Is Visible Aminolevulinic Acid-Induced Fluorescence an Independent Biomarker for Prognosis in Histologically Confirmed (World Health Organization 2016) Low-Grade Gliomas?

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BACKGROUND: Approximately 20% of low-grade gliomas (LGG) display visible protoporphyrin fluorescence during surgery after 5-aminolevulinic acid (5-ALA) administration.
OBJECTIVE: To determine if fluorescence represents a prognostic marker in LGG.
METHODS: Seventy-four consecutive patients with LGG (World Health Organization 2016) were operated on with 5-ALA. Fluorescent tissue was specifically biopsied. Tumor size, age, Karnofsky index, contrast-enhancement, fluorescence, and molecular factors (IDH1/IDH2-mutations, Ki67/MIB1 Index, 1p19q codeletions, ATRX, EGFR, p53 expression, and O^6-methylguanine DNA methyltransferase promotor methylation), were related to progression-free survival (PFS), malignant transformation-free survival (MTFS) and overall survival (OS).
RESULTS: Sixteen of seventy-four LGGs (21.6%) fluoresced. Fluorescence was partially related to weak enhancement on magnetic resonance imaging and increased (positron emission tomography)PET-FET uptake, but not to Karnofsky Performance Score, tumor size, or age. Regarding molecular markers, only EGFR expression differed marginally (fluorescing vs nonfluorescing: 19% vs 5%; $P = .057$). Median follow-up was 46.4 mo (95% confidence interval [CI]: 41.8-51.1). PFS, MTFS, and OS were shorter in fluorescing tumors (PFS: median 9.8 mo, 95% CI: 1.00-27.7 vs 45.8, 31.9-59.7, MTFS: 43.0 [27.5-58.5] vs 64.6 [57.7-71.5], median not reached, $P = .015$; OS: 51.6, [34.8-68.3] vs [68.2, 62.7-73.8], $P = .002$). IDH mutations significantly predicted PFS, MTFS, and OS. In multivariate analysis IDH status and fluorescence both independently predicted MTFS and OS. PFS was not independently predicted by fluorescence.
CONCLUSION: This is the first report investigating the role of ALA-induced fluorescence in histologically confirmed LGG. Fluorescence appeared to be a marker for inherent malignant transformation and OS, independently of known prognostic markers. Fluorescence in LGG might be taken into account when deciding on adjuvant therapies.

KEY WORDS: Diffuse astrocytoma, 5-ALA, Fuorescence-guided resection, Malignization transformation-free survival (MTFS), PET, Low-grade glioma

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Five-aminolevulinic acid (5-ALA) induces the accumulation of fluorescing porphyrins in malignant glioma tissue and has recently been approved by the US Food and Drug Administration for fluorescence-guided resection of these tumors. Porphyrins can be

ABBREVIATIONS: 5-ALA, -aminolevulinic acid; **CI,** confidence interval; **DNA,** deoxyribonucleic acid; **LGG,** low-grade gliomas; **EoR,** extent of resection; **MGMT,** O^6-methylguanine DNA methyltransferase; **FET-PET,** fluoroethyl-L-tyrosine positron emission tomography; **KPS,** Karnofsky performance score; **MLPA,** multiplex ligation-dependent probe amplification; **MR,** magnetic resonance; **MRI,** magnetic resonance imaging; **MTFS,** malignant transformation-free survival; **OS,** overall survival; **PFS,** progression-free survival

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visualized with specially adapted surgical microscopes during surgery.[1-13]. In gliomas harboring anaplastic foci, regions with a higher proliferation rate can be identified by the accumulation of fluorescence, which can be specifically interrogated pathologically.[7,13,14] Spectrographically detectable porphyrin fluorescence has been related to malignancy in gliomas.[15]

In confirmed low grade diffuse gliomas (LGG, WHO grade II), on the other hand, visible fluorescence can be detected in only about 20% of patients.[7,13,14,16] In an earlier study LGG with fluorescence were found not to differ histologically from LGG without fluorescence.[14] The reasons for the nonuniform accumulation in LGG are not understood. Simply assuming porphyrin accumulation to be a hallmark of malignancy may be erroneous, as benign tumors such as meningeomas,[15,17-20] or grade II ependymomas have been found to accumulate fluorescence.[21-24] We therefore questioned whether patients with LGG with visible fluorescence have a prognosis different to that of patients without fluorescence. We interrogated our database regarding LGGs operated on using 5-ALA to determine prognosis, and related prognosis to known clinical and molecular factors.

METHODS

Consecutive patients entered into a prospective data base formed the basis of this study. This retrospective analysis was approved by the ethical committee of the University of Münster (reference: 2014-560-f-N). Due its retrospective nature, no informed consent was available or necessary. However, informed consent had been obtained from all patients regarding the use of their tissues for scientific purposes prior to their entry into the data base.

We identified 74 consecutive patients operated on using 5-ALA between October 2010 and January 2016 with a final diagnosis of WHO 2016 grade II glioma, for whom follow-up data greater than 3 mo was available.

Surgery

We have adapted a generous policy toward using 5-ALA in patients with assumed LGG since a number of these tumors will ultimately turn out to harbor grade III pathologies, especially if there is any weak or indistinct contrast-enhancement. It has previously been demonstrated that ALA induced fluorescence can be used to detect anaplastic foci in tumors that appear as low grade lesion on the magnetic resonance imaging (MRI).[13,14,16,25]

Patients received 5-ALA (Gliolan®, medac, Wedel, Germany) at a dose of 20 mg/kg dissolved in 50 mL of tap water 3 to 4 hr prior to induction of anaesthesia.[11] During surgery surgeons frequently toggled between white light and the fluorescence mode of the microscope (OPMI Pentero, Carl Zeiss Meditech, Oberkochen, Germany, equipped with the BLUE400 fluorescence option) for detecting fluorescence. Neuronavigation was used to correlate areas with weak enhancement on MRI or regions with increased uptake ratios, as derived from O-(2-[^{18}F]-fluoroethyl)-L-tyrosine positron emission tomography (FET-PET). Neuronavigation was used as early as possible in order to minimize brainshift. Fluorescence, if present, was either found in a diffuse fashion throughout the tumor, or focally (Figure 1) and specifically biopsied. Fluorescence was confirmed visibly after extraction of tissue before securing samples in prepreparedvials for processing.

Surgery was only performed by 1 of 3 surgeons (W.S., C.E., J.W.).

Preoperative Imaging

Magnetic resonance (MR) imaging and FET-PET imaging were performed as described in detail previously.[14,26]

Neuropathology

Tumor tissues were graded in accordance with 2016 WHO criteria.[27] Neuropathologists were blinded as to whether tumors had fluoresced during surgery. The Ki-67/MIB-1 proliferation index, ATRX expression, EGRFR expression and the IDH1 (R132H) mutation status were determined using immunohistochemistry.[28] All tumors showing negative staining for mutated IDH1 (R132H) protein were sequenced for non-R132H-IDH1 and IDH2 hotspot mutations.[29] In tumors with possible oligodendroglial differentiation, 1p/19q co-deletions were determined by multiplex ligation-dependent probe amplification (MLPA) using probes (SALSA MLPA P088 Oligodendroglioma 1p-19q probemix) and protocols provided by the manufacturer (MRC Holland, Amsterdam, The Netherlands) in all tumors, in which an oligodendroglial differentiation was suspected histopathologically.

O^6-methylguanine DNA methyltransferase (MGMT) promoter methylation status was determined by methylation-specific polymerase chain reaction of bisulfite converted deoxyribonucleic acid (DNA) (EZ DNA Methylation-Gold Kit; Zymo Research, Orange, California) as previously described.[30]

Clinical Data

We recorded age, gender, Karnofsky Performance Score (KPS), extent of resection (EoR) based on the MRI fluid-attenuated inversion-recovery image, and presence of weak contrast-enhancement on preoperative MRI. Treatment with radiotherapy or chemotherapy after initial surgery (adjuvant therapy) was documented.

Treatment decisions regarding adjuvant therapies were in all cases based on evaluations in our multidisciplinary tumor board. In the face of progression or malignant degeneration patients were treated according to accepted guidelines.[31]

Patients were followed for overall survival (OS), progression-free survival (PFS), or malignant transformation-free survival (MTFS). Progression was defined according to RANO criteria by an independent neuroradiologist and was based on imaging with or without histological corroboration.[32] PFS consequently incorporated increases in size, malignant degeneration, or death. Malignant degeneration was only assumed when histology, obtained during surgery or from stereotactic biopsy, proved high-grade glioma pathology. Tissue was collected in all patients in whom malignant degeneration was assumed by resection or stereotactic biopsy.

The present cohort partially coincides with patients analyzed for an earlier study without follow-up, but included only patients from that cohort in whom follow-up data of >3 mo were available and that were not lost to follow-up.

Statistical Methods

Commercially available software (Statistical Package for Social Sciences, version 23.0; IBM Inc, Armonk, New York) was used for all statistical analyses. The Person Chi^2 square statistic was calculated for categorial variables. Difference in average values for independent

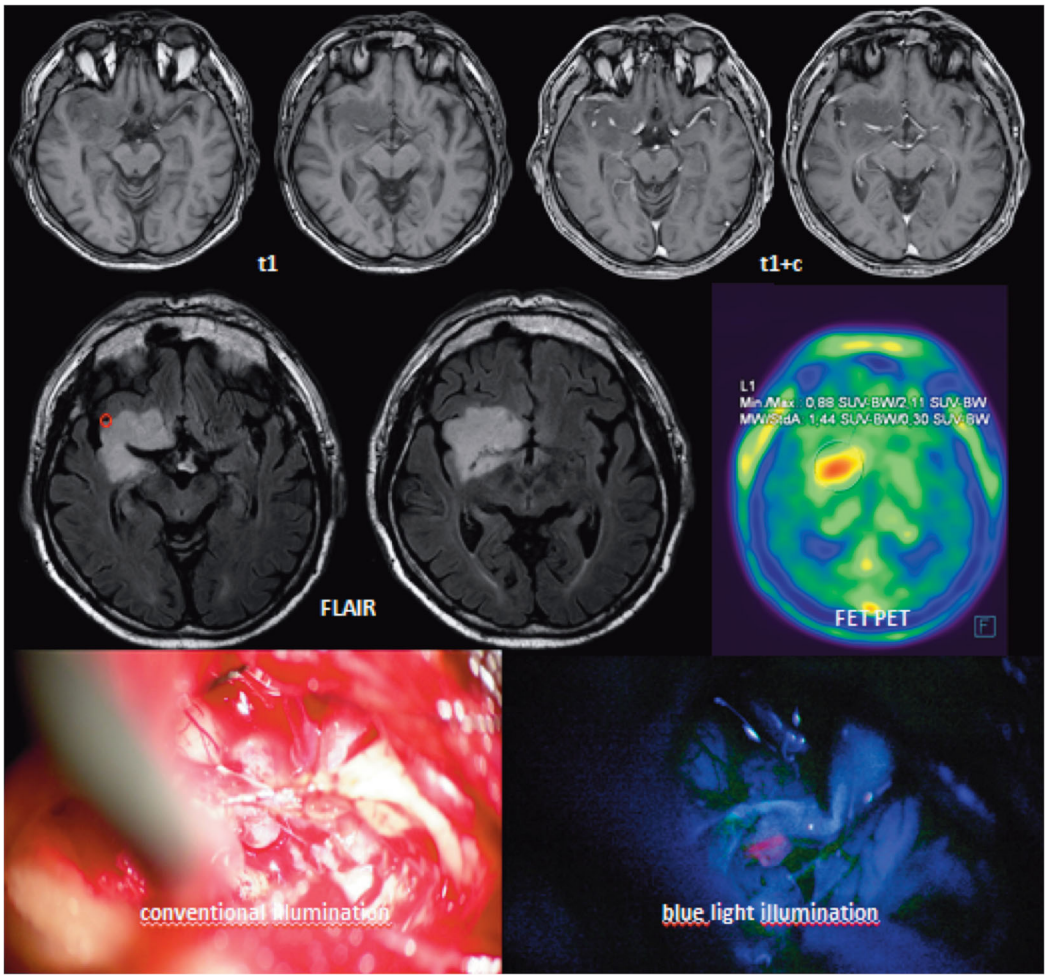

FIGURE 1. *Example for MRI of a LGG glioma of the right insula and perisylvian structures (top row: t1 + /− GD, middle row: FLAIR images and representative FET-PET image). Bottom row left: intraoperative white light image of insula viewed from top with partially resected tumor, right: blue white image. Note greenish fluorescence of insular M3 branch of middle cerebral artery. The circle on the FLAIR image marks the region of focal fluorescence encountered during surgery.*

groups were test using analysis of variance for testing the significance of differences in mean values between independent groups. For testing univariate differences in outcome data, the Kaplan–Meier estimator was employed with the log rank test statistic for assessing significance for categorical variables and cox regressions for continuous variables. Cox regressions with stepwise forward inclusion were used for testing the effects of multiple variables in time to event analyses. Multinomial logisitic regression was employed for testing possible relationships between independent variables and fluorescence as outcome. We assumed a 2-sided error probability level *P* of less than .05 to indicate significance.

RESULTS

Patient and Imaging Characteristics

A typical case example is given in Figure 1, which summarizes MR, PET, and intraoperative findings in a patient with an IDH1 mutated diffuse astrocytoma. MRI indicated weak, spotty enhancement. Fluorescence was found in a focal distribution. Pathologically, no differences were detected between fluorescing and nonfluorescing tissue. The patient harbored an IDH1 mutated diffuse astrocytoma WHO (2016) grade II. Both a sample from fluoresceing as well as a sample from nonfluorescing tumor tissue had a MIB-Index of approximately 1%.

Table 1 summarizes the clinical characteristics of patients stratified by the presence or lack of fluorescence. Among 74 patients fluorescence was observed in 16 (21.6%) cases. No significant differences were noted between patients with fluorescing and nonfluorescing tumors regarding age, tumors size, gender, and EoR. Table 2 summarizes the results of our molecular analyses stratified by fluorescence findings. 1p19q co-deletions/IDH1

TABLE 1. Clinical Characteristics

							ALA									
		All (n = 74)					No fluorescence (n = 58)					Fluorescence (n = 16)				
Variable		Avg	SD	M	n	n/N	Avg	SD	M	n	n/N	Avg	SD	M	n	n/N	P[a]
Age		42.8	13.4	41.0			42.8	13.1	41.0			43.0	14.7	41.1			.956
Gender	male				42	56.8%				35	60.3%				7	43.8%	.236
	female				32	43.2%				23	39.7%				9	56.3%	
KPS	80				4	5.4%				3	5.2%				1	6.3%	.030
	90				12	16.2%				6	10.3%				6	37.5%	
	100				58	78.4%				49	84.5%				9	56.3%	
Volume	ccm	11.4	20.1	6.00			13.0	22.5	6.00			5.79	3.16	6.0			.207
Enhancement	no				56	75.7%				49	84.5%				7	43.8%	.001
(weak, indistinct)	yes				18	24.3%				9	15.5%				9	56.3%	
FETmax[b]		2.45	1.29	2.35			2.24	1.29	2			3.21	0.952	3			.009
EoR	>90%				24	32.4%				21	36.2%				3	18.8%	.187
	<90%				50	67.6%				37	63.8%				13	81.3%	
Volume	ccm	11.4	20.1	6.00			13.0	22.5	6.00			5.79	3.16	6.0			.207
Adj.	none				39	52.7%				32	55.2%				7	43.8%	.335
Cytotoxic	chemo				9	12.2%				8	13.8%				1	6.3%	
Therapy	RT				24	32.4%				16	27.6%				8	50.0%	
	missing				2	2.7%				2	3.4%				0	0.0%	

[a]Chi[2] for categories and ANOVA for continuous data; avg, average; SD, standard deviation; M, median; EoR, extent of resection; KPS, Karnofsky Performance Score; ccm, cubic centimeters; FETmax, maxium standard uptake ratio; RT, radiotherapy; chemo, chemotherapy; [b]n = 70 patients; non-fluorescing tumors: 55, fluorescing tumors 15.

TABLE 2. Molecular Data

							ALA									
		All (n = 74)					No fluorescence (n = 58)					Fluorescence (n = 16)				
		Avg	SD	M	n	n/N	Avg	SD	M	n	n/N	Avg	SD	M	n	n/N	P[a]
MIB-index %		4.3	4.0	3.0			4.0	3.3	3.0			5.3	5.9	4.0			.242
WHO 2016 oligo					12	16.2%				9	15.5%				3	18.7%	.859
DA	IDH wildtype				16	21.6%				12	20.7%				4	25.0%	
	IDH mutated				46	62.1%				37	63.8.%				9	56.3%	
EGFR expression	yes				6	8.1%				3	5.2%				3	18.8%	.057
	no				53	71.6%				44	75.9%				9	56.2%	
	missing				15	20.3%				11	18.9%				4	0.25%	
P53 mutation	yes				22	29.7%				16	27.6%				6	37.5%	.294
	no				43	58.1%				36	62.1%				7	43.7%	
	missing				9	12.2%				6	10.3%				3	18.8%	
ATRX loss	yes				20	27.0%				18	31.0%				2	12.5%	.100
	no				38	51.4%				27	46.6%				11	68.8%	
	missing				16	21.6%				13	22.4%				3	18.7%	
MGMT meth.	yes				40	54.1%				33	56.9%				7	43.8%	.813
	no				20	27.0%				16	27.6%				4	25.0%	
	missing				14	18.9%				9	15.5%				5	31.2%	

[a]Chi[2] for categories and ANOVA for continuous data; avg, average; SD, standard deviation, M, median; DA, diffuse astrocytoma; oligo, oligodendroglioma (1p19q co-deleted); EGFR,epidermanl growth factor receptor; MGMT meth, meythlation of the MGMT promotor gene.

mutated tumors were observed in 12 patients (16.2%), indicating oligodendroglioma according to the 2016 WHO classification. Of the remaining 62 diffuse astrocytomas, IDH1 mutations were observed in 46 (74.2%) cases. No IDH2 mutations were observed. Median MIB-Index was 4.3% in the pooled cohorts, which did not differ between fluorescing and nonfluorescing tumor samples. No significant differences were found regarding p53, ATRX expression or MGMT promotor methylation. Interestingly, EGFR expression was three times more common in fluorescing LGG than in nonfluorescing LGG, but not significantly so (19 vs 5%; $P = .057$).

We did observe differences in the distribution of KPS, with more patients in the group with tumor fluorescence having a KPS of 90 than in the group of patients without fluorescence.

Overall, in 18 (24.3%) tumors, weak, indistinct, or spotty enhancement was observed on the MRI. No strong enhancement was observed. Patients with tumors showing fluorescence more frequently had MR images with such weak contrast-enhancement (9/16 patients, 56.3% vs 9/49, 15.5%, $P = .001$).

Patients with intraoperative fluorescence had a slightly increased PET maximal standardized uptake value (SUV) ratio of 3.21 ± .952 (SD) compared to patients without fluorescence 2.24 ± 1.29 ($P = .009$).

No differences regarding adjuvant therapies after initial surgery (radiotherapy or chemotherapy) were observed.

Multivariate Analysis of Factors Predicting Fluorescence

We considered contrast-enhancement, tumor volume, 1p19q co-deletions, IDH1, MIB1 Index, and the FET-PET to possibly influence the accumulation of fluorescence. In multinomial logistic regression analysis only FET SUV maximum and preoperative contrast-enhancement predicted fluorescence independently (Table 3).

TABLE 3. Factors Affecting Fluorescence

Factor/covariate	HR	95% CI	P
Volume[a]	1.09	0.894-1.335	.388
Max. SUV ratio PET[a]	2.103	1.04-4.26	.039
1p19q	2.88	0.402-20.6	.293
IDH1 mutation	2.74	0.562-13.3	.213
MIB index	0.903	0.785-1.040	.157
Enhancement	8.31	1.83-37.7	.006

[a]Continuous.

Univariate Outcome Analyses

Median follow-up was 46.4 mo (95% CI: 41.8-51.1).

Kaplan–Meier (univariate) analysis showed WHO grade II subtypes (IDH mutated diffuse astrocytomas, IDH wildtype diffuse astrocytomas, IDH mutant/1p19q codeleted oligodendrogliomas) to predict PFS, MTFS, and OS to varying extents (Figure 2A-2C).

Patients with IDH1 wild type diffuse astrocytomas tumors tended to have a shorter PFS compared to IDH 1 mutated tumors (median, 95% CI: 16.0, 0,0-47.2 vs 50.7, 38.0-63.3, $P = .099$). Regarding MTFS and OS, median survivals were not reached in either group. MTFS was shorter in IDH 1 wild type tumors than in tumors with IDH1 mutations (median not reached; average, 95% CI: 39.0, 25.6-52.4; 64.6 vs 57.3-71.9 mo, $P = .003$). Similarly, average OS was shorter for patients with IDH1 wildtype tumors (49.0, 32.3-65.6 mo) compared to IDH1 mutated tumors (71.5, 64.4-78.6 mo, $P = .004$).

Prognosis in 1p19q codeleted/IDH mutant oligodendrogliomas was similar to IDH mutated diffuse astrocytomas (median PFS: 24.4, 19.8-29.0, average MTFS: 62.3, 55.9-68.7; average OS: 52.2, 43.5-60.9 mo).

Importantly, fluorescence was prognostic for PFS, MTFS and OS. PFS in fluorescing was shorter in fluorescing vs. nonfluorescing tumors (9.8, 0.00-27.7 vs 45.8, 31.9-59.7 mo; $P = .019$), as was MTFS (43.0, 27.5-58.5 vs 64.6, 57.7-71.5 mo, median not reached, $P = .015$) and OS (51.6, 34.8-68.3 vs 68.2, 62.7-73.8 mo, $P = .002$; Figure 3A-3C).

On the other hand, no significant influence of contrast-enhancement, maximum PET SUV ratios or EoR on MTFS was found in univariate analysis. Average MTFS was 37.0 (39.7-43.4) mo in enhancing tumors and 60.3 (52.7-67.9) mo in nonenhancing tumors ($P = .635$). The hazard ratio for maximum PET SUV ratios for predicting MTFS, tested as a continuous variable, was 1.20 ($P = .312$). EoR had no significant influence on PFS (hazard ratio [HR]: .548, $P = .065$) OS (OS: HR .713, $P = .817$) or MTFS (HR = .636, $P = .363$). The low hazard ratios for resection indicated underpowering for this analysis.

Multivariate Outcome Analysis

For multivariate cox regression analysis of OS and MTFS we considered four factors, which were significant on univariate survival analysis, that is age, fluorescence, IDH1 satus, and weak contrast-enhancement on MRI (Table 4). We found fluorescence, IDH1 status and age to be prognostic for OS, and only IDH1 status and fluorescence to be prognostic for MTFS. Malignant transformation was highly predictive for survival (HR 5.70, $P = .001$). Figure 4 illustrates the independent effects of fluorescence and IDH1 mutation status.

DISCUSSION

Demographic and Molecular Factors

ALA-induced tumor fluorescence has been linked to malignancy in presumed LGG.[10,14,16] Previously however, looking exclusively at a population of histologically proven LGG patients, we observed a subpopulation of LGG with macroscopic fluorescence.[14] Importantly, in this series histology was based on samples taken from fluorescing tissue, and corroborated by samples from nonfluorescing tumor, if available. According to institutional policy, we took great care to specifically collect samples from any

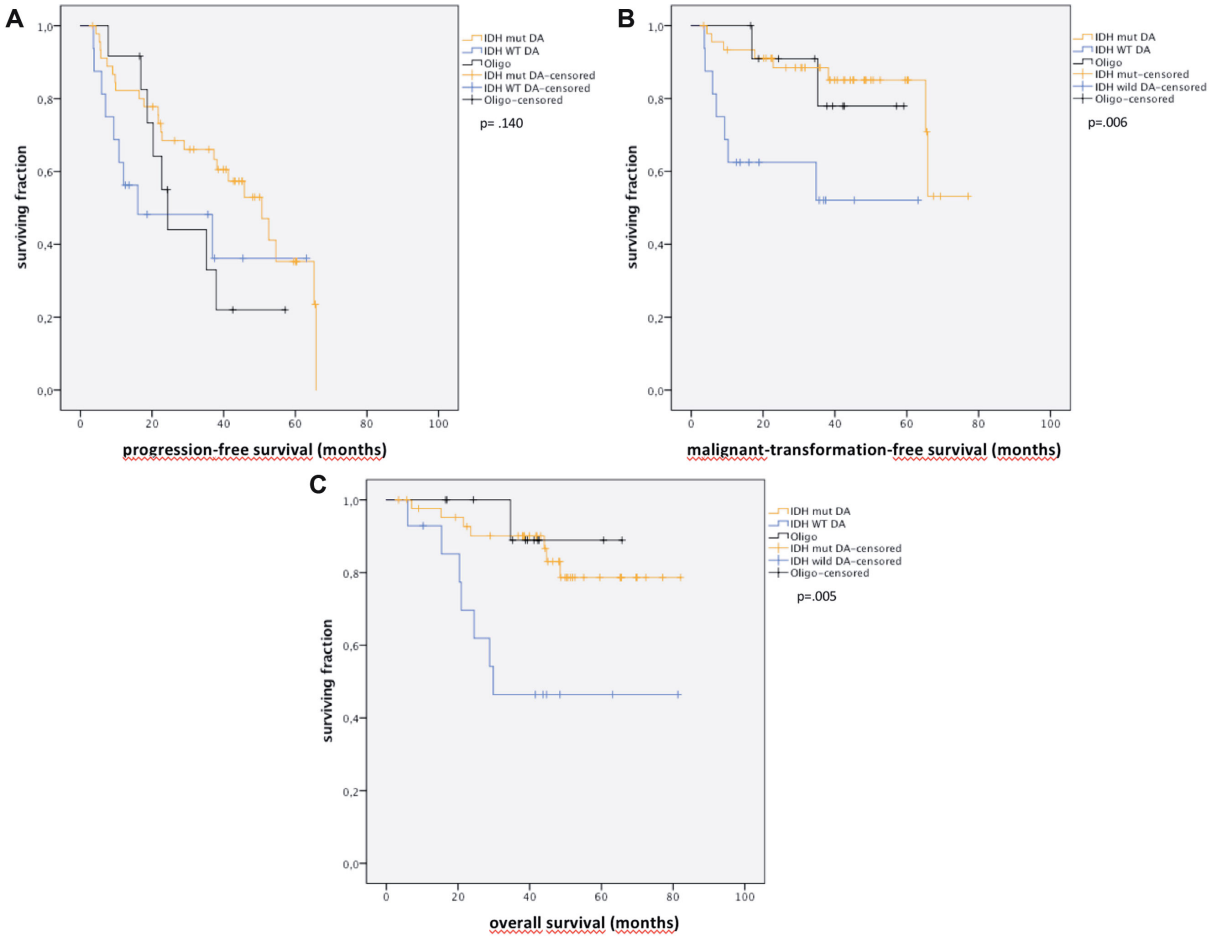

FIGURE 2. *Kaplan–Meier curves for* **A**, *PFS,* **B**, *malignant transformation-free survival, MTFS, and* **C**, *OS, stratified by WHO 2016 grade II pathology (Oligo: oligodendrglioma; IDHmut DA: IDH1 mutated diffuse astrocytoma; and IDH WT DA: IDH1 wildtype diffuse astrocytoma).*

suspicious regions based on enhancement, PET uptake or fluorescence to ensure tissue to be representative for the true tumor dignity and to rule out sampling errors in possibly anaplastic gliomas. All other extracted tissue was also given to the pathologist for histological analysis.

Fluorescence was not predicted by factors previously associated with worse prognosis, ie, higher MIB Index, IDH1 wildtype pathology or lack of 1p19q co-deletions.[33,34] These molecular factors now play an integral part in the 2016 modification of the WHO classification of brain tumors.[27] We now address the question of whether patients with fluorescing LGG differ prognostically from patients without fluorescence. We found no differences in the MIB index or light morphological features between fluorescing and nonfluorescing samples. However, we found a (marginally significant) difference in the expression of EGFR. This molecule has been associated malignancy and angiogenesis in gliomas.[35]

We observed a shorter time to malignant deterioration and a shorter survival in patients with fluorescing LGG, identifying visible fluorescence in LGGs as a possible intraoperative, independent biological marker for incipient unfavorable outcome.

Given the discussions regarding that patients with LGG are to be considered high risk,[36] the observation of such fluorescence supports the recommendation of adjuvant cytotoxic therapies and shorter surveillance intervals especially in IDH wildtype diffuse LGG with fluorescence. This worse prognosis was independent of 1p19q-codeletion status, age, tumor size, or IDH1 mutations.

Stratified by fluorescence, our cohorts were found to be very similar. Young age and generally favorable KPS (90 or 100) are characteristic for LGG patients, as were the low median MIB values and the high fraction of patients with IDH1 mutated tumors. We found no IDH2 mutations, which are much rarer than IDH1 mutations. KPS, although somewhat different in our

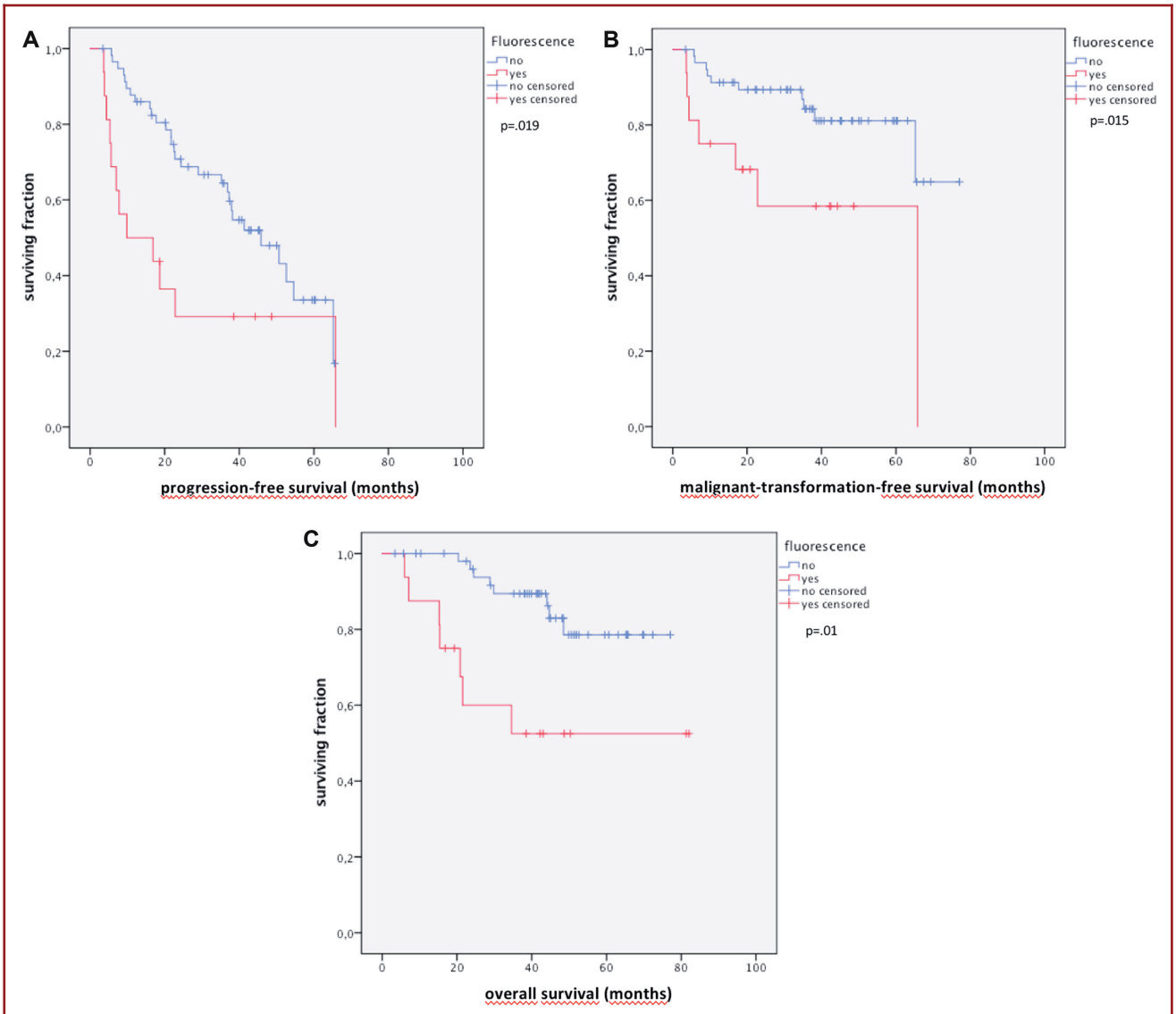

FIGURE 3. *Kaplan–Meier curves for* **A**, *PFS,* **B**, *MTFS, and* **C**, *OS, stratified by the presence of tumor fluorescence.*

cohorts (but with minor exceptions either 90 or 100), did not influence outcomes. EoR was similar in both groups. We did not find a significant influence of EoR on outcome, as defined by PFS, MDFS, or OS. The respective hazard ratios of .548, .636, and .71, indicted that our cohorts were most likely underpowered for corroborating existing assumptions on the influence of EoR on outcome in LGG.[37,38]

Due to the similarities in prognostic variables between groups, adjuvant therapies were very comparable, consisting of observation in over 50% of cases, and radiotherapy or chemotherapy in the remaining cases, as expected. The observation of fluorescence did not lead to different adjuvant therapies in this series, which might have confounded our results.

In sum, therefore, we regard fluorescence to be an early indicator of malignant transformation, possibly related to EGFR expression and incipient angiogenic changes, which can not necessarily be determined by enhancement, higher uptake of amino acids, conventional light microscopy, or immunohistochemistry.

Mechanisms of Fluorescence Accumulation in LGG

Three mechanisms might be considered regarding fluorescence accumulation in a subgroup of low-grade glioma. Firstly, it has been suggested that IDH1 mutations might negatively influence porphyrin accumulation after in vitro exposure of tumor cells

TABLE 4. Cox Regression Analysis of Factors Predicting Outcomes in Univariate Analysis.

Factor/covariate	PFS HR	0.000[b] P	MDFS HR	0.003[b] P	OS HR	0.000[b] P
Age	1.01	.294	1.03	.135	1.06	.011
Enhancement	3.09	.012	1.34	.673	3.07	.062
IDH1 wildtype	5.30	.000	3.17	.043	5.007	.006
Visible fluorescence	1.45	.312	4.44	.010	3.60	.026
Max. SUV ratio PET[a]	1.41	.009	–	–	–	–

[a]maximal standardized uptake ratio on FET-PET; univariate only significant for PFS.
[b]omnibus test for analysis.

to 5-ALA[39] because energy metabolism is impaired in IDH1 mutant cells. However, we found no relationship between visible fluorescence and IDH1 mutation status. Our observation does not necessarily contradict the in vitro data, since ALA does not cross the intact blood brain barrier and entry of ALA into the brain is a prerequisite of ALA metabolism by tumor cells.[40]

Secondly, fluorescence accumulation may be the result of very early angiogenic changes, a hallmark of malignancy. We observed a relationship between fluorescence, weak contrast-enhancement on the MRI and FET-PET, respectively, as summarized in Table 3. The common denominator may well be initial changes in vascular permeability, intravascular volume or vessel density within tumors. ALA for itself does not cross the blood-brain barrier,[40] whereas FET uptake appears to depend on a blood-brain barrier bound L-type amino acid transporter.[41] ALA derived porphyrins are observed if FET uptake in tumors exceeds

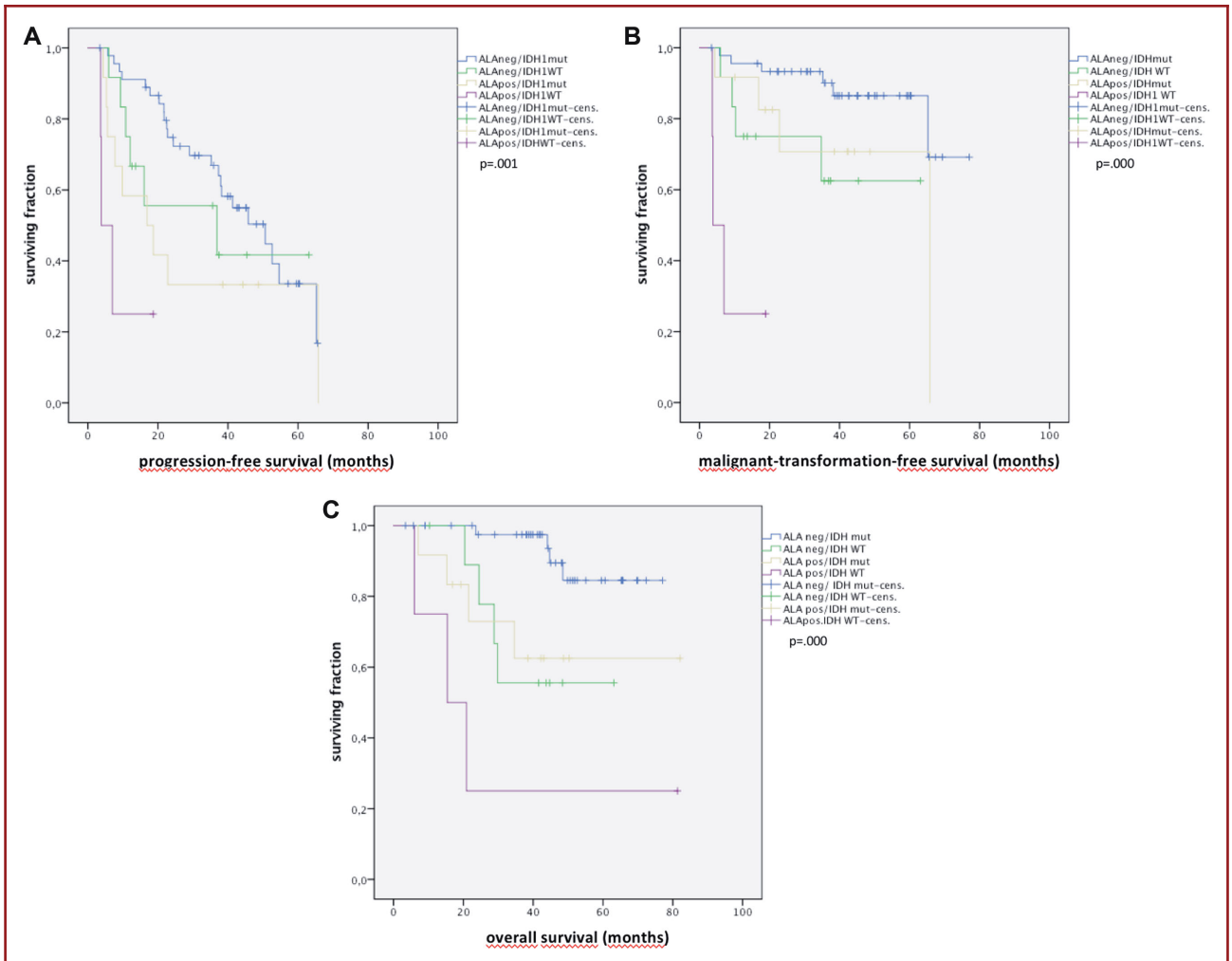

FIGURE 4. *Kaplan–Meier curves for* **A**, *PFS,* **B**, *MTFS, and* **C**, *OS, stratified by IDH1 mutation (mutated: IDHmut, wildtype: IDHWT) and presence of fluorescence (fluorescing: ALApos, nonfluorescing: ALAneg) demonstrating the independent influence of both factors on outcome.*

a certain level,[14,25] suggesting permeability changes to play a role for increased accumulation of both amino acids in tumors with early angiogenic changes. The threshold of FET uptake for predicting fluorescence in our cohort was 1.85. The values for 1.37 were observed by Stockhammer et al[25] in their cohort, which also contained a number of high-grade gliomas.

Thirdly and finally, porphyrin accumulation in response to ALA has been linked to proliferation, cell cycle, and abnormal heme metabolism in tumors.[42-44] However, cellular mechanisms of accelerated porphyrin synthesis can only become operational when ALA crosses a mildly dysfunctional blood-barrier, even if permeability increases do not have to be great to allow passage of ALA, which has a molecular weight of 131 Daltons, again supporting the permeability hypothesis.

Overall, the underlying mechanisms for FET and ALA uptake and ALA metabolism in LGG require further study.

Outcome Analyses

In multivariate analysis, PFS was not independently predicted by ALA induced fluorescence, whereas MTFS and OS were, indicating a role of fluorescence for heralding malignant deterioration and ultimately death but not simple low grade tumor growth. On the other hand, FET-PET was not prognostic for MTFS. We did however observe only a limited number of events, with 17 of 74 patients (23.0%) experiencing malignant deterioration. In total,15 of 74 patients (20.2%) died during the observation period. The relatively small number of events limits our conclusions on FET-PET. Possibly a longer follow-up will further clarify the role of FET-PET maxima for OS and MTFS.

FET accumulation has been associated with worse prognosis in LGG.[45,46] and may better be assessed by dynamic PET imaging[45,47] that was not performed in this study. Maximal tumor to brain ratios of FET have been shown to correlate with outcome with and without IDH1/2 mutations, and depend mostly on WHO grade.[48] In our cohort we only studied patients with LGG and not HGG.

Limitations

Overall, we acknowledge the comparably small number of patients as a limitation of this study. Also, we did not objectively determine porphyrin fluorescence using spectrometry[12,15,49] that might be considered another limitation. Spectrometry allows quantitative estimations of tissue Protoporphyrin IX (PPIX) concentrations rather than relying on than visual identification of fluorescent regions alone. Low concentrations of PPIX in tumor not visible to the eye cannot be ruled out even in completely non-conspicious LGG without visible fluorescence as previously demonstrated by Sanai et al, 2011.[50] However, in routine use, fluorescence-guided resections rely on optical visualization. Thus, the conclusions from our present observations will still be of value for the practicing neurosurgeon, and fluorescence quantification would not add further value regarding the prognosis and treatment of LGG.

We acknowledge the retrospective nature of our study to limit our conclusions, as well as the relatively short follow up of 46 mo in the context of LGG. Also, we did not gather dynamic information on FET-PET, which might have better reflected the true role of FET-PET in predicting porphyrin accumulation or outcome.

CONCLUSION

Together, our cohort of LGG patients with visible fluorescence during surgery had a worse prognosis than those without visible fluorescence, independently of known molecular factors. This observation, which should be prospectively validated, may justify more aggressive adjuvant therapies in this distinct subgroup. Porphyrin accumulation requires a certain level of blood-brain barrier permeability increase and appears to herald malignant degeneration prior to changes becoming evident on histology.

Disclosures

Dr Stummer has received consultant and speaker's fees from medac and speakers fees from Zeiss, NXDC, Leica. 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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COMMENTS

In this study, the authors present data using 5-ALA fluorescence during resection of low-grade gliomas and report a correlation between visible fluorescence and both overall survival and progression to a more malignant phenotype. The role of 5-ALA fluorescence has been well-documented in the resection of high-grade gliomas. However, its role in low-grade gliomas remains to be fully elucidated.

Approximately 16 (or 20%) low-grade gliomas in this study displayed fluorescence. Fluorescence was partially related to weak enhancement on MRI and increased uptake in PET. In this cohort with a median follow-up period of 46.6 months, low-grade gliomas with visible fluorescence had shorter overall survival (51.6 months vs 68.2 months) and had a shorter time to malignant transformation (43 months vs 64 months). Statistical analysis revealed fluorescence to be an independent predictor of both overall survival and malignant transformation-free survival. This is an interesting finding with potential clinical implications especially in cases where the aggressiveness of subsequent treatment may be nuanced. Overall, this study augments the body of literature on the role of 5-ALA fluorescence in low-grade gliomas.

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The current article is timely as it will add to the growing use of fluorescence to guide glioma surgery, and suggests that (ALA-positive, ALA +) fluorescence can be viewed as an independent biomarker for prognosis in histologically confirmed low grade gliomas (LGGs). This interesting, well-done, and provocative study will expand the value of fluorescence-guided surgery in the surgeon's armamentarium. The work, however, raises a few questions:

1) In the ALA + tumors, were these really LGGs? Or were these anaplastic foci, representing early malignant transformation, not yet detectable by histology, but detectably by pathophysiology?[1]

2) It is known that contrast enhancement is the functional byproduct of angiogenesis, with the formation of permeable, new vessels and breakdown of the blood-brain barrier.[2] Likewise, there is an "angiogenic switch" in the stepwise transformation of LGG to secondary glioblastomas.[3] The authors found no relationship to known prognostic molecular factors (MIB, 1p19q codeletions, IDH1/2 mutations), but it would be of interest if the ALA + tumors showed increased markers of angiogenesis: microvascular density, VEGF165, CD31, CD45, Tie2, etc.

3) The authors found no correlation with IDH-1 protein (R132) or proliferative markers (Ki67/MID1), but other determinants of malignancy, eg, TERT promoter, IL-6, EGFR, p53,[4-6] or the TCGA atlas, were not interrogated. Is it possible that there is a molecular or genomic biomarker specifically linked to ALA-fluorescence?

The current study shows that ALA-positivity may be a tool to help the surgeon exploit that opportunity and potentially eradicate a premalignant or an "in situ" malignancy within a larger zone of LGG, moving closer to the ideal of a 'preventive surgical neurooncology'.[1,7,8] The current study will also stimulate the development of other fluorescent modalities, such as indocyanine green (ICG), which is highly sensitive,[9] and could potentially detect premalignant foci within a larger LGG. Taken together, the data reported here will strengthen the argument for aggressive, complete surgical removal of LGGs whenever possible.[10] The silent evolution of LGGs to high-grade gliomas provides a window of opportunity to detect these tumors earlier[7] that the neurosurgeon can utilize to improve the outlook for patients with low-grade glioma.

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