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Abstract

The extent of resection in high grade glioma is increasingly been shown to positively effect survival. Nevertheless, heterogeneity and migratory behavior of glioma cells make gross total resection very challenging. Several techniques were used in order to improve the detection of residual tumor. Aim of this study was to analyze advantages and limitations of fluorescence and image guided resection. A multicentric prospective study was designed to evaluate the accuracy of each method. Furthermore, the role of 5-aminolevulinc acid and neuronavigation were reviewed.
Twenty-three patients harboring suspected high grade glioma, amenable to complete resection, were enrolled. Fluorescence and image guides were used to perform surgery. Multiple samples were obtained from the resection cavity of each lesion according to 5-ALA staining positivity and boundaries as delineated by neuronavigation. All samples were analyzed by a pathologist blinded to the intra-operative labeling. Decision-making based on fluorescence showed a sensitivity of 91.1% and a specificity of 89.4% ($p < 0.001$). On the other hand, the image-guided resection accuracy was low (sensitivity: 57.8%; specificity: 57.4%; $p = 0.346$). We observed that the sensitivity of 5-ALA can be improved by the combined use of neuronavigation, but this leads to a significant reduction in specificity. Thus, the use of auxiliary techniques should always be subject to critical skills of the surgeon. We advocate a large-scale study to further improve the assessment of multimodal approaches.

Keywords

5-Aminolevulinic acid; 5-ALA; Neuronavigation; High grade glioma surgery; Gross total resection; GTR

1. Introduction

Surgery in high grade gliomas plays a major role both for cytoreduction and for the possibility of establishing a histopathological diagnosis. Furthermore, surgery enhances the efficacy of adjuvant therapies [1]. The main goal is the achievement of surgical radicality, since a significant median survival advantage is associated with resection of 98% or more of the tumor volume [2]. Recently, protoporphyrin IX fluorescence, induced by 5-aminolevulinic acid (5-ALA) oral administration, has been introduced to improve the detection of residual tumor in order to achieve a gross total resection (GTR).
Stummer et al. showed that the use of fluorescence leads to a significant increase of the GTR rate [3] and [4], but further studies observed several limitations. False positive fluorescent tissues may be detected in cases of recurrent gliomas [5], in radiation necrosis and in neurodegenerative diseases [6]. On the other hand, the heterogeneity of porphyrin IX fluorescence may reduce the ability to detect pathological tissue and this affects the sensitivity of the method [7].
Image-guided resection has been widely used in glioma surgery, but its accuracy is hampered by the brain shift phenomenon. Aim of this paper was to analyze advantages and limitations of both fluorescence- and image-guided resection techniques. We reported our experience and reviewed the literature.

2. Materials and methods

2.1. Study protocol

Prospective cohort study carried out by the University of Turin and Geneva – Period: November 2008–March 2010. The project was approved by the Ethics Committee – Consent form signed by all patients.
Fifty-four patients harboring high grade gliomas were consecutively operated on in the Neurosurgical Division of Turin. Twenty-three aged 43–79 years met the inclusion criteria and were enrolled for the study.

2.2. Inclusion criteria

Patients with suspected, newly diagnosed, untreated malignant gliomas and who were amenable to complete resection of contrast-enhancing tumor.

2.3. Exclusion criteria

Karnofsky Performance Scale of less than 70%. Gliomas involving eloquent areas. More than one contrast-enhancing lesion. Tumors of the midline, basal ganglia, cerebellum and brain stem. Substantial, non-contrast-enhancing tumor areas.

2.4. Imaging protocol

Thin-layer, contrast-enhanced Computed Tomography (CT) images, T1-weighted magnetic resonance imaging (MRI) with and without contrast enhancement sequences, T2-weighted sequences, diffusion-weighted images, and fluid-attenuated inversion recovery sequences. Imaging data were merged and loaded into a neuronavigation system (Brainlab, Feldkirchen, Germany) and used intraoperatively.

2.5. 5-ALA protocol

Pretreatment with 4 mg dexamethasone three times a day for at least 2 days before operation. Oral administration of 5-ALA (20 mg/kg body-weight), diluted in 50 mL of water 3 h (range 2–4) before induction of anesthesia.

2.6. Surgery protocol

We used a modified neurosurgical microscope (OPMI Pentero, Carl Zeiss), equipped with a fluorescence kit. All the operations were carried out by a single surgeon, trained in the use of 5-ALA.
Each resection was initially performed using a conventional illumination. After the exeresis was judged as complete, the cavity was systematically inspected in the violet-blue light and with the neuronavigation system in order to identify any residual tumor. Subsequently, the surgeon has sampled multiple biopsies and has labeled them according to the 5-ALA fluorescence status, positive or negative, and the neuronavigation data, inside or outside the limits tracked (Fig. 1). Four specimens were biopsied for each patient according to the scheme summarized in Table 1.

2.7. Neuropathology

The designated neuropathologist was blinded to the intra-operative classification of each specimen. The histopathological criteria were established according to the World Health Organization 2007 diagnostic consensus criteria [8]. MIB-1 antibody (anti-Ki-67) was used to assess the tumor cell proliferation.
Histological criteria to define the samples as positive were: presence of anaplastic foci, diffused atypies and pallisading necrosis, increased mitotic activity (KI-67 > 5%) and microvascular proliferation. Non-pathological specimens were defined as negative.

2.8. Statistical analysis

Chi2 contingency tables were calculated using SPSS 19.0 (IBM). Significance was accepted for $p < 0.05$.

3. Results

Conventional surgical excision was performed in all cases without newly diagnosed permanent deficit. We did not observe adverse effects related to 5-ALA administration and fluorescent tumor tissue was well visualized in all cases. All patients harbored Glioblastoma Multiforme (GBM).

3.1. Histopathology

Decision-making based on 5-ALA fluorescence positivity alone had a sensitivity of 91.1% and a specificity of 89.4% for a pathological finding (significant at $p < 0.001$). Neuronavigation delineated tumor tissue with a sensitivity of 57.8% and a specificity of 57.4% (not significant, $p = 0.346$). All statistical analyses were calculated according to the data shown in Table 2.

3.2. 5-ALA positive

In the first group (5-ALA and neuronavigation-positive) all samples were pathological. In group 2 (5-ALA positive, but neuronavigation-negative), 18/23 (78.3%) samples proved to be tumoral tissue. Nonspecific reactive gliosis associated with peritumoral inflammation was observed in the 5 fluorescence positive samples (21.7%) without pathological features. Adding neuronavigation to 5-ALA positivity increased the specificity to 100%, but lowered the sensitivity to detect malignant tissue to 51.1%. However, this finding was not significant ($p = 0.13$). Statistical analysis is according to data in Table 1.

3.3. 5-ALA negative

If no 5-ALA staining is present, 20/23 (87%) of samples obtained within tumor boundaries as delineated by neuronavigation proved to be normal white matter, whereas only 3/23 specimens (13%) were pathological. In the last group (5-ALA negative and neuronavigation-negative), only one sample out of 23 (4.3%) showed pathological features. A low cellularity was observed in the four 5-ALA negative specimens resulted as pathological tissue (Table 1).

4. Discussion

4.1. 5-ALA: an overview

5-ALA is a necessary component for the production of protoporphyrin IX in the mitochondria. The addition of a ferrous ion to protoporphyrin IX leads to the ferrochelatase-mediated synthesis of the heme moiety of hemoglobin. Some cells can produce larger amounts of protoporphyrin IX when exogenous 5-ALA is added [9]. Due to the relatively slow rate of heme production by ferrochelatase however, an oversupply of 5-ALA in these cells leads to accumulation of protoporphyrin IX [10]. This phenomenon has been found to be particularly marked in malignant cells of different origin. Protoporphyrin-fluorescence of malignant cells is thus exploited for visualization of pathological tissue in surgery for malignant glioma [11]. Low grade gliomas do not show intraoperative fluorescence after application of 5-ALA [12]. A modified surgical microscope with the ability to switch from white illumination to violet-blue excitation can be used to detect protoporphyrin IX fluorescence at 635 and 704 nm [3].
At its currently recommended dose of 20 mg 5-ALA/kg body weight in combination with dexamethasone, it has a very low rate of undesired effects. Side effects were reported in particular for other indications where higher doses are used (more than 40 mg/kg body weight) and include hypotension, nausea, vomiting and elevated liver enzymes. Moreover, 5-ALA leads to sensitization of the skin. It is therefore recommended to avoid direct bright light or sunlight exposure for 24 h after administration of 5-ALA.
During surgery, the slightly reduced resolution of the fluorescence-mode may require the surgeon to occasionally switch to white light for better visualization when performing critical steps. Moreover, autofluorescence of normal tissue may be overamplified and give a misleading image of pathological fluorescence. These and other helpful technical details and potential pitfalls of 5-ALA in glioma surgery are summarized in a recent review [13].
5-ALA is gaining popularity and the technical equipment required can be mounted as a supplementary module on commercially available high-end neurosurgical operating microscopes. It is possible that the limited indications and additional costs currently involved in its acquisition have so far prevented its widespread use.

4.2. 5-ALA to improve localization of residual glioma

The randomized clinical trial carried out by Stummer et al. showed an increased rate of contrast-enhancing tumor resection compared to conventional surgery [4]. Some surgeons report difficulties in interpreting 5-ALA when performing surgery on recurrent glioma, but a recent phase II study found a 93% positive predictive value for 5-ALA to indicate pathological tissue in recurrent malignant glioma [14].
5-ALA accuracy is reduced in several cases. Perinecrotic area in radiation necrosis or neurodegenerative disease can be labeled as fluorescence positive [6]. Inflammatory cells and reactive astrocytes may appear fluorescent, especially in recurrent glioma [5]. Moreover, the sensitivity of 5-ALA is hampered by the significantly change of fluorescence intensity due to heterogeneity of gliomas, invasion beyond the resection cavity and intercellular heterogeneity of porphyrin IX fluorescence [7]. Therefore, the surgical strategy in case of vague positive 5-ALA fluorescence is uncertain.
In our experience, fluorescence-guided resection showed a sensitivity of 91.1%. A low cellularity was observed in the false negative samples. Thus, we speculate that a low number of cells could not be able to generate a detectable signal. The specificity was 89.4%. The false positive cases may be due to an increased reactive mitotic activity in the peritumoral area.

4.3. Neuronavigation

Frameless neuronavigation is a popular and widely used tool in modern neurosurgery to aid the surgeon in intra-operative localization and orientation [15]. While most surgeons agree on its general utility, there is little evidence on the precise circumstances under which neuronavigation adds a benefit for the patient. Randomized clinical trials on surgical methods such as neuronavigation are difficult to perform. One such trial was carried out by Willems and colleagues from Tübingen [16]: in cases where the surgeon was uncertain about the use of neuronavigation, it was used (or not) during surgery in a randomized manner. Although surgeons in three quarter of the surgeries subjectively reported that navigation was useful, the extent of resection was no better in the navigated than in the un-navigated group. The obvious limitations of that study were a small patient sample, and the fact that this trial was based on solitary cerebral lesions for which the surgeon already decided that neuronavigation is not essential.
One of the technical limitations of classical neuronavigation is that it is based on preoperative imaging. Thus, accuracy is decreased when controlling the resection cavity after surgery due to distorted anatomy and released CSF. This explains the apparent lack of accuracy of neuronavigation in this study to provide data on tumor remnants. First, the resection cavity at the end of surgery is more distorted than any other region and shifts in brain parenchyma are significant. Although brain shift has been addressed in a number of seminal studies [17], no algorithm has yet provided reliable and accurate modeling based on preoperative data [18]. Some surgeons even argue if it is even possible to achieve such a compensation without intra operative imaging. Second, in most cases at the end of surgery the small rim of suspected remaining tumor tissue is so slim that it is likely to fall within the margin of error reported for neuronavigation accuracy.

4.4. Neuronavigation to improve localization of residual glioma

Kurimoto et al. [19] and Wirtz et al. [20] observed a significant impact of neuronavigation to achieve GTR, but their works bear the disadvantages of retrospective analyses and limited

cohorts. On the other hand, the prospective randomized study carried out by Willems et al. [16] showed that the extent of resection is not enhanced by the use of a frameless stereotactic system.
We previously remarked that the effectiveness of neuronavigation is biased by the brain shift phenomenon [17]. Intra-operative MRI and ultrasonography were used in order to update the navigation system during surgery [21]. Nevertheless, MRI is costly and not widely available [22], and ultrasonography shows an overestimation of tumor tissue during resection and it is not able to detect tumor remnants in surgical cavity [23].
Thus, alternative techniques should be developed to improve the intra-operative neuronavigation accuracy.
In our study, sensitivity and specificity of the image-guided system used alone were 57.8% and 57.4% respectively. These suboptimal results may be explained by the decreased accuracy of neuronavigation within the resection cavity. All fluorescent specimens collected within the limits of neuronavigation were pathological, but the rate of pathological tissue outside the navigated tumor boundaries is high (19/46 = 41.3%). Therefore, the fluorescence-guided assistance is mandatory.

4.5. Results of a combined approach

The use of 5-ALA and neuronavigation led to increase the extent of resection obtained with a conventional surgical strategy. The image-guided resection may improve the sensitivity of 5-ALA. As a matter of fact, the rate of non-fluorescent tissue collected outside the limits delimitated by neuronavigation, resulted as pathological, is low (4.3%).
On the other hand, the specificity is reduced if we assume as pathological the tissue showing positivity at least one technique (Table 2). Therefore, it is important to remember that surgery in critical brain regions cannot be guided by images or fluorescence alone. Thus, resection should be guided by the surgeon's capacity to integrate surgical anatomy with auxiliary techniques.

4.6. Limitations of the study

In this prospective pilot study, patient numbers were relatively low due to strict application of inclusion criteria (newly diagnosed, macroscopically fully resectable high grade glioma). Although the accuracy of 5-ALA to assess tumor remnant achieved statistical significance, the benefits of a combined approach were possibly underestimated and subgroup analyses were difficult to ascertain statistically. We therefore advocate a large-scale prospective study evaluating multimodal approaches.

5. Conclusions

Fluorescence- and image-guides can be used to detect pathological tissue hidden to conventional surgical strategy. 5-ALA is a safe, reliable and highly sensitive technique. Neuronavigation shows several limits, but it can improve the sensitivity of 5-ALA. The specificity in case of combined approach is reduced. Therefore, surgery should always be guided by surgical anatomy, even with the undoubted aid of auxiliary techniques.

References

[1] M.D. McCarter, Y. Fong
Role for surgical cytoreduction in multimodality treatments for cancer
Ann Surg Oncol, 8 (2001), pp. 38–43

[2] M. Lacroix, D. Abi-Said, D.R. Fourney, Z.L. Gokaslan, W. Shi, F. DeMonte, et al.
A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival
J Neurosurg, 95 (2001), pp. 190–198

[3] W. Stummer, H. Stepp, G. Möller, A. Ehrhardt, M. Leonhard, H.J. Reulen
Technical principles for protoporphyrin-IX-fluorescence guided microsurgical resection of malignant glioma tissue
Acta Neurochir, 140 (1998), pp. 995–1000

[4] W. Stummer, U. Pichlmeier, T. Meinel, O.D. Wiestler, F. Zanella, H.J. Reulen, et al.
Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial
Lancet Oncol, 7 (2006), pp. 392–401

[5] S. Utsuki, H. Oka, S. Sato, S. Shimizu, S. Suzuki, Y. Tanizaki, et al.
Histological examination of false positive tissue resection using 5-aminolevulinic acid-induced fluorescence guidance
Neurol Med Chir (Tokyo), 47 (2007), pp. 210–213

[6] S. Miyatake, T. Kuroiwa, Y. Kajimoto, M. Miyashita, H. Tanaka, M. Tsuji
Fluorescence of non-neoplastic, magnetic resonance imaging-enhancing tissue by 5-aminolevulinic acid: case report
Neurosurgery, 61 (2007), pp. 1101–1103

[7] M. Hefti, G. von Campe, M. Moschopulos, A. Siegner, H. Looser, H. Landolt
5-Aminolevulinic acid induced protoporphyrin IX fluorescence in high-grade glioma surgery: a one-year experience at a single institution
Swiss Med Wkly, 138 (2008), pp. 180–185

[8] D.N. Louis, H. Ohgaki, O.D. Wiestler, W.K. Cavenee, P.C. Burger, A. Jouvet, et al.
The 2007 WHO classification of tumours of the central nervous system
Acta Neuropathol, 114 (2007), pp. 97–109

[9]B. Krammer, K. Plaetzer
ALA and its clinical impact, from bench to bedside
Photochem Photobiol Sci, 7 (2008), pp. 283–289

[10] J.C. Kennedy, R.H. Pottier, D.C. Pross
Photodynamic therapy with endogenous protoporphyrin IX: basic principles and present clinical experience
J Photochem Photobiol B, 6 (1990), pp. 143–148

[11] W. Stummer, S. Stocker, A. Novotny, A. Heimann, O. Sauer, O. Kempski, et al.
In vitro and in vivo porphyrin accumulation by C6 glioma cells after exposure to 5-aminolevulinic acid
J Photochem Photobiol B, 45 (1998), pp. 160–169

[12] C. Ewelt, F.W. Floeth, J. Felsberg, H.J. Steiger, M. Sabel, K.J. Langen, et al.
Finding the anaplastic focus in diffuse gliomas: the value of Gd-DTPA enhanced MRI, FET-PET, and intraoperative, ALA-derived tissue fluorescence
Clin Neurol Neurosurg, 113 (2011), pp. 541–547

[13] J.C. Tonn, W. Stummer
Fluorescence-guided resection of malignant gliomas using 5-aminolevulinic acid: practical use, risks, and pitfalls
Clin Neurosurg, 55 (2008), pp. 20–26

[14] A. Nabavi, H. Thurm, B. Zountsas, T. Pietsch, H. Lanfermann, U. Pichlmeier, et al.
Five-aminolevulinic acid for fluorescence-guided resection of recurrent malignant gliomas: a phase II study
Neurosurgery, 65 (2009), pp. 1070–1076

[15] G.H. Barnett, D.W. Kormos, C.P. Steiner, J. Weisenberger
Use of a frameless, armless stereotactic wand for brain tumor localization with two-dimensional and three-dimensional neuroimaging
J Neurosurg, 33 (1993), pp. 674–678

[16] P.W. Willems, J.W. van der Sprenkel, C.A. Tulleken, M.A. Viergever, M.J. Taphoorn
Neuronavigation and surgery of intracerebral tumours
J Neurol, 253 (2006), pp. 1123–1136

[17] N.S. Litofsky, A.M. Bauer, R.S. Kasper, C.M. Sullivan, O.H. Dabbous, Glioma Outcomes Project Investigators
Image-guided resection of high-grade glioma: patient selection factors and outcome
Neurosurg Focus, 20 (2006), p. E16

[18] C. Nimsky, O. Ganslandt, S. Cerny, P. Hastreiter, G. Greiner, R. Fahlbusch
Quantification of, visualization of, and compensation for brain shift using intraoperative magnetic resonance imaging
Neurosurgery, 47 (2000), pp. 1070–1079

[19] M. Kurimoto, N. Hayashi, H. Kamiyama, S. Nagai, T. Shibata, T. Asahi, et al.
Impact of neuronavigation and image-guided extensive resection for adult patients with supratentorial malignant astrocytomas: a single-institution retrospective study
Minim Invasive Neurosurg, 47 (2004), pp. 278–283

[20] C.R. Wirtz, F.K. Albert, M. Schwaderer, C. Heuer, A. Staubert, V.M. Tronnier, et al.
The benefit of neuronavigation for neurosurgery analyzed by its impact on glioblastoma surgery
Neurol Res, 22 (2000), pp. 354–360

[21] C. Nimsky, B. von Keller, S. Schlaffer, D. Kuhnt, D. Weigel, O. Ganslandt, et al.
Updating navigation with intraoperative image data
Top Magn Reson Imaging, 19 (2009), pp. 197–204

[22] H. Hirschberg, E. Samset, P.K. Hol, T. Tillung, K. Lote
Impact of intraoperative MRI on the surgical results for high-grade gliomas
Minim Invasive Neurosurg, 48 (2005), pp. 77–84

[23] O.M. Rygh, T. Selbekk, S.H. Torp, S. Lydersen, T.A. Hernes, G. Unsgaard
Comparison of navigated 3D ultrasound findings with histopathology in subsequent phases of glioblastoma resection
Acta Neurochir (Wien), 150 (2008), pp. 1033–1041

Fig. 1.
Multiple sampling protocol. For each patients we collected one specimen from each of the following areas: (C + A) fluorescent sample within the neuronavigation area; (C + B) fluorescent sample outside the neuronavigation area; (D + A) non-fluorescent sample within the navigation area; (D + B) non-fluorescent sample outside the navigation area.

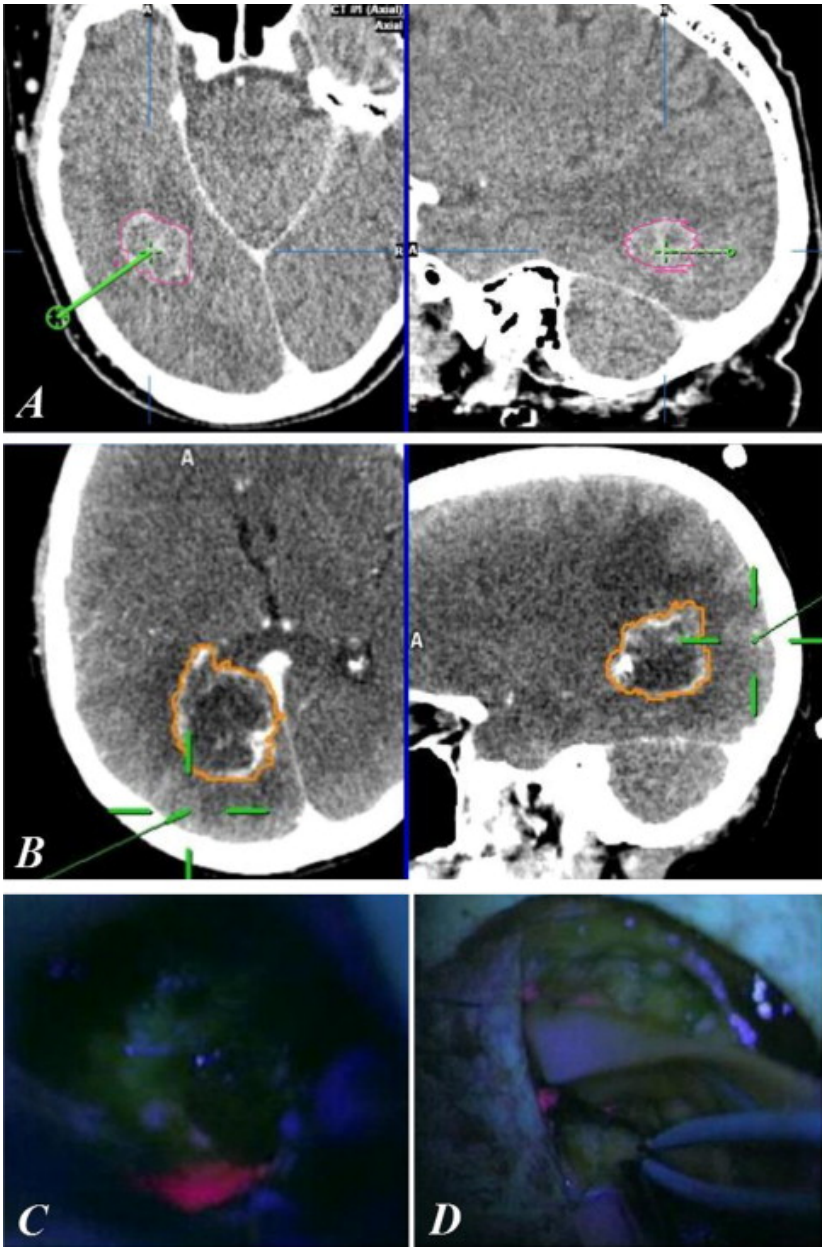

Table 1
Study protocol and histopathological results. Fluorescent specimens collected within tumor boundaries as delineated by neuronavigation (group 1) and outside (group 2). Non fluorescent samples obtained in the limits (group 3) and outside (group 4) the boundaries set by neuronavigation. Histopathological results and correlations with each group.

	Fluorescence – image guides		Histopathological results		Total
	5-ALA	Neuronavig.	Pathological	Non-pathol.	
Group 1	+	Inside	23 (100%)	0	23
Group 2	+	Outside	18 (78.3%)	5 (21.7%)	23
Group 3	–	Inside	3 (13%)	20 (87%)	23
Group 4	–	Outside	1 (4.3%)	22 (95.7%)	23
Total	/	/	45	47	92

Table 2
Histopathological results of (A) fluorescence positive and negative specimens; (B) samples biopsied inside and outside the limits marked by neuronavigation; (C) specimens obtained according to at least one technique (5-ALA or neuronavigation or both).

	Fluorescence – image guides	Pathological	Non-pathological	Total
A	5-ALA +	41	5	46
	5-ALA –	4	42	46
B	Neuronavigation +	26	20	46
	Neuronavigation –	19	27	46
C	5-ALA and/or Navigation +	44	25	69
	5-ALA and Navigation –	1	22	23
	Total	45	47	92