World Neurosurgery: X 23 (2024) 100379

Contents lists available at ScienceDirect

World Neurosurgery: X

journal homepage: www.journals.elsevier.com/world-neurosurgery-x

Resection of supratentorial high-grade gliomas availing of neuronavigation matched intraoperative ultrasound and Fluorescein: How far is it safe to push the resection?☆

Alessandro Pesce [a], Mauro Palmieri [b,*], Andrea Pietrantonio [a], Silvia Ciarlo [a], Maurizio Salvati [c,#], Angelo Pompucci [a,#]

[a] A.O. "Santa Maria Goretti", Neurosurgery Division - Latina, Via Lucia Scaravelli, 04100, Latina, LT, Italy
[b] Università "La Sapienza" di Roma, Neurosurgery Division - Roma, Viale del Policlinico 155, 00161, Roma, RM, Italy
[c] Department of Neurosurgery, Policlinico "Tor Vergata", University of Rome "Tor Vergata", 00133, Rome, RM, Italy

ARTICLE INFO

Keywords:
Prognostic factor
Long term survival
Glioblastoma
IDH
EGFR
Ki67
p53

ABSTRACT

Background: High-Grade Gliomas are the most common primary brain malignancies and despite the multimodal treatment, and the increasing amount of adjuvant treatment options the overall prognosis remains dismal. The present investigation aims to analyze the safety profile of the use of intraoperative ultrasounds (Io-US) in a homogeneous and matched cohort of patients suffering from High-grade gliomas (HGG) operated on with or without the aid of Io-US and Fluorescein in specific relation to the incidence of neurological and functional status sequelae.

Methods and materials: A retrospective analysis was performed on 74 patients affected by HGG. 22 patients were treated with Io-US matched with neuronavigational system (Group A); 15 patients were treated both with the use of Io-US and Fluorescein matched with neuronavigational system (Group B); 37 patients were treated with the use of the neuronavigational system only (Group C). Primary endpoints were the extent of resection and functional outcome (measured with Karnofski Performance Status)

Results: Significative differences were observed in terms of a higher extent of resection in Group B. In a multivariate analysis, this data appears to be independent of the location (eloquent/non-eloquent) of the lesion and from its histology. Regarding functional outcomes, no differences were detected between the two groups.

Conclusions: The present study is the first that analyzes the simultaneous use of Io-US and Fluorescein, and the results demonstrate that these two instruments together could improve the extent of resection in HGG while ensuring good outcomes in terms of functional status.

Abbreviations: **5-ALA**, 5-aminolevulinic acid; **AA**, Anaplastic Astrocytoma; **BBB**, Brain–Blood Barrier; **CEUS**, contrast-enhanced ultrasound; **DTI**, Diffusion Tensor Imaging; **DWI**, Diffusion Weighted Imaging; **EGFR**, Epidermal Growth Factor Receptor; **EOR**, Extent Of Resection; **FLAIR**, Fluid Attenuated Inversion recovery; **fMRI**, Functional Magnetic Resonance Imaging; **GBM**, Glioblastoma; **GTR**, Gross Total Resection; **HGG**, High Grade Gliomas; **IDH**, Isocitrate Dehydrogenase; **IDH-WT GBM**, IDH Wild-type Glioblastoma; **IoN**, Intraoperative Neurophysiological monitoring; **IoNT**, Intraoperative Neuropsicological testing; **Io-US**, Intraoperative Ultrasounds; **LGG**, Low Grade Gliomas; **KPS**, Karnofsky Performance Status; **MPRAGE**, Magnetization-Prepared Rapid Gradient-Echo; **MRI**, Magnetic Resonance Imaging; **NTR**, Near Total Resection; **STR**, Subtotal Resection; **ROI**, region of interest; **OS**, Overall Survival; **PFS**, Progression Free Survival.

☆ The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript. The authors confirm their adherence to ethical standards and have NO financial disclosures that would be a potential conflict of interest with this publication.

* Corresponding author. AOU "Policlinico Umberto I", University of Rome "Sapienza", Via del policlinico 155, Roma, (RM), Italy.
E-mail address: Mauro.palmieri10@gmail.com (M. Palmieri).

Both Pompucci and Salvati are Senior Authors.

https://doi.org/10.1016/j.wnsx.2024.100379
Received 2 May 2023; Received in revised form 30 March 2024; Accepted 2 April 2024
Available online 16 April 2024
2590-1397/© 2024 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC license (http://creativecommons.org/licenses/by-nc/4.0/).

1. Introduction

1.1. Background

High-grade gliomas (HGG) are the most common primary brain malignancies[1] and despite the multimodal treatment,[1–3] and the increasing amount of adjuvant treatment options[4,5] the overall prognosis remains dismal. Although the role of the molecular pattern of the malignant clone has been thoroughly investigated in the last decade, with, for instance, the MGMT methylation and the IDH mutation status,[6–9] it is still ascertained and worldwide accepted that surgery plays a critical role in prolonging both Progression Free (PFS) and Overall Survival (OS), namely the extent of resection (EOR), demonstrates an obvious association with increased oncologic outcomes. This finding brought several authors to propose a "supramarginal" or "supratotal" resection policy to maximize the expected survival.[10–13] Such lesions are infiltrative per nature: HGG cells display a migrating behavior, by sliding across the white matter bundles[14] and therefore their margins are irregular, in some cases poorly distinguishable from the normal surrounding brain parenchyma. To surgically address such specific features of HGG by preserving the function while increasing the EOR, several approaches have been proposed, such as the intraoperative neuronavigation matched with neuromonitoring and awake surgery,[15,16] fluorescence (5-aminolevulinic acid, known as 5-ALA, and Fluoresceine),[17,18] the intraoperative imaging (MRI and Ultrasound). The intraoperative use of Ultrasound (Io-US) dates back to the early 80's[19] as a promising dynamic intraoperative imaging to aid neuro-oncological surgery. In recent years its role has been reappreciated,[20,21] because of its easy and quick use, and reduced costs, while being less time-consuming compared to intraoperative MRI and intraoperative CT.[22] The main limit of Io-US is the operator-dependent accuracy of the imaging and thus its reliability[23]: in such context the critical question is, considering the surgical alteration created on the brain parenchyma to which US is particularly sensible, how much should a surgeon rely on the information derived from this method before the neurological status and therefore the functional status is at risk? The use of Io-US and Fluorescein was never in-depth assessed as far as its safety profile is concerned.

1.2. Objective

The present investigation aims to analyze the safety profile of the use of IoUS in a homogeneous and matched cohort of patients suffering from HGG operated on with or without the aid of IoUS and Fluorescein in specific relation to the incidence of neurological and functional status sequelae.

2. Material and methods

2.1. Participants and eligibility

We performed an Institutional retrospective review of a consecutive series of surgically treated patients suffering from histologically confirmed High-Grade Gliomas (HGG), operated on in the Department of Neurosurgery of Santa Maria Goretti University Hospital. We collected a total of 86 patients suffering from HGGs. Histological diagnoses were performed according to the updated version of the WHO guidelines.[24]

We selected a total of 86 patients affected by HGG who underwent surgery, radiation, and chemotherapy in our Institution in the period ranging between June 2019 and December 2022 meeting the following inclusion criteria:

Patients were included in the study if their pre- and post-operative MR imaging was either performed at our institution or available on the picture archiving and communication system (PACS) for review.

The estimated target of the surgical procedure was the *total or subtotal resection of the lesions*: no biopsies were included.

A total of 7 patients were excluded for incomplete or wrong data on clinical, radiological, and surgical records and/or lost to follow-up. All the patients who met the inclusion criteria, were assigned on the ground of the operative strategy to the following subgroups:

Group A: patients who were operated on with the aid of the Neuronavigation matched with Io-US, in this subset of patients Fluorescein was not used (22 patients)

Group B: patients who were operated on with the aid of the Neuronavigation matched with Io-US and administration of intravenous Fluorescein (15 patients)

Group C: patients who were operated on without the use of the Io-US, but with the use of the Neuronavigation system (37 patients)

The approach we chose, and subsequently, the assignment to each subgroup, was related to the progressive introduction of operative technologies in our Institution: after the introduction and routine use of the Neuronavigation system (Group A), we then introduced the Neuronavigation matched with the US system (Group C) and, lastly, our operative microscope was upgraded with fluorescein filter (Group B).

For all the included patients we recorded age, sex, location, Tumor volume, and clinical onset, were recorded. IDH and Ki67 statuses were routinely performed in the Department of Neuropathology of our Hospital.

A special focus was on the KPS results and most importantly on the variation of the neurological status: such parameter was considered, as previously observed[8,9] as associated with Survival and the accessibility to adjuvant treatments. It was recorded in three different moments: 1. Before surgery, 2. At 7 days after surgery. The functional status was coded with KPS results, subsequently compared to the postoperative, and re-coded in a three-step ordinal variable as follows: 0. Worsened, 1. Stable, 2. Improved.

Before surgery all the patients included underwent a preoperative brain MRI scan included a high field 1.5 T volumetric study with the following sequences: T2w, FLAIR, isotropic volumetric T1-weighted magnetization-prepared rapid acquisition gradient echo (MPRAGE) before and after intravenous administration of paramagnetic contrast agent; diffusion tensor sequences (DTI) with 3D tractography for the reconstruction of the white matter bundles. The volume of the contrast-enhancing lesion was recorded: and calculated drawing a region of interest (ROI) in a Volumetric enhancing post-contrast study weighted in T1 (a multi-voxel study).

All the procedures were performed with an infrared-based Neuronavigator (Brainlab, Curve® Purely Navigation), in a standard neurosurgical theatre, with a standard operative microscope (Leica, model OH4). In 15 patients operated on since the update of the microscope, intravenous Fluorescein was administered at 5 mg/kg, as previously reported by other authors.[25] On the first postoperative day, the patients underwent a CT scan to evaluate major early complications. Lesions involving the motor function cortical and subcortical structures were operated on with the aid of Intraoperative Neuromonitoring realized with the use of bi- and monopolar stimulating probes respectively for the cortical and subcortical mapping. If intraoperative neuromonitoring was performed, no muscle relaxants were administered (representative case in Fig. 1). The standard intraoperative neuromonitoring included MEPs, SSEPs, a continuous EEG with a cortical stripe, Phase Reversal was routinely acquired for lesions located nearby the central sulcus and motor pathways. Direct cortical and subcortical stimulation and negative motor mapping was routinely performed in each of the cases in which we used intraoperative neuromonitoring.

In general, it was intra-operatively judged necessary to stop tumor excision when the white matter appeared free of disease in any aspect of the surgical cavity, and in Group A, the Io-US disclosed the complete resection of the lesion, and/or despite a direct visualized or a Navigation proven remnant, neuromonitoring outlined a risk for postoperative motor morbidity.

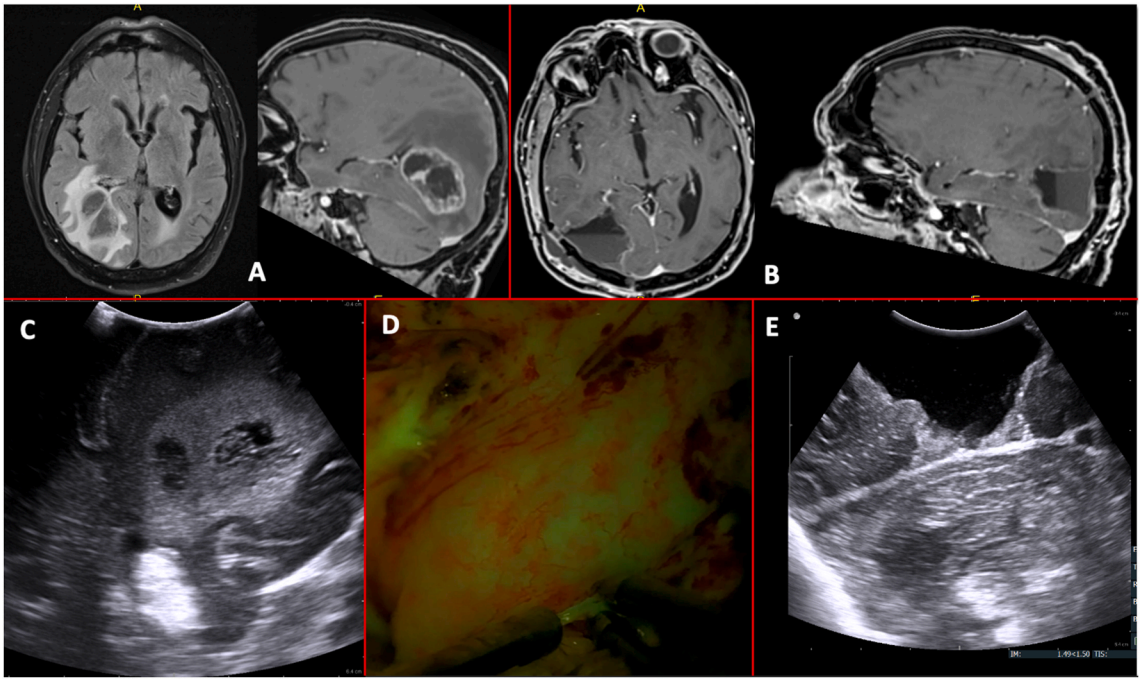

Fig. 1. Representative case. **A.** T1-weighted axial without- and sagittal scans with contrast enhancement showing a right temporal-occipital HGG (maximum dimension 46 × 30 × 39 mm) before surgery.
B. T1-weighted axial and sagittal scans with contrast enhancement after surgery. No residual disease is detectable and perilesional edema is reduced.
C. Intra-operative ultrasounds footage showing both cystic and solid components of the lesion.
D. Intra-operative footage caught from the surgical microscope while using Fluorescein. The lesion is well highlighted by the fluorescent agent.
E Intra-operative ultrasounds footage at the end of the procedure. Lesion's component is no longer detectable.

2.2. Data sources and quantitative variables

The extent of resection (EOR) was determined through a comparison between the MR images obtained before surgery and the 30th post-operative day MRI. EOR was calculated as a percentage by comparing the preoperative and early postoperative imaging, with the aforementioned software. Gross Total Resection (GTR) was defined as a confirmed reduction of the preoperative volume of the tumor of at least 95 % conversely a Near or Subtotal Resection was the surgical result on radicality (NTR/STR) identified with contrast-enhancing tissue in gadolinium-enhanced T1-Weighted imaging and Perfusion Weighted Imaging.[8,9] The EOR was also estimated by independent observers, certified radiologists, blind to the aims and design of the present paper, who produced specific reports concerning the general findings of the MRI scan and of the resection. The 30th-day re-evaluation was considered the turning point from the surgical to the adjuvant treatment phase.

2.3. Statistical methods

The sample was analyzed with SPSS version 18. Comparisons between nominal variables have been made with Chi2 test. EOR and PFS means were compared with One-way and Multivariate ANOVA analysis along with Contrast analysis and Post-Hoc Tests. Continuous variable correlations have been investigated with Pearson's Bivariate correlation. The threshold of statistical significance was considered $p < 0.05$.

2.4. Potential source of bias and study size

We addressed no missing data since incomplete records were an exclusion criterion. A potential source of bias is expected from exiguity of the sample, which nevertheless, regarding the endpoints selected (use of IoUS), presents an excellent post-hoc statistical estimated power (1- β = 0.921 for α 0.05 and effect size = 0.70), thus providing extremely reliable conclusions.

The informed consent was approved by the Institutional Review Board of our Institution. Before the surgical procedure, all the patients gave informed written explicit consent after appropriate information. Data reported in the study have been completely anonymized. No treatment randomization has been performed. This study is perfectly consistent with the Helsinki declaration of Human Rights.

3. Results

3.1. Descriptive data

The final cohort is composed of 74 patients whose clinical, radiological, and surgical records were retrospectively reviewed for the present investigation: a total of 42 males and 32 females whose average age was 62.04 ± 12.80 (Range 30–84, $p = 0.379$). From a histological perspective, the most common lesion was Glioblastoma (GBM) (52 patients, 71.62 %), followed by 20 (27.02 %). Anaplastic Astrocytoma (AA), 1 case of grade III anaplastic oligodendroglioma and 1 case of grade IV gliosarcoma (diagnosed up according to the 2016 WHO Guidelines). To simplify, there were 53 grade IV lesions and 21 grade III lesions (see Table 1). A total of 28 lesions were in eloquent areas, of which 11 involved the motor pathways and 17 the language cortices and arcuate fasciculus. There was no statistically significant difference between the three subgroups and the incidence of lesions in eloquent areas ($p = 0.928$, 0.633 and 0.476).

As previously stated, a total of 37 and 22, 15 patients belonged to Group C, A, and B respectively, namely were operated on without or with the use of Io-US and the use of Io-US and Fluorescein. Thirty-two patients underwent a GTR, 42 patients experienced a near total resection, whereas in 2 cases we recorded a major residual disease (STR). The postoperative functional performance status was stable and improved in 66.21 % and 24.32 % of the total respectively, whereas 9.45 % of the total were worsened. All the relevant details, concerning the final cohort are included in Table 1.

Table 1
Patient's demographics.

Subgroups	A (22 Patients)	B (15 Patients)	C (37 Patients)	*P* Value
Sex	11 M/11 F	11 M/4 F	M 20/17 F	0.303
Age	63.27 ± 12.64	63.73 ± 6.97	58.82 ± 16.61	0.379
Eloquent Language Areas	5 (22.72 %)	4 (26.67 %)	8 (21.62 %)	0.633
Eloquent Motor Areas	2 (9.09 %)	3 (20.00 %)	6 (16.21 %)	0.476
Functional Status	Improved 7 (31.81 %) Stable 13 (59.09 %) Worsened 2 (9.09 %)	Improved 5 (33.33 %) Stable 7 (46.67 %) Worsened 3 (20.00 %)	Improved 6 (16.21 %) Stable 29 (78.37 %) Worsened 2 (5.40 %)	0.178
GTR Rate	GTR 7 (31.81 %) STR/NTR 15 (68.18 %)	GTR 12 (80.00 %) STR/NTR 3 (20.00 %)	GTR 13 (35.13 %) STR/NTR 24 (64.86 %)	0.012
WHO Grade	IV 13 Patients III 9 Patients	IV 12 Patients III 3 Patients	IV 28 Patients III 9 Patients	0.036
KPS Pre	85.10 ± 11.60	86.67 ± 15.05	79.28 ± 15.91	0.447
KPS Post	87.85 ± 16.72	75.01 ± 32.09	84.28 ± 14.52	0.404

AA: Anaplastic Astrocytoma; **GBM:** Glioblastoma; **GTR**: Gross Total Resection; **IoUS:** Intraoperative Ultrasounds; **NTR**: Near Total Resection; **STR**: Subtotal Resection.

3.2. Main results

3.2.1. Extent of resection analysis

Per se, the simple use of Io-US did not demonstrate, in our experience and our cohort of patients a clear association with an increased resection ($p = 0.223$), nevertheless, interestingly, administering the intravenous Fluorescein demonstrated a statistically significant association with a gross total resection ($p = 0.012$, Fig. 2). In a multivariate analysis, this data appears to be independent of the location (eloquent/non-eloquent) of the lesion ($p = 0.556$) and from its histology ($p = 0.159$). These results, although clear, should be interpreted cautiously because we added the fluorescein filter several months after the introduction of Io-US in our division, and therefore in the meantime the surgeons could have experienced a learning curve.

3.2.2. Functional status analysis

There was no significant association between the functional status codified as a dichotomous variable ($p. = 0.178$ Fig. 3) and the three

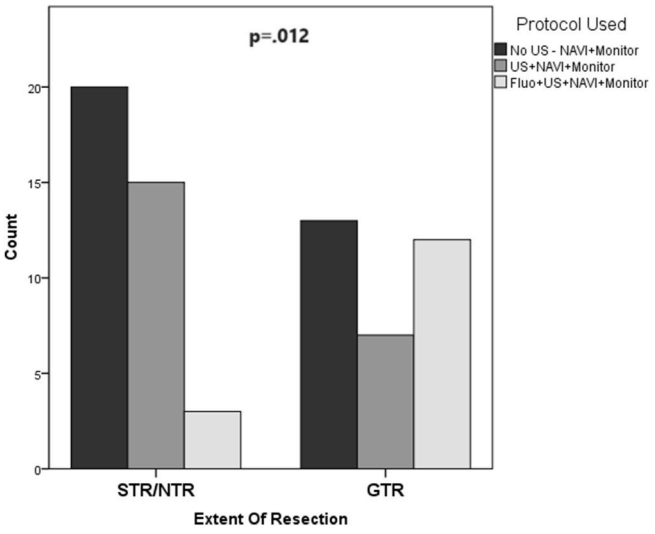

Fig. 2. Bar Chart showing the Extent of Resection in each group.

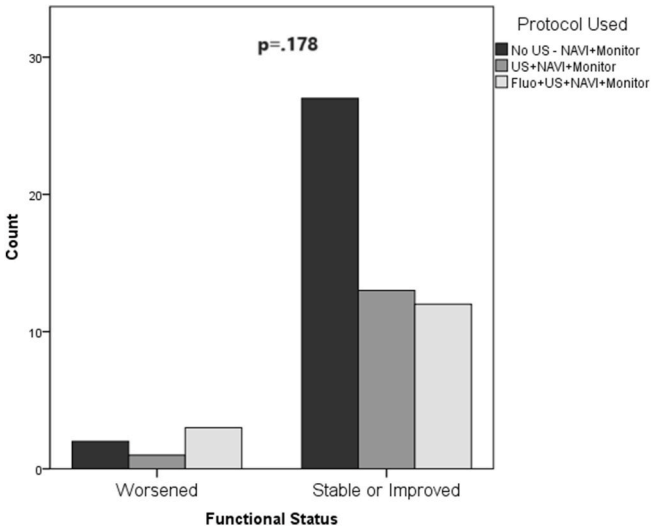

Fig. 3. Bar Chart showing the functional outcome in each group.

subgroups of patients. The single comparisons confirmed the findings (respectively, $p = 0.566$, 0.795, 0.514). A set of multivariate analyses was demonstrated to be independent concerning sex ($p = 0.131$), eloquent/non-eloquent location of the lesion ($p = 0.648$), and histology ($p = 0.659$).

4. Discussion

Considering the dismal prognosis of HGG regardless of the multimodal treatment, the current neurosurgical objective towards these lesions is to reach a balance between maximal surgical resection and preservation of functional status.[16] It has been demonstrated widely that greater extent of resection correlates with longer survival, however reaching GTR or supra-marginal resection with aggressive surgery can lead to permanent neurological impairment and consequent worsening of functional status. Gross total or even supratotal resections have been proven to be associated with prolonged survival.[2,9,10] Apart from the modern intraoperative imaging technologies, modern microscopes, and dyes to specifically discriminate between neoplastic and normal tissues, intraoperative neuromonitoring is a milestone to pursue a safe maximal resection, while preserving the function, in the context of maintaining the neuro-oncological balance.[13] Unfortunately, patients with poor functional status cannot reach the required autonomy to sustain adjuvant treatment, such as radiotherapy and chemotherapy. In this scenario, the research for technological tools capable of aiding the maximal safe resection possible during surgery has led to the introduction of several instruments that are now considered essential during practice,[15–22] including Io-US and fluorescence.

The use of Io-US was described for the first time in 1982[26] and during the decades the use of this instrument has been improved with the development of new probes and the possibility to use contrast-enhanced ultrasound, doppler function and merging with CT/MRI scans.[21] Io-US demonstrated to be a useful and safe instrument to obtain a greater extent of resection,[21,27] even though it represents two major pitfalls: the impossibility of complete removal of artifacts, especially in deep surgical fields, and the variability of its use between different operators. Moreover, in the present study, it is demonstrated that the simultaneous use of Io-US with the navigation system and IoN does not provide advantages over the single use of navigation and IoN, both in terms of extent of resection and functional status.

The other tool evaluated in the present study is intraoperative fluorescence. The use of intraoperative fluorescence for the resection of HGG was described for the first time in 1948[28] and nowadays there are two major fluorescent agents used in daily practice: 5-ALA and Fluorescein [29]

Even though 5-ALA is the only agent approved by the FDA for the resection of HGG, the high cost for a single application, the necessity of being ingested hours before surgery and to keep away patients from sunlight due to photo-sensibility[28–31] represent important pitfalls that prevent its application compared to Fluorescein. Even though Fluorescein is not specific for HGG since it spreads in every zone where the Brain–Blood Barrier (BBB) is compromised, the application of this fluorescent agent has been demonstrated widely to be safe and effective in the resection of HGG.[25,29–31] Moreover, the cost for a single application is low and it can be easily injected after performing anesthesia.[32] However, the use of both fluorescent agents has common pitfalls: when lesions do not present contrast enhancement in MRI studies and when blood or surgical debris is present in the surgical field, fluorescent agents cannot be detected using the appropriate filter under microscopical magnification.[30] Nevertheless, in the present study, better results in terms of the extent of resection have been demonstrated in the group of patients treated with the application of fluorescence, confirming the data widely reported in current Literature.[30]

The single application of Io-US and Fluorescein has been widely discussed in several papers[21,30] and both instruments present evident and well-known strengths and weaknesses.[32] However, the simultaneous use of these tools has not been described yet, and suggested by the present results, it could lead to better surgical outcomes in terms of the extent of resection, suggesting that one tool could overcome the pitfalls related to the use of the other one, even though no significative evidence were detected in terms of functional outcome. In all the three groups of patients presented in this study, navigation and IoN were used and this specific aspect of study design might justify these findings, suggesting that these two instruments have a major role in preserving functional status, more than Io-US and Fluorescein. According to our results, the combination of US and Fluoresceine-guided surgery enhanced the resection of supratentorial HGG. Since HGG are infiltrative by nature, remnants, especially late in the resection, could be easily misinterpreted as normal brain parenchyma. This reason is linked to the introduction, and progressive diffusion, of the fluorescent dyes.[29–32] Among these molecules, metabolized by tumor cells and therefore staining the neoplastic tissue, 5-aminolevulinic Acid (5-ALA) and Fluorescein, appear to be the most widely used in neurooncological surgery.[33] The first is more expensive, has the necessity of patient-specific preparation could be associated with several side effects, while the second appears to be less specific for glioma cells, being sensible to the blood–brain barrier damage, and thus being associated with a potentially increased risk of damage to the healthy tissues 34. We found nevertheless that a prudent use of intraoperative imaging and dyes could significantly increase the safety of the resection.

5. Limitations

The main limitations of the present investigation are its retrospective nature and the possible biases derived from the relative exiguity of the cohort, which nevertheless presents a fair statistical power of the study. Moreover, when the Fluorescein filter was introduced in our Institution, Io-US was used for several months and therefore a possible “learning curve” effect could to some extent bias the results. Nevertheless, such statistical significance ($p = 0.012$, concerning EOR), weighed for the width of our cohort is worth reporting. Further investigations are already ongoing, in our Institution, to confirm such powerful effects on wider cohorts.

6. Conclusion

Even when availing of advanced intraoperative protocol, the risk of permanent or long-lasting postoperative morbidity in HGG surgery remains significant. It is important to preserve the function while maximizing the resection. Intravenous Fluorescein matched with a neuronavigated Io-US system significantly increases the resection rate, but the clues derived from the staining should always be carefully weighed with those from the anatomy (advanced neuronavigation) and neuromonitoring.

Funding

This research received no external funding

Institutional Review Board statement

Ethics Committee/Institutional Review Board approval is not required for this manuscript because it is a retrospective, observational data collection, the data collected are purely retrospective, devoid of any treatment randomization, the radiological and clinical data, and the analyzed data are completely anonymous, no variation in respect to the world recognized gold standard treatments has been performed, it is just an analyzed series of retrospective data concerning oncologic and functional prognosis. Of course, all the included patients signed a formal informed consent after appropriate information both for letting the data be analyzed in research and concerning surgical information.

Informed consent statement

Written informed consent has been obtained from the patients to publish research papers concerning their clinical conditions. The surgical and research informed consent was approved by our institution. The blank copy of the consent is available on request.

CRediT authorship contribution statement

Alessandro Pesce: Writing – review & editing, Supervision, Formal analysis, Data curation, Conceptualization. **Mauro Palmieri:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Andrea Pietrantonio:** Methodology, Investigation. **Silvia Ciarlo:** Data curation. **Maurizio Salvati:** Validation, Supervision, Investigation. **Angelo Pompucci:** Supervision, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Zhao Z, Zhang KN, Wang Q, et al. Chinese glioma genome Atlas (CGGA): a comprehensive resource with functional genomic data from Chinese glioma patients. *Dev Reprod Biol.* 2021 Feb;19(1):1–12. https://doi.org/10.1016/j.gpb.2020.10.005. Epub 2021 Mar 2. PMID: 33662628; PMCID: PMC8498921.
2. Silantyev AS, Falzone L, Libra M, et al. Current and future trends on diagnosis and prognosis of glioblastoma: from molecular biology to proteomics. *Cells.* 2019 Aug 9; 8(8):863. https://doi.org/10.3390/cells8080863. PMID: 31405017; PMCID: PMC6721640.
3. Mallick S, Benson R, Hakim A, Rath GK. Management of glioblastoma after recurrence: a changing paradigm. *J Egypt Natl Cancer Inst.* 2016 Dec;28(4):199–210. https://doi.org/10.1016/j.jnci.2016.07.001. Epub 2016 Jul 28. PMID: 27476474.
4. Smith C, Lineburg KE, Martins JP, et al. Autologous CMV-specific T cells are a safe adjuvant immunotherapy for primary glioblastoma multiforme. *J Clin Invest.* 2020 Nov 2;130(11):6041–6053. https://doi.org/10.1172/JCI138649. PMID: 32750039; PMCID: PMC7598048.
5. Frederico SC, Hancock JC, Brettschneider EES, Ratnam NM, Gilbert MR, Terabe M. Making a cold tumor hot: the role of vaccines in the treatment of glioblastoma. *Front Oncol.* 2021 May 10;11, 672508. https://doi.org/10.3389/fonc.2021.672508. PMID: 34041034; PMCID: PMC8141615.
6. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med.* 2005 Mar 10;352(10):997–1003. https://doi.org/10.1056/NEJMoa043331. PMID: 15758010.
7. Hartmann C, Hentschel B, Wick W, et al. Patients with IDH1 wild type anaplastic astrocytomas exhibit worse prognosis than IDH1-mutated glioblastomas, and IDH1 mutation status accounts for the unfavorable prognostic effect of higher age: implications for classification of gliomas. *Acta Neuropathol.* 2010 Dec;120(6):

707–718. https://doi.org/10.1007/s00401-010-0781-z. Epub 2010 Nov 19. PMID: 21088844.
8. Armocida D, Pesce A, Frati A, Santoro A, Salvati M. EGFR amplification is a real independent prognostic impact factor between young adults and adults over 45yo with wild-type glioblastoma? *J Neuro Oncol*. 2020 Jan;146(2):275–284. https://doi.org/10.1007/s11060-019-03364-z. Epub 2019 Dec 30. PMID: 31889239.
9. Salvati M, Pesce A, Palmieri M, Floriana Brunetto GM, Santoro A, Frati A. The role and real effect of an iterative surgical approach for the management of recurrent high-grade glioma: an observational analytic cohort study. Jan 3:S1878-8750(18)32941-3 *World Neurosurg*. 2019. https://doi.org/10.1016/j.wneu.2018.12.118. Epub ahead of print. PMID: 30610982.
10. Vivas-Buitrago T, Domingo RA, Tripathi S, et al. Influence of supramarginal resection on survival outcomes after gross-total resection of IDH-wild-type glioblastoma. *J Neurosurg*. 2021 Jun 4;136(1):1–8. https://doi.org/10.3171/2020.10.JNS203366. PMID: 34087795; PMCID: PMC9248270.
11. Certo F, Altieri R, Maione M, et al. FLAIRectomy in supramarginal resection of glioblastoma correlates with clinical outcome and survival analysis: a prospective, single institution, case series. *Oper Neurosurg (Hagerstown)*. 2021 Jan 13;20(2):151–163. https://doi.org/10.1093/ons/opaa293. PMID: 33035343.
12. Certo F, Stummer W, Farah JO, et al. Supramarginal resection of glioblastoma: 5-ALA fluorescence, combined intraoperative strategies and correlation with survival. *J Neurosurg Sci*. 2019 Dec;63(6):625–632. https://doi.org/10.23736/S0390-5616.19.04787-8. Epub 2019 Jul 29. PMID: 31355623.
13. Duffau H. Is supratotal resection of glioblastoma in noneloquent areas possible? *World Neurosurg*. 2014 Jul-Aug;82(1–2):e101–e103. https://doi.org/10.1016/j.wneu.2014.02.015. Epub 2014 Feb 15. PMID: 24534058.
14. Seker-Polat F, Pinarbasi Degirmenci N, Solaroglu I, Bagci-Onder T. Tumor cell infiltration into the brain in glioblastoma: from mechanisms to clinical perspectives. *Cancers*. 2022;14(2):443. https://doi.org/10.3390/cancers14020443.
15. Gogos AJ, Young JS, Morshed RA, Hervey-Jumper SL, Berger MS. Awake glioma surgery: technical evolution and nuances. *J Neuro Oncol*. 2020 May;147(3):515–524. https://doi.org/10.1007/s11060-020-03482-z. Epub 2020 Apr 8. PMID: 32270374.
16. Frati A, Pesce A, Palmieri M, et al. Hypnosis-aided awake surgery for the management of intrinsic brain tumors versus standard awake-asleep-awake protocol: a preliminary, promising experience. *World Neurosurg*. 2019 Jan;121:e882–e891. https://doi.org/10.1016/j.wneu.2018.10.004. Epub 2018 Oct 10. PMID: 30315969.
17. Schwake M, Stummer W, Suero Molina EJ, Wölfer J. Simultaneous fluorescein sodium and 5-ALA in fluorescence-guided glioma surgery. *Acta Neurochir*. 2015 May;157(5):877–879. https://doi.org/10.1007/s00701-015-2401-0. Epub 2015 Mar 28. PMID: 25820632; PMCID: PMC4477944.
18. Koc K, Anik I, Cabuk B, Ceylan S. Fluorescein sodium-guided surgery in glioblastoma multiforme: a prospective evaluation. *Br J Neurosurg*. 2008 Feb;22(1):99–103. https://doi.org/10.1080/02688690701765524. PMID: 18224529.
19. Dohrmann GJ, Rubin JM. Use of ultrasound in neurosurgical operations: a preliminary report. *Surg Neurol*. 1981 Nov;16(5):362–366. https://doi.org/10.1016/0090-3019(81)90279-2. PMID: 7336322.
20. Pino MA, Imperato A, Musca I, et al. New hope in brain glioma surgery: the role of intraoperative ultrasound. A review. *Brain Sci*. 2018 Nov 19;8(11):202. https://doi.org/10.3390/brainsci8110202. PMID: 30463249; PMCID: PMC6266135.
21. Dixon L, Lim A, Grech-Sollars M, Nandi D, Camp S. Intraoperative ultrasound in brain tumor surgery: a review and implementation guide. *Neurosurg Rev*. 2022 Aug;45(4):2503–2515. https://doi.org/10.1007/s10143-022-01778-4. Epub 2022 Mar 30. PMID: 35353266; PMCID: PMC9349149.
22. Kaale AJ, Rutabasibwa N, Mchome LL, et al. The use of intraoperative neurosurgical ultrasound for surgical navigation in low- and middle-income countries: the initial experience in Tanzania. *J Neurosurg*. 2020 Feb;28:1–8. https://doi.org/10.3171/2019.12.JNS192851. Epub ahead of print. PMID: 32109864.
23. Trevisi G, Barbone P, Treglia G, Mattoli MV, Mangiola A. Reliability of intraoperative ultrasound in detecting tumor residual after brain diffuse glioma surgery: a systematic review and meta-analysis. *Neurosurg Rev*. 2020 Oct;43(5):1221–1233. https://doi.org/10.1007/s10143-019-01160-x. Epub 2019 Aug 14. PMID: 31410683.
24. Wen PY, Packer RJ. The 2021 WHO classification of tumors of the central nervous system: clinical implications. *Neuro Oncol*. 2021 Aug 2;23(8):1215–1217. https://doi.org/10.1093/neuonc/noab120. PMID: 34185090; PMCID: PMC8328017.
25. Acerbi F, Broggi M, Schebesch KM, et al. Fluorescein-guided surgery for resection of high-grade gliomas: a multicentric prospective phase II study (FLUOGLIO). *Clin Cancer Res*. 2018 Jan 1;24(1):52–61. https://doi.org/10.1158/1078-0432.CCR-17-1184. Epub 2017 Oct 10. PMID: 29018053.
26. Chandler WF, Knake JE, McGillicuddy JE, et al. Intraoperative use of real-time ultrasonography in neurosurgery. *J Neurosurg*. 1982;57:157–163.
27. Woydt M, Krone A, Becker G, Schmidt K, Roggendorf W, Roosen K. Correlation of intra-operative ultrasound with histopathologic findings after tumour resection in supratentorial gliomas. A method to improve gross total tumour resection. *Acta Neurochir*. 1996;138(12):1391–1398. https://doi.org/10.1007/BF01411117. PMID: 9030345.
28. Moore GE, Peyton WT. The clinical use of sodium fluorescein and radioactive diiodofluorescein in the localization of tumors of the central nervous system. *Minn Med*. 1948;31:1073–1076.
29. Schebesch K-M, Proescholdt M, Höhne J, et al. Sodium fluoresceine guided resection under the YELLOW 560 nm surgical microscope filter in malignant brain tumor surgery—a feasibility study. *Acta Neurochir*. 2013;155:693–699.
30. Smith EJ, Gohil K, Thompson CM, Naik A, Hassaneen W. Fluorescein-guided resection of high grade gliomas: a meta-analysis. *World Neurosurg*. 2021 Nov;155:181–188.e7. https://doi.org/10.1016/j.wneu.2021.08.126. Epub 2021 Sep 4. PMID: 34492388.
31. Teixidor P, Arráez MÁ, Villalba G, et al. Safety and efficacy of 5-aminolevulinic acid for high grade glioma in usual clinical practice: a prospective cohort study. *PLoS One*. 2016;11, e0149244.
32. Maugeri R, Villa A, Pino M, et al. With a little help from my friends: the role of intraoperative fluorescent dyes in the surgical management of high grade gliomas. *Brain Sci*. 2018;8:31.
33. Zeppa P, De Marco R, Monticelli M, et al. Fluorescence-guided surgery in glioblastoma: 5-ALA, SF or both? Differences between fluorescent dyes in 99 consecutive cases. *Brain Sci*. 2022;12(5):555.