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'Dark Corridors' in 5-ALA resection of High Grade Gliomas: combining fluorescence guided surgery and contrast enhanced ultrasonography to better explore the surgical field

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ABSTRACT

BACKGROUND: To increase the extent of resection (EOR) is considered a main goal in High Grade Glioma (HGG) surgery. Significant advancements have been recently made to assist surgery: namely the use of 5-aminolevulinic acid (5ALA) and the application of contrast-enhanced ultrasound (CEUS) embody two of the most recently introduced tools in the neuro-oncology field. A combined approach including the two techniques has been suggested in literature.

Our primary aim is to identify in which conditions CEUS final survey has a real impact in a 5-ALA guided context and assess which preoperative tumour characteristics, with specific attention to working corridors can predict strains of the fluorescence guided procedure and hence recommend the use of the combined technique.

METHODS: 49 HGG glioma surgeries were performed at our institution with the abovementioned protocol between January 2016 and June 2016. Based on pre-operative MRI, we stratified glioma characteristics according to 3 determinants: localization (deep vs superficial), size (<3.5cm vs >3.5cm) and shape (regular vs irregular).

RESULTS: CEUS modified 5-ALA guided resection in 11 cases (22.45%): this appeared to be associated with statistically significance to deep tumour localization (p=0,04) and irregular/multi-lobulated margins (p=0,003). On the other hand, tumour size alone did not appear as a statistically significant determinant.

CONCLUSION: When dark corridors are presents or when overlying brain parenchyma hinders illumination, drawbacks to the 5-ALA assistance can be expected, hence CEUS final survey has a crucial role of 'refinement'. In those selected cases, an integrated 5ALA+CEUS protocol was shown as advisable in EOR improvement.

INTRODUCTION

Significant advances have been recently made in High Grade Glioma (HHG) surgery aiming to enhance the identification of abnormal tissue at tumour margins and at to detect inadvertent residuals after gross resection[1-4].

5-aminolevulinic acid (5-ALA) has shown itself to be a major improvement within this framework. Several studies demonstrated it provides an increase in the extent of resection, resulting in a noteworthy change in the surgical technique[5-8]. However, 5-ALA in some specific working condition, as shown in a number of literature reports, can fail to identify some pathological areas[9-11].

More recently, contrast enhanced ultrasound (CEUS) has been successfully applied to the neurosurgical field as well[12-16]; it has been proven as effective and specific in identifying remnants in HGG surgery[15, 17-26]. A synergistic use of the two techniques has been already suggested in literature[13].

In this background, we aim to investigate when this integrated approach is best applied. Based on preoperative magnetic resonance imaging (MRI), we aim to identify a series of anatomical characteristics (namely tumor *localization*, *size* and *shape*, with a specific focus on *working corridors*), that might predict drawbacks of 5-ALA surgery and, hence, anticipate that CEUS assistance to the 5-ALA resection is advisable and, conversely, those in which 5-ALA alone is sufficient.

Discussing their strengths and strains, the paper also aims to identify which information are provided by the two techniques while favouring residuals identification.

METHODS

Study Design

Between January 2016 and June 2019, 49 HGG consecutive resections applying 5-ALA + CEUS protocol were performed at our institution. Only primary surgeries were included in the study, for which all patients provided informed consent.

Stratification of Gliomas Characteristics according preoperative MRI was as follows:

- *Localization:* Superficial vs deeply seated lesions;

Lesions reaching the cerebral cortex or within one centimetre from the surface were defined as superficial, whilst lesions with most superficial part > 1 cm from the surface were considered as deep.

- *Tumor size*: < 3,5 cm vs >3,5 cm.
- *Shape*: regular shape vs irregular/multi-lobulated shape

Intraoperative Protocol was the following (figure 1):

1. Initial CEUS evaluation to identify tumour characteristics, vascular features (feeders, drain, overall enhancement), anatomical relationships with cerebro-vascular structures and cisternal spaces.
2. 5-ALA guided resection under blue-filter illumination to achieve gross total resection (GTR); surgical field inspection with blue-light to identify residual fluorescence; completion of haemostasis.
3. Final CEUS survey of the surgical cavity is performed to identify potential residuals; if suspicious residual: supplementary inspection with blue-light to evaluate for fluorescence presence. If neurophysiological feasible, the area is resected.

Our primary outcome was to assess whether the final CEUS survey had an impact on the 5-ALA resection.

Hence, cases in which CEUS final survey identified inadvertent residuals after 5ALA resection (confirmed after subsequent blue-filter inspection) were recorded and statistically analysed in relationship with the aforementioned preoperative tumoral characteristics (location, size, shape).

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 neuronavigation system were used in all cases. According surgeon’s request, CUSA was used in most of the procedures.

5-ALA

5-ALA (Medac GmbH, Wedel, Germany) was administered 3 to 5 hours prior to surgery at a dose of 20 mg/kg body weight p.o. Intraoperatively, regions of interest were defined under violet-blue illumination (Blue 400 filters).

CEUS

The CEUS technique was performed according to the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) guidelines[27, 28].

The surgical field was examined with ultrasound (MyLab™Twice, Esaote, Italy— linear-array multifrequency 3–11 MHz device) through the craniotomic window. The probe was covered in a sterile plastic sheet, with sterile ultrasound coupling gel. First, B-mode was used to identify the lesion along with surrounding parenchyma. Second, sulfur hexafluoride-filled lipidic microbubbles ecographic contrast agent (SonoVue®—Bracco, Italy) was prepared by the anaesthesiologist; a bolus of 2.4 mL (5 mg/mL), followed by a flush of 10 ml of saline solution was later injected intravenously. Switching to specific contrast-tuned imaging algorithm automatically decreased the ultrasound mechanical index value needed; only the specific echo signal deriving from microbubble resonance was thus shown. Tumour characteristics dynamically displayed 25–30 s after injection included: solid or cystic components, arterial feeders, degree of vascularization, venous drainage. The scan was repeated later after completion of 5-ALA resection. A potential area for residual was identified in those enhancing parenchymal nodule with more rapid and persistent enhancement compared to surrounding brain tissue.

Statistical analysis

Continuous variables (i.e. diameters) were summarized by mean and standard deviation, or median and range, as appropriate. Dichotomous or categorical variables were summarized by frequencies and percentages. Statistically significant differences on distribution were evaluated performing t-test or Wilcoxon rank-sum tests for continuous variables and chi-squared test for categorical variables, as appropriate. Univariate and multivariate logistic regression were performed to identify the predictive variables for CEUS modifying 5-ALA surgery as outcome variable. Variables with p-value < 0.10 were included in the multivariate logistic model. Statistical significance for all tests was set at a p-value of 0.05. All statistical analyses were performed by Stata/IC 13.0 (StataCorp LP, College Station, USA).

RESULTS

Baseline characteristics of the studied population are summarised in table 1. In the analysed period of time, 49 patients (27 [56%] males, 22 [44%] females, mean age 62 yrs [27-84]) were analysed in the study. Tumour median size on preoperative MRI was 3,8 cm [2-6,8 cm]. Tumour size categorically divided into <3,5 cm and >3,5 cm was respectively 22 cases (44.90%) and 27 (55.10%). Localization was superficial in 31 cases (63.27%) and deep in 18 (36.73%). Tumoral margins were regular in 30 cases (61.22%) and irregular/multi-lobulated in 19 cases (38.78%).

CEUS modified 5-ALA guided resection in 11 cases (22.45%). When a suspicious area was identified at CEUS this was confirmed as fluorescent in all 11 cases.

At univariate logistic regression, this appeared to be associated with statistically significance when localization was deep (p=0,043) and margins were irregular/multi-lobulated (p=0,003). While a stronger statistical significance was associated with the latter, no association was found with tumour size, considering it both as categorical (p=0,53) and continuous variable (p=0,06).

At multivariate regression model, statistically significant association with the irregular/multi-lobulated margins exists with an OR= 0.095 IC95% (0.017 - 0.536) p=0.008.

Identification of the predictive variables of 5-ALA surgery modification after CEUS survey at univariate and multivariate logistic regression analysis are reported in table 2.

DISCUSSION

An integrated protocol: enhancing vision to improve radicality

Several studies confirmed that increasing EOR in glioma surgery improves both overall and progression free survival[29-33]. A resection above the anatomical limits of enhancing area after complete microsurgical resection is nowadays established as paramount determinant enabling significantly local tumor control compared to standard gross total resection[1, 3, 9, 34-37]. Because of that, supramarginal resection has to be seen as the primary goal in HGG surgery: it must be always sought within the bounds of functional-integrity[1, 6, 8, 19, 38-40].

5-ALA has a paramount role in extending resection minimizing the risk of inadvertent residuals; high diagnostic accuracy and real-time tumour visualization provided have been well established[1, 5, 6, 9].

However, several papers demonstrated there are cases in which technical limitations of tumor visualization by 5-ALA (based on microscopic light cone or craniotomy) might impede complete fluorescence visualization. Endoscopic assistance[10, 11], employment of 400-nm wavelength fiber-optic lighted suction instrument[41] and red light illumination technique[9], have been suggested as tools combinable with 5ALA to overcome this strain.
Within this framework, CEUS may play a major role. Its applications in neurosurgery remained unknown until quite recently [15, 20, 23-26, 42, 43]. In respect to HGG, the group of DiMeco in Milan demonstrated its feasibility in highlighting remnants after GTR[17, 19, 21, 22]. Indeed, CEUS assessment is suitable, rapid and does not modify the overall surgical flow.
Pertinent literature showed the association of 5ALA and CEUS techniques having an impact on EOR if compared to standard microsurgical procedure and to 5-ALA vs CEUS alone[13]. On the heels of these observations, we developed our integrated protocol. In our series, core resection was 5-ALA guided; CEUS instead enhanced visualization before and after resection. CEUS initial evaluation identified lesion's vascular supply[44-46]: it gave further insight into the surgical strategy, facilitated vascular deafferentation and maximized resection minimizing neurological sequelae (fig. 2, 3); after resection, CEUS final survey was crucial to increase the possibility of detecting unexpected residuals (fig.4).

Exploring Dark Corridors: when 5-ALA alone and when 5-ALA + CEUS?

Our primary objective was to identify when CEUS final survey has a real impact in a 5-ALA guided context. Based on preoperative MRI, we established 3 main anatomical tumour features: *localization*, *size* and *shape*. We aimed to identify whether those preoperative tumour characteristics, with specific attention to working corridors can predict strains of the fluorescence guided procedure and hence recommend the use of 5ALA+CEUS or, conversely, suggest 5-ALA alone as sufficient to pursue resection.
In our series, 11 cases showed CEUS inadvertent residual identification after 5-ALA guided resection. Even if the figure was not impressive, this 22,4% rate still has significance on the overall resection rate.

Regarding the application of the integrated approach, data analysis showed *tumour morphology* as the strongest a priori determinants; indeed, an irregular, multi-lobulated tumour was the condition in which 5-ALA presented major drawbacks and thus the 5-ALA + CEUS was the most effective. An assistance to the standard 5-ALA resection was advisable also for *deep localized lesions*. In the subset of patients displaying an association of the two abovementioned determinants (irregular margins + deep localisation), we noticed the greatest utility of the combined technique.

In multi-lobulated irregular shaped lesions, conditions of non-orthogonal working corridors are mostly likely: microscope light might fail to thoroughly illuminate the surgical field, resulting in blind corners.

This is equally true in deep localisations, where overlying brain edges might cover up residuals determining the so called ‘***gutter effect***’.

In both scenarios, tumoral edges tend to collapse within the surgical cavity, thus creating shadowing where microscope light might fail to identify pathological fluorescence. The issue of ***dark corridors*** is especially true in cases of distant non-exposed nodules, covered by a layer of normal tissue, or in those cases in which direction of resection radically changes[47, 48]. Analogously, haemostasis is a crucial point in HGG glioma surgery: exceedingly difficult haemostasis or clots might occult neoplastic tissue at blue light [47, 48].

Our data confirm that in these conditions, especially when associated, a CEUS intraoperative final assessment can improve EOR.

On the other hand, *tumour size* alone does not appear to be a statistically significant determinant. Our data suggested as approachable with 5-ALA alone a superficial and regular lesion, even if large. Figure 5 and figure 6 exemplifies the different conditions in which this model applies.

From our result we can conclude that CEUS final survey has a crucial role of ‘refinement’ of the 5-ALA procedure when working channel is oblique, non-orthogonal or deep or when surgical cavity edges tend to collapse hindering illumination. In other situations, such as superficial regular-shaped lesions, 5-ALA guided resection alone is appropriate and integration with other methods can be regarded as optional (fig. 5, fig. 6 and fig.7).

A feasible and effective association

5-ALA and CEUS look to the surgical field from two complementary perspectives; they explore the cerebral tissue in a different way: microscope light has to directly illuminate the tumour cells in order observe 5-ALA fluorescence[9], whereas CEUS is capable of showing unexposed, hidden, parenchymal tissue also at a distance from the exploring probe. In addition, the two observe two different phenomena: on one side, 5-ALA reflects pathological metabolism; on the other, CEUS uptake is related to tumoral augmented vascularisation and blood-brain-barrier disruption[17, 22]. These essential differences make their association particularly effective as the two techniques truly complement each other.

Our series revealed some key characteristics of remnants at CEUS final survey. Residual tumors appeared as enhancing nodule, usually at the margins of the surgical cavity. Located in a parenchymatous area, it displayed an early and persistent enhancement compared to the adjacent brain tissue (fig.4). When a suspicious area is identified it should be double checked under 5-ALA filter and eventually removed. Our series showed a complete concordance between CEUS and 5-ALA at residual inspection, thus underlining the specificity of the approach.

Several studies have demonstrated better outcomes in terms of EOR when 5-ALA guided-resection is assisted with CT or MRI intraoperative imaging[48, 49]. Despite the undisputed value of these techniques, they are expensive, time demanding, and hardly repeatable during surgery.
Conversely, CEUS is readily inexpensive, dynamic rand easy repeatable any time during surgery. Given the feasibility of the method, our experience showed which are the conditions in which a multimodal 5-ALA+CEUS setting is necessary and should be planned *a priori* and when it can be regarded as option instead.

CONCLUSIONS

Several studies demonstrated how 5-ALA-guided resection could be improved and refined by CEUS final survey. Indeed, CEUS has been confirmed as valuable tool in remnants identification. Our observations underlined the relevance of combined approach when **dark corridors** undermining fluorescence detection can be expected, such as in case of deeply seated and/or irregular lesions.
Providing complementary information to the surgeon, the two techniques well integrate. Their real-time multimodality integration allows to pursue the goal of supramarginal resection in HGG surgery.

TABLES

TABLE 1: BASELINE CHARACTERISTICS OF THE STUDIED POPULATION

General description		
Characteristics		**N**
N. of cases		49
Mean age [range]		62 [27-84]
Sex, n (n%)	M	27 (56%)
	F	22 (44%)
Diameter (cm) modelled as continuous variable, median (range)	3.8 (2.0%– 6.8%)	
Diameter (cm) modelled as dichotomous variable, n (n%)	< 3,5	22 (44.90%)
	> 3,5	27 (55.10%)
Localization	Superficial	31 (63.27%)
	Deep	18 (36.73%)
Margins	Regular	30 (61.22%)
	Multi-lobated	19 (38.78%)
CEUS Modifying 5-ALA procedure (identification of residual)	Yes	11 (22.45)
	No	38 (77.55)

<u>Table 2</u>. Univariate and multivariate logistic regression analysis. Identification of the predictive variables of 5-ALA surgery modification after CEUS survey.

Predictive variables	Univariate			Multivariate		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
Max diameter cm (modelled as dichotomous variable)						
< 3,5	1	-	-			
> 3,5	0.635	0.159 – 2.533	0.520			
Localization						
Superficial	1	-	-	1	-	-
Deep	0.233	0.057 – 0.958	**0.043**	0.333	0.069 – 1.611	0.172
Margins						
Regular	1	-	-	1	-	-
Irregular (poli-lobated)	0.079	0.015 – 0.432	**0.003**	0.095	0.017 – 0.536	**0.008**

FIGURE LEGEND

FIG.1: Diagrammatic representation of the surgical protocol that integrates the two techniques. Before resection, the lesion is explored with CEUS and B-Mode ultrasound, revealing key features of the lesion (solid/cystic components, vascular supply, overall vascularisation and anatomical relationships with surrounding neuro-vascular structures).

The glioma is then resected under 5-ALA guidance as for standard procedures, till the resection is considered completed; haemostasis is then performed.

Last step before dural closure is the final survey with CEUS possibly revealing inadvertent residuals.

FIG. 2: CEUS frame line of a HGG. The microbubble contrast medium allows to visualize the feeding arteries (red arrow) in the early phase, whilst in the central arterial phase the tumor parenchyma (green asterisk) is well visualized with its necrotic non-enhancing component (red asterisk), followed by the initial venous phase with major draining vessels (blue arrow) of the lesion.

FIG. 3: CEUS frame line of a right temporal HGG. The microbubble contrast medium allows to visualize the surrounding brain anatomy also at a distance, well depicting the cisternal and liquoral spaces (CS). Brainstem (BS) parenchyma appears hypoechoic in standard B-Mode and palely enhancing after CEUS, whilst liquoral spaces do not enhance; surrounding vessels are also well studied with CEUS such as Basilar artery (BA) and posterior communicating artery (PCA). In the central arterial phase after CEUS injection, the tumor parenchyma (single asterisk) is well visualized with its necrotic non-enhancing component (double asterisk). Lesional enhancement persists also in the later CEUS phases

FIG.4: CEUS scans depicting an inadvertent residual. Surgical cavity (green asterisk), partially collapsed, is well evident under CEUS algorithm after microbubbles injection an enhancing nodule con be observed already at the early phase after CEUS microbubbles injection (red arrows); enhancement is persistent also in the later phases after injection. After CEUS injection cerebral sulci become evident (blue arrow).

FIG. 5: artistic representation of three model cases in which pre-operative tumour characteristics recommended the use of 5-ALA alone or suggested an implementation with final CEUS survey.

In superficial and regular shaped lesions (*upper*) microscope light thoroughly illuminates the surgical field ensuing a potentially complete resection.

Deep seated lesions (*center*) and irregular/multi-lobulated shaped ones (*bottom)*, favour conditions of non-orthogonal working corridors where microscope light might fail to thoroughly illuminate the surgical field, resulting in blind corners where fluorescence can be not elicited. In these conditions possible strains of the 5-ALA fluorescence can be expected. In these specific situations CEUS can play a refinement role as ensues a more complete control of the surgical field as it insights through unexposed, hidden, parenchymal tissue.

FIG. 6 three paradigmatic cases in which pre-operative tumour characteristics recommended the use of 5-ALA alone or suggested an implementation with final CEUS survey. In deep seated lesions and in those with irregular/multi-lobulated margins, possible drawbacks of the fluorescence can be expected.

On the heel of these observations, synergistic approach was regarded as unnecessary in the first case, being it a superficial and unilobate lesion.

5ALA+CEUS protocol was instead advisable in the second, which shows a superficial and multilobate tumor. Same consideration applies to the last case, describing an unilobate but deep lesion.

FIG.7 flow-chart representation summarizing the decision of a 5-ALA guided procedure vs an integrated 5-ALA + CEUS protocol, based on preoperative tumor characteristics.

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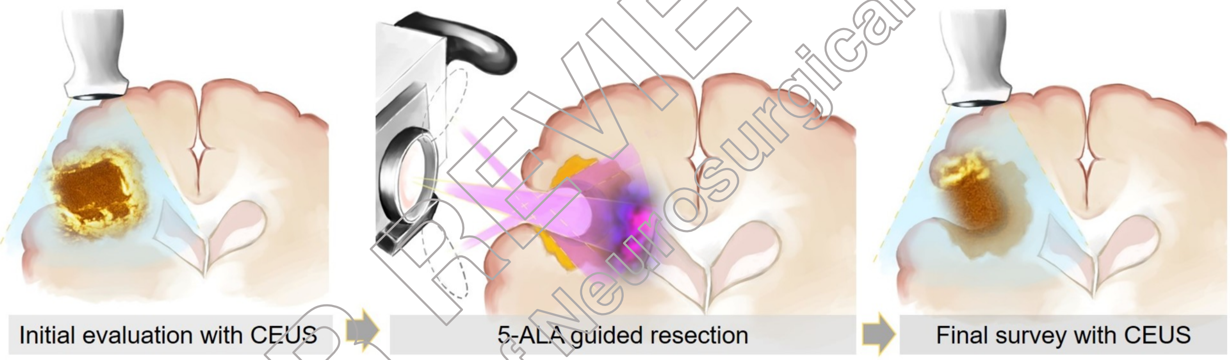
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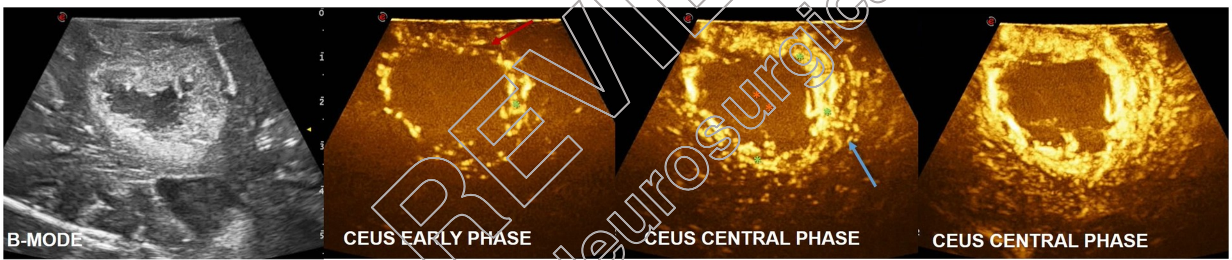
5-ALA guided resection
Final survey with CEUS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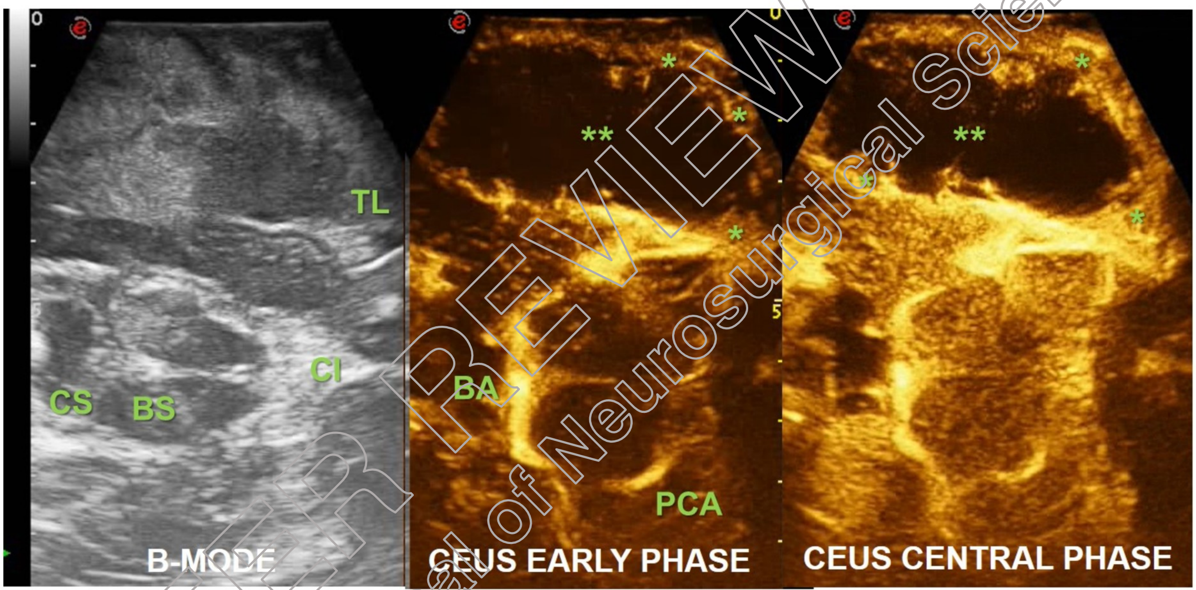
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CEUS EARLY PHASE
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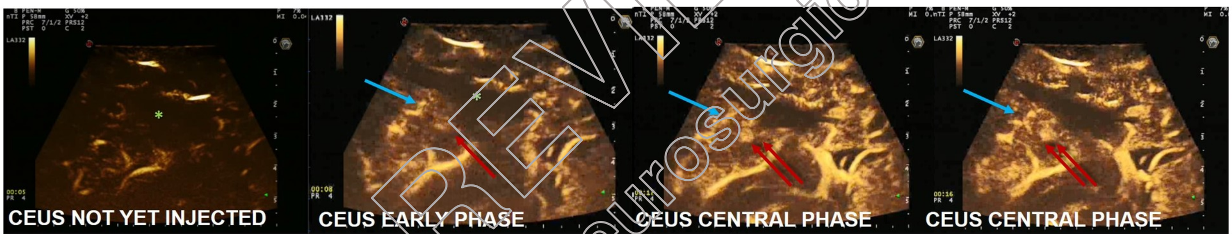
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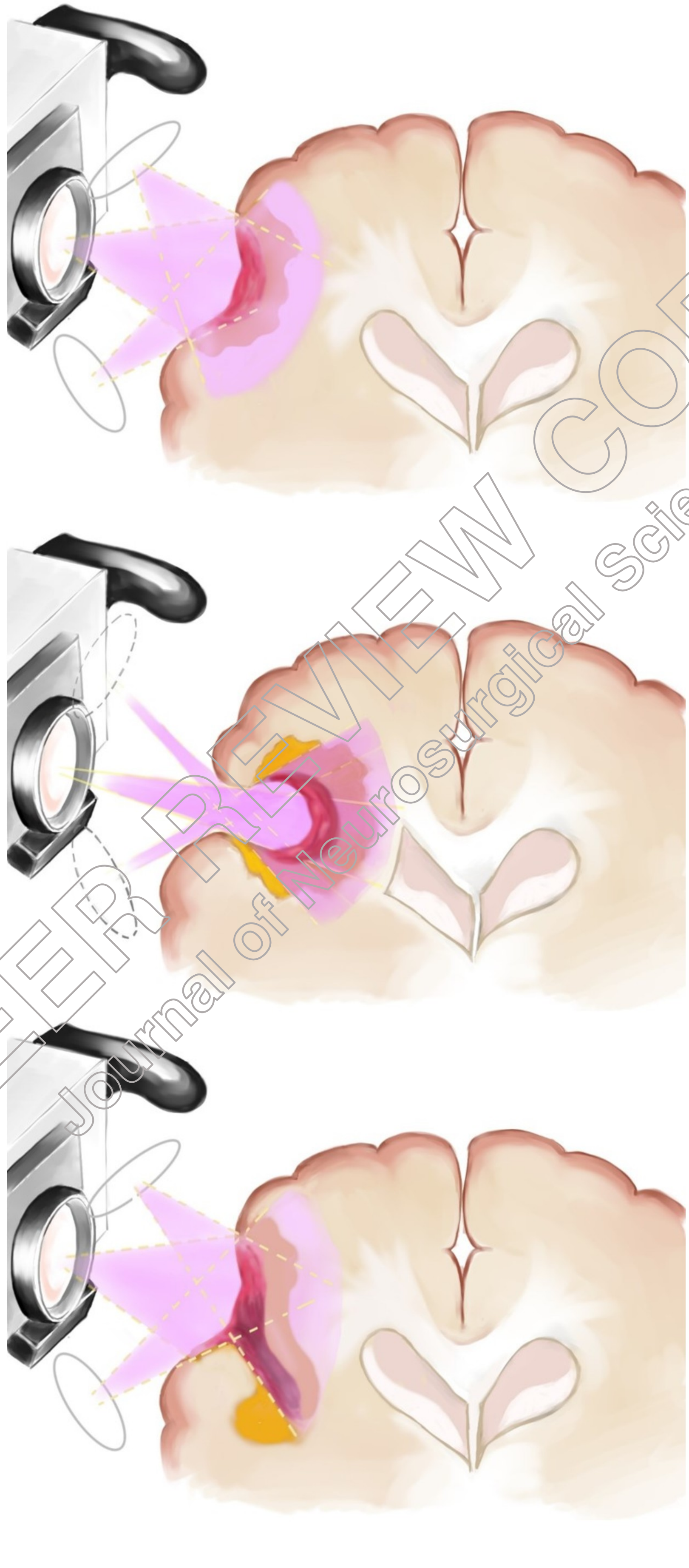

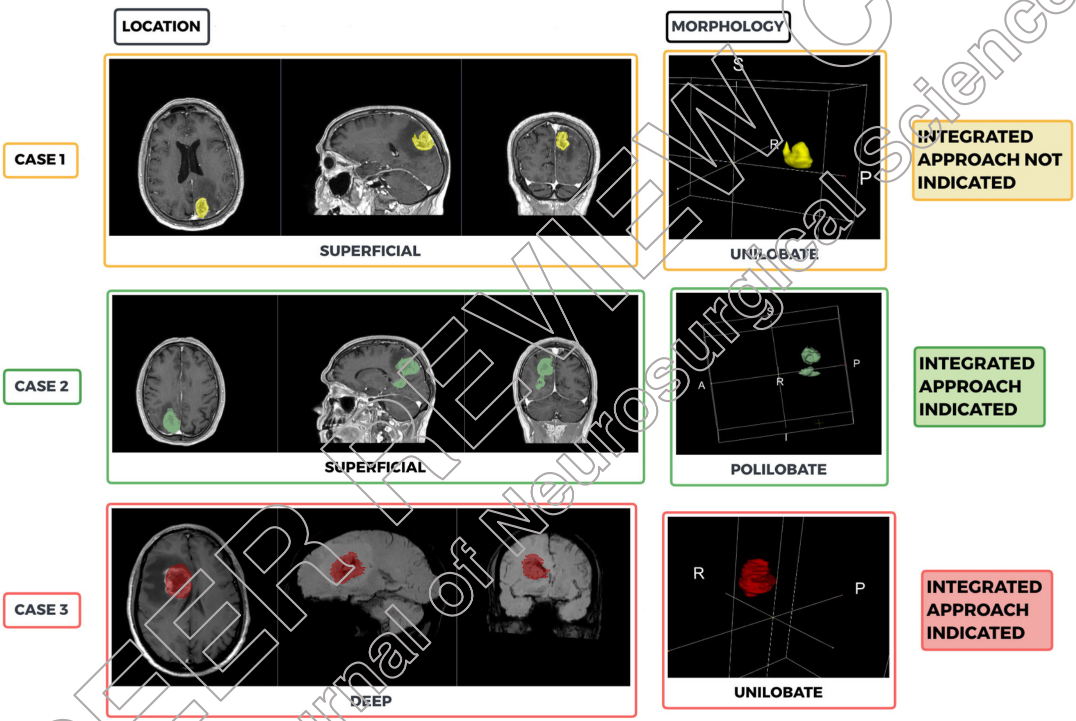
LOCATION
MORPHOLOGY
CASE 1
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INTEGRATED APPROACH NOT INDICATED
CASE 2
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CASE 3
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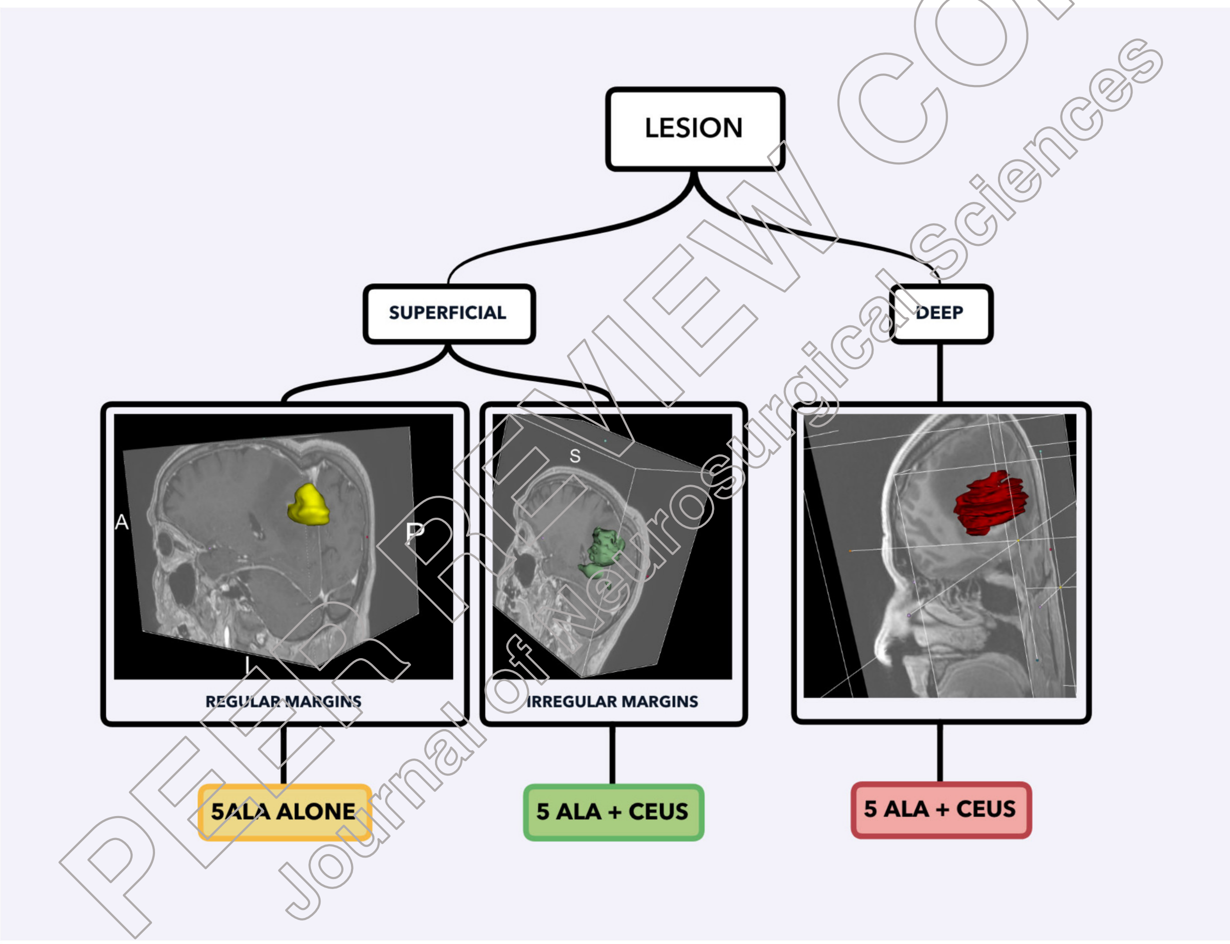
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