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FLAIRectomy in Supramarginal Resection of Glioblastoma Correlates With Clinical Outcome and Survival Analysis: A Prospective, Single Institution, Case Series

BACKGROUND: Extent of tumor resection (EOTR) in glioblastoma surgery plays an important role in improving survival.

OBJECTIVE: To analyze the efficacy, safety and reliability of fluid-attenuated inversion-recovery (FLAIR) magnetic resonance (MR) images used to guide glioblastoma resection (*FLAIRectomy*) and to volumetrically measure postoperative EOTR, which was correlated with clinical outcome and survival.

METHODS: A total of 68 glioblastoma patients (29 males, mean age 65.8) were prospectively enrolled. Hyperintense areas on FLAIR images, surrounding gadolinium-enhancing tissue on T1-weighted MR images, were screened for signal changes suggesting tumor infiltration and evaluated for supramaximal resection. The surgical protocol included 5-aminolevulinic acid (5-ALA) fluorescence, neuromonitoring, and intraoperative imaging tools. 5-ALA fluorescence intensity was analyzed and matched with the different sites on navigated MR, both on postcontrast T1-weighted and FLAIR images. Volumetric evaluation of EOTR on T1-weighted and FLAIR sequences was compared.

RESULTS: FLAIR MR volumetric evaluation documented larger tumor volume than that assessed on contrast-enhancing T1 MR (72.6 vs 54.9 cc); residual tumor was seen in 43 patients; postcontrast T1 MR volumetric analysis showed complete resection in 64 cases. O6-methylguanine-DNA methyltransferase promoter was methylated in 8/68 (11.7%) cases; wild type Isocytrate Dehydrogenase-1 (IDH-1) was found in 66/68 patients. Progression free survival and overall survival (PFS and OS) were 17.43 and 25.11 mo, respectively. Multiple regression analysis showed a significant correlation between EOTR based on FLAIR, PFS ($R^2 = 0.46$), and OS ($R^2 = 0.68$).

CONCLUSION: EOTR based on FLAIR and 5-ALA fluorescence is feasible. Safety of resection relies on the use of neuromonitoring and intraoperative multimodal imaging tools. FLAIR-based EOTR appears to be a stronger survival predictor compared to gadolinium-enhancing, T1-based resection.

KEY WORDS: 5-ALA, Extent of resection, FLAIR, *FLAIRectomy*, Glioblastoma, i-CT, Survival, Neuronavigation

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Treatment of glioblastoma, is challenging.[1] Although biomolecular differences between glioblastoma might account for the different clinical outcome in patients, other elements, including extent of tumor resection (EOTR), chemotherapy, and radiotherapy are

ABBREVIATIONS: 5-ALA, 5-aminolevulinic acid; **11C-MET-PET,** 11C-methionine positron emission tomography; **DTI,** diffusion tensor imaging; **DWI,** diffusion-weighted imaging; **EOTR,** Extent of tumor resection; **FLAIR,** fluid-attenuated inversion-recovery; **Gad-T1,** Gadolinium-enhanced, T1-weighted; **HGG,** high grade gliomas; **i-CT,** intraoperative computed tomography; **IDH-1,** Isocytrate Dehydrogenase-1; **i-US,** intraoperative ultrasound; **KPS,** Karnofsky performance score; **LGG,** low-grade glioma; **MGMT,** O^6-methylguanine-DNA methyltransferase; **MR,** magnetic resonance; **OS,** overall survival; **PFS,** progression free survival; **RANO,** response assessment in neuro-oncology; **ROIs,** regions of interest; **SMR,** supramarginal resection

considered important factors associated with progression free survival (PFS) and overall survival (OS).[2-7]

The concept of supramarginal resection (SMR) for low-grade glioma (LGG), diffuse LGG,[8] and metastases,[9] as a strategy providing improved PFS and OS, has been reported.[8-10] The rationale for glioblastoma SMR is based on the observation that recurrences usually take place within 2 centimeters of the initial tumor edges.[6,11,12]

Gadolinium-enhanced, T1-weighted (Gad-T1) magnetic resonance (MR) images allow tumor volume measurement and edge delineation.[13] T2-weighted fluid-attenuated inversion-recovery (FLAIR) MR images help in determining tumor anatomical margins thanks to cerebrospinal fluid suppression and improved anatomy visualization.[14,15] FLAIR sequences usually show tumors larger than Gad-T1 sequences, and can also show peritumoral areas, where tumor cells are thought to be located.[16] However, specificity of FLAIR sequences is reduced by the limitations in distinguishing tumor areas (with real tumor infiltration) from perilesional edema.[15] Moreover, like other preoperative imaging techniques, accuracy of neuronavigation based on preoperative FLAIR sequences is also plagued by brain shift.[17,18]

Following the study by Stummer et al,[19] 5-aminolevulinic acid (5-ALA) fluorescence is widely used to improve tumor visualization and resection. Clinical experience with 5-ALA has demonstrated the presence of fluorescent tissue beyond enhancing tumor edges.[20,21]

FLAIR sequences and 5-ALA fluorescence both improve tumor visualization, also detecting those peritumoral areas where small tumor cell nests hide[11,22]; these are associated with the high frequency of tumor recurrence and the consequent low survival expectancy.[23,24]

This study aimed to evaluate if FLAIR based SMR (*FLAIRectomy*) based on the combined use of T2-weighted FLAIR navigation, 5-ALA fluorescence and neurophysiological monitoring is feasible, safe, and effective in improving patients' OS and PFS.

METHODS

Study Design, Setting, and Population

This is a prospective, single institution case-series of 68 patients (29 males), with a mean age of 65.8 yr (range 49-82), with MR appearance of glioblastoma (including positive MR spectroscopy) enrolled from June 2015 to December 2018 and followed-up until June 2019 (Table 1). Patients with recurrent tumors, previous tumor biopsy or expected partial resections (ie, tumors infiltrating eloquent areas) were not considered for inclusion. In all included patients, a complete resection of the enhancing tumor was deemed feasible preoperatively. Inclusion and exclusion criteria are summarized in Table 2. This study was approved by the local ethics committee and all patients had signed a specific informed consent for the study before surgery. The informed consent form for study participation was also approved by the local ethics committee and all signed informed consents were archived in the study files.

TABLE 1. Demographic and Clinical Characteristics of Patients Included in the Study

Characteristic	Value
Patients enrolled	68
Sex	
Male	29
Female	39
Age (yr)	
Mean	65.8
Range	49-82
Follow-up	
Mean	24.5
Range	10-38
Histology	
Glioblastoma	66/68
Gliosarcoma	2/68
Tumor location	
Non-eloquent brain	22/68
Near-eloquent brain	29/68
Eloquent brain	17/68
Survival (mo)	
Mean OS	25.11
Mean progression-free survival	17.43
KPS	
Preoperative	75.4
Postoperative (5 d after surgery)	64.8
Postoperative (last follow-up visit)	76.6
Neurological outcome	
No deficits	60/68
Transient deficits (recover within 90 d)	5/68
Permanent deficit	3/68
Postoperative Radiotherapy	
Number of patients	67/68
Mean dose (Gy)	60.91
Range of doses (Gy)	55-62
Postoperative Chemotherapy	
Stupp protocol (number of patients)	56/68
More than 6 cycles of TMZ (number of patients)	11/68
Mean number of cycles	8.86
Range of cycles	4-45

TABLE 2. Inclusion and Exclusion Criteria Considered for Patients' Enrollment

Inclusion criteria	Exclusion criteria
Magnetic resonance imaging spectroscopy suggesting HGG	Recurrent tumors
Complete resection of enhancing tumor preoperatively deemed feasible	Patients already treated with biopsy or partial resections
	Pediatric patients
	Multifocal disease
	Pathological findings not compatible with glioblastoma (postsurgery exclusion)

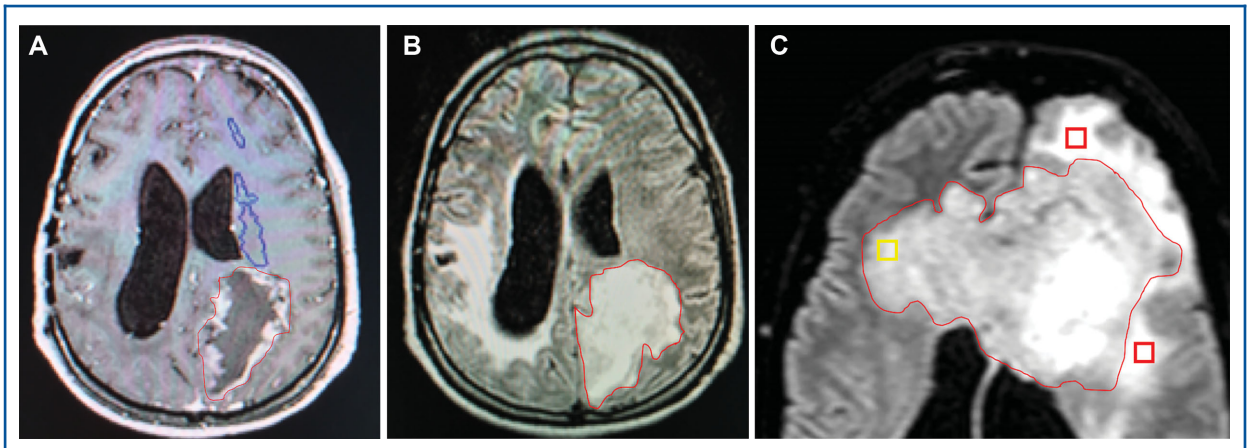


FIGURE 1. *Manual segmentation based on T1-Gad* **A** *and FLAIR* **B** *sequences for volumetric evaluation of HGG. The segmented pathological area on FLAIR sequences was selected according to the hyperintensity value recorded by multiple ROIs placed in different peritumoral areas, in each slice used for segmentation* **C**. *In this case the yellow ROI revealed a lower hyperintensity value than 2 red ROIs peripherically placed. This differentiates tumor (yellow ROI), included in segmented areas, from edema (red ROIs) excluded from the segmented lesion.*

Preoperative Radiological Evaluation

The MR protocol included volumetric T1-weighted, with and without gadolinium enhancement, volumetric FLAIR sequences, and T2-weighted, diffusion-weighted imaging (DWI)- and MR-spectroscopy images. In 50/68 patients MR-diffusion tensor imaging (DTI) sequences were also acquired and tractography was obtained. All MR scans were performed using a Philips 1.5T MR system by 2 neuroradiologists with proven experience in brain tumor diagnosis. Volumetric evaluation of preoperative MR was performed both on Gad-T1 and FLAIR sequences. Volumetric reconstruction and measurement were carried out using a manual segmentation method. The StealthViz® (Medtronic Inc, Dublin, Ireland) software was used for segmentation, rendering, and reconstruction as well as for volumetric measurements (Figure 1). On Gad-T1 images, all slices showing pathological contrast enhancement were segmented and reconstructed. On FLAIR images segmentation was based on the differences in signal intensity found in the peritumoral areas. The brain area surrounding the contrast-enhancing tumor has a higher signal intensity compared to the more distant white matter, which shows a FLAIR signal similar to the edema observed in noninfiltrating tumors (ie, meningioma and metastases).[14,22] The edges of peritumoral areas with different signal intensity were determined by placing regions of interest (ROIs) in the hyperintense brain parenchyma surrounding the tumor (Figure 1). This method establishes a threshold to distinguish areas of presumed "true" tumor infiltration from areas of edema/brain reaction. Based on such thresholds, manual segmentation of the tumor, including peri-tumoral infiltrated areas, was performed on FLAIR images (Figure 1C). Tumor volume calculated on the FLAIR sequence was greater than the volume calculated on Gad-T1 images and included both the enhancing nodule and all peritumoral hyperintense areas segmented as tumor according to the threshold established by ROIs. Therefore, for each patient a double volumetric evaluation was performed on preoperative MR scans. FLAIR-based differentiation between edema and tumor was reported by Tsuchiya et al in 1996[14]; however, these authors did not describe any quantitative method to distinguish hyperintensity due to edema or tumor. Therefore, we adopted a quantitative assessment of FLAIR hyperintensity using multiple ROIs, as described by Jha et al in 2014.[25] We performed FLAIR segmentation of the tumor both preoperatively, to plan the resection, and postoperatively to evaluate EOTR. Both T1 and FLAIR sequences, after segmentation and rendering, were made available for neuronavigation.

In 43/68 (63.2%) cases 11C-methionine positron emission tomography (11C-MET-PET) was also preoperatively obtained and merged with FLAIR and T1 sequences in the neuronavigation system.

Intraoperative 5-ALA Fluorescence

All patients underwent 5-ALA fluorescence-guided tumor resection. 20 mg per kilogram of 5-ALA hydrochloride (5-ALA HCL, Gliolan, Medac GmbH, Germany) were orally administered 3 h before anesthesia. An ultraviolet light filter integrated in the microscope (OPMI Pentero, Zeiss, Germany) was used to stimulate fluorophores, making fluorescent tissue visible. Intensity of fluorescence was recorded by the first surgeon, according to direct microsurgical vision, and classified as intensely fluorescent, vaguely fluorescent or non-fluorescent.

Neuromonitoring

Intraoperative monitoring was used in 46/68 (67.6%) cases, when tumors were involved near-eloquent or eloquent areas. Tumor location in relation to brain eloquence was graded according to the functional-topographic classification proposed by Sawaya et al in 1998[11] and also applied by Li et al in 2016.[11] In 22 (32.3%) patients the tumor was located in noneloquent areas, in 29 (42.6%) in near-eloquent brain and in 17 (25%) in eloquent areas. The neuromonitoring protocol included Motor Evoked and SomatoSensory Evoked Potentials and direct cortical and sub-cortical electrical stimulation.

Two different devices were used for neuromonitoring: Nimbus I-Care (Innopsys, Carbonne, France) and NIM-Eclipse® Nerve Monitoring System (Medtronic).

Three of the enrolled patients underwent awake craniotomy: one with a tumor involving the motor area and two with clear infiltration of the language area, preoperatively documented by DTI and functional MR.

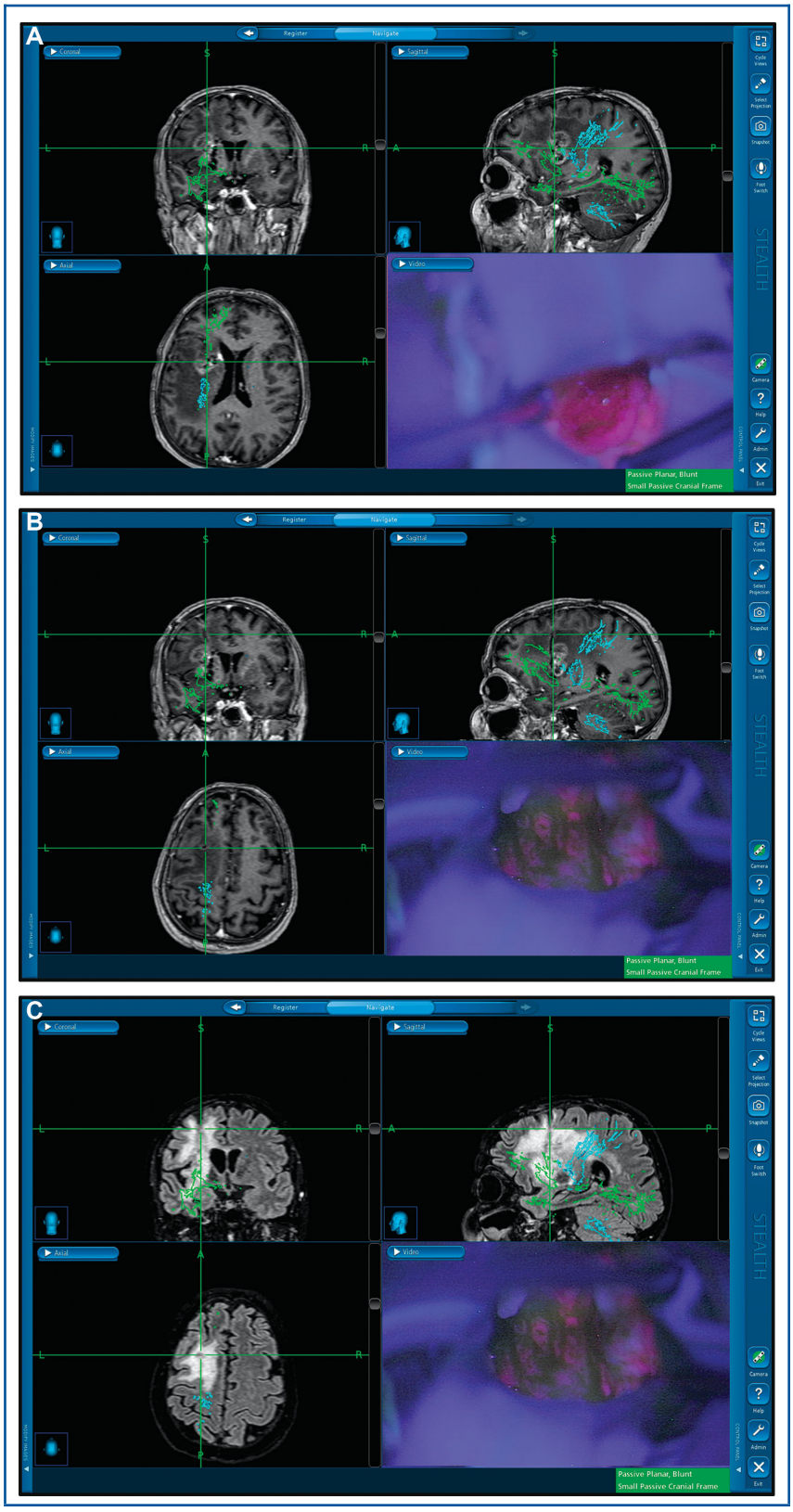


FIGURE 2. *Correlation between intraoperative 5-ALA fluorescence and MR features based on neuronavigation. Gadolinium-enhanced regions on T1 sequences commonly correspond to the intensely fluorescent tumor portion* **A**, *whilst the areas beyond the gadolinium margins, located within an apparently normal T1 region,* **B** *are hyperintense on FLAIR images* **C** *and correspond to a vaguely fluorescent tumor portion.*

These patients were selected for awake craniotomy on the basis of preoperative images and specific patients' characteristics (ability to psychologically tolerate awake surgery, absence of preoperative neurological deficits, age, co-existing cardiac, and respiratory pathologies)

Intraoperative Neuronavigation and Imaging Protocol

The StealthStation S7® (Medtronic Inc) navigation system was used in all cases. Gad-T1 and FLAIR sequences were used for intraoperative navigation. The two datasets were merged before patient registration using the software provided by the navigation system. T1-sequences were used as reference images and either an optic or electromagnetic surface tracer was used for patient registration. Microsurgical tumor resection was performed under continuous navigation using the microscope focus as the navigation pointer. Microscope-generated videos (obtained by alternating white and ultraviolet light) were also visualized on the navigation system screen to obtain immediate comparison between MR images (Gad-T1 and FLAIR) and intraoperative fluorescence findings (Figure 2). In particular, intensely fluorescent and vaguely fluorescent areas were localized by navigation and visualized both on Gad-T1 and FLAIR sequences. When resection of the T1-weighted, contrast-enhancing tumor was deemed complete, peripheral residual white matter areas still showing fluorescence were identified and localized with navigation on FLAIR sequences, then resected (Figure 2). Comparison between pathological area extension based on Gad- T1 and FLAIR images was then performed (Figure 2). Intraoperative imaging tools were also used and combined with the navigation protocol: intraoperative computed tomography (i-CT) was used in 65/68 (95.5%) cases and combined with intraoperative ultrasound (i-US) in 53/68 (77.9%) cases. The surgical protocol combining the use of i-CT, i-US, and 5-ALA has already been described.[26]

Postoperative Radiological Evaluation

All patients underwent postoperative MR within 48 h after surgery, with a protocol including volumetric T1-weighted, with and without gadolinium, volumetric FLAIR sequences, and T2- and DWI-weighted images. Hyperintense areas in the surgical cavity, visualized on T1-weighted images before and after gadolinium infusion, were interpreted as blood clots and not considered for the EOTR analysis. Volumetric measurement of residual tumor was made using the same method described above for preoperative assessment on Gad-T1 and FLAIR images. EOTR analysis was then performed by calculating the rate of tumor resection, considering the values of pre- and postoperative tumor volumes on Gad-T1 and FLAIR sequences, according to the following formula: [(preoperative tumor volume–postoperative tumor volume)/preoperative tumor volume] $\times 100$[7].

Evaluation of MGMT Promoter Methylation and IDH1 Mutation

For O^6-methylguanine-DNA methyltransferase (MGMT) promoter methylation analysis, bisulfite modification was performed on 100 ng of genomic DNA with the EpiTech Bisulfite kit (Qiagen). To detect Isocytrate Dehydrogenase-1 (IDH-1) mutations, amplicons spanning the entire gene sequence were obtained from 50 ng of genomic DNA using Platinum PCR SuperMix HiFi (Life Technologies). Both MGMT and IDH1 amplicons were resolved on agarose gel electrophoresis, purified and then sequenced using the conventional Sanger method.

Survival Data and Statistical Analysis

OS and PFS were recorded. For patients who are still undergoing clinical and radiological follow-up every 3 mo, survival data were calculated at last follow-up visit (June 2019). Tumor progression was defined according to the response assessment in neuro-oncology criteria (RANO Criteria)[3] based on a quarterly follow-up scheme. 11CMet-PET was also used during follow-up to differentiate real tumor progression from radionecrosis or pseudoprogression in 52/68 (76.4%) patients. Karnofsky performance score (KPS) was used to assess patients' clinical status pre- and postoperatively, as well as during follow-up.

Statistical analysis was performed using the SPSS 22.0 software (IBM, New York). Student's *t*-test was used to verify the presence of statistically significant differences between preoperative tumor volumes measured on T1 and FLAIR sequences. Linear regression analysis investigated the statistical correlation between survival and EOTR. Multiple regression analysis assessed the impact of other factors (age, RT dose, number of Temozolomide cycles, MGMT promoter methylation status, and IDH1 mutation) on survival parameters.

RESULTS

Postoperatively, all but one patient underwent the Stupp chemotherapy protocol. Radiotherapy with concomitant temozolomide was started 37.4 (range 28-51) d after surgery. All patients but one received the standard RT dose. Mean radiation dose was 60.91 (range 55-62). Temozolomide was continued after 6 cycles in 11 (16%) patients; mean temozolomide cycles were 8.86 (range 4-45). Details of adjuvant therapies are summarized in Table 1.

Histologic examination revealed glioblastoma in 66/68 (97%) and gliosarcoma in 2/68 (3%) cases, respectively. Biomolecular analysis revealed the presence of MGMT-promoter methylation in 8/68 (11.7%) patients; IDH-1 mutation was detected in 2/68 (2.9%) patients, whereas wild type IDH-1 was found in 66/68 (97%) patients.

Mean preoperative tumor volume on Gad- T1 MR sequences was 54.9 cc (range 33.4-89.7 cc); mean preoperative tumor volume on FLAIR sequences was 72.6 cc (range 39.5-103.8 cc). Paired Student's *t*-test revealed statistically significant differences between the 2 series of data ($P < .05$).

KPS changed from a mean preoperative value of 73.2 to a mean postoperative value of 67.4. The KPS registered at the last

TABLE 3. Summary of Volumetric Evaluation of Extent of Resection Based on Gad-T1 and FLAIR Sequences of MR Images and 5-ALA Fluorescence Guided Resection Data

	5-ALA	T1-GAD	FLAIR
Patients with residual tumor	60/68	4/68	43/68
Residual tumor volume (cc)	/		
Mean		1.54	3.7
Range		0.9-1.8	0.3-8.7
Extent of resection (%)	/		
Mean		99.8	92.43
Range		96.2-100	69.8-100

follow-up control was 76.6. No statistically significant differences in KPS variations were documented during follow-up.

Neurological worsening related to surgery was documented in 8/68 patients (11.7%). We observed motor impairment in 7 patients and language deficit in 1 patient. Motor deficits were related to primary motor cortex involvement in 4/7 cases and to supplementary motor area involvement in 3/7 cases. Three of the eight patients suffered from permanent neurological deficits (all patients had deficits related to the primary motor area); in the other five patients, neurological impairment was transient and recovered within 3 mo after surgery.

Intensely fluorescent areas surrounded by vaguely fluorescent tissue were found in all cases. Fluorescent areas were always entirely resected if neuromonitoring ruled out the presence of eloquent tissue. Complete resection of all fluorescent tissue (both intensely and vaguely fluorescent) was achieved in 60/68 patients (88.2%). In cases with incomplete fluorescent tumor removal, the resection was stopped in close proximity to eloquent areas detected by navigation and neuromonitoring. Evidence of fluorescent tissue beyond the contrast-enhancing edges of the tumor, according to navigation data, was documented in 65/68 patients (95.6%). Interestingly, in such cases neuronavigation based on FLAIR images revealed a constant correspondence between 5-ALA fluorescent and hyperintense areas preoperatively segmented as tumor and not edema (Figures 1 and 2).

EOTR based on Gad-T1 sequences documented residual tumor in 4/68 (5.9%) patients. Mean residual tumor volume was 1.54 cc (range 0.9-1.8 cc). Conversely, EOTR evaluation based on FLAIR sequences revealed residual tumor in 43/68 (63.2%) patients. Mean residual tumor volume measured on FLAIR images was 3.7 cc (range 0.3-8.7 cc). Mean EOTR rate, calculated on the 2 different imaging modalities, was 99.8%

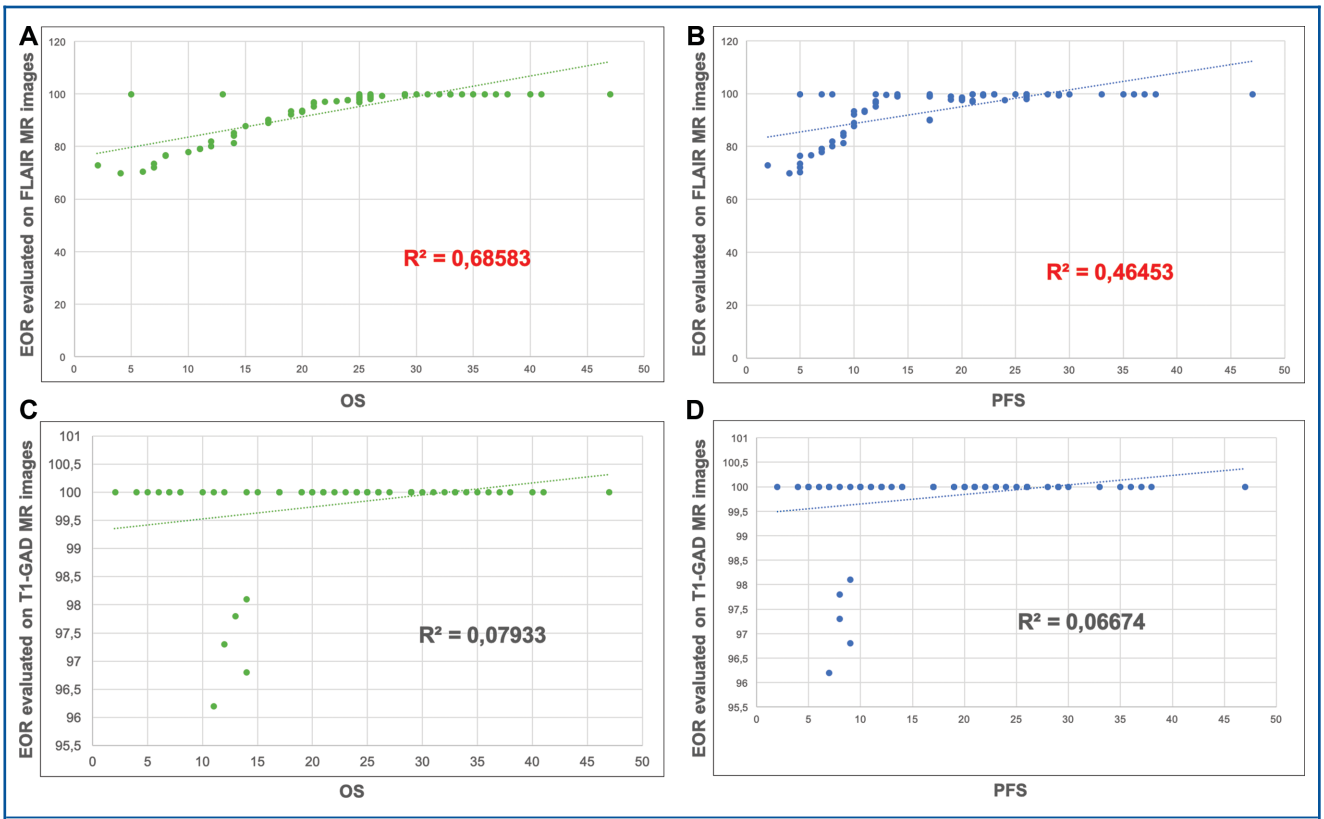


FIGURE 3. *Multiple regression analysis demonstrated the presence of a positive correlation between FLAIR-based EOTR values and OS* **A**, *and PFS* **B**. *No significant correlation between survival parameters and T1-Gad-based Extent of resection evaluation was documented* **C** *and* **D**.

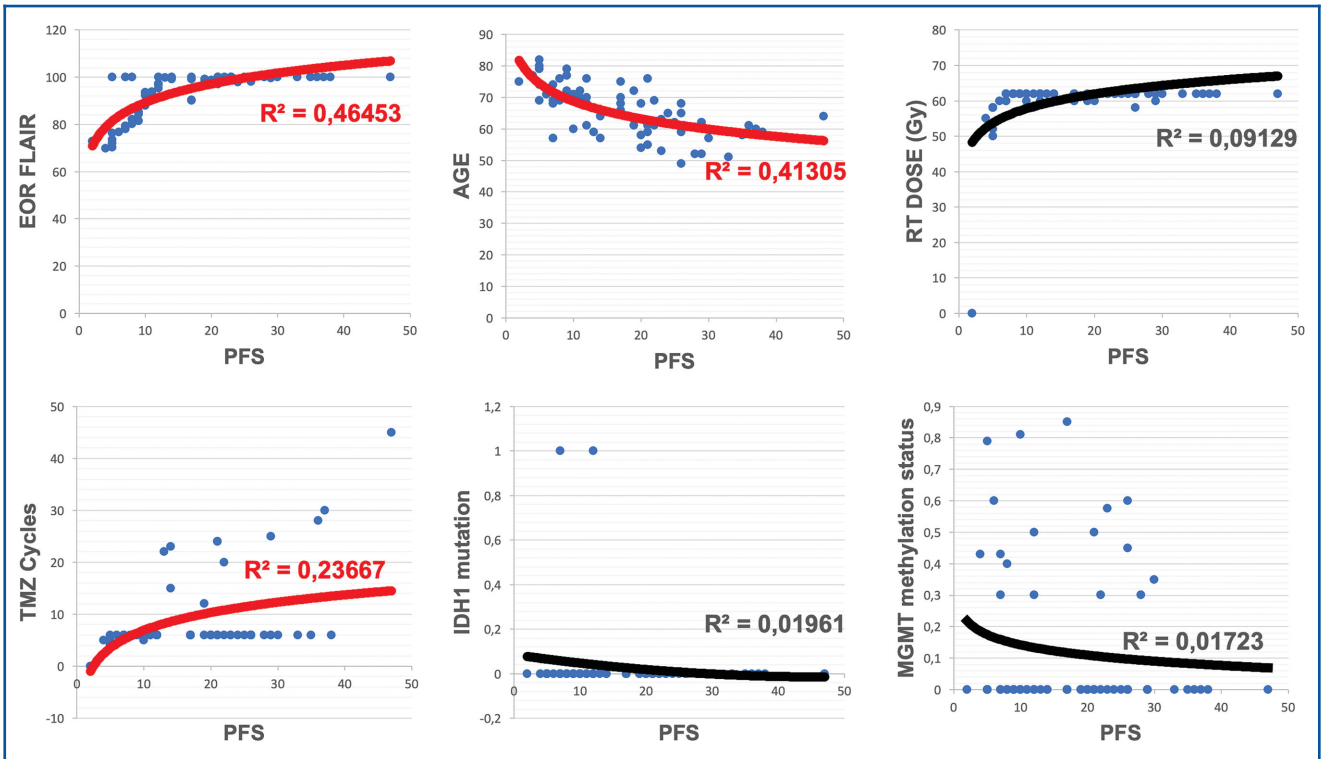


FIGURE 4. *Multiple regression analysis is summarized by scatter plots illustrating the different power of correlation between PFS and different factors (EOR FLAIR resection, age, TMZ cycles, RT cycles, MGMT promoter methylation status, and presence of IDH1 mutation); strength of correlation is expressed by the R^2 value (a statistically significant correlation is indicated in red), which is higher in correlation between EOR measured in FLAIR and PFS.*

(range 96.2%-100%) and 92.43% (range 69.8%-100%), respectively (Table 3). Comparison of the data revealed a statistically significant difference in postoperative residual tumor volume according to the paired Student's *t*-test ($P < .05$). New FLAIR alterations (ie, hyperintense areas documented in postoperative MR in the brain area appearing as normal in the preoperative scan) were observed in 2/68 cases, with small ischemic postoperative lesions due to vascular injuries. This finding was also confirmed by comparison of FLAIR images with DWI sequences.

Mean OS and PFS were, respectively, 25.11 and 17.43 mo. Twenty-five patients (22%) were still alive and under follow-up in June 2019 (Table 1). In 65/68 (95.5%) patients, progression was radiologically documented according to RANO criteria[3] at the last follow-up MR. The presence of recurrent tumor was confirmed by 11C-MET-PET in 45/68 (66.1%) cases. Recurrent tumors were located in the wall of the previous surgical cavity in 12/45 (26.6%) patients, within 2 cm of the surgical cavity in 15/45 (33.3%) and in other sites (ie, periventricular, other lobes, contralateral hemisphere) in 18 (40%) patients. Sixteen patients underwent second surgery, whereas the remaining 29 patients were treated with second line chemotherapy or prolonged temozolomide administration.

Linear regression analysis (Figure 3) revealed a positive correlation between PFS and FLAIR-based EOTR rate ($R^2 = 0.46$) as well as between OS and FLAIR-based EOTR rate ($R^2 = 0.68$). We found weak correlations between PFS and OS and Gad-T1-based EOTR ($R^2 = 0.028$ and 0.006, respectively). A stronger inverse correlation was also found between residual tumor volume measured on FLAIR with respect to Gad-T1 MR images and OS ($R^2 = 0.07$ and 0.08, respectively). This difference in R^2 determination coefficients is small, but it still suggests a role for the FLAIR residual tumor as a survival predictor. Multiple linear regression analysis, investigating the impact of FLAIR-based EOTR rate, age, radiation dose, number of Temozolomide cycles as well as OS and PFS, documented a statistically significant correlation ($P < .05$) between age and Temozolomide cycles and PFS; conversely, radiation dose did not statistically correlate ($P > .05$) with survival parameters (Figures 4 and 5). Statistically significant correlations were not found between OS, PFS, and MGMT promoter methylation status as well as IDH-1 mutation ($P > .05$, Figures 4 and 5).

DISCUSSION

EOTR is a survival predictor in brain glioma surgery.[4,6,26] However, glioblastoma have intrinsic radiological, histological and biological heterogeneity, which make the oncological approach difficult. In fact, glioblastoma, often identified as

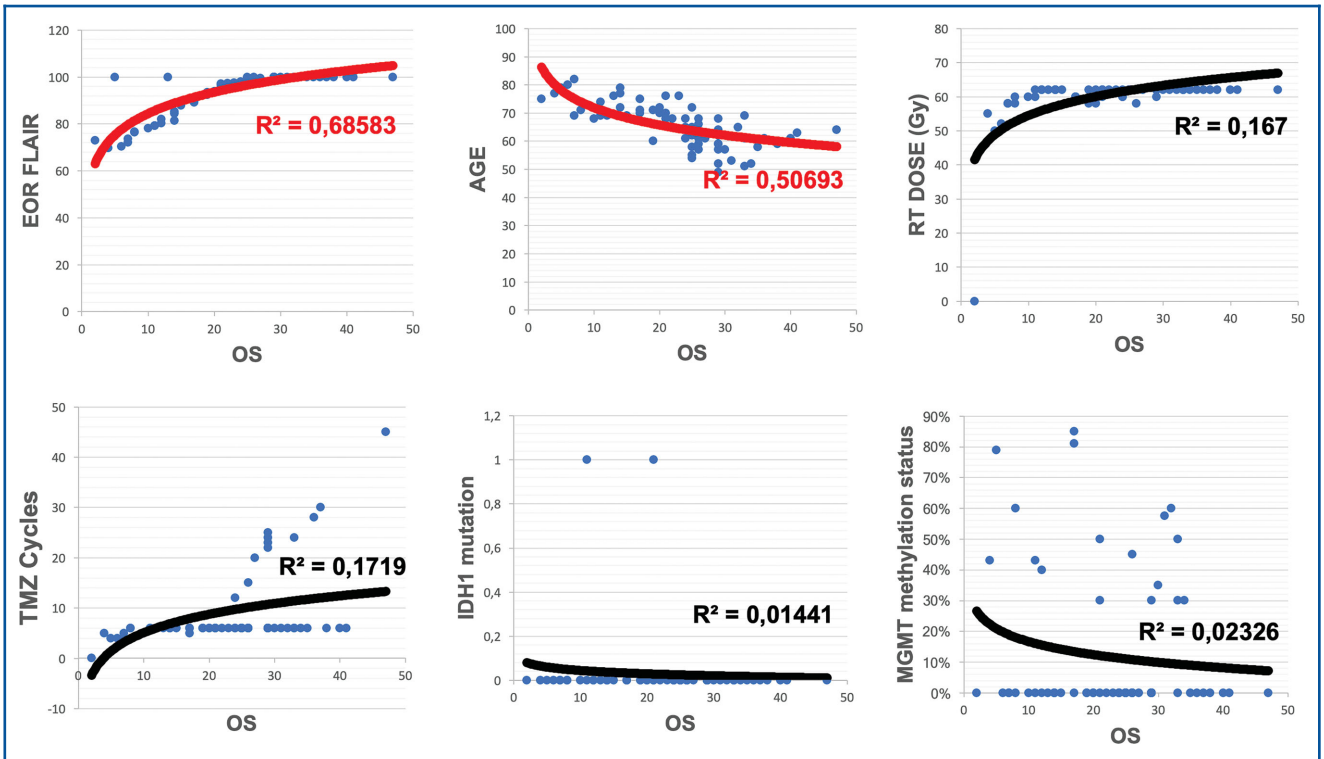


FIGURE 5. *Multiple regression analysis is summarized by scatter plots illustrating the different power of correlation between OS and different factors (EOR FLAIR resection, age, TMZ cycles, RT cycles, MGMT promoter methylation status, and presence of IDH1 mutation); strength of correlation is expressed by the R^2 value (a statistically significant correlation is indicated in red), which is higher in correlation between EOR measured in FLAIR and OS.*

the most infiltrating and aggressive brain tumors, should be considered as the manifestation of a complex process also involving the peritumoral areas.[16,22-24,27,28]

Resection of glioblastoma aims to safely remove all visible tumor while respecting neurological functions. However, strategies aiming to increase EOTR (ie, 5-ALA and intraoperative imaging tools)[19,21,29,30] have the potential risk to mistakenly facilitate resection of eloquent brain, particularly in peritumoral infiltrated areas.[31]

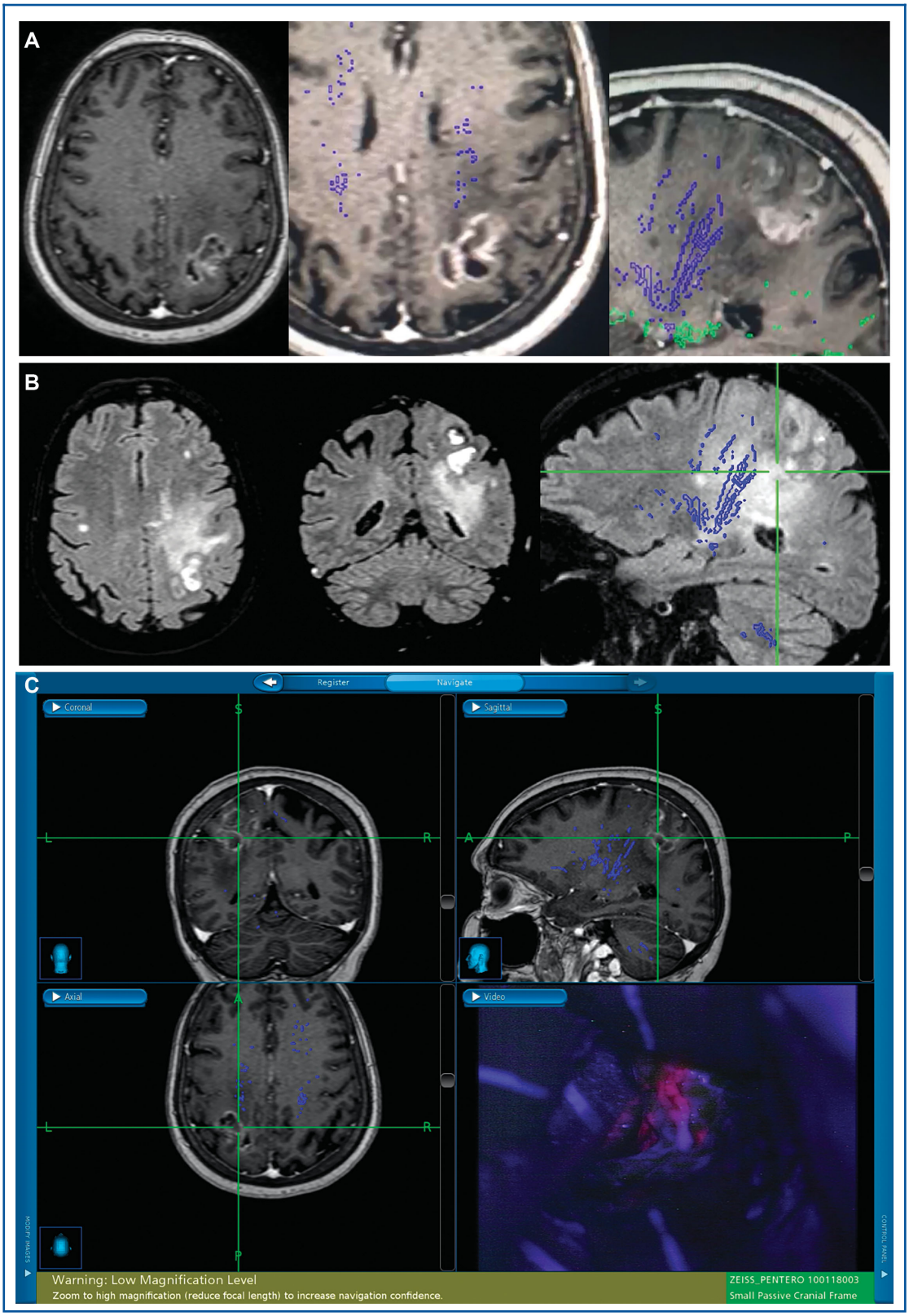
The role of 5-ALA in detecting infiltrating tumor areas has recently been investigated. In 2016, Lau et al[32] published a prospective phase II clinical trial, which correlated cellularity with fluorescence intensity in brain high grade gliomas (HGG). This study demonstrated a direct correlation between 5-ALA fluorescence intensity and rate of glioma cell infiltration. Interestingly, the authors also highlighted some limitations in the use of 5-ALA, which can generate false positives, particularly in peritumoral areas.[32]

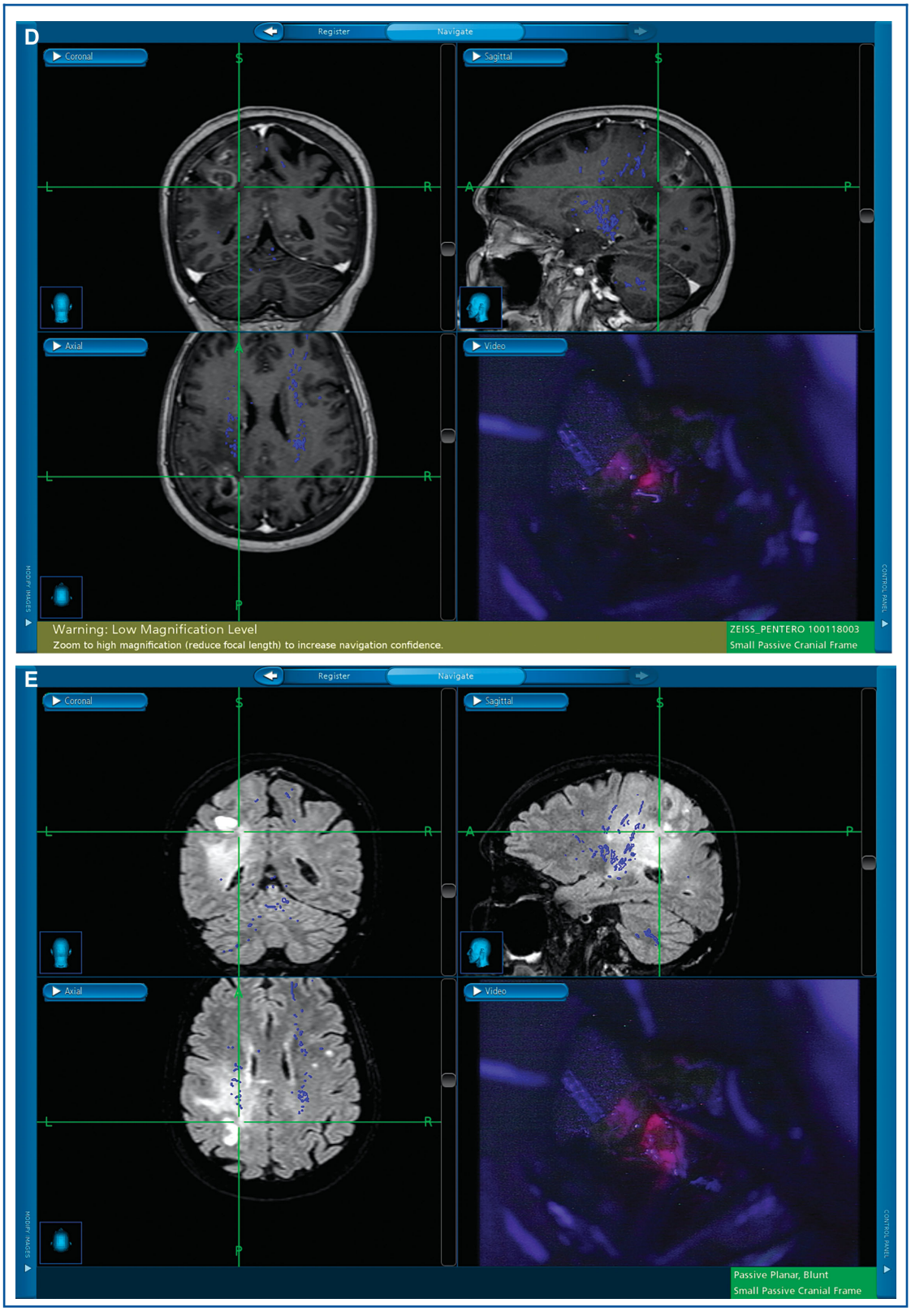
Evidence of the positive correlation between tumor resection pushed beyond the margins of enhancing tumor and survival parameters comes from recently published studies.[33] Eyüpoglu et al[33] reported the results of a series of 105 patients, 30 of them had undergone GBM supra-complete resection, based on 5-ALA vaguely positive areas surrounding the enhancing tumor. In 2015, Li et al[1] published the results of a retrospective analysis on a large cohort of patients who had undergone surgery for HGG. A subgroup of these patients had received a resection of ≥53.21% of the abnormal FLAIR area beyond the 100% T1-weighted, contrast-enhancing resection, obtaining a significant survival prolongation.[11]

Our study was designed to prospectively evaluate the impact of intended *FLAIRectomy* on patients' safety and clinical outcome. We hypothesized that pre-operative planning of FLAIR-based resection and navigation of FLAIR sequences, coupled with intraoperative neurophysiological monitoring, i-US and i-CT imaging, may be a more reliable approach to guarantee safe and accurate SMR (Figure 6).[34]

Peritumoral areas, not included in the enhancing area, are composed of tumor-reactive edema and low-density tumor-cell infiltration. The greatest problem is the inability of FLAIR sequences to clearly distinguish the latter component from the former.[14,15,27,28] Several studies have tried to solve this problem, improving details of MR acquisition techniques and their elaboration.

In our series, the FLAIR segmentation to distinguish between edema and tumor infiltration was verified analyzing MR signal differences detected by multiple ROIs placed in different peritumoral areas (Figure 1). This method has also been validated and





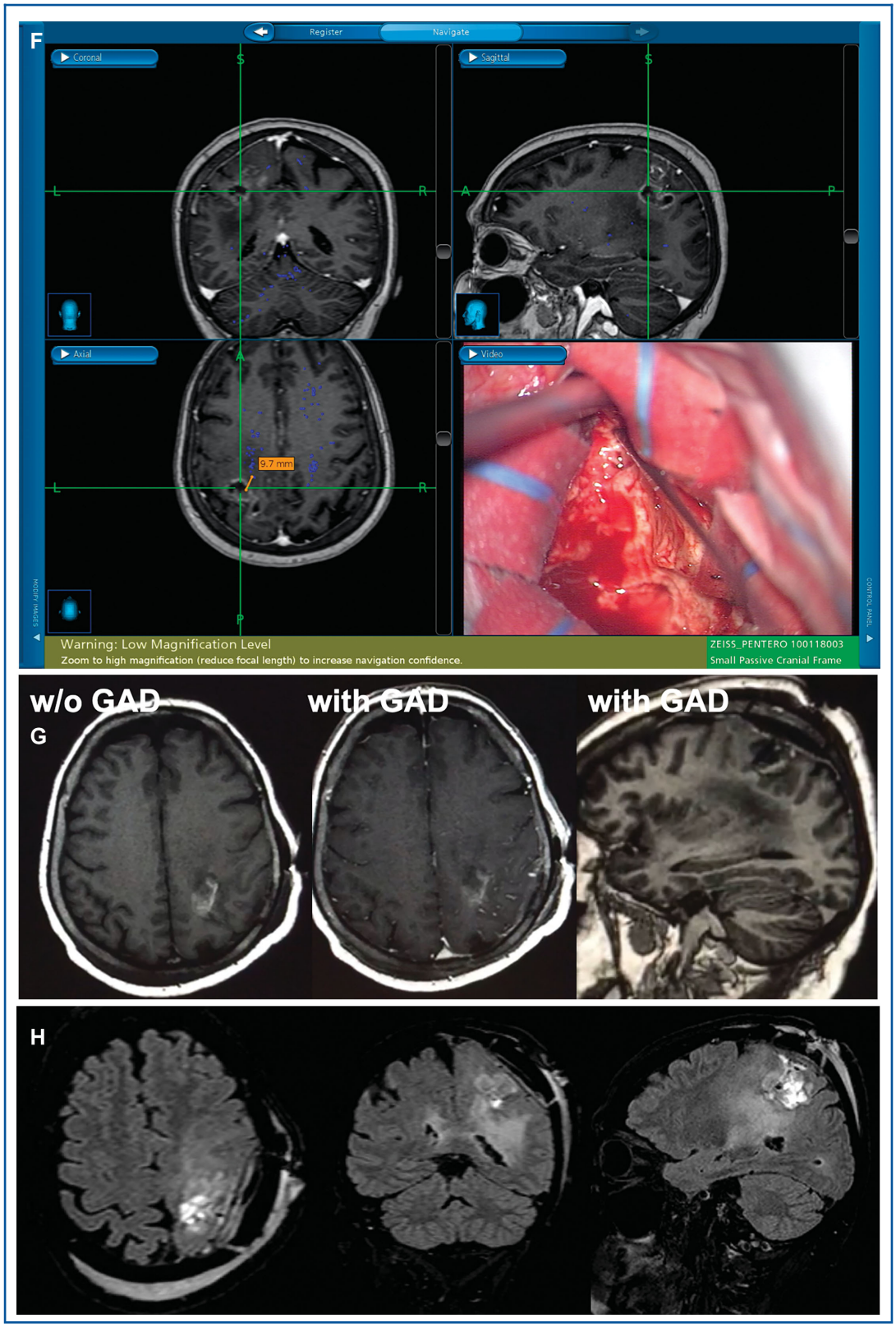


FIGURE 6. *Illustrative case. A 60-yr-old female presented with moderate right hemiparesis and seizures. Preoperative brain MR scans showed a multi-lobulated lesion involving precentral and postcentral gyri, with MR-spectroscopic features compatible with glioblastoma* **A**. *FLAIR sequences* **B**, *integrated with reconstruction of tractography demonstrated that the anterior portion of the hyperintense areas infiltrated the cortico-spinal tract. The patient was scheduled for awake craniotomy. After removing all intensely fluorescent tissue, corresponding to gadolinium-enhanced nodules* **C**, *SMR of FLAIR positive areas was started. All the posterior and lateral pathological hyperintense areas, showing vague fluorescence, were removed after verifying the absence of a positive functional response with subcortical direct stimulation* **D**. *The resection of the fluorescent anterior wall of the surgical cavity, corresponding to a FLAIR positive area beyond the Gadolinium-enhanced tumor margins and infiltrating the cortico-spinal tract, was preserved as subcortical stimulation documented its proximity to the eloquent subcortical pathway* **E**. *Interestingly, the estimated distance of cortico-spinal tract fibers calculated on the basis of stimulation parameters was the same as shown by the navigation system* **F**. *Postoperative MR performed 24 h after surgery* **G** *and* **H** *documented the complete resection of the Gadolinium-enhanced tumor (T1-Gad EOR: 100%) also showing the presence of the intentionally left small anterior residual (red arrows) nodule detected on FLAIR sequences (FLAIR EOR: 96.4%).*

extensively used in radiosurgery,[35] demonstrating good reliability in the definition of tumoral infiltration pathways.

The application of FLAIR-based volumetric evaluation, as well as the most accurate definition of FLAIR-positive tumor areas are likely to be associated with more accurate preoperative planning and postoperative evaluation of EOTR. As a consequence of using this method, we observed a high rate of FLAIR-based EOTR (92.43%), also related to the use of FLAIR navigated images during surgery, which allow an aware and safe SMR. Indeed, our results, though limited to a small patient cohort, demonstrate the presence of a direct and constant correlation between *FLAIRectomy* and survival. In particular, we found that a higher rate of FLAIR-based EOTR is associated with prolonged OS (Figures 3-5). This suggests a possible positive impact of both our treatment strategy and an intraoperative, multimodal protocol on clinical outcome.

Another interesting finding in our study is the analysis of residual tumor assessed on FLAIR images. As demonstrated by a recent study,[36] residual tumor is a negative survival predictor factor in glioblastoma and a residual tumor volume smaller than 2 cm^3 can guarantee a significant improvement in clinical outcome. We did not find a statistically significant correlation between residual tumor measured on T1 or FLAIR sequences and survival (Figure 3). However, statistical correlation was stronger for the FLAIR-based evaluation of residual tumor volume. This finding can be explained by the fact that a complete (ie, 100% EOTR) resection of the abnormal FLAIR area around the enhancing tumor is not achievable (because it is necessary to preserve neurological functions) in most cases where 100% of enhancing tumor resection can be performed.

Furthermore, these findings open the way to another discussion on the possibility to also use intraoperative imaging tools to update neuronavigation and perform *FLAIRectomy*, always under neuromonitoring guidance, even without 5-ALA fluorescence, should the latter not be available.

Limitations

This study has some limitations: the sample size is small and the follow-up of patients has not been completed yet, as 25/68 (36.7%) patients are alive and still under follow-up. MGMT methylation status and IDH-1 mutational status were retrospectively obtained and adjuvant therapies were not targeted according to biomolecular data.

CONCLUSION

There is mounting evidence that evaluation of glioblastoma EOTR based only on Gad-T1 MR is not accurate enough and does not correspond to the real tumor extension and its EOR. FLAIR MR sequences could be more accurate and reliable in identifying both the main tumor nodule and the surrounding tumor cell-infiltrated areas; however, if coupled with neuromonitoring and intraoperative imaging tools, they can allow surgeons to perform *FLAIRectomy* as effectively as under 5-ALA fluorescence guidance.

Given the above findings, future studies could also investigate the feasibility and reliability of neuromonitoring and intraoperative imaging tools-based *FLAIRectomy* without 5-ALA fluorescence.

Although our results on the correlation between FLAIR MR-based EOTR and survival data deserve further investigation in larger studies, they are promising and support our approach in the management of glioblastoma.

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