5-Aminolevulinic Acid and Contrast-Enhanced Ultrasound: The Combination of the Two Techniques to Optimize the Extent of Resection in Glioblastoma Surgery

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BACKGROUND: The survival benefit in maximizing resection in glioblastomas (GBMs) has been demonstrated by numerous studies. The true limit of infiltration of GBMs has been an overwhelming obstacle, and several technological advances have been introduced to improve the identification of residual tumors.

OBJECTIVE: To evaluate whether the integration of 5-aminolevulinic acid (5-ALA) with microbubble contrast-enhanced ultrasound (CEUS) improves residual tumor identification and has an impact on the extent of resection (EOR), overall survival (OS), and progression-free survival (PFS).

METHODS: A total of 230 GBM procedures were retrospectively studied. Cases were stratified according to the surgical procedure into 4 groups: 5-ALA- and CEUS-guided surgeries, 5-ALA-guided surgeries, CEUS-guided surgeries, and conventional microsurgical procedures.

RESULTS: Patients undergoing conventional microsurgical procedures showed the worst EORs compared to the assisted techniques (5-ALA and CEUS procedures). Both 5-ALA and CEUS techniques improved the EOR compared to conventional microsurgical procedures. However, their combination gave the best results in terms of the EOR ($P = .0003$). The median EOR% and the number of supramarginal resections are hence superior in the 5-ALA + CEUS + group compared to the others; this observation had consequences on PFS and OS in our series.

CONCLUSION: In terms of the EOR, the best results can be achieved through a combination of both techniques, where the 5-ALA-guided procedure is followed by a final survey with CEUS. Compared with other intraoperative imaging techniques, CEUS is a real-time, readily repeatable, safe, and inexpensive technique that provides valuable information to the surgeon before, during, and after resection.

KEY WORDS: 5-ALA, CEUS, fluorescence, High-Grade Glioma, resection, residuals, ultrasound

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The survival benefit of maximizing local control for patients with glioblastoma (GBM) has been demonstrated by numerous studies.[1-4]

Several studies have shown an association between the extent of resection (EOR) with progression-free survival (PFS) and overall survival (OS). The challenge in GBM surgery relies on the infiltrative nature of the disease and the related difficulty in defining the true limit of oncological infiltration.[5]

By virtue of the infiltrative nature of the disease, abnormal tumor cells can be found well beyond the margins of what is radiographically

ABBREVIATIONS: 5-ALA, 5-aminolevulinic acid; **CEUS,** contrast-enhanced ultrasound; **EOR,** extent of resection; **GBMs,** glioblastomas; **KM,** Kaplan-Meier; **KPS,** Karnofsky performance scale; **MRI,** magnetic resonance imaging; **OS,** overall survival; **PFS,** progression-free survival; **ROIs,** regions of interest

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evident as well as what is grossly abnormal to the unassisted eye. Several technological advances have been recently made to improve the identification of abnormal tissue at tumor margins and at the periphery of a gross resection.[1,6-8]

Enthusiasm has grown around 5-aminolevulinic acid (5-ALA), which has been popularized to define the limits of surgical resection for GBMs[9] 5-ALA has been shown in prospective randomized trials to significantly improve the EOR and OS of patients with GBM.[3,5,10]

Conversely, contrast-enhanced ultrasound (CEUS) has shown an effective and specific role in identifying residual tumors in GBM surgery, and several papers have shown several applications of this real-time, readily repeatable technique.[11-22]

Often, these tools are used simultaneously.[23]

The aim of the paper is to evaluate whether the combination of 5-ALA and CEUS in GBM surgery has an impact on the EOR and OS. Ultimately, the paper aims to identify which information is provided by the abovementioned techniques that favor residual tumor identification and to discuss strengths and limitations. The combination of the two techniques has not yet been investigated.

METHODS

Study Design

This was a single-institution retrospective study. All patients provided informed consent for the analysis of clinical data. After obtaining ethical committee approval, the study was conducted under the EQUATOR standards for reporting qualitative research.[24]

We reviewed the records of consecutive patients from the clinical database who underwent resection of a GBM between January 2015 and January 2018. Eligibility criteria were as follows: histopathologically verified glioblastoma multiforme (WHO grade IV) and follow-up > 12 mo. Needle biopsies, recurrent gliomas/second surgeries, and those who did not complete the postoperative Stupp protocol or underwent other treatments were excluded.

The patients were thus stratified according to the surgical procedure:

- 5-ALA- and CEUS-guided surgeries (5-ALA + CEUS+, group 0)
- 5-ALA-guided surgeries (5-ALA + CEUS-, group 1)
- CEUS-guided surgeries (5-ALA- CEUS+, group 2)
- Conventional microsurgical procedures (5-ALA- CEUS-, group 3)

Independently of the present study, patients were directed to a 5-ALA, CEUS, or conventional microsurgical procedure according to the surgeon's choice.

The primary outcomes were the EOR, PFS, and OS differences among the 4 groups.

Clinical Characteristics

Medical records were assessed for information on gender, age, neurological deficits, and Karnofsky performance scale (KPS) pre- and postoperatively and at FU and for pre- and postoperative gadolinium-enhanced tumor volume, survival, and PFS. Tumor localization, MGMT methylation analysis, and IDH1 mutation were also recorded in our database.

Eloquent areas were defined as the sensorimotor strip (precentral and postcentral gyri), the dominant hemisphere perisylvian language areas (superior temporal, inferior frontal, and inferior parietal areas), and the calcarine area; deep localizations were defined as all lesions reaching the basal ganglia/internal capsule, the thalamus or the corpus callosum and not surfacing to the cortex.[25,26]

All patients underwent postoperative magnetic resonance imaging (MRI) before discharge. If patients were lost to follow-up, the most recent clinical information was entered in the analysis.

TABLE 1. Baseline Characteristics of the Study Population

Parameters	Value (N and %, mean ± sd or median and range)
No. of patients	230
Sex	
Female	134 (58.26%)
Male	96 (41.74%)
Age	65 yr (20-85)
Tumor side	
Left	105 (45.65%)
Right	125 (54.35%)
Tumor site	
Precentral	96 (41.74%)
Retrocentral	70 (30.43%)
Temporal + Insular	64 (27.83%)
Preoperative Tumoral Volume computed on postcontrast T1-weighted images, cm3	31.15 (3-96.3)
Intraoperative protocol	
5ALA + CEUS +	39 (16.96%)
5ALA + CEUS -	33 (14.35%)
5ALA - CEUS +	28 (12.17%)
5ALA - CEUS -	130 (56.52%)
EOR %	94 (48-100)
EOR >= 100%	97 (42.17%)
99% <= EOR <= 90%	44 (19.12)
89% <= EOR <= 80%	52 (22.61%)
89% <= EOR <= 80%	37 (16.09%)
MGMT methylation *yes vs no*	153 vs 77 (66.52% vs 33.48%)
IDH1/2 mutation *yes vs no*	19 vs 211 (8.26% vs 91.74%)
Ki-67	20% (5-70)

Characteristics of the study population are described using means ± s.d. (standard deviation) or median and range for continuous variables, number of cases with relative percentages reported in parentheses for categorical variables.

Operative Setup

All surgeries were performed with an OPMI Pentero (Carl Zeiss, Oberkochen, Germany) or a Leica M720 OH5 (Leica Microsystems, Wetzlar, Germany) microscope. Electrophysiological monitoring (Nicolet Endeavor CR, Cardinal Health, Dublin, Ireland) and a neuronavigation system were used in all cases. A CUSA was used in most procedures, according to the surgeon's request.

5-ALA

5-ALA (Medac GmbH, Wedel, Germany) was administered 3 to 5 h prior to surgery at a dose of 20 mg/kg body weight p.o. Intraoperatively,

TABLE 2. Differences in Tumor Localization in the 4 Analyzed Groups

	Group 0 5-ALA +; CEUS + [39 cases]	Group 15-ALA +; CEUS -[33 cases]	Group 2 5-ALA -; CEUS + [28 cases]	Group 3 5-ALA -; CEUS -[130 cases]	*P*
Eloquent/non-eloquent	7/32	5/28	5/23	20/110	>.05
Deep/superficial	9/30	6/27	6/22	22/108	>.05

Eloquent areas were defined as the sensorimotor strip (precentral and postcentral gyri), dominant hemisphere perisylvian language areas (superior temporal, inferior frontal, and inferior parietal areas), calcarine area; *deep localisations* were defined as all lesions reaching basal ganglia/internal capsule, the thalamus or corpus callosum.

TABLE 3. Intraoperative Protocol and Median EOR % Value

Intraoperative Protocol	Median EOR% value	Range
5ALA + CEUS +	100%	56%-100%
5ALA + CEUS -	94%	70%-100%
5ALA - CEUS +	96%	58%-100%
5ALA - CEUS -	90%	48%-100%

Statistically significant difference among the 4 groups (Kruscan Wallis test, $P = .0003$)

regions of interest (ROIs) were defined under violet-blue illumination (Blue 400 filters).

CEUS

The surgical field was examined with ultrasound (MyLab™Twice, Esaote, Genoa, Italy, a linear-array multifrequency 3-11 MHz device) through the craniotomic window, first using B-mode. The probe was covered in a sterile plastic sheet with sterile ultrasound coupling gel.

A sulfur-hexafluoride-filled lipidic microbubble agent as ecographic contrast (SonoVue®, Bracco, Milan, Italy) was used. The protocol was based on an injection, a 2.4-mL (5 mg/mL) bolus, in addition to a subsequent 10-ml flush of saline. Tumor features are real-time dynamically shown, generally 25 to 30 s after injection, using a specific image algorithm based on contrast.

The contrast-tuned algorithm automatically decreases the ultrasound mechanical index, and only the specific echo signal from the microbubble resonance is displayed. The CEUS technique was performed according to the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) guidelines.[27,28]

The scan was repeated in the same way after the 5-ALA resection was completed. An area suspicious for residual tumor was defined as an enhancing parenchymal nodule in the surgical cavity adjacencies, with a more rapid and persistent enhancement compared to the surrounding brain tissue.

Our protocol included multiple B-mode and CEUS scans prior to, during, and after resection.

Tumor Volumetry and the EOR

Imaging data available from the preoperative imaging were retrospectively analyzed. Postsurgical MRI was performed before home discharge. Volumetric analyses of pre- and postsurgical MR images were performed by an experienced investigator based on freehand-drawn ROIs with the semiautomatic system. The volumes of the resection cavities resulting from surgery were assessed in the same way. Neuroradiologists were blinded to the different groups.

A residual tumor was defined as contrast-enhanced tissue on 3D-T1-weighted sequences. Tumor volumes were calculated with a dedicated workstation (Advantage Windows, GE Healthcare).

The EOR was then acquired but as a consecutive variable expressed as a percentage of resection and stratified categorically into 4 classes:

- Total or supramarginal (≥100%)
- Near-total (90-99%)
- Subtotal (80-89%)
- Other EORs (<80%).

Statistical Analysis

Categorical variables were reported as percentages; continuous variables were reported as mean ± standard deviation or median and range as appropriate, according to the data distribution. Normality of the continuous variables was tested using the Shapiro-Wilk test. Normal distribution was tested using the Shapiro-Wilk test. Continuous variables between groups were compared, as appropriate with Student's t or Mann–Whitney *U* test. Categorical variables were compared with chi-square test or Fisher's exact test, as appropriate.

The OS was defined from surgery until patient death; PFS time was defined as extending from surgery until the demonstration of gadolinium enhancement on follow-up imaging. OS and PFS were estimated using the Kaplan-Meier (KM) approach. The association between variables and survival distribution were tested with univariate and multivariate analysis with Cox proportional hazard models. Patients with unknown survival were censored to the last scan date.

Patients with unknown survival were censored as of their last scan date.

In univariate analysis, variables considered as possible prognostic factors were age, sex, KPS score, preoperative tumor volume, tumor location, tumor side, intraoperative protocol used, and the EOR.

The EOR was modeled both as a continuous and an ordinal variable (Total or Supramarginal [≥100%], Near-Total [90-99%], Subtotal [80-89%], and Other EORs [<80%]) in univariate analysis to ensure consistency with previous studies that focused on the impact of glioma resection in terms of volumes.

The multivariate analysis included all variables determined to exhibit a significance level of $P = .05$ via the conducted univariate analysis.

To analyze the impact of each intraoperative protocol used on the EOR, the nonparametric Kruskal-Wallis test was used.

Retention in the stepwise model required the variable to be significant at a level of $P = .05$ in a multivariate analysis.[29]

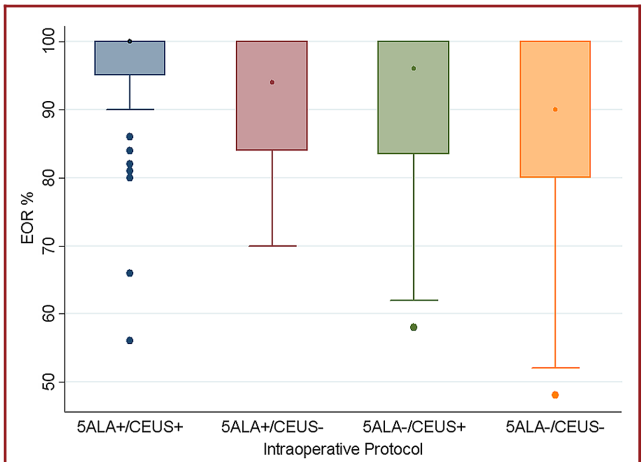

FIGURE 1. *Boxplot representation displaying the distribution of the EOR% in the 4 groups (5-ALA + CEUS+, group 0; 5-ALA + CEUS-, group 1; 5-ALA- CEUS+, group 2; 5-ALA- CEUS- group 3).*

All analyses were conducted using Stata/SE software (version 14.0 StataCorp), and data were presented as HRs and 95% CIs. A $P < .05$ was considered to indicate (2-tailed) statistical significance.

RESULTS

In the analyzed period of time, 230 patients (96 males, 134 females, mean age 65 yr) who met the inclusion criteria were analyzed in the study. The baseline characteristics of the studied population are summarized in Table 1.

The intraoperative protocol was as follows:

- 5-ALA- and CEUS-guided surgeries (5ALA + CEUS+, group 0): 39 patients (16.9%)
- 5-ALA-guided surgeries (5ALA + CEUS-, group 1): 33 patients (14.3%)
- CEUS-guided surgeries (5ALA- CEUS+, group 2): 28 patients (12.2%)
- Conventional microsurgical procedures (5ALA- CEUS-, group 3): 130 patients (56.5%).

The 4 groups appeared to be homogeneous in terms of tumor localization (eloquent areas and deep localizations, Table 2).

EOR and Surgical Protocol

The median EOR (regardless of surgical protocol) was 94% (48-100), distributed as follows (Table 1): Total or Supramarginal (≥100%), 97 patients (42,2%); Near-total (90-99%), 44 patients (19,1%); subtotal (80-89%), 52 patients (22,6%); and Other EORs, 37 patients (16,1%).

The EOR % median value in the 4 groups is summarized in Table 3 and showed a statistically significant difference among the 4 groups (Kruskal-Wallis test, $P = .0003$). Patients undergoing conventional microsurgical procedures were those who displayed the worst EORs, while patients undergoing assisted techniques (5-ALA- and CEUS-assisted procedures) displayed increased median EOR% values.

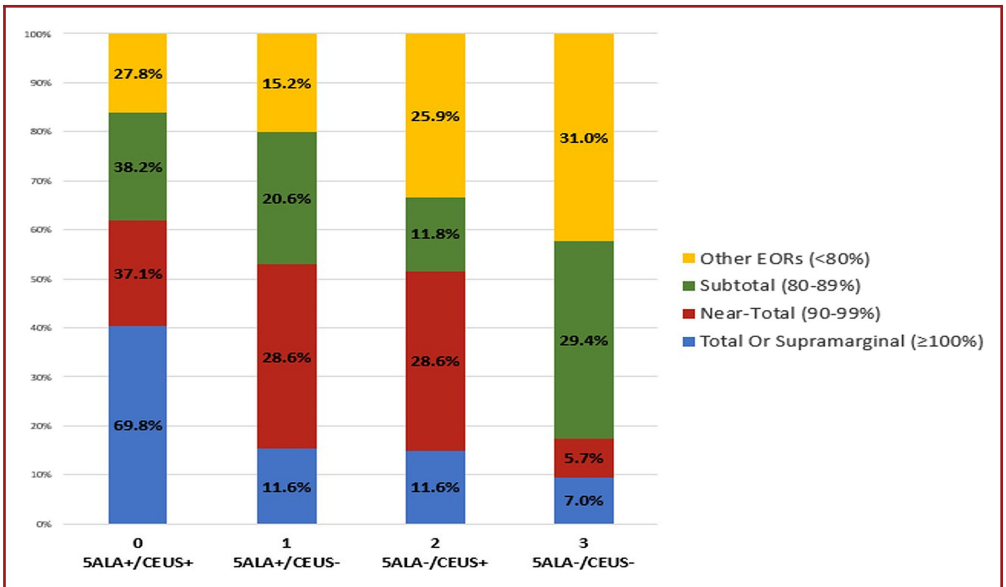

FIGURE 2. *Bar chart representing the EOR as a categorical variable (total or supramarginal [≥100%], near-total [90-99%], subtotal [80-89%], other EORs [<80%]) in the 4 surgical protocols (5-ALA + CEUS+, group 0; 5-ALA + CEUS-, group 1; 5-ALA- CEUS+, group 2; 5-ALA- CEUS-, group 3).*

TABLE 4. OS and PFS in the General Population and According to Surgical Protocol

	1 yr estimated OS	2 yr estimated OS
General OS	60.59%	24.96%
General PFS	37.10%	16.34%
OS 5ALA + CEUS +	76.84%	44.53%
OS 5ALA + CEUS -	71.62%	55.42%
OS 5ALA - CEUS +	63.91%	19.31%
OS 5ALA - CEUS -	52.18%	18.04%
	1 yr estimated PFS	**2 yr estimated PFS**
PFS 5ALA + CEUS +	53.85%	36.94%
PFS 5ALA + CEUS -	39.88%	26.58%
PFS 5ALA - CEUS +	28.57%	21.48%
PFS 5ALA - CEUS -	33.08%	12.06%

Figure 1 shows boxplot representations that stratify the distribution of the EOR% according to the surgical protocol. The combination of 5-ALA and CEUS displayed a higher rate of supramarginal resections than 5-ALA alone and CEUS alone. These differences are significant among the 4 groups. Additionally, if analyzed categorically, the EOR distribution varied significantly among the 4 groups, confirming the shift towards supramarginal and near-total resections in the 5-ALA + CEUS + group (Figure 2).

OS, PFS, and Surgical Protocol

The general OS in the whole population was 60.6% at 1 yr and 24.7% at 2 yr. General PFS was 37.1% at 1 yr and 16.3% at 2 yr.

Data on OS and PFS in the general population (230cases) and stratified according to surgical protocol are summarized in Table 4.

The results of multivariate analysis (Table 5) show that OS is independently associated with age ($P = .001$), EOR ($P = .001$), surgical protocol (5ALA + CEUS + $P = .015$ and 5ALA + CEUS– $P = .012$), and MGMT methylation ($P = .04$), while no correlation was observed with other observed variables such as sex, tumor size and site, IDH-1 status, and Ki67%.

TABLE 5. Predictors of OS, Univariate and Multivariate Analyses

	Univariate analysis			Multivariate analysis		
Variable	**Odds ratio**	**95% CI**	***P* value**	**Odds ratio**	**95% CI**	***P* value**
Age (yr)	1.029	1.011-1.047	**.001**	1.035	1.017-1.052	**.000**
Sex						
Male	1					
Famale	0.837	0.596-1.176	.306			
Tumor side						
left	1					
right	1.077	0.222-2.578	.848			
Tumor site						
Precentral	1					
Retrocentral	1.064	0.717-1.579	.759			
Temporal and Insular	1.223	0.817-1.832	.328			
Preoperative Tumoral Volume computed on postcontrast T1-weighted images, cm^3	0.997	0.989-1.006	.543			
Intraoperative protocol						
ALA -/CEUS -	1					
ALA +/CEUS +	0.518	0.305-0.881	**.015**			
ALA +/CEUS -	0.447	0.239-0.835	**.012**			
ALA -/CEUS +	0.722	0.431-1.210	.217			
% EOR *Continuous variable*	0.953	0.941-0.965	**.000**	0.946	0.934-0.959	**.000**
% EOR *Categorical variable*						
EOR = 100%	1					
99% < = EOR < = 90%	1.556	0.969-2.497	.067			
89% < = EOR < = 80%	2.325	1.485-3.640	**.000**			
EOR < = 79%	4.999	3.152-7.925	**.000**			
MGMT methylation *yes vs no*	0.693	0.488-0.983	**.040**	0.823	0.575- 1.176	.285
IDH 1-2 mutation *yes vs no*	0.872	0.521-1.459	.602			
Ki-67	0.999	0.987-1.010	.822			

Boldfacing represent statistical significant results ($P < .05$).

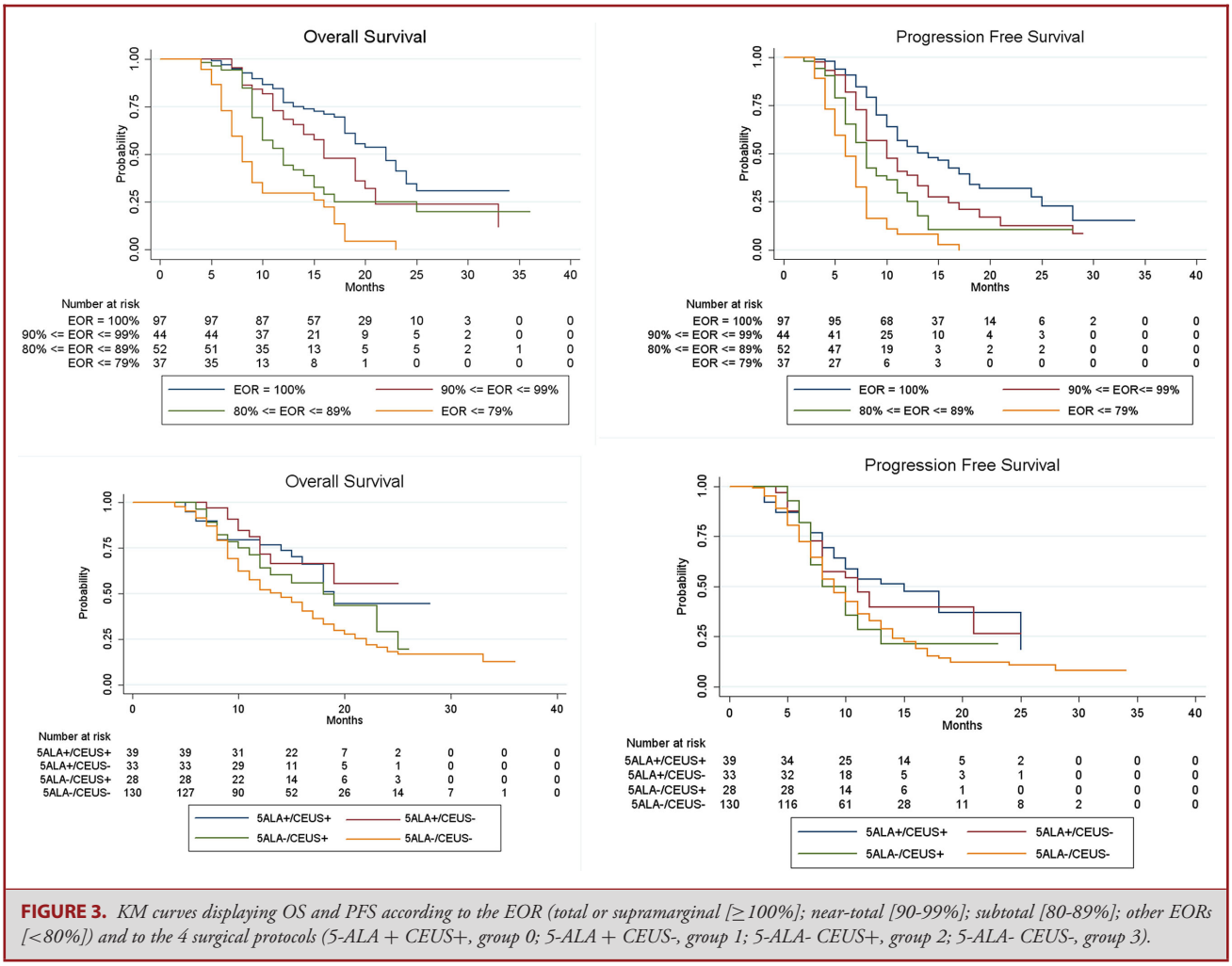

FIGURE 3. *KM curves displaying OS and PFS according to the EOR (total or supramarginal [≥100%]; near-total [90-99%]; subtotal [80-89%]; other EORs [<80%]) and to the 4 surgical protocols (5-ALA + CEUS+, group 0; 5-ALA + CEUS-, group 1; 5-ALA- CEUS+, group 2; 5-ALA- CEUS-, group 3).*

Our data confirm that EOR significantly affects OS and PFS in patients with GBM, as represented in the KM curves of Figure 3. Conversely, as shown in Figure 3, KM OS is affected by the surgical protocol; the protocols that displayed the highest EORs, namely the 5ALA + CEUS + and 5ALA + CEUS– groups, distinctly showed the best survivals, and the EORs were very similar between these two groups.

Similarly, PFS is shown to be significantly higher in patients in the 5ALA + CEUS + and 5ALA + CEUS– groups. The integration of the two methods shows the best survival curves, as displayed in the KM curves of Figure 3. The results of multivariate analysis on PFS (Table 6) show that PFS is independently associated with the EOR ($P = .0001$), surgical protocol (5ALA + CEUS + $P = .011$), and MGMT methylation ($P = .028$), while no correlation was observed with other observed variables such as age, sex, tumor size and site, IDH-1 status, and Ki67%.

DISCUSSION

Significance of Supramarginal Resection in GBM Surgery

Over the past decade, evidence has demonstrated that the EOR in glioma surgery translates to improvements in OS and PFS: an extended resection above the anatomical limits of the enhanced area after complete microsurgical resection is currently established as a paramount determinant in terms of both OS and of PFS.[1,30-34] Hence, supramarginal resection has to be considered the goal in GBM resection and pursued whenever possible with respect to functional integrity.[1,2,5,13,35]

Our results confirm the general observations that the EOR is the strongest independent predictor for OS and PFS in GBM; our data corroborate that patients undergoing supramarginal or near-total resections display significant survival benefit in terms of both OS and PFS (Figure 3).

TABLE 6. Predictors of PFS, Univariate, and Multivariate Analyses

	Univariate analysis			Multivariate analysis		
Variable	**Odds ratio**	**95% CI**	***P* value**	**Odds ratio**	**95% CI**	***P* value**
Age (yr)	1.011	0.997-1.026	.133	1.012	0.998- 1.027	.093
Sex						
Male	1					
Famale	0.791	0.584-1.072	.130			
Tumor side						
left	1					
right	1.223	0.625-2.432	.861			
Tumor site						
Precentral	1					
Retrocentral	1.134	0.799-1.608	.481			
Temporal and Insular	1.025	0.716-1.468	.893			
Preoperative Tumoral Volume computed on postcontrast T1-weighted images, cm^3	1.001	0.994-1.008	.852			
Intraoperative Protocol						
ALA -/CEUS -	1					
ALA +/CEUS +	0.558	0.356-0.874	**.011**			
ALA +/CEUS -	0.693	0.430-1.116	.132			
ALA -/CEUS +	0.931	0.589-1.472	.790			
% EOR *Continuous variable*	0.954	0.944-0.965	**.000**	0.955	0.944- 0.966	**.000**
% EOR *Categorical variable*						
EOR = 100%	1					
99% < = EOR < = 90%	1.566	1.039-2.363	**.032**			
89% < = EOR < = 80%	2.266	1.567-3.363	**.000**			
EOR < = 79%	4.428	2.905-6.750	**.000**			
MGMT methylation *yes vs no*	0.706	0.517-0.963	**.028**	0.858	0.623- 1.181	.347
IDH 1-2 mutation *yes vs no*	0.872	0.521-1.459	.602			
Ki-67	0.999	0.987-1.01o	.822			

Boldfacing represent statistical significant results ($P < .05$).

5-ALA and CEUS: 'Enhancing Vision' to Improve Radicality

The drive to reduce unexpected residual tumors has led to the recent introduction of several surgical tools designed to 'enhance vision' to improve radicality.[36-41]

Since its introduction in 2007 in Europe and in 2017 in the United States, several qualitative analyses have pointed out a benefit of 5-ALA for improving the resection in GBMs This investigation highlighted the undoubtable role of 5-ALA in optimizing tumor resection in patients with GBMs by allowing a real-time accurate visualization of the residual tumor during surgery.[1,3,5,30,42-44]

Analogously, the ongoing rediscovery of ultrasonography in neuro-oncological surgery is promising with the introduction of CEUS, which embodies one of the most recent innovations in the field of GBM intraoperative imaging. CEUS has been demonstrated to be valuable in guiding resection, allowing it to highlight residual tumor tissue with great accuracy and overcoming the difficulties of ultrasound interpretation caused by artifacts, edema, and surgical manipulation.[11,13,15,16] The potential applications of this technique are currently growing in neurosurgery, including brain and spinal cord oncology and vascular purposes.[14,17-21,45]

This technique has often been used in combination with 5-ALA in GBM surgery at our institution for several years.

On the heels of this experience, we reported the integration of 5-ALA and CEUS to further enhance the chances of supramarginal resection in GBM. The integration of these techniques has not yet been investigated.

This study shows that both 5-ALA and CEUS techniques improve the EOR with respect to standard microsurgical procedures (Table 3, Figure 1, and Figure 2). Specifically, the data show that 5-ALA is superior to CEUS in improving the EOR. However, it is their combination that gives the best results in terms of the EOR. Median EOR% and the number of supramarginal resections are hence superior in the 5-ALA + CEUS + group compared to the 5-ALA + CEUS- group. In other words, 5-ALA is the pillar on which the extension of resection is mainly built,

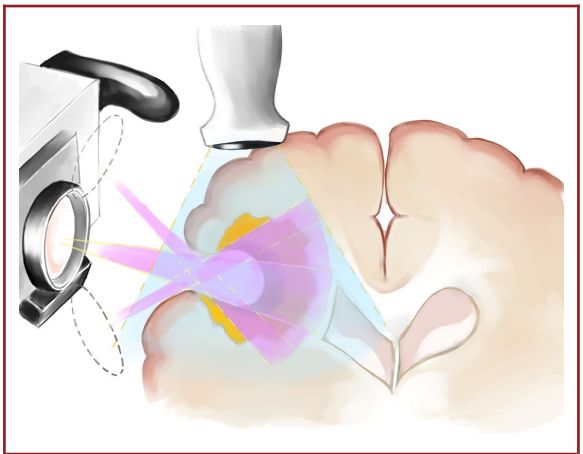

FIGURE 4. *Artistic representation of the integration of the two techniques. The final survey with CEUS possibly reveals inadvertent residual tumors in the 5-ALA-guided procedure: In deep fields or in conditions of nonorthogonal working corridors, microscope light might fail to thoroughly illuminate the surgical field, resulting in blind corners. Similarly, distant nonexposed nodules and the presence of blood/hemostasis can impair 5-ALA fluorescence. The 2 techniques are well integrated because they investigate the surgical field in two different perspectives: 5-ALA sees what is directly illuminated by the microscope light, whereas ultrasound and CEUS sees through unexposed, hidden, or parenchymal tissue.*

while CEUS provides a final survey of the surgical field, further increasing the possibility of detecting unexpected residual tumors.

Although our data did not show a robust difference in terms of PFS and OS between the 5-ALA + CEUS + and 5-ALA + CEUS- groups, probably in relation to the small size of the examined population, the PFS and OS of these two groups were superior to those of the other 2 groups (5-ALA- CEUS + and 5-ALA- CEUS-).

The increased gross total resection rate for the combination of 5-ALA and CEUS is notable, and based on our observations, it is possible to assume that the integration of the two techniques improves the chances of a supramarginal resection, and this has repercussions on survival and recurrence. The intraoperative protocol that combines the two techniques is, hence, a feasible support to the surgeon to improve radicality in GBM surgery.

5-ALA and CEUS: 2 Different Perspectives of the Surgical Field

The 2 techniques integrate well because "conceptually" they are different: one sees what is directly illuminated by the microscope light, and the other sees through unexposed, hidden, parenchymal tissue. In addition, these techniques observe two different phenomena: 5-ALA represents a means of making viable tumor cells directly visible to the surgeon's eye through cellular porphyrin metabolism,[30] while CEUS uptake is basically an expression of tumoral augmented vascularization and blood-brain barrier disruption.[16]

It is as if the 2 techniques offer 2 thoroughly different perspectives of the same surgical field, hence increasing the chance of identifying neoplastic residual tumor tissue.

Tumors that are covered by blood, cottonoid, or overlapping normal brain tissue will not illuminate under blue-light conditions and can be missed, resulting in incomplete tumor resections also in the 5-ALA-guided procedures.[36,46] Especially in deep fields or in conditions of nonorthogonal working corridors, microscope light might fail to thoroughly illuminate the surgical field, resulting in blind corners where the *gutter effect* might conceal residual tumor tissue. Similarly, distant nonexposed nodules covered by a layer of normal tissue could also be missed under 5-ALA fluorescence.[46,47]

Analogously, hemostasis is a crucial point in 5-ALA surgery, as exceedingly difficult hemostasis or clots might occult neoplastic

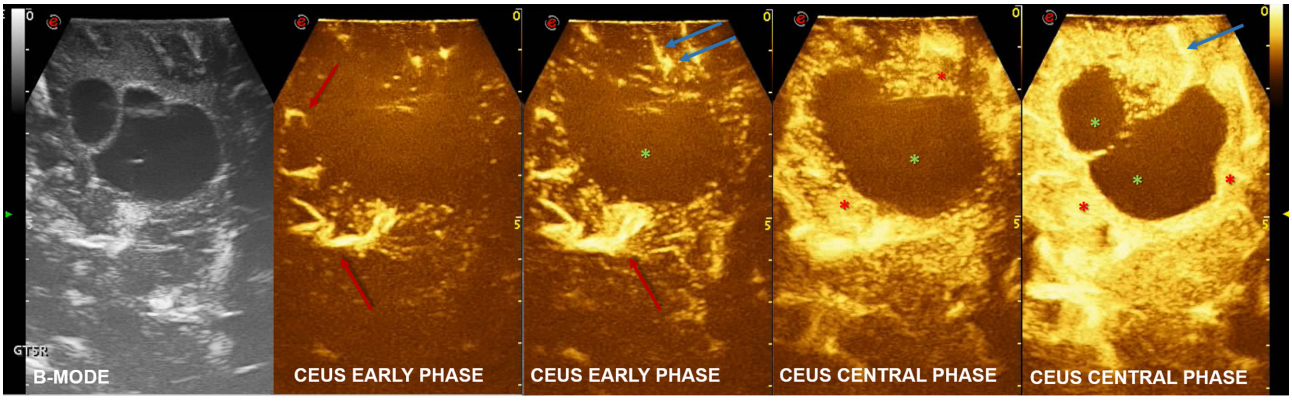

FIGURE 5. *CEUS frame line of a grade 4 high-grade glioma. The microbubble contrast medium allows the visualization of the feeding arteries (red arrow) in the early phase, while in the central arterial phase, the tumor parenchyma (red asterisk) is well visualized with its necrotic nonenhanced component (green asterisk), followed by the initial venous phase with the major draining vessels (blue arrow) of the lesion.*

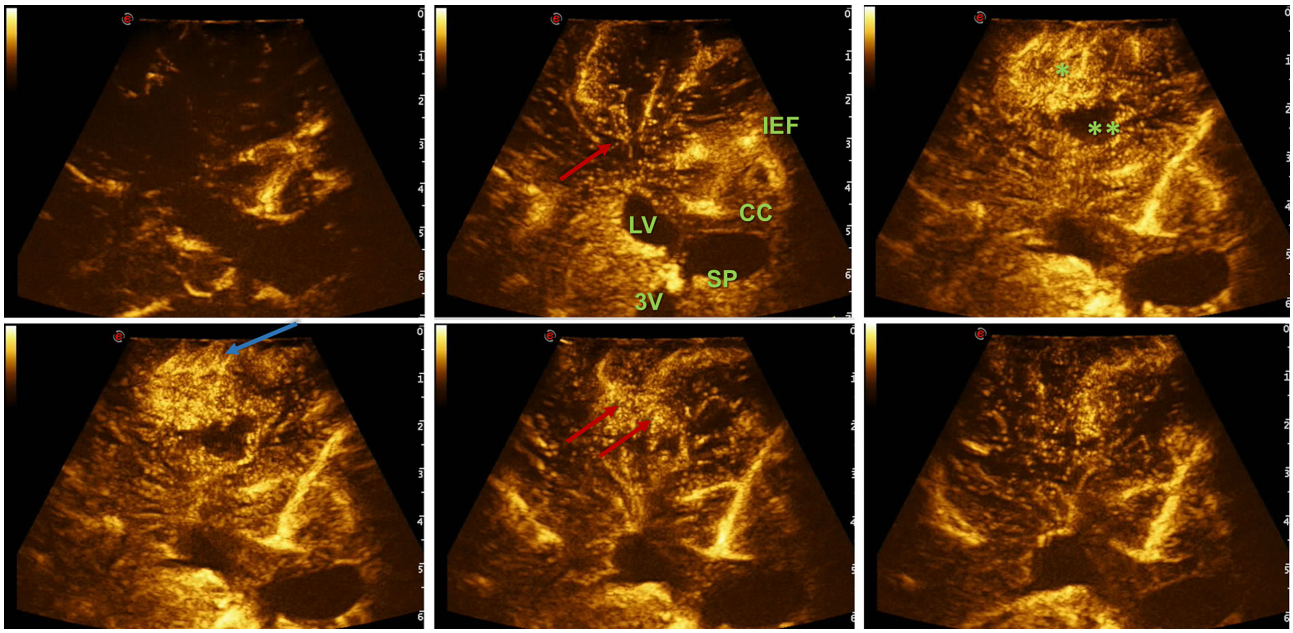

FIGURE 6. *CEUS frame line of a right frontal glioblastoma. The microbubble contrast medium also allows the visualization of the surrounding brain anatomy at a distance, well depicting the cisternal and liquoral spaces and highlighting fissures and sulci. The brain parenchyma appears slightly enhanced, while liquoral spaces are not enhanced. Tumoral feeding vessels (red arrows) are evident in the early CEUS phases, while in the central arterial phase, the tumor parenchyma (single asterisk) is well visualized with its necrotic nonenhanced component (double asterisk), followed by the initial venous phase with the major draining vessels (blue arrow) of the lesion. Lesional enhancement also persists in the later CEUS phases (red arrows). IEF: interhemispheric fissure, CC: corpus callosum, LV: lateral ventricle, SP: septum pellucidum, 3V: third ventricle.*

tissue under blue light. Photobleaching, which results in an impairment of the fluorescence signal that might occur after prolonged work under normal microscopic light, can reduce 5-ALA sensitivity.[46,47]

From this point of view, the final survey with CEUS possibly overcomes the abovementioned limitations, possibly revealing inadvertent residual tumors (Figure 4).

Intraoperative Imaging as Support to 5-ALA Surgery

Comparative studies have demonstrated high supramarginal resection rates when 5-ALA-guided resection is integrated with CT or MRI intraoperative imaging.[47-49]

Despite the undisputed value of these techniques, CT scan and MRI undeniably have several limitations, including their costs and the facts that surgical procedures must temporarily be stopped to conduct them and they are time-consuming. Intraoperative CT and MRI are time-demanding and hardly repeatable during surgery.

Conversely, CEUS is readily repeatable, dynamic, inexpensive, and provides a real-time dynamic visualization of tumor characteristics and vascular patterns. Assessment is rapid and can be performed at any time during surgery.

In glioma surgery, CEUS is more capable of highlighting the lesions and defining their margins than standard B-mode ultrasound. CEUS displays an important contrast enhancement in proliferating areas and can also be helpful in differentiating between tumor and edematous brain tissue[13,16] (Figures 5-6).

CEUS has been shown to be an important intraoperative toll in detecting the residual tumor. It can be helpful in the management of a potential residual tumor unless the tumor has not been devascularized in the early surgical phase[13] (Figures 7-8).

Our observations underline that this is true also in a 5-ALA-guided setting; we believe that CEUS might prove useful, particularly as a support to other complementary intraoperative techniques such as fluorescence imaging.

In addition, CEUS provides other valuable information to guide surgeons during resection. Our experience, along with other experiences in the literature, confirms CEUS's ability to show rapid and dynamic events such as the arterial and venous phases, intraoperatively defining the intrinsic vascular characteristics of the lesion[11,15,19-21] (Figure 8).

This information orients surgical strategy to a selective vascular deafferentation and tumor excision, maximizing resection and avoiding neurological vascular sequelae.

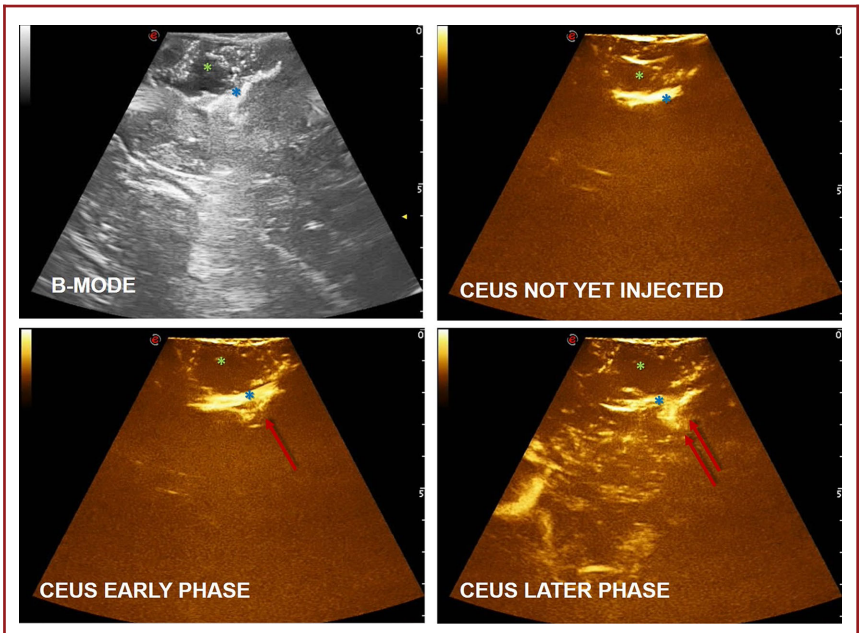

FIGURE 7. *The B-mode and CEUS scans depicting an inadvertent residual tumor. The surgical cavity (green asterisk) is well evident in B-mode and under the CEUS algorithm before microbubble injection. Hyperechoic hemostatic material can also be observed at the bottom of the cavity (blue asterisk). Under the hemostatic material, an enhanced nodule was observed at the early phase after CEUS microbubble injection (red arrows); enhancement persisted in the later phases after injection.*

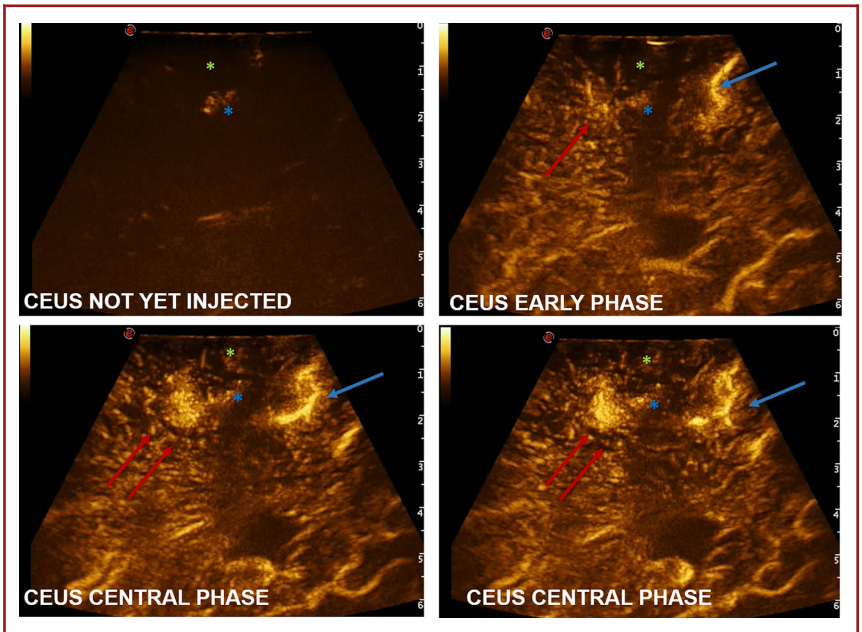

FIGURE 8. *CEUS identification of a residual tumor. The surgical cavity is well evident when switching to the CEUS algorithm before microbubble injection (green asterisk). Hyperechoic hemostatic material can also be observed at the bottom of the cavity (blue asterisk). After CEUS injection, cerebral sulci become evident (blue arrow). An enhancing nodule can be observed at the central CEUS phases after microbubble injection (red arrows).*

Limitations

The limitation of the CEUS technique, similar to traditional ultrasounds, is that the procedure is operator dependent and the scanning visualizes only a portion of the lesion at a time. Neurosurgeons are often not familiar with ultrasound image interpretation, and their application and correct setting requires a learning curve. A contrast-specific algorithm, present only in high-end ultrasound equipment, is mandatory to obtain the required CEUS imaging.

A number of factors can limit CEUS imaging interpretation, including tumor devascularization, along with the presence of artifacts due to hemostasis or blood. The standardization of the method with rigorous surgical field exploration, precise preparation and injection of the microbubbles, and accurate setting of the echograph (frequency and power of echoes) are mandatory for a correct interpretation of the images.

Other drawbacks involve the design of the study: the retrospective setting represents the major limitation of the paper. Hence, there are surely differences due to different surgeons' experience and different tumor localizations. In addition, as the study includes patients since 2015, it is not possible to retrieve those patients in whom a subtotal removal was planned a priori. These limitations should be taken into account when evaluating the study data. Future prospective studies could overcome these drawbacks.

CONCLUSION

This study demonstrated that the intraoperative combination of 5-ALA and CEUS allows to optimize the EOR in GBM surgery. The combination of 5-ALA and CEUS provides an improved real-time estimation of residual tumor volume compared to standard surgery, allowing the improvement resection. Compared with other intraoperative imaging techniques, CEUS is a real-time, readily repeatable, safe, and inexpensive technique that provides a series of valuable information to the surgeon, without a significant deviation from standard operative workflow.

Disclosures

The other authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

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