Acta Neurochirurgica (2019) 161:2595–2605
https://doi.org/10.1007/s00701-019-04094-x

TECHNICAL NOTE - BRAIN TUMORS

Ultrasound-based real-time neuronavigated fluorescence-guided surgery for high-grade gliomas: technical note and preliminary experience

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Received: 15 July 2019 / Accepted: 1 October 2019 / Published online: 28 October 2019

Abstract

Background The extent of resection (EOR) plays a fundamental role in the prognosis of patients with high-grade gliomas (HGG). One of the main challenges in achieving a complete resection is the distinction between tumor and normal brain. Nowadays, several technologies are employed to obtain a higher tumor removal rate and respect the normal tissue in glioma surgery and in the last decades, fluorescein sodium (FS) and intraoperative ultrasound (IOUS) have been widely used. The aim of our technical note is to demonstrate how combining these two tools offers an ultrasound-based real-time neuronavigated fluorescence-guided surgery in order to optimize HGG removal.

Methods Five patients (3 males, 2 females; mean age 55.2 years, range 36–68 years) undergoing craniotomies for removal of intraaxial lesions suggestive of high-grade gliomas on preoperative MRI were included in the study. Intraoperative navigated B-mode and CEUS associated with sodium fluorescein were used in all cases; white light appearance, IOUS, and fluorescence findings were recorded immediately after each surgery. Also, extent of resection was evaluated on postoperative Gd-enhanced MRI performed within 72 h.

Results All tumors effectively stained yellow with fluorescein sodium during the surgical procedure and four were well delineated by IOUS. IOUS was repeated frequently (average 2.6 time) to obtain an orientation of the gross residual tumor with respect to anatomical landmarks as the surgery proceeded. Tumor removal was completed under Yellow 560 filter.

Conclusions In our technical report, we demonstrate that combining intraoperatively fluorescein sodium and IOUS improves the information and facilitates making decisions during the HGG surgery. Further experience gained in larger studies will help confirm these findings

Keywords High-grade gliomas · Fluorescence-guided surgery · Sodium fluorescein · Yellow 560 · Intraoperative ultrasound · CEUS

Introduction

Gliomas are the most common primary malignant brain tumors in adults and patients with high-grade gliomas (HGGs) have a poor prognosis. Many variables may play a role in the prognosis for patients including patient age, location and some neuroimaging features, recurrence, and the ability to perform an adjuvant treatment after surgery. The extent of resection (EOR) is extremely important in the prediction of progression-free or overall survival and recurrence in glioma surgery [10, 44]. One of the main challenges in achieving a complete resection without causing damage to the surrounding normal brain parenchyma is the distinction between tumor

This article is part of the Topical Collection on *Brain Tumors*

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and normal tissue. Unfortunately, the appearance of the tumor and the surrounding functional brain, even under operating microscope, is often very similar. This distinction is especially challenging in primary brain tumors, which often have a diffuse growth pattern.

In the last decades, several technologies have been employed to obtain a higher tumor removal rate respecting the normal tissue in glioma surgery, including neuronavigation [52] and intraoperative magnetic resonance imaging [15]. Image-guided intraoperative navigation has become a basic component of modern neurosurgery; however, MRI and CT scans are unable to account for intraoperative brain shifts consequent to patient positioning and gravity, CSF loss, tumor debulking, and cerebral edema [24, 31]. On the contrary, intraoperative MRI (iMRI) offers imaging, although statics, in real time but costs, poor resolution, and increased operative times have limited its extensive use [3].

In daily practice, fluorescence-guided surgery and intraoperative ultrasound [48], useful, real-time adjunct to the above-mentioned technical tools, have emerged as a promising new modalities to increase the extent of resection in brain tumor surgery distinguishing tumor from normal brain.

5-Aminolevulinicacid (5-ALA), a natural precursor of hemoglobin, which is metabolized by some enzymes in the highly fluorescent protoporphyrin IX in tumor tissues, was used as a fluorescent dye in glioma surgery with good results [42, 44]. A prospective, randomized, controlled phase III trial confirmed its effectiveness in treatment of gliomas [43]. Despite the specificity and sensitivity of 5-ALA for the tumor tissues, there are various elements that limit its clinical application in brain tumor surgery: the method of administration (it should be orally administrated 3 h prior to the induction of general anesthesia [2, 42]); the increased risk of skin sensitivity and eye irritation if the patient is exposed within 24 h to sunlight or intense artificial light [2]; its high cost (about 900 EUR per ampoule) [33]. Due to these limitations, fluorescein sodium (FS) has recently become an attractive alternative to 5-ALA. FS is a sodium salt and an organic fluorescent dye with peak when excited by a light whose wavelength is in the range 460–500 nm, it emits a fluorescent radiation with a wavelength of 540–690 nm. Its tumor specificity is 0% because FS does not selectively localize in tumor cells, but in the extracellular matrix within the tumor site, suggesting its role as a marker for areas of damaged blood–brain barrier (BBB). It has been safely used in ophthalmic surgery for many years [16, 23]. Fluorescein sodium is administered intravenously before glioma resection, is virtually free of side effects, and has low costs (about 5 EUR each ampule) [1, 34]; it is usually visible by the naked eye with a high dose (20 mg/kg) but, at lower doses, with a special filter (YELLOW 560 nm) applied to the microscope PENTERO 900 (Carl Zeiss, Meditec, Oberkochen, Germany), it allows an optimal discrimination of the normal brain [11, 35].

Furthermore, we are recently observing an increased use of conventional ultrasounds (US) in neurosurgery to obtain costless real-time feedback of changes occurring during surgical intervention and the extent of resection [7, 19, 32, 36, 41, 47, 53, 54]. However, many neurosurgeons lack specific US training and have difficulties recognizing anatomic structures with the same confidence as for CT or MR preoperative imaging. Indeed, the quality of US images is operator dependent, and it often shows sections and views that are not in standard axial, coronal, or sagittal planes, thus making it difficult to compare the true anatomical location and the ultrasound image. Accordingly, an ultrasound-based real-time neuronavigation system that fuses intraoperative ultrasound (IOUS) with preoperative MRI has been developed. This technology simultaneously displays an MRI slice coplanar to an IOUS image and allows adjustments to correct for brain shift and tissue distortion. Various IOUS modalities such as B-mode and Doppler have been applied during neurosurgical procedures. However, although both of them are excellent for tumor localization, little information is provided concerning microcirculation and perfusion dynamics [6, 18, 21, 51]. Thus, intraoperative contrast-enhanced ultrasound (CEUS) is being increasingly used to provide dynamic imaging and functional or perfusion data to help differentiate pathology from surrounding normal brain. CEUS allows real-time assessment of contrast enhancement and vascularization of focal lesions during the different dynamic phases, after injection of an intravenous contrast agent. The dye consists of microbubbles (5-μm diameter) of air or inert gas encapsulated in a layer of protein or polymers that are retained in the vessels, thus depicting the arterial and venous network. Second generation US contrast agents are clinically safe and well tolerated [25] and previous investigations have described the benefits of CEUS in neurosurgery [28, 30, 49, 50].

Table 1 Demographic and clinico-radiological features of the patient cohort

Case no.	Sex	Age	Symptoms at presentation	Location	Histological analysis
1	M	61	Hemiparesis	Right occipital	Glioblastoma (grade IV)
2	F	58	Headache	Right temporo-insular	Glioblastoma (grade IV)
3	M	53	Seizures	Left frontal	Anaplastic astrocytomas (grade III)
4	M	68	Confusion	Right frontal	Glioblastoma (grade IV)
5	F	36	Seizures	Right temporal	Glioblastoma (grade IV)

Table 2 Magnetic resonance imaging, intraoperative white light, intraoperative ultrasound, and fluorescence findings in the series of tumors

Case no.	MRI findings	Intraoperative white light findings	IOUS findings	Fluorescence findings
1	Uniformly strong enhancement	Well-delineated sharp margins	Circumscribed and discrete margins	Strong and discrete fluorescence
2	Uniform strong enhancement with central necrosis	Well-delineated but infiltrative edge	Circumscribed but equivocal margins	Strong fluorescence including the margins
3	Uniform but poor enhancement	Diffuse ill-defined margins	Diffuse and poorly demarcated	Strong and discrete fluorescence
4	Uniformly strong enhancement	Well-delineated but infiltrative edge	Circumscribed and discrete margins	Strong fluorescence including the margins
5	Uniform strong enhancement with central necrosis	Well-delineated but infiltrative edge	Circumscribed but equivocal margins	Strong fluorescence including the margins

In this preliminary experience, we describe high-grade glioma removal under fluorescence dyeing and ultrasonography in B-mode and CEUS methodic. We aim to demonstrate how combining both tools can help the surgeon recognize the boundary between the normal brain and pathological tissue. In addition, we stress the effectiveness and safety, accuracy and feasibility, time and cost sparing of ultrasound-based intraoperative navigated fluorescein-guided surgery in the treatment of high-grade gliomas.

Materials and methods

Patient population

After the approval of Local Ethic Committee, a prospective study was designed and carried out. Five patients (3 males, 2 females; mean age 55.2 years, range 36–68 years) undergoing craniotomies between September 2017 and December 2017 for removal of intraaxial lesions suggestive of high-grade gliomas on preoperative MRI were included (Table 1).

Intraoperative navigated B-mode and CEUS associated with sodium fluorescein were used in all cases as specified below.

All patients were informed about the surgical procedure, the trans-operative use of sodium fluorescein and sulfur hexafluoride–filled lipidic microbubbles (SonoVue®); in all cases, written consent was obtained.

Clinical presentation was non-urgent in all cases, allowing elective surgery. The inclusion criteria were as follows: (1) age over 18; (2) suspect of high-grade glioma (grade III or IV according WHO classification) based on postcontrast MRI findings; (3) tumor location allowing a total removal of the enhanced area.

Exclusion criteria included severe heart, liver or kidney disease, recent acute ischemic stroke; prior history of adverse reaction to fluorescein sodium or ultrasonographic contrast agents, or severe reactions to other contrast agents; women during the first trimester of pregnancy; specific brain tumor locations such as corpus callosum, basal ganglia, brain stem, posterior cranial fossa; preoperative KPS score of 60 or less; tumor diameter < 1 cm or > 5 cm.

Fluorescein protocol

In all cases, 5 mg/kg bodyweight of fluorescein sodium was injected intravenously via the central venous line, after the induction of anesthesia according to FLUOGLIO phase II trial [1]. Vital parameters were monitored for 15 min. The fluorescent dye was visible under the YELLOW 560-nm filter, on the OPMI PENTERO 900 surgical microscope (Carl Zeiss Meditec, Oberkochen, Germany).

Ultrasound platforms, contrast agents, and IOUS-CEUS procedures

The last generation US equipment device (MyLab, Esaote, Italy), furnished with a software application based on an electromagnetic tracking system for Virtual Navigation (MedCom, Germany), was used in our study. As exhaustively

Table 3 Summary of the results of the series

Case no.	Preoperative KPS	Postoperative KPS	Duration (min)	IOUS use	Follow-up (months)
1	70	90	125	2	7.8
2	100	100	140	2	6.8
3	80	100	210	4	5.8
4	70	80	180	3	5.8
5	90	100	135	2	3.9

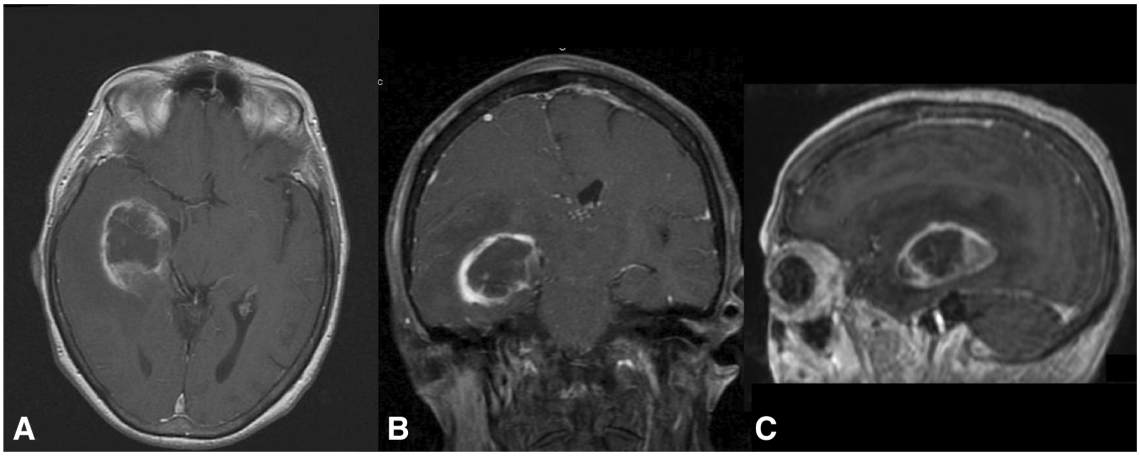

Fig. 1 Axial (**a**), coronal (**b**), and sagittal (**c**) preoperative postcontrast MRI scans showing the illustrative case of a large right temporal intraaxial mass

described in a recent article by Prada et al. [27], volumetric T1-weighted contrast-enhanced MR images were used as preoperative reference and transferred to the system in DICOM format using LAN network. Once images were acquired, the software generated a 3D volume reconstruction to allow the operator perform the registration, as in a standard neuronavigation system. After the patient was positioned and the head fixed in a three-pin head-holder, the transmitter, considered the origin of the reference system, was placed on a support fastened to the surgical table, 15–20 cm away, and the initial registration started. The same 5–8 anatomical landmarks chosen on the 3D reconstruction (tip of the nose, glabella, lateral canthus, tragus, ear attachment) were marked on the patient's skin in this phase with a receiver attached to a pointer; fiducials were not commonly used. After registration, it was possible to simultaneously display MRI slices coplanar to IOUS images. During surgery, we used a variable band linear array probe with an operating bandwidth of 3–11 MHz (Esaote-LA332) or a 3–9-MHz microconvex probe (CA123; Esaote, Italy), covered with a sterile plastic sheath (Civco, USA) with 5-mL US transducing gel. After craniotomy, misalignments of MRI slices compared with real-time US secondary to the brain shift (using major anatomical structures such as ventricles, big vessels, mid-brain, falx, or tentorium as landmarks) could be corrected with the so-called fine-tuning freezing the images [29].

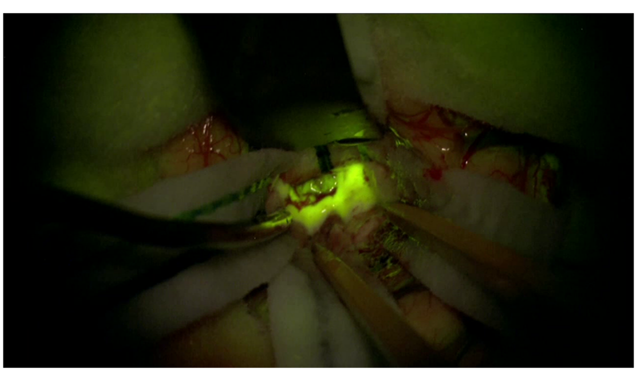

Fig. 2 Intraoperative view of a right frontal glioblastoma at the beginning of tumor removal under the YELLOW 560-nm filter. During tumor removal on the Pentero 900 surgical microscope, there is a clear delineation of the tumor area which shows significant fluorescein sodium enhancement revealing the boundary between the bright yellow tumor and the surrounding brain

Concerning the use of CEUS, we followed the criteria of European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) guidelines on CEUS in an off-label setting as described by Prada et al. [5, 9, 26, 28, 40]. As ultrasound contrast agent (UCA), we used sulfur hexafluoride-filled lipidic microbubbles (SonoVue, Bracco, Italy).

Before microbubble contrast agent injection, the lesion was visualized with B-mode imaging, positioning the focus on the lesion. Then, the contrast agent was injected intravenously by the anesthesiologist as a bolus (2.4 mL [5 mg/mL]), followed by a flush of saline solution (10 mL). Our surgical protocol included at least two administrations: before dural opening and after total tumor removal, anyway repeatable on request.

Intraoperative and postoperative assessment

All surgeries were performed by the senior author (N.F.). Fluorescence and IOUS were both used in all cases. Standard microsurgical principles were applied during tumor removal. The intraoperative white light appearance, IOUS, and fluorescence findings were recorded immediately after each surgery.

Extent of resection was evaluated on postoperative Gd-enhanced MRI performed within 72 h; it was classified as gross total (complete resection of the tumor with no evidence of residual at postoperative MRI), subtotal (> 70% resection), and partial (< 70% resection).

Each patient general physical performance was recorded using the KPS during the clinical evaluation at the admission to our Neurosurgical Unit. The median preoperative KPS score was 82 (range 70–100). Surgery was followed by

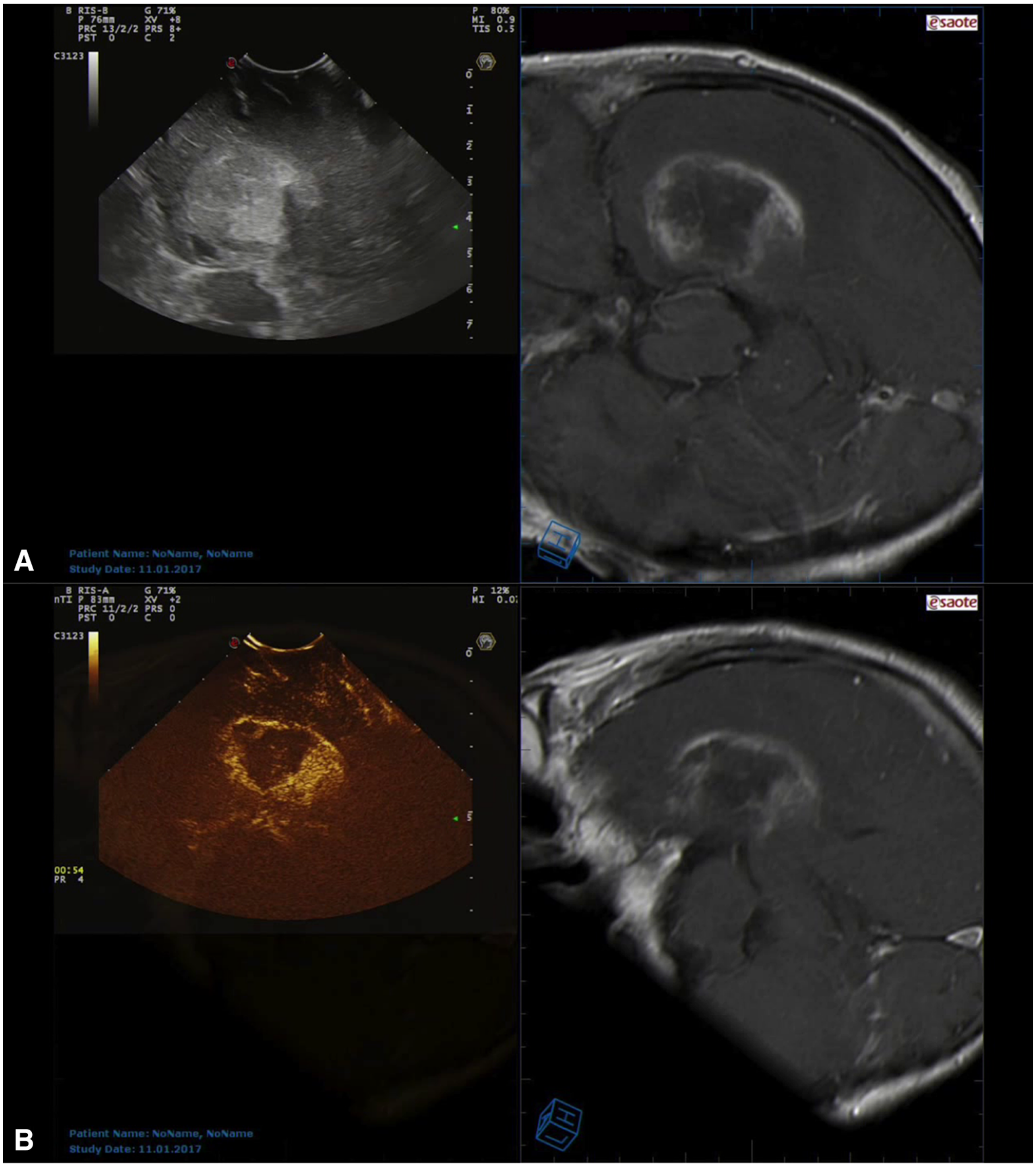

Fig. 3 **a** Intraoperative US in B-mode before tumor removal on the left side of the screen aligned to preoperative T1 gadolinium-enhanced MRI on the right side. Note a minimal misalignment of mid-brain subsequently corrected by fine-tuning registration. **b** Intraoperative screenshot showing the tumor highlighted with contrast-enhanced ultrasound (CEUS) on the left side matched to preoperative T1 gadolinium-enhanced MRI on the right side

radiotherapy with concomitant and adjuvant temozolomide in all cases, according to the Stupp protocol [45].

Histological analyses were performed by the team of neuropathologists.

Results

The mean duration of the entire surgical procedure (skin to skin) was 158 min (range of 125–210 min). The MRI findings and intraoperative appearance of all tumors under white light and IOUS, as well as fluorescence, are shown in Table 2. All tumors effectively stained yellow with fluorescein sodium during the surgical procedure and four were well delineated by IOUS. However, in one case, the tumor appeared diffusely hyperechogenic, and margins could not be well defined.

The average duration of a single IOUS examination was of 5.3 min both in B-mode and CEUS methodic. IOUS was frequently repeated (average 2.6 time) to obtain an orientation of the gross residual tumor with respect to anatomical

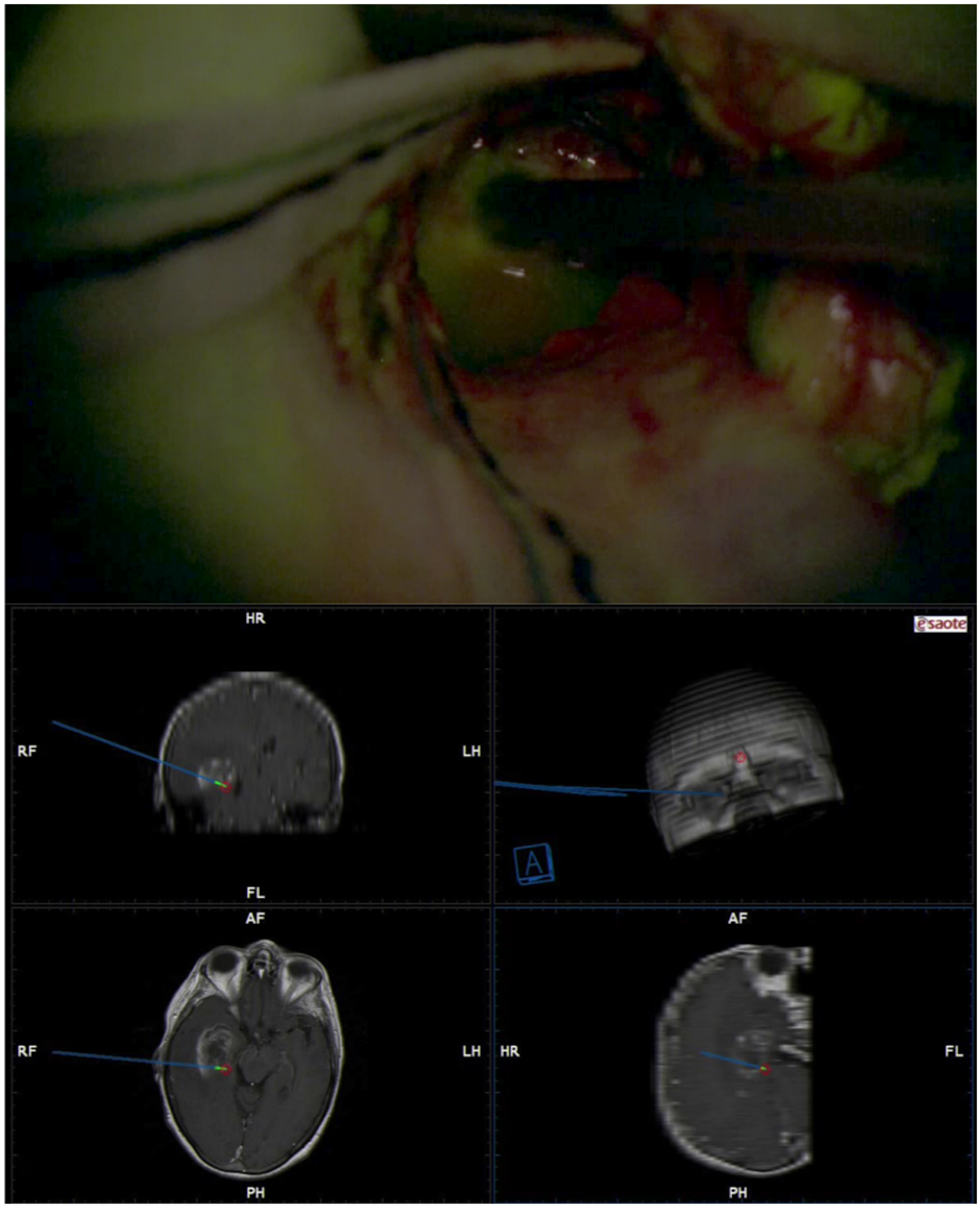

Fig. 4 Intraoperative virtual navigation to confirm the gross total resection of the tumor at the end of procedure. **a** Image depicting the use of the pointer stick mounted on the US probe at the deepest point of the surgical field. **b** The three corresponding MRI orthogonal planes and the 3D volume generation showing the exact point of the probe's tip

landmarks as the surgery proceeded. The tumor removal was completed under Yellow 560 filter.

Histological examination showed four glioblastomas and one anaplastic astrocytoma.

No adverse effect associated with the combined administration of fluorescein sodium and SonoVue® was observed. There were no technical failures of either modality. No abnormal changes have been detected in routine blood or urine examinations, nor in liver and kidney function tests. Overall, no serious complications emerged and there was no peri-operative mortality. One patient with a right occipital glioblastoma experienced a transient worsening of his hemiparesis that improved within a week.

The median postoperative KPS score was 94 (range of 80–100) and gross total resection was achieved in all patients (100%) (Table 3). The overall median follow-up was 6 months (range of 3–8 months).

Illustrative case

A 58-year-old woman (case no. 2 of the series) with an 8-month history of headache was admitted to our division. On neurological examination, she demonstrated a left arm weakness (grade 4+/5). MRI showed a large right temporal intraaxial mass with surrounding edema and midline shift suggestive of glioblastoma multiforme (Fig. 1).

The patient underwent a right fronto-temporal craniotomy with the help of IOUS system with virtual navigation. Once registration had been verified, the navigated US probe was used as a pointer to plan the craniotomy. After bone flap

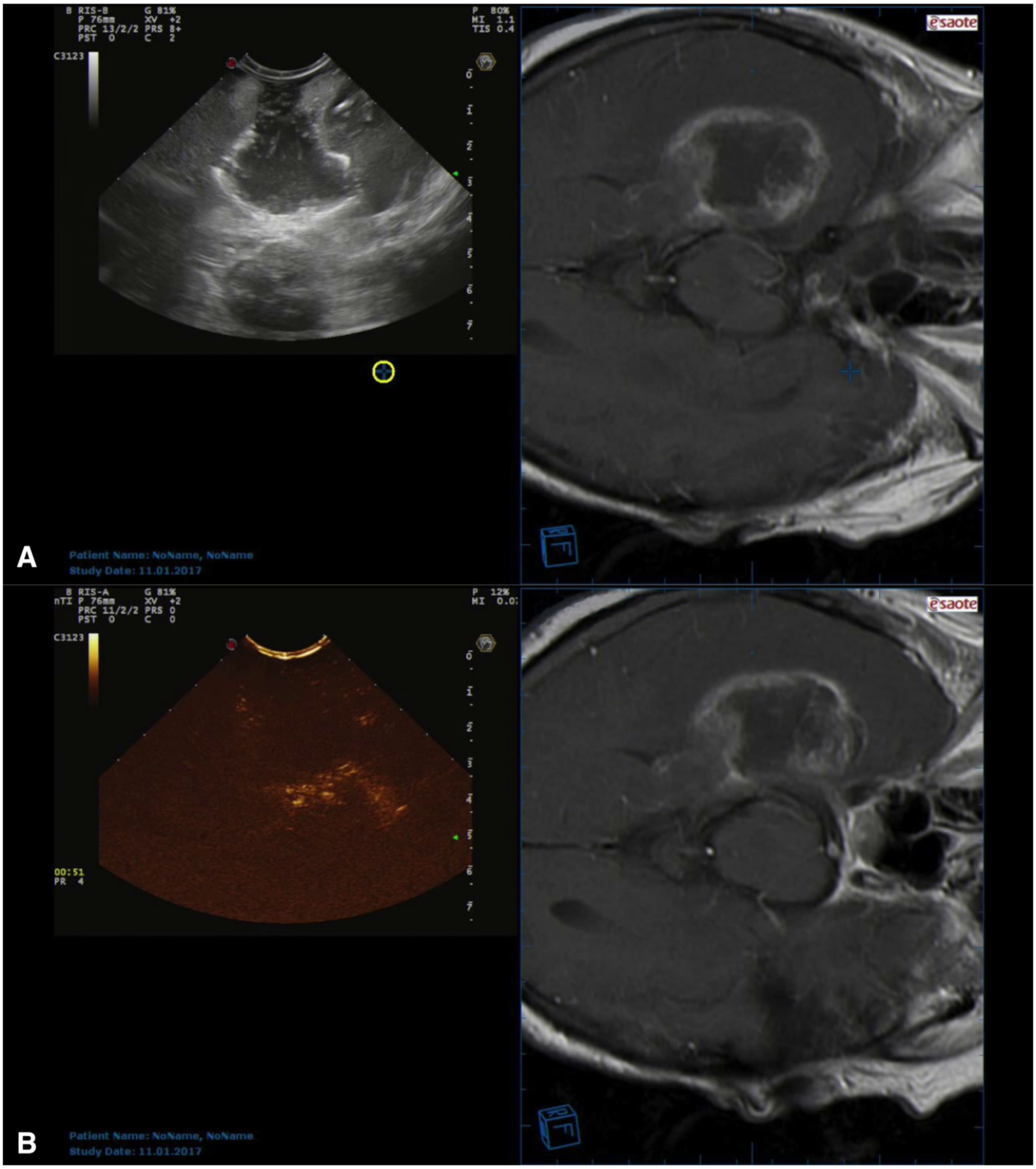

Fig. 5 Intraoperative screenshots showing US in B-mode (**a**) and in CEUS mode (**b**) after tumor removal on the left side of the screen matched to preoperative T1 gadolinium-enhanced MRI on the right side

removal and dural opening, the tumor was not visible on the brain surface despite fluorescein sodium administration. But when corticectomy was performed under the YELLOW 560 filter, the fluorescent tumor was clearly identified (Fig. 2). The brain surface was then scanned with standard B-mode US modality, and the lesion was analyzed: on the screen of the virtual navigation system, the US imaging and the correspondent preoperative MRI were displayed merged together (Fig. 3a). Intraoperative CEUS was used to better localize the tumor and define its relationship to surrounding brain tissue: a heterogeneous pattern of enhancement was observed (Fig. 3b).

Dissection of the tumor from normal brain tissue was performed with the filter on and it proceeded since the yellow-stained tumor could be identified. At the end of procedure, the use of a pointer stick mounted on the US probe helped the surgeon to confirm the gross total resection of the tumor (Fig. 4). B-mode and CEUS confirmed the absence of residual tumor at the end of procedure (Fig. 5).

Postoperative MRI (48 h after surgery) also validated GTR (Fig. 6) and the histopathological examination showed a glioblastoma.

Discussion

Radical resection in high-grade gliomas can be challenging, and neurosurgeons tend to overestimate the degree of resection achieved. Therefore, in the last decades, several technologies have been developed to optimize the extent of resection.

Several fluorescent agents have been studied in glioma surgery to improve intraoperative identification of tumors. 5-aminolevulinic acid (5-ALA) and fluorescein sodium (FS) are the most used [20] but many others (indocyanine green, hypericin, 5-aminofluorescein labeled to human serum albumin, etc.) have been clinically tested in the literature [37, 46]. The ideal fluorescent agent should be safe and easy to use, tumor-specific, with a strong and evident signal. Currently, 5-ALA is the only agent that has been tested in a multicenter randomized controlled trial [43]. On the other hand, fluorescein sodium has been used in medical applications over the past 70 years [22]: it is a fluorophore that penetrates in those areas of the brain where the blood–brain barrier (BBB) is damaged; despite its non-specificity for tumor cells, it makes intraoperatively visible what gadolinium enhances on MRI. Its lower cost and an easier way of administration together with a bright working area under Yellow 560 filter explain how fluorescein sodium has become an alternative to 5-ALA in the last years.

Concerning intraoperative ultrasound, its use has been introduced in neurosurgery in the 1960s [13, 14]. Le Roux et al. [17] were the first to report that also low-grade gliomas (LGG) were amenable to IOUS but still the role of ultrasound in neurosurgery has been limited because of the difficulty in interpreting the images [27]. For this purpose, more recent systems were developed to fuse IOUS with preoperative MRI images: the result is an ultrasound-based real-time neuronavigation. In addition, intraoperative contrast-enhanced ultrasound (CEUS) using microbubbles offers functional and perfusion data that help differentiate tumor from normal tissue: this overcomes some limitations of IOUS, including difficulty to discern between edema and tumor tissue or to detect small lesions. Although technological innovations have improved ultrasound image quality, a long learning curve is still required to be familiar with IOUS. In the literature, some series report the use of IOUS by experienced neurosurgeons: GTR was obtained in up to 90–95.5% of patients [38].

Among different intraoperative fluorescent dyes, we chose fluorescein sodium; in our experience, its intrinsic features (easy way of administration, steady-state fluorescence, bright working area, moderate cost, virtual free of side effects, marker for compromised blood–brain barrier areas) make it the best association with IOUS. Moreover, other authors failed to demonstrate advantages using combination of IOUS and 5-ALA considering the latter “the two faces of the same medal” [4]. On the contrary, we found FS and IOUS to have complementary advantages. Their combination facilitates resection of malignant gliomas; while IOUS is crucial in a real-time assessment of the tumor residual under the brain surface, fluorescence enhances the tumor during progressive removal layer by layer.

In our preliminary experience, we confirm the utility of IOUS in the initial steps of surgery. Ultrasound-based neuronavigation provides intraoperative support in planning the craniotomy, localizing the lesion, choosing the best point for the corticectomy (especially if deep tumors) as well as for resection control checking the boundaries structures. Fluorescence-guided surgery, in contrast, is basically a surface phenomenon. It is very useful to identify and demarcate the tumoral tissue once it is sufficiently exposed; so, fluorescein sodium is more important in the latest steps of resection.

Our results seem to confirm the central role of fluorescence in achieving a GTR. Furthermore, this small case series emphasizes the concept that CEUS is a fast, safe, and relatively economic tool in maximizing tumor resection. The possibility to correct brain shift during surgery and to evaluate dynamic

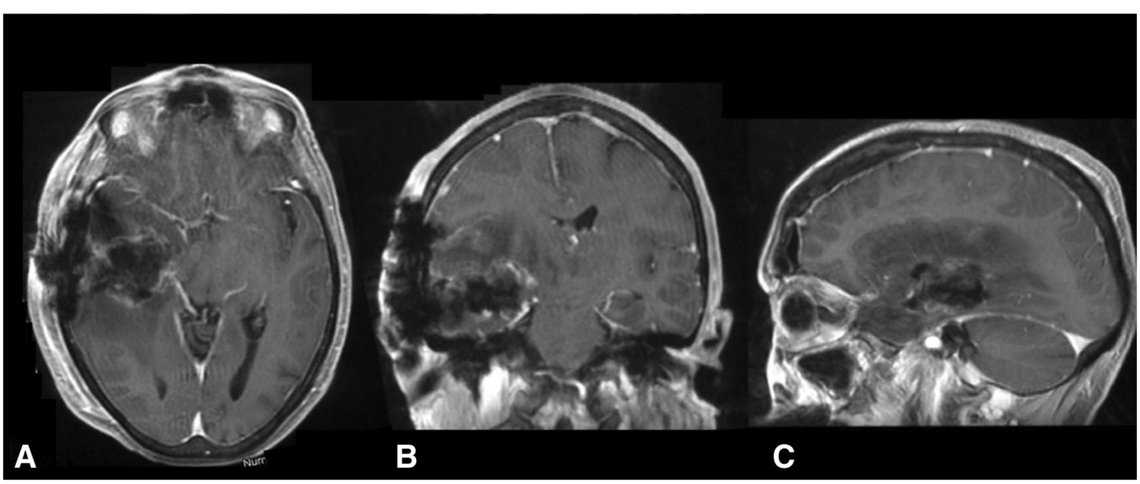

Fig. 6 Axial (**a**), coronal (**b**), and sagittal (**c**) postoperative postcontrast MRI scans showing the removal of the large right temporal intraaxial mass

contrast enhancement of the tumor and its associated feeding arteries and draining veins makes CEUS more reliable than conventional neuronavigation. CEUS efficacy remains unknown compared with intraoperative MRI (iMRI), but still it offers the advantage of real-time, dynamic imaging with the option of repeating infusion.

In a recent study, the authors compare the cost-effectiveness of the four cited technologies: 5-ALA, fluorescein sodium (FS), intraoperative ultrasound (IOUS), and intraoperative MRI (iMRI) [12]. They calculated the incremental cost per quality-adjusted life-year (QALY) and the cost/QALY was $16,218, $3181, $6049, and $32,954 for 5-ALA, FS, IOUS, and iMRI respectively. It is clear that FS and IOUS are the less expensive technologies and even their combination does not reach the cost of 5-ALA or iMRI alone.

Concerning time loss, our results show that the average duration of a single IOUS examination was of 5.3 min; on the contrary, an analysis of the literature shows that iMRI has been associated with an increased duration of surgery of 1–4 h, although multiple series report that iMRI is the most accurate tool for margin assessment [8, 39]. Thus, ultrasound-based real-time neuronavigated fluorescence-guided surgery for high-grade gliomas is a fast, safe (no adverse effects associated with fluorescein or microbubbles), and relatively economic tool in maximizing tumor resection.

Although these two technologies are widely used in neurosurgery, their combined use has never been described in the literature. Therefore, this technical note represents a starting point: although limited to five patients, this series demonstrates that combining both tools could intraoperatively increase the available information and facilitate the decisions-making process during the surgery. Further experience from wider studies is needed to confirm these promising findings.

Conclusions

The intraoperative identification and resection of high-grade gliomas are a significant and important challenge in neurosurgery. In our technical report, we described an US-assisted fluorescence-guided surgery of HGG using fluorescein sodium and microbubbles. In our opinion, this combination is safe and effective to achieve gross total resection and to discriminate between tumoral and normal brain tissue. Larger scale studies are now needed, in order to determine the efficacy of navigated ultrasound-assisted fluorescence-guided surgery for high-grade gliomas in improving the extent of resection as well as the progression-free and overall patient survival.

Compliance with ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the ARNAS Civico Hospital and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All patients included in the study were informed about the surgical procedure and in all cases, written consent was obtained.

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

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