generation photosensitizer that preferentially accumulates in cancer cells. However, it has to be administered 2 days before surgery and induces long-lasting skin photosensitivity. Photofrin[®] is also less selective than 5-ALA and emits very dark red fluorescence wavelengths that are difficult to visualize [48, 49]. Foscan[®] (bioLitec Pharma, Edinburgh, UK), which is also known as m-tetrahydroxuphenyl chlorine (m-THPC), has also been used for FGR [50]. M-THPC is known to cause higher skin toxicity levels combined with a longer half-life in comparison with 5-ALA. M-THPC is preferred for photodynamic therapy but not for photodiagnoses [51]. Fluorescein sodium is another passive targeting photosensitizer, which can be used, with good sensitivity and specificity, for GBM resections. Fluorescein sodium's main advantage is its low cost and it has recently been successfully used in the FLUOGLIO study in Italy [52]. However, fluorescein sodium is not yet approved for brain tumor resections [53].

Concerning specific operative room equipment, FGR requires a neurosurgical microscope that is upgraded to detect 5-ALA fluorescence, for example Carl Zeiss's OPMI[®] Pentero[™] or Leica[®] M720 OH5. Additionally, routinely used neuronavigation, micro-instruments, and ultrasonic dissectors are also required. Anesthesiologists and nurses need to be aware that patients should avoid continuous pulse-oxymetry and fundal retinal examinations during the 24 h following 5-ALA administration to prevent photosensitivity related lesions.

Surgical procedure

Resection is driven by fluorescence intensity and is limited by eloquent areas. FGR is best used as an additional modality, combined with neuronavigation, functional MRI, and intraoperative electrophysiological monitoring [38]. In spite of such innovative techniques, it is mandatory that the surgical technique itself is not neglected and to maintain a high level of neuroanatomy and neurocognitive function topography knowledge. Image-guided technology allows for location of craniotomy optimization. After opening the dura and evaluating the cortex, the surgeon begins to debulk easily recognizable tumors. If the tumor is not visible on the brain surface, the best dissection route is planned using an image guidance system. Then, after switching off the operative room lights to darken the environment, the surgeon can switch to FGR mode (Fig. 1). Excitation is initiated with a filtered violet-blue light source (400 – 410 nm bandwidth) [54]. The excitation light intensity diminishes with distance, so for maximal effect, the light source should be close to the cavity [22]. A long-pass filter that is mounted on the surgical microscope, which is switchable from white light to blue, allows for tumor visualization and dissection (Fig. 1). The remainder of the tumor shines bright red through the blue surgical field (Fig. 1). Thus, the neurosurgeon can remove the tumor tissue and, if necessary, in overlaying fluorescence bleeding cases, the surgeon can switch back to white light. Indeed, hemostasis is not properly achievable under blue light exposure, and the control of bleeding points is mandatory before undergoing FGR [48]. Maximal fluorescence occurs 6 h after 5-ALA intake [22]. As 5-ALA is administered 3 hours before anesthesia induction, the neurosurgeon has sufficient time to perform brain dissection before the definitive resection stage [42]. However, under preoperative conditions (6 h after oral 5-ALA administration at a dose of 20 mg/kg), visible PpIX fluorescence decays to 36% of the peak intensity within 25 min. under blue light (400-410 nm) that is emitted by the microscope, and 87 min. under white light due to photobleaching [22, 55]. This exclusively concerns the small brain area under direct light exposure, while PpIX is useful in other areas that are covered with pads or blood is not affected. Photobleaching only affects superficial layers of the exposed brain area, as the excitation light penetration is limited to a few millimeters [55]. In areas with low PpIX fluorescence that may become completely photobleached, the surgeon can restore the fluorescence signal by removing superficial cell layers [42].

During FGR, HGGs (and especially GBM) are expected to present strong fluorescence. As previously reported, fluorescence intensity is correlated with the histological grade, tumor activity markers (including the differentiation level), high proliferation index, tumor cell density and microvessel density (CD31) [24, 47, 56]. Strong PpIX fluorescence is associated with an elevated Ki 67 index, as reported by Diez Valle [57]. Adjacent structures, such as vessels, blood, normal brain tissue, the olfactory tract, the cavernous sinus, dura, and optic nerves, do not

fluoresce [48]. However, some non-invaded anatomical structures, such as the choroid plexus, ventricular ependymal, and pia mater are known to accumulate PpIX [54, 58]. Concerning the ventricle wall, preoperative MRIs cannot predict its fluorescence. Periventricular GBM was known to be an independently poor prognostic factor for progression free (PFS) and overall survival (OS) [59]. Nevertheless, ventricle wall fluorescence was not reported as a survival or complications predictor [59].

Figure 1: Microsurgical dissection of a glioblastoma that is located in the right ventricular crossroad and associative areas. On the left, the surgical cavity is shown in white light. On the right, the same site illuminated area is shown in blue light. We can clearly distinguish tumor remnants in the posterior region of the cavity.

Surgical outcomes, limits, and future prospects:

Photodynamic diagnoses, with its 89% sensitivity and 96% specificity, offers an excellent chance of visualizing tumor cells, even in areas that received prior treatment, such as radiation or chemotherapy, according to Stummer [12, 60]. Recently, a set of studies validated this report, as reported in a systematic review and metanalysis. 5-ALA guided resections demonstrated an overall sensitivity of 0.87 (95%, CI 0.81-0.92) and a specificity of 0.89 (95% CI, 0.79-0.94) [61, 62]. Furthermore, Roberts demonstrated a significant association between contrast enhancements regarding preoperative MRIs and intra-operative observable PpIX fluorescence [47]. According to previous results, FGR has an excellent positive predictive value but a low negative predictive value [57, 63]. In other words, an intense fluorescence is correlated with an aggressive tumor region, whereas a weak fluorescence or no fluorescence does not indicate the absence of tumor tissue. Thus, FGR helps surgeons differentiate between HGG tissue from surrounding brain tissue and maximize safe resections.

Surgical outcomes:

FGR has been reported to significantly improve the glioblastoma EOR and progression free survival (PFS). Concerning the overall survival (OS) and PFS increase when using FGR versus WL, the data still remain sparse [64, 65]. Table 1 compiles the recent clinical studies with prospective or retrospective designs, and it depicts the gains in terms of EOR, OS, and PFS when using FGR for newly diagnosed GBMs [38, 39, 64-72].

In 2006, Stummer assessed, in a randomized controlled multicenter phase III trial, the effect of FGR with 5-ALA for surgery of malignant gliomas [65]. He reported that FGR enabled more complete resections of contrast-enhancing tumor versus conventional microsurgery resection (difference between two groups 29% [95% CI 17-40] $p < 0,0001$), leading to improved PFS (the difference between two groups was 19.9% [9,1-30,7] $p = 0,0003$). Eloquent area tumors were not included. However, the influence of age was observed on the resection degree, which confounded the interpretation concerning the causal influence of resection on survival. To consider the pretreatment prognostic variables, the Radiation Therapy Oncology Group (RTOG) proposed a nonparametric recursive partitioning analysis (RPA). The patient survival rate from the ALA study was correctly predicted by the RTOG-RPA classes. These classes took into account the patient's age, their Karnofsky scale, neurological status, and the treatment type, between resection or biopsy treatment. The differences in survival were dependent upon their resection status, especially in RPA classes IV and V, which strongly supported a causal influence of resection on survival [73]. Based on this work, the Cochrane Collaboration very recently released a review regarding "image guided surgery for the resection of brain tumors". In this review, tumor remnants were significantly smaller in the FGR group in comparison with the "white light" (WL) resection group, (RR 0.55, 95% CI 0.42 to 0.71) [21].

Two comparative studies confirmed the significant impact of FGR in improving the EOR (Table 1). In a 2013 national Spanish study called VISIONA, which was led by Diez Valle, complete resections were noted in 67% of patients in the FGR group compared with 45% in the WL group, ($p < 0.001$) [74]. Slotty et al reported that FGR was associated with complete EORs in comparison with standard WL resections, (48.50% vs. 27%, $p < 0.01$) [64]. More observational studies reported higher total tumor removal rates using FGR [69, 71], considering no more contrast enhancement on early post-operative MRI was utilized. Examples of this were shown in a Schucht et al study, which reported an 89% complete resection rate when they combined FGR and brain mapping [75], or in a Della Puppa et al study that observed a 93% complete resection rate [67]. Such astonishing results bring to light that experienced centers are able to reach a very high standard of resection. For instance, Diez Valle et al reported a 83% total tumor removal rate in their center [57]. This is in contrast with the VISIONA study, in which Diez Valle was involved. VISIONA also incorporated less experienced centers, which presented "only" a 67% total tumor resection rate [74]. Most recently, a meta-analysis from Su et al confirmed the significant EOR increase when FGR was utilized, in which they reported a 71.9% gross total resection rate vs. 36.5% in the control group [76].

Glioblastomas in the vicinity of eloquent areas, even when they are infiltrating them, are not an absolute contraindication to FGR anymore. While using multimodal functional imaging data, Feigl et al reported a 64% glioblastoma EOR rate in eloquent areas without worsening neurological deficits [38]. Della Puppa et al

reached an 81% EOR rate for the same location type, with only a 3% severe morbidity rate at 3 months [39]. This morbidity corresponded with severe motor deficits (i.e., no spontaneous movement against gravity) or severe speech impairments (i.e., great difficulty in understanding and producing spoken and written language) [39].

Furthermore, FGR can be used for early re-excisions if post-operative imagery reveals an unintended tumor remnant. As Schucht reported, 5-ALA fluorescence remained highly sensitive and specific for early reoperations and provided the same results as intra-operative MRIs (iMRI) for complete resection [77].

Regarding PFS, two studies reported a significant gain using FGR in comparison with WL resections. Stummer reported a 41% PFS rate at 6 months using FGR vs. 21% with WL resections ($p = 0.0003$) [65]. Diez Valle, in the VISIONA study, reported a 69% PFS rate in their FGR group vs. 48% in their WL group ($p = 0.002$). One of the key data concepts was the significant impact of FGR on OS. Slotty et al reported, in 2013, on a retrospective series of 253 patients and observed a significant OS increase in their FGR group, (20.1 vs. 16.6 months, [$p < 0.01$]). When the median OS was available, it was always more than 15 months in the FGR group. Stummer [68], Diez Valle [57], and Jacquesson [71] reported median OS rates of 16.3 months, 15.7 months, and 17 months in their FGR groups, respectively. Unfortunately, in these last three studies, the lack of comparison with a WL group did not permit for significantly increased OS rate conclusions.

According to Stummer, in his work regarding the elderly and adverse events, older patients (over 60 years old) should not be excluded from receiving FGR followed by concomitant radio-chemotherapy [68]. Surgery risks were not increased amongst these patients [68]. Additionally, FGR did not hamper the efficiency of actual adjuvant therapies. Stummer also reported that patients who underwent FGR required fewer re-interventions ($p = 0.03$) [65].

Limits:

Bright red-pink fluorescence is easy to distinguish from the normal parenchyma; however, weak fluorescence on the tumor margins or in infiltrated edema might be more difficult to distinguish. Until very recently, photodiagnoses were only performed subjectively, while using a surgical microscope. High-resolution intraoperative microscopy and spectroscopy tools were then developed in order to increase PpIX detection [53, 78]. A Swedish team recently perfected a hand-held optical touch pointer using a fluorescence spectroscopy system to quantitatively distinguish healthy brain from malignant tissue, intraoperatively [79]. They obtained an effective suppression of low power lamp background light from the recorded spectra in addition to a significant reduction of high-powered surgical lights. They reported a ratio number of 6.0 in the spectrum between the PpIX intensity in

GBM tissue compared with normal brain tissue. Valdes et al also reported clinically profound findings using an intraoperative fiber-optic probe that was connected to a spectrometer [80]. Valdes showed that diagnostically significant but visually imperceptible PpIX concentrations could be quantitatively measured in vivo and be used to discriminate normal from neoplastic brain tissue, not only for HGG but also for a wide range of tumor histologies, including low grade gliomas, meningiomas, and metastases [80]. More than 81% (57 of 70) of the quantitative fluorescence measurements that were below the threshold of the surgeon's visual perception were classified correctly in an analysis of all tumor types [80]. In this way, peroperative optical spectroscopy helps to evaluate tumor cells beyond human visual perception. More recently, Valdes et al reported a precise and sensitive system for quantitative spectroscopy that was based on a high-dynamic-range arrays for performing hyperspectral fluorescence and diffuse reflectance detection [81]. This imaging system, which was tested on a rodent glioma model, was able to detect a very low PpIX concentration (20 ng/ml) and generated wide field fluorescence images. Such a detection method could be an answer for the issue of fluorescence intensity subjectivity.

Fluorescence heterogeneity also hampers FGR. Inside a single GBM, the PpIX concentration differs widely between vital tumor regions that harbor bright fluorescence, which mostly become superimposed with contrast enhanced areas, and lower fluorescence levels in infiltrative zones or in necrotic regions [82, 83]. Grade III tumors present even lower PpIX concentrations [83]. No PpIX concentration differences were found between de novo and recurrent GBMs according to Johansson [23]. However, Hefti reported a case of a non-fluorescent GBM that was already operated on and treated with radio-chemotherapy [62]. Although intraoperative recurrent tumors proved to be of a very fibrous and hard consistency, the histological features were found to be of a typical glioblastoma multiforme [62]. In the same way, Tsugu et al reported some initial operation GBM cases that did not fluoresce at all [84]. Among 20 WHO grade IV gliomas, 3 were not fluorescent. Using iMRI, Tsugu reported a 20.5% resection rate increase in patients harboring non-fluorescent GBMs [84]. Tsugu concluded that FGR and iMRI should be used together in order to obtain the best resection rate, especially when the tumor does not fluoresce. To predict if an HGG would fluoresce or not, Ewelt proposed the use of preoperative ¹⁸F-fluoro-ethyl-tyrosine (¹⁸F-FET) positron emission tomography (FET-PET) approach [83]. The combination of FET-PET with FGR, using 5-ALA, yielded a high-grade glioma foci identification sensitivity of 70.5% and a specificity of 92.3% [83]. FET-PET appeared to be valuable for preoperative identification of anaplastic foci and hot spots that were strongly predictive for ALA-derived fluorescence, which highlighted the anaplastic foci during resections [83].

Utsuki reported that 5-ALA fluorescence also indicated areas of inflammatory cell infiltration and edema with or without tumor cells [85]. According to Utsuki, during initial GBM resection, FGR was very sensitive

and specific, whereas there were more false positive results during recurrent GBM surgeries. He explained that this loss of specificity occurred from 5-ALA leakage through the damaged brain-blood barrier, due to previous surgery or radio-chemotherapy [85]. 5-ALA FGR does not have 100% specificity. Rapp et al reported that radiation induced necrosis could exhibit fluorescence [86]. Other necrosis forms, such as necrotizing multiple sclerosis, have been shown to be 5-ALA positive [87].

A major fear for neurosurgeons regarding FGR is that it will drive surgeries into eloquent areas by following deeper fluorescence levels. The invasion of functional areas or cortical tracts by malignant cells represents one of the major restrictions for total tumor removal. The combination of FGR and multimodal intra-operative monitoring provides additional safety for neurosurgeons [38, 39]. Preoperative neuro-radiological examinations, including 3D volume MRIs, functional MRs, and diffusion tensor imaging sequences to visualize functional areas and fiber tracts and preoperative motor mapping, evoked potential help neurosurgeons avoid critical zones minimizing risk of morbidity [38, 39].

In spite of surgical precautions, patients who underwent FGR presented with initial transient neurological deterioration levels more frequently. Stummer reported that 24% of patients presented with one to three points of deterioration on the NIH-SS at 48 h in their FGR group vs. 15% in the WL group; however, no statistical significance was observed at 7 days, 6 weeks or 6 months [40]. Patients with a preoperative deficit and who were unresponsive to steroids were more likely to present a transient neurological deficit after FGR [60]. In the VISIONA study, Diez Valle reported that a significant increase of hemianopia and aphasia occurred in their FGR group [74]. Nevertheless, most of these deficits are not described anymore, one month after surgery.

Future prospects

Microsurgical FGR that is associated with neuronavigation and multimodal functional imaging data should be the new standard of treatment for primary high-grade gliomas.

For deep brain lesions, an operating endoscope that is suitable for PpIX fluorescence visualization has been developed [22]. It allows for fluorescence guided malignancy biopsies in the thalamus or third ventricle and represents an additional tool for deep glioma resections [88, 89].

FGR could be applied for other cerebral tumor types, such as meningiomas, low-grade tumors, and metastases. Regarding meningiomas, Coluccia et al reported a series of 33 FGR operation cases [90]. They observed a high level of fluorescence in 31 cases. However, fluorescence did not correlate with their histological findings (n=30 WHO I-II, n=1 WHO grade III) or with preoperative brain edema and steroid administration [90].

According to them, certain tumor tissues that infiltrate the cortex and compress cortical veins, including bony infiltrations, could only be visualized under fluorescence [90]. In the Valdes study, 80% (12/15) of meningiomas harbored a strong red-pink fluorescence [91]. Quantitative fluorescence was exploited to measure PpIX concentrations in both visible and non-visible fluorescent tissues, and significantly higher PpIX concentrations were observed in both visibly fluorescent tissues ($p = 0.001$) and tumor tissues ($p = 0.002$) [91]. It allowed up to 90% accuracy for differentiating tumors from normal dura. Cornelius et al also reported that 5-ALA FGR improved meningiomas EORs [90-92]. Especially in high-grade meningiomas, additional information regarding brain and neurovascular infiltration was provided. Cornelius et al suggested adding an item to the Simpson grading to rate any residual fluorescence.

Concerning metastases, several authors clearly demonstrated that the PpIX concentrations were significantly increased [80, 93]. Using a quantitative approach, Valdes et al succeeded in discriminating metastatic tissue from normal parenchyma in 95% of subjects, with 92% sensitivity and 100% specificity [80]. Thus, the PpIX concentration appeared to be a highly specific and sensitive biomarker for intracranial tumors, and for brain metastases as well [80]. Albeit, the extent of metastatic lesion infiltration into brains is far less pronounced compared with the majority of primary brain tumors, and diffuse gliomas in particular, Kamp et al suggested that a fraction of intracerebral metastases locally invade into the adjacent brain tissue [94]. Therefore, the surrounding parenchyma is also a therapeutic target to enhance local control. Yo et al reported prolonged local control by extending the intracerebral metastasis resections in non-eloquent areas to a depth of approximately 5 mm [95]. In the Kamp et al study, 52 metastases were operated on with FGR [94]. Thirty-two (62%) exhibited a positive inhomogeneous fluorescence pattern. 5-ALA induced fluorescence was neither associated with the histological type nor with the metastasis site of origin [94]. Eighteen patients harboring positive fluorescence metastases, presented residual fluorescent areas in the resection cavity after macroscopic total white light resections. However, residual tumor tissue was histologically confirmed in only 6 of the 18 (33%) patients with available tissue specimens from such fluorescence positive areas. Then, Kamp concluded that FGR improved metastatic tumor tissue visualization within the brain; nonetheless, residual fluorescence after macroscopically complete metastasis resections needed to be interpreted with caution because of the limited specificity of the detection technique [94].

So far, very few authors have reported on pediatric FGR use. Beez et al recently reported a pilot study of 16 pediatric brain tumor cases that were infra or supratentorial treated with 5-ALA [96]. These 16 cases, in which the subjects had a mean age of 9 years, included 7 pilocytic astrocytomas, 4 medulloblastomas, 1 anaplastic astrocytoma, 3 glioblastomas, and 1 anaplastic ependymoma. Among them, Beez observed positive fluorescence

in one glioblastoma, one anaplastic astrocytoma and one medulloblastoma. He concluded that 5-ALA appeared to be capable of inducing fluorescence in high-grade pediatric tumors [96]. However, hepatic functions have shown to be disturbed with a slight increase of alanine aminotransferase and gamma glutamyl transferase, which correlated with younger age [96]. With the same 5-ALA ratio in adults, 20 mg/Kg was administered 3 to 5 hours before induction. The authors explained a lack of fluorescence among other high-grade tumors occurred in infancy because they are known to differentially express certain genes, which might influence 5-ALA metabolism, that are not expressed in pediatric and adult glioblastomas [96]. These specific “infant” genes are involved in glucose metabolism and tissue hypoxia [97]. In another study, Barbagallo et al reported 3 pediatric cases, including one de novo glioblastoma, one recurrent glioblastoma, and one medulloblastoma case [98]. According to their work, 5-ALA FGR was very useful in better identifying tumor tissue and achieving gross total resections in GBMs, whereas inconstant fluorescence was detected in medulloblastoma [98]. This was also observed in a Preuss et al study, which reported a consequent series of 18 children that were operated on with FGR [99]. The Preuss data even suggested that improved survival parameters were observed after total GBM resections using FGR [99]. No adverse effect was observed in these studies [98, 99]. None of these publications reported positive fluorescence levels in pilocytic astrocytomas [96, 98, 99]. Actually, FGR is still used off-label in pediatric subjects [98, 99]. More studies are needed to elucidate the pharmacokinetic and pharmacodynamic properties of 5-ALA in children and its impact on resection.

Conclusion

FGR is a cost effective innovation [21, 100] that allows for significant EOR and PFS increases for glioblastoma patients. FGR has shown its superiority versus conventional white light resections in terms of EOR with a nearly doubled total removal rate [40, 64]. Fewer reinterventions are needed and FGR delayed neurological deterioration [40]. FGR was correlated with gadolinium uptake, FET-PET uptake and histological grades in primitive brain tumors [56]. Very few data are currently available concerning the OS increase [40, 64]. Indeed, regarding Stummer’s data, if an increase of PFS was observed in the FGR group, then no impact on OS was demonstrated. Similarly, Slotty’s data showed a marginal increase in OS (20.1 vs. 16.6 months). Finally, although FGR results in more complete tumor resections, its impact on OS requires complementary clinical evaluations. Thus, randomized controlled trials are needed to evaluate FGR outcomes and especially to assess the impact of FGR on OS and its role in recurrent GBM. Currently, such a study is recruiting patients in France, which is called RESECT [101]. This is a randomized controlled multicenter phase III trial comparing FGR vs. conventional intraoperative

neuronavigation WL resections, which aims to assess the EOR, PFS and, particularly, the OS rates at 24 and 60 months [101].

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