



## 5-Aminolevulinic Acid Fluorescence Indicates Perilesional Brain Infiltration in Brain Metastases

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■ **BACKGROUND:** In glioma surgery, 5-aminolevulinic acid (5-ALA) fluorescence reflects tumor infiltration, and fluorescence-assisted resection correlates with higher removal rates and improved progression-free survival. Recent studies report that a sizable proportion of brain metastases exhibit peritumoral infiltration on the cellular level. There is little information regarding whether 5-ALA is useful to guide surgery in the peritumoral zone in metastases. The aim of this study was to assess histologically whether 5-ALA fluorescence accurately reflects metastatic brain infiltration.

■ **METHODS AND MATERIALS:** Fluorescence-assisted tumor resection was performed in 27 patients with brain metastases. Patients received 20 mg/kg 5-ALA 3 hours before anesthesia. After resection, biopsy specimens of the surrounding parenchyma were analyzed for 5-ALA fluorescence and histologic evidence of infiltrating tumor cells. The correlation between 5-ALA positivity and immunohistochemical evidence of tumor in the peritumoral zone was also assessed.

■ **RESULTS:** Of 27 metastases, 23 (85%) were 5-ALA positive. For qualitative tissue analysis, 110 of 125 samples were collected. Metastatic infiltration was present in 49 samples with faint or red fluorescence; 33 samples without fluorescence were tumor-free. The presence of metastatic infiltration correlated with fluorescence ( $P < 0.001$ ). Tumor

infiltration correlated with fluorescence (blue fluorescence  $0.09\% \pm 0.04\%$  and red or faint fluorescence  $3.26\%$ ;  $P = 0.003$ ).

■ **CONCLUSIONS:** Infiltration of surrounding brain tissue is a common finding in brain metastases in selected primary tumors. 5-ALA fluorescence correlates with tumor cell infiltration and might guide more radical resection.

### INTRODUCTION

The prognosis for patients with metastatic brain disease is grim. About 1 in 10 patients with advanced solid tumors develop brain metastases over the course of their disease. We and others recently demonstrated that the majority of brain metastases infiltrate the adjacent brain tissue.<sup>1-5</sup> Most importantly, we demonstrated that this infiltration correlated with a worse prognosis compared with metastases without any evidence of infiltration. Most remarkable was the difference for 2-year overall survival, with only 6.6% of the infiltrative cohort surviving versus 43.5% of cases with only displacing growth.<sup>6</sup>

Surgical resection in an oligometastatic situation with up to 3 metastatic lesions has been described as effective.<sup>7-9</sup> In palliative settings with multiple metastases, resection of single foci might be useful to control leading neurologic symptoms or if a mass effect is present, particularly in the case of infratentorial metastatic masses. However, after gross total tumor resection without

### Key words

- 5-ALA
- Brain metastasis
- Brain tumor
- Fluorescence-guided surgery
- Histology
- Tumor infiltration

### Abbreviations and Acronyms

**5-ALA:** 5-Aminolevulinic acid  
**NSCLC:** Non—small cell lung cancer

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**Table 1.** Tumor Types, Fluorescence, and Biopsy Results

Tumor Type	Number of Tumors	5-ALA Fluorescence	Number of Biopsies	Number of Tumor-Positive Biopsies
NSCLC	10 (37%)	7 (70%)	14	8 (57%)
SCLC	3 (11.1%)	3 (100%)	39	17 (44%)
Squamous cell Ca	1 (3.7%)	1 (100%)	7	6 (86%)
Breast Ca	4 (14.8%)	4 (100%)	12	8 (67%)
Carcinoma	1 (3.7%)	1 (100%)	6	4 (67%)
Adeno CUP	1 (3.7%)	1 (100%)	3	3 (100%)
Adeno GI	4 (14.8%)	3 (100%)	18	9 (50%)
Melanoma	3 (11.1%)	3 (100%)	8	4 (50%)

NSCLC, non–small cell lung cancer; SCLC, small cell lung cancer; Ca, cancer; Adeno, adenocarcinoma; CUP, cancer of unknown primary; GI, gastrointestinal.

radiotherapy, local recurrence rates reach 57% at 12 months<sup>7</sup> owing to suboptimal resection.<sup>10</sup> Depending on the tumor's origin, metastases infiltrate the adjacent brain parenchyma, and thus infiltrative islets could well be the origin of local recurrence.<sup>6</sup> Addressing this infiltration zone might improve local metastatic control.<sup>11</sup> In contrast to brain metastases, it has been known for a long time that infiltration must be taken into account during surgical resection of malignant glioma. Oral administration of 5-aminolevulinic acid (5-ALA) causes intratumoral accumulation of protoporphyrin IX and a red fluorescence staining that is visible when the tissue is illuminated by a filtered light spectrum of short wavelength. Use of 5-ALA prolonged progression-free survival in several studies.<sup>12,13</sup> Retrospective analyses of metastatic tissues also describe 5-ALA fluorescence brain metastasis.<sup>14–19</sup> Here we report on the prospective MetaStaSys study data including patients operated for metastatic brain disease.

## MATERIALS AND METHODS

### Patient Cohort

All patients in whom magnetic resonance imaging could not clearly differentiate between malignant glioma and metastasis received 20 mg/kg 5-ALA 3 hours before anesthesia and were included in the MetaStaSys trial. The ethical committee of the University Hospital Göttingen, Germany, authorized obtaining biopsy specimens of the perimetastatic brain parenchyma after resection of brain metastases for analysis of tumor cell infiltration. Informed consent was obtained from the patients. After

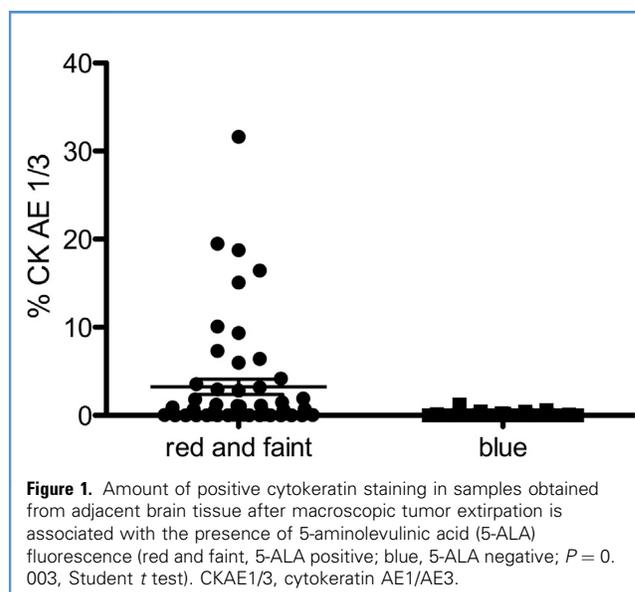
intraoperative confirmation of a metastasis by frozen section analysis, up to 8 samples of 4- to 5-mm diameter were obtained by forceps from surrounding brain tissue, provided that it was located in noneloquent tissue. Using the microscope built-in blue light filter, each sample was categorized as blue, faint red, or red, in accordance with the seminal 5-ALA trial.<sup>13</sup> Afterward the samples were fixed with 0.5% formalin and embedded in paraffin. Immunohistochemical staining of 4- $\mu$ m slices was performed using a monoclonal antibody against cytokeratin AE1/AE3 in cases of carcinoma and MelanA in cases of melanoma, as previously described.<sup>6</sup>

### Qualitative Analysis of Metastatic Infiltration in Biopsy Specimens of Adjacent Brain Parenchyma

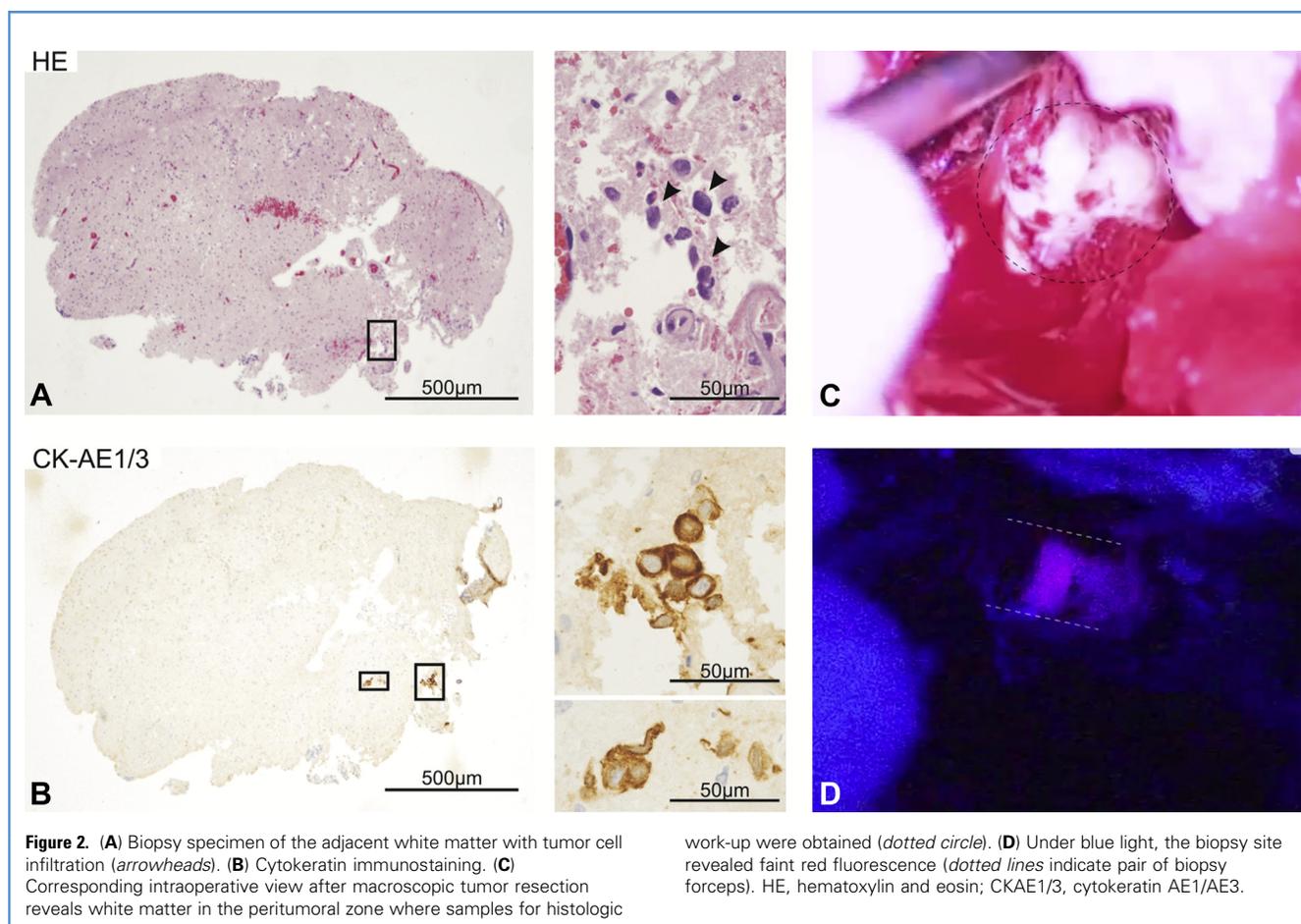
For qualitative analysis, all samples were scanned for any evidence of metastatic infiltration by an experienced neuropathologist (A.B.-F.). A sample was considered positive when metastatic infiltration was detected.<sup>6</sup>

**Table 2.** Tumor Infiltration Findings

	No Infiltration	Metastatic Infiltration	Total
No fluorescence	33	13	46
Fluorescence	15	49	64
Total	48	62	110



**Figure 1.** Amount of positive cytokeratin staining in samples obtained from adjacent brain tissue after macroscopic tumor extirpation is associated with the presence of 5-aminolevulinic acid (5-ALA) fluorescence (red and faint, 5-ALA positive; blue, 5-ALA negative;  $P = 0.003$ , Student  $t$  test). CKAE1/3, cytokeratin AE1/AE3.



### Quantitative Analysis of Metastatic Infiltration in Biopsy Specimens of Adjacent Brain Parenchyma

Digital images were captured of representative areas with a maximum of metastatic infiltration of the immunohistochemistry slides. The JPEG images were stored at a resolution of  $1360 \times 1024$  pixels and segmented for the brownish stain of 3,3'-diaminobenzidine immunohistochemistry using the open source software ImageJ 1.8.0 for Windows (National Institutes of Health, Bethesda, Maryland, USA; <https://imagej.nih.gov>). The pixel count of the segmented area was set in ratio to the total image as a measure of the amount of tumor infiltration given in percent. This quantitative, semiautomated analysis was omitted for melanoma cases because of the different immunohistochemistry technique used in melanomas.

### Statistics

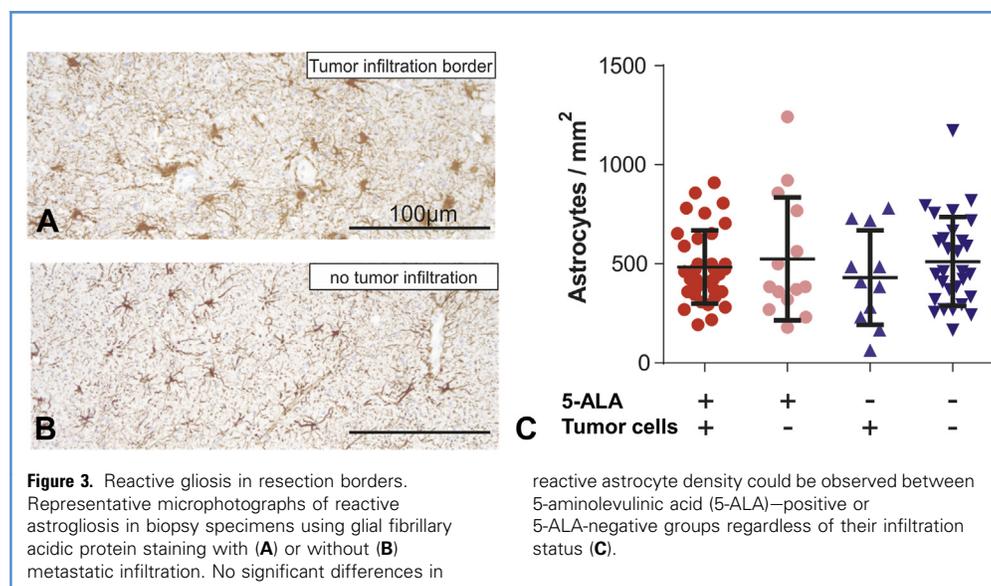
Data were analyzed using Fisher exact test, Student t test, and receiver operating characteristic curve by GraphPad Prism Mac 5 (GraphPad Software, La Jolla, California, USA).

### RESULTS

Between 2011 and 2015, 27 patients were enrolled in this study. Mean age was  $62 \pm 8$  years, and 14 patients (52%) were women.

All patients underwent fluorescence-guided resection of tumor based on the suspicion of a malignant glioma and a differential diagnosis of cerebral metastasis. Metastasis of a solid tumor was ultimately proven by frozen section analysis. Ten patients (37%) had non-small cell lung cancer (NSCLC), 4 patients had breast cancer (14.5%), 4 patients had colorectal cancer (14.5%), 1 patient had carcinoma of unknown origin (4%), 3 patients had melanomas (11%), 3 patients had small cell lung cancers (11%), 1 patient had an undifferentiated cancer with known lung cancer (4%), and 1 patient had squamous cell carcinoma of the lung (4%). Red or faint fluorescence of the tumor was recorded in 23 patients (85%) (Table 1). In 3 patients with NSCLC and 1 patient with colorectal metastasis, no fluorescence was detected intraoperatively. After macroscopic tumor resection, fluorescence of the adjacent brain parenchyma was assessed followed by assessment of 125 biopsy samples. Fluorescence was rated as faint or red (i.e., positive) in 75 (60%) and as blue (i.e., negative) in 50 (40%) samples.

For qualitative analysis, 110 samples of 25 patients were analyzed. Fifteen samples could not be analyzed owing to lack of tissue or insufficient tissue quality. Metastatic infiltration was demonstrated in 62 samples (57%); no metastatic cancer cell infiltration was demonstrated in 48 samples (43%). In this qualitative analysis, fluorescence was associated with tumor



infiltration ( $P < 0.0001$ , Fisher exact test) (Table 2). Sensitivity, specificity, and positive predictive value were 79%, 69%, and 77%, respectively. Except for 1 patient with breast cancer, metastatic infiltration into the adjacent brain was found in at least 1 biopsy sample of the resection cavity (24 of 25 patients [96%]).

There were 92 samples eligible for quantitative tissue analysis. In 9 samples, the quality was insufficient for analysis by digital segmentation, and a further 9 samples were excluded because of melanoma origin. The mean ratio of tumor infiltration was 7.12% (range, 0%–31.6%). The amount of tumor infiltration was associated with the presence of fluorescence (blue fluorescence 0.09%  $\pm$  0.04% and red or faint fluorescence 3.26%  $\pm$  0.86;  $P = 0.003$ , Student *t* test) (Figure 1). Receiver operating characteristic curve analysis showed a sensitivity of 97.3% (95% confidence interval 85.84%–99.93%) for tumor infiltration (Figure 2). Post hoc evaluation of reactive astroglia showed no correlation between tumor infiltration and astrocytic activation (Figure 3).

## DISCUSSION

In recent years, the concept of brain metastasis as circumscribed, noninfiltrating lesions had declined. Siam et al.<sup>6</sup> proved by biopsy specimens taken from the peritumoral zone that most metastases indeed have an infiltration zone. A relationship between intensity and depth of infiltration and the primary tumor entity could be seen.<sup>2</sup> Yoo et al.<sup>11</sup> performed total resection of metastases (as confirmed by tumor-free resection margins) and were able to lower the recurrence rate without irradiation from 43.1% to 23.3%. This stimulated the idea of investigating if 5-ALA fluorescence allows intraoperative visualization of infiltration zone and fluorescence-guided resection.

The present study supports previous reports showing that a high percentage of brain metastases are positive for 5-ALA

fluorescence.<sup>15,17–19</sup> However, the metastatic tissues themselves appear to be highly heterogeneous and are usually also highly vascularized. As blood absorbs any fluorescence, intraoperative impressions may vary. Additionally, there are instances where the interior of a tumor lacks, while the surrounding brain tissue exhibits, fluorescence.<sup>17</sup> In our study, 85% of tumors were 5-ALA-positive. This ratio is higher compared with a previous series in mainly patients with NSCLC with a reported 5-ALA fluorescence positivity of 57%. However, in our series also, only 7 of 10 patients with NSCLC showed no fluorescence, which was the lowest percentage among primary entities. The relatively low patient number and selection bias may have led to a limited generalizability of our study. Only patients with morphology compatible with malignant glioma were included. Of note, no renal cell carcinoma, a tumor that is known for a predominantly displacing growth pattern, was present in this series. However, we were able to confirm clearly that brain metastases lead to a conversion to 5-ALA fluorescence in the adjacent brain tissue of the pseudocapsule in a high percentage of patients.

Furthermore, our study demonstrates that 5-ALA fluorescence correlates with metastatic infiltration. However, the intensity of the signal did not seem to derive from the metastatic cells. In our study, fluorescence was detected even in samples of very scarce tumor infiltration. It is unlikely that the single infiltrative tumor cells alone provoked the fluorescence. Fluorescence might be enhanced owing to the infiltration of tumor cells, but is not restricted to the presence of tumor cells.<sup>19,20</sup> 5-ALA fluorescence was reported in tumor-free yet reactive brain tissue after glioma resection in about one third of specimens.<sup>21</sup> Owing to the nonglial origin of carcinoma metastasis, reactive changes of adjacent brain tissue are more frequent.<sup>22</sup> We and others have already reported that the brain metastasis/brain parenchyma is the area with the most glial reaction of astrocytes, microglia, and macrophages.<sup>4–6</sup> Moreover, the glial reaction is different and seems to depend on

the growth pattern of the metastatic tissue (i.e., displacing vs. infiltrating). The brain metastasis/brain parenchyma interface of 1 metastasis is also heterogeneous.<sup>5,6</sup> Whereas a metastasis with displacing growth pattern often leads to a very dense and structured pseudocapsule or a moderate glial activity, infiltrating metastasis often provokes a very heavy glial reaction. Thus, glial reaction also varies in the same metastasis.

As discussed in previous reports, it is possible that the fluorophore protoporphyrin IX also leaks from the metastatic tumor cells in the adjacent tissue and amplifies the visibility.<sup>19</sup> Likewise, protoporphyrin IX leakage might also be the reason why specificity of peritumoral 5-ALA fluorescence to contain tumor cells in malignant glioma is only 89%.<sup>23</sup> This prospective clinical trial was not randomized. Therefore, we refrain from the tempting evaluation

whether the fluorescence-guided resection affects local metastatic control or outcome.

## CONCLUSIONS

This study confirms that infiltration is a common finding in brain metastases in selected primary tumors. 5-ALA fluorescence of peritumoral brain tissue correlates with the presence of tumor cells beyond the pseudocapsule.

## DECLARATION OF COMPETING INTEREST

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