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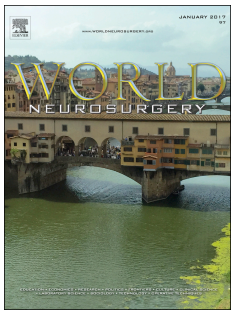

5-ALA *'False Positives'* in cerebral neurooncology: not all that fluorescences is tumor. A case-based update and literature review.

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5-ALA *'False Positives'* in cerebral neurooncology: not all that fluorescences is tumor. A case-based update and literature review.

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5-ALA '*False Positives*' in cerebral neurooncology: not all that fluorescences is tumor. A case-based update and literature review.

ABSTRACT

Background One of the most valuable innovations in the field of high-grade glioma (HGG) surgery is 5 aminolevulinic acid (5-ALA). Florescence is a specific and sensitive indicator of metabolically active tumoral tissue. In published literature the main focus was placed on false negative cases, with only few papers addressing the issue of false positivity. The aim of the paper is to highlight those settings in which 5-ALA flourescence does not necessarily mean tumor, and to point out those conditions in which intraoperative 5-ALA fluorescence has to be critically considered.

Methods

Via PubMed access, a review of pertinent literature was aimed to specifically investigate all those conditions, including non-neoplastic and other metabolically active lesions which can mimic HGGs, causing a misleading intraoperative diagnosis. In addition, an institutional case characterized by strong 5-ALA fluorescence in radionecrosis is presented

Results:

Literature results were grouped in two main categories according to the field of application, namely oncological setting (9 papers + institutional case) and non-oncological settings (5 papers).

Discussion and Conclusion As reported, 5-ALA-induced fluorescence is not limited to glioma but is also evident in non-glioma and non-neoplastic conditions. Critical interpretation of the intraoperative fluorescence is therefore mandatory in recurrences and in atypical cases that might hinder alternative diagnoses.

Keywords: 5-ALA; fluorescence; false positive; High grade glioma; glioblastoma; radionecrosis.

INTRODUCTION

A wide range of intraoperative tools aimed to improve High Grade Gliomas (HGG) surgical identification have been developed in the last decades[1].

One of the most valuable innovations in the field of enhancing vision surgery is 5 aminolevulinic acid (5-ALA): several papers demonstrated its indisputable value in improving the extent of resection (EOR), which in turn positively correlates with increased survival in HGG patients[2, 3].

5-ALA accumulates in metabolically active glioma cells, which fluorescence as opposed to surrounding healthy brain parenchyma: it allows the surgeon to identify neoplastic tissues also in areas where tumor margins discrimination can be challenging[4-6].

Approximately 20% of grade II and most grade III and IV gliomas are fluorescent after 5-ALA application [6, 7]. In literature, most of the attention was placed on false negative cases; only few papers specifically addressed the issue of false positive fluorescence instead[4].

Highlighting those settings in which it does not necessarily mean tumor, aim of our case - based review is to point out those conditions in which intraoperative 5-ALA fluorescence has to be critically considered.

A review of pertinent literature was aimed to specifically investigated all those including non-neoplastic, metabolically active lesions which can mimic HGGs, causing a misleading intraoperative diagnosis; secondly, an institutional case characterized by strong 5-ALA fluorescence in radionecrosis is presented.

METHODS

Literature review

An online literature research was conducted using PubMed access. The algorithm used the terms “5ALA” “radionecrosis” “false positives” “gliomas” “glioblastoma” “recurrences” in various combination. To be included, studies had to involve human, had to report original data and had to have used 5-ALA in a clinical neurosurgical setting depicting intraoperative fluorescence without evidence of tumoral tissue. By the same token, reviews and general discussion papers not reporting original data were excluded. Cross check of references of the selected articles was performed in order to complete bibliographical research. Results were grouped in two main categories according to the setting, namely **oncological setting** and **non-oncological setting**.

Institutional Case

Our patient was a 47-year-old lady who underwent surgery for an infiltrative left frontal lesion one year before the reported event. At the time, no residual pathology was shown. Pathological diagnosis was anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted.

After multidisciplinary board, it was decided to administer adjuvant chemo-RT. It has long been known that oligodendroglial gliomas are highly chemiosensitive to the Procarbazine, Lomustine and Vincristine (PCV) scheme even if severe and long-lasting hematological toxicities are present. In light of this, the patient followed the PVC scheme together with fractionated radiotherapy.

During neuro-radiological follow-up a surgical cave relapse was observed on magnetic resonance imaging (**MRI**). On the basis of this finding, the patient underwent a second surgical procedure. Pathological diagnosis revealed radionecrosis. Post-operative course was uneventful. Post -operative brain MRI showed complete lesion removal without pathological enhancement.

Surgery

Patient was pretreated with 5-ALA (Gliolan; Medac, Wedel, Germany), 20 mg/kg body weight, administered 6 hours before anesthesia. Surgery was performed using Leica M530 OH6 microscope equipped with BLUE 400 for visualizing fluorescence together with Brainlab neuronavigation (Brainlab AG, Feldkirchen, Germany). Two certified surgeons for fluorescence-guided surgery performed surgery (GS and GLR). Frozen sections were obtained before and during final resection. Tissue were sampled from strong fluorescence area during tumor resection

Neuropathology

Surgical specimen was whitish and soft. It was formalin fixed, sectioned, and entirely embedded for histological examination and immunohistochemistry.

Immunohistochemistry

Immunohistochemistry was performed using the automatic stainer Leica Bond-Max (Leica Biosystems, Germany) and the following primary antibodies: GFAP (clone 6F2; dilution 1: 250, Dako, Denmark), OLIG-2 (clone EPR2673; dilution 1:100, Abcam, United Kingdom), IDH1 R123H (clone H09; dilution 1:40; Dianova, Germany), ATRX (clone AX1; dilution 1:100; Dianova, Germany), P53 (clone D07; dilution 1:200; Monosan, Netherlands), Ki-67 (clone MIB-1, dilution 1:100, Dako, Denmark), CD68 (clone PGM1, Dako, Denmark). Microscopic examination showed brain parenchyma with diffuse vacuolization, calcifications and a huge number of foamy macrophages. Glial cell density was

increased, with some cells showing mild nuclear atypia. We observed microvascular proliferation, necrosis and vessels with thrombosis or hyalinization. Focal neuronal eosinophilic degeneration was seen as well.

GFAP staining highlighted that increased glial cell density was due to reactive astrocytosis. Olig-2 staining was positive in the cells with mild nuclear atypia, confirming their glial nature. No cells were stained by anti-IDH1 R132H antibody in the whole tissue. P53 and Ki-67 were positive in few atypical cells. CD68 stain highlighted diffuse macrophage infiltrate in the tissue.

In view of the absence of IDH1 R132H stain (that was positive in the primary tumor), low Ki-67 labelling index and vascular hyalinization, we interpreted the histopathological findings as late effects of chemo-radiotherapy treatment and the clinical suspicion of a recurrent tumor was not confirmed.

RESULTS

Literature review

A total of 14 papers matching the aforementioned criteria were published from 2007 to 2019. Papers corresponding to application domains were as follows: **oncological setting**, 9 articles (reported in *Table 1*); **non-oncological,** 5 articles (reported in *Table 2*). The herein presented case should be included to the oncological ones and was added to *table 1*

Figure 1 Schematically shows findings (true positives, false negatives, true negative, false positive) highlighting the evidences found in literature;

Figure 2 visually highlights main findings of the institutional case of fluorescence in radiation necrosis.

DISCUSSION

5-ALA fluorescence in non glial tumors

5AA actively accumulates in malignant glial cells, where is converted to a fluorescent substance, protoporphyrin IX (PpIX). This phenomenon has a high tumor specificity: hence 5-ALA has been adopted as a specific intraoperative marker for HGG and low grade gliomas (LGG) with anaplastic foci.

Indeed, it provides neurosurgeons with a real-time tool to distinguish malignant from normal or non-malignant tissue[4].

Several papers have described 5-ALA synergistic use with other intraoperative techniques as a means to increase EOR in HGG surgery, as later confirmed by randomized, controlled studies[3, 8, 9].

Lower evidence is present regarding LGG: only a small percentage of LGG (less than 20%) have a visible porphyrin accumulation useful for 5-ALA guided surgery. Nonetheless, it is worth mentioning the feasibility of the technique to guide surgeon to most LGG anaplastic foci as their sampling its of paramount importance.

In addition to glioma surgery, 5-ALA application has been described also in brain metastases, lymphomas, meningioma as in other non-glial tumors. These can display different degrees of fluorescence and 5-ALA has been purposed as a guide for tissue sampling or to extend resection to infiltrated white matter. However, the exact mechanism of 5-ALA metabolism and accumulation of fluorescent PpIX in these different subsets has been not yet clarified[10, 11].

False positive fluorescence in oncological cases

Few reports showed 5-ALA fluorescence as not associated to malignant tumor tissue.

Most of these papers reported the fluorescence in the area **near to the surgical cavity**, extremely close to the vital tumor cells or tumor core [12-17], but not in normal brain tissue far from the gross tumor.

As widely suggested by different studies[18-20] the presence of **peri-tumoral inflammatory** state and an **increased reactive mitotic activity** could explain those false positive findings.

Actually, specificity of 5-ALA-induced fluorescence is thought to be 100% during initial resection, unless **inflammatory cell infiltration is present**. Indeed, out of 6 reported false positives by Utsuki et al.[21], only one was a first diagnosis glioblastoma (GBM) with remarkable infiltration of neutrophils. **Responsive astrocytosis associated to surgical and radiation intervention** accounted for the remaining false positives associated with recurrent HGGs instead.

Association between false positive 5-ALA fluorescence and radiation necrosis was first as reported by Miyatake et al.[22]. Kamp et al.[23] described about 3% of radio-necrotic tissue with positive fluorescence out of 313 patients that undergone surgery for suspected relapse of GBM.

As demonstrated by Ji et al [24] reported on treatment effect-related tissue fluorescence in radiation necrosis and reactive gliosis is showed with an interval between radiation therapy and surgery ranging from 1 to 20 months.

False positive 5 ALA fluorescence findings in oncological setting were thus associated to:

1) **Immediate vicinity of tumor cells** [12-14, 25]
2) **Autofluorescence of normal brain tissue** due to peritumoral inflammation, a rare cause reported by Panciani et al [18] and later demonstrated in other papers [19]
3) HGGs cases with **high inflammatory infiltrations**[13, 21]
4) **Radiation necrosis and reactive gliosis** in patients who underwent adjuvant therapy before recurrent HGGs surgery[14, 24]

On the heels of these experiences, our report confirms that a strong 5-ALA uptake can be also observe in the specific setting of **radionecrosis / late effects of chemo-radiotherapy treatment**.

With a **histopathological view**, **infiltration of reactive astrocytes, immune cells presence and 5-ALA extracellular accumulation** can be responsible for false positive cases. Indeed, histiocytes/macrophages (which have function of phagocytosis) and lymphocytes can internalize 5-ALA: this may lead to a significant buildup of porphyrin precursors, making the tissue fluorescent. Regarding 5-ALA leakage and extracellular fluorescence accumulation, the latter is true mostly for lesions with pronounced perifocal edema[15, 21].

However, acknowledging that not all fluorescence means tumor, this information can be, nevertheless, valuable to surgeon, especially in the context of radionecrosis or pseudoprogression. In this setting, **non tumor-related 5-ALA positivity can be used as a guide to target surgical excision to areas of inflammatory infiltrations or reactive gliosis, when surgery is indicated for edema relief**. Besides, non HGG-related fluorescence can be helpful to **identify and sample inflammatory lesions**.

Finally, one aspect which needs to critically be appraised when analyzing literature concerning non HGG-related fluorescence, especially in oldest reports, is the fact neuropathological examination in many reports can actually underestimate the presence of tumor cells. As a matter of fact, in many cases, hematoxylin eosin staining depicting reactive changes only, can be actually converted to the diagnosis of infiltrating tissue after additional immunohistochemistry investigations.

False positive fluorescence in Non-oncological cases

From literature search fluorescence has been observed also in non-oncological settings, such as in multiple sclerosis[26], neurodegenerative diseases[22], infectious conditions[27-30]. Notably, all these conditions share a conspicuous number of immune cells that, as mentioned before, can display different degrees of fluorescence due to 5-ALA uptake and metabolism.

Moreover, several bacterial species have the capability to elaborate porphyrin precursors being useful cofactors for growth: this accumulation in bacterial cells has been already postulated in literature [27-30], explaining the reason behind bacterial abscesses macroscopic fluorescence.

CONCLUSIONS

As reported, 5-ALA-induced fluorescence is not limited to glioma but is also evident in non-glioma and non-neoplastic conditions. Further studies should establish the utility in various areas, as the biosynthetic mechanism of fluorescent PpIX in non-glioma tumors has not yet been fully elucidated. [38]
Our experience confirmed 5-ALA may be positive upon re-resection of glioblastoma patients following combined radio-/chemotherapy even in the absence of histologically proven recurrent cellular tumor tissue.
To conclude, a critical interpretation of the intraoperative fluorescence is mandatory in case of recurrences and in atypical cases that might hinder alternative diagnoses.

FIGURES

Figure 1: Schematic diagram stratifying 5-ALA fluorescence findings as: 1) true positives 2) false negatives 3) true negative 4) false positive, highlighting the latter with a view of the aforementioned evidences found in literature. In particular, it is emphasized how false positive findings could be related to both oncological and not oncological setting, thus pointing out the importance of a critical interpretation of intraoperative fluorescence.

Figure 2: *upper row* – MRI with gadolinium depicting the anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted at first diagnosis (*left*) and at relapse (*center*, *right*), with evidence of area suspected for disease recurrence.
Central row – intraoperative appearance of surgical cavity, showing strong 5-ALA fluorescence.
Lower row- hystopatological examination showing glial cells with mild nuclear atypia (*left)* and microvascular proliferation and necrosis at hematoxylin and eosin stain (*center*) along with abundant macrophage infiltrate positive for CD68 (*right),* findings consistent with diagnosis of radiation necrosis.

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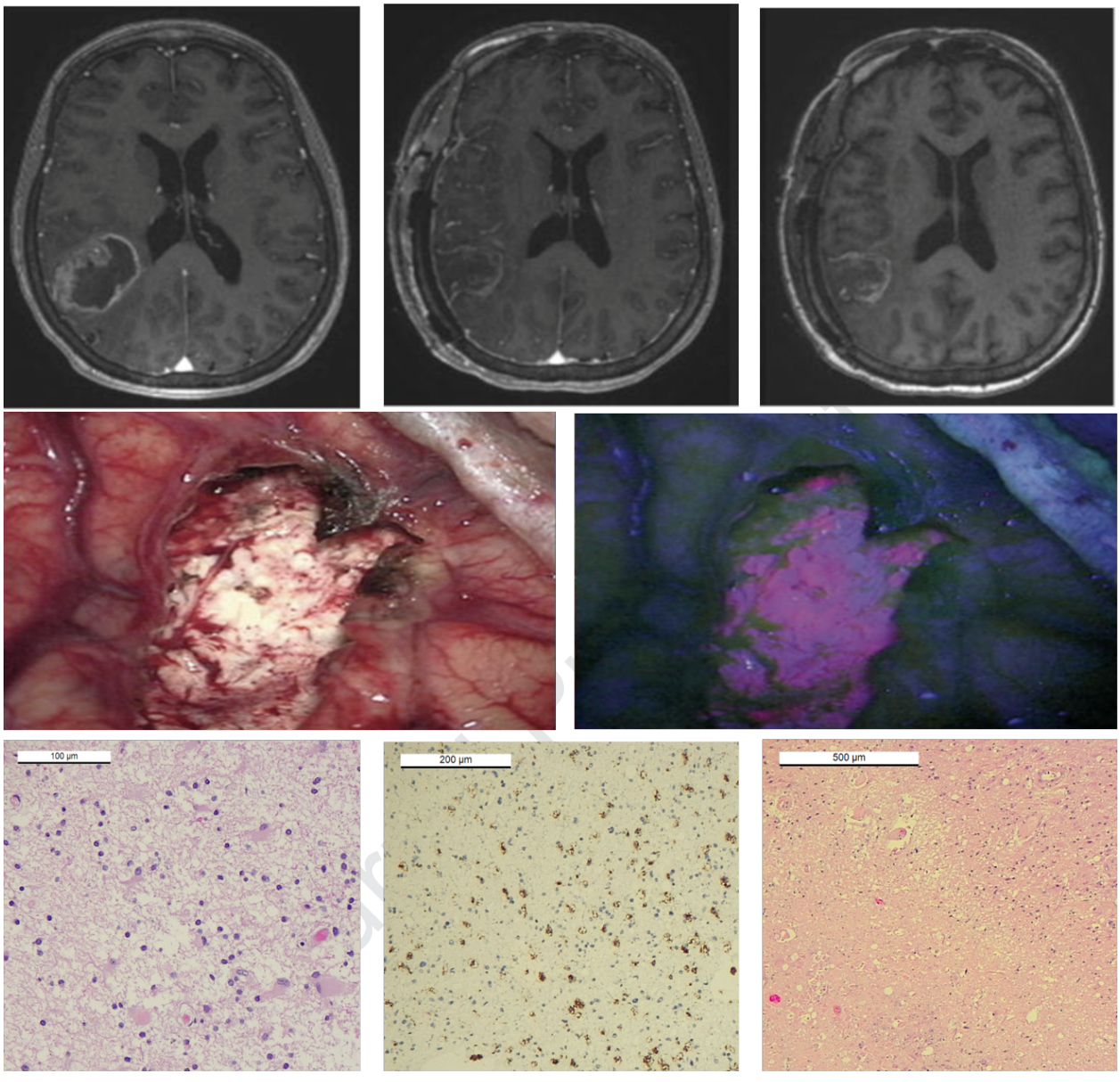
Table 1: FALSE POSITIVE FLUORESCENCE IN ONCOLOGICAL CASES

AUTHOR	YEAR	TYPE OF STUDY	NUMBER OF TOTAL CASES	FALSE POSITIVES	COMMENTS
Miyatake et al.	2007	Case series	3 [1 possible recurrence, 1 with radiotherapy for salivary duct carcinoma, 1 underwent surgery for suspected GBM]	3	2 **radionecrosis** 1 **neurodegenerative disease**
Utsuki et al	2007	Case series	42 gliomas	6 [1 first diagnosed GBM, 5 recurrent cases]	1 GBM with remarkable **inflammatory cell infiltration** 5 **recurrent HGG** with responsive astrocytosis due surgical and **radiation** intervention.
Nabavi et al.	2009	Multicenter prospective single arm uncontrolled phase II study	36 gliomas → 354 biopsies	1 patient 12 biopsy	In **biopsy based** analysis 12 sections did not reveal any tumor cells (false-positive specimens). 9 → weak fluorescence 3 → strong fluorescence **Attributable to infiltration of reactive astrocytes and macrophages, leaked and fluorescence into surrounding edema.**
Ando et al.	2010		5 → 13 biopsies	1 biopsy	No tumor cell but **increased reactive astrocytosis** in strong fluorescent area.
Roberts et al	2011	Prospective study	11 newly diagnosed GBM → 124 biopsies [86 fluorescence positive]	4 biopsies [out of 86]	3 → fully necrotic or with abnormal vasculature 1 → no abnormalities
Panciani et al	2012	Prospective study	18 suspected HGG	4	Increased reactive mitotic activity associated with the **peri-tumoral inflammation**.
Kamp et al., 2014	2014	Retrospective	303	13 [7 solid AIF, 5 vague AIF, 1 no AIF]	Solid AIF → 2 finished **adjuvant radiation** 1 and 3 months before; 5 suffered from one or more previous recurrences and had carmustin wafer implantation and previous TMZ second line chemo (4) or **re-irradiation** (1) Vague AIF → finished the **reirradiation** 4 to 9 months before 1 no AIF → multiple recurrences

					without radiation therapy
Hickmann et al, 2015	2015	Retrospective	58	7	7 false positive fluorescence according to post-operative MRI → **scar tissue and surrounding edema was observed when comparing intraoperative impression**. **No separate histological samples available** from the margins of fluorescing area, **determination of the actual sensitivity and specificity in this series was not possible.**
Lau et al., 2016	2016	Prospective phase II clinical	59 [52.5 % recurrences] → 211 biopsies	35.4% **of biopsies**	Biopsy with no tumor cells (cellularity Grade 0) → 48.4% contained **normal brain tissue** and 51.6% of biopsies contained **areas of abnormal (inflammatory) brain parenchyma**.

Table 2: FALSE POSITIVE FLUORESCENCE IN NON - ONCOLOGICAL CASES

		TYPE OF STUDY	NUMBER OF CASES	TYPE OF LESION
Nestler et al.,	2012	Case report	1	Multiple sclerosis
Voellger at al., 2014	2014	Case report	1	Listeria abscess
Solis et al,	2017	Case report	1	Cryptococcoma
Omoto et al, 2017	2017	Case report	1	Inflammatory infiltrates
De Laurentis et al, 2019	2019	Case report	1	Aggregatibacter bacterial abscess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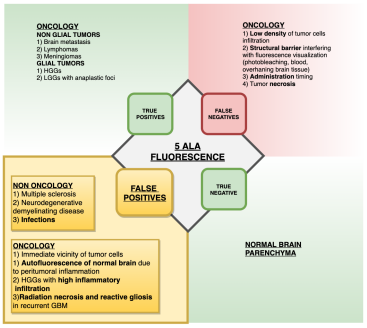

ABBREVIATIONS

5-ALA 5 aminolevulinic acid

EOR Extent of Resection

HGG High Grade Gliomas

GBM Glioblastoma

LGG Low Grade Gliomas

MRI Magnetic Resonance Imaging

PCV Procarbazine, Lomustine and Vincristine

5-ALA *'False Positives'* in cerebral neurooncology: not all that fluorescences is tumor. A case-based update and literature review.

Declaration of interests

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

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