

5-aminolaevulinic acid-induced protoporphyrin IX fluorescence in high-grade glioma surgery

A one-year experience at a single institution

M. Hefti, G. von Campe, M. Moschopoulos^a, A. Siegner^b, H. Looser^c, H. Landolt

^a Dept. of Neurosurgery Kantonsspital Aarau, Dept. of Neuropathology Kantonsspital Aarau

^b Biophotonics AG Arbon

^c Fachhochschule Nordwestschweiz

Summary

Objective: Among the factors determining prognosis in patients with malignant glioma, the extent of resection has long been controversial. However, recent data have shown that patients derive a survival benefit from extensive tumour resection. 5-aminolaevulinic acid (5-ALA)-induced fluorescence renders more complete resection possible in malignant glioma. We report on the feasibility of the method in daily clinical practice, the benefits for patients and surgeons, the technical limitations and the methods we have devised of overcoming these limitations.

Methods: We describe our initial experience in 74 cases undergoing gross total resection, partial resection and biopsy. Fluorescence intensity and histological data are analysed, specificity and sensitivity are calculated according to fluorescence intensity, and the pitfalls and limitations are defined. The fluorescence signal was quantified via digital video data and by single photon count.

Results: Solid fluorescence signals define tumours with a sensitivity of 0.98 and a specificity of 1.0. Vague fluorescence reduces sensitivity to 0.76 and specificity to 0.85. Limitations of 5-ALA-

assisted surgery are apparent within the inter-observer interpretation of solid or vague fluorescence, heterogeneity of gliomas, invasion beyond the resection cavity and intercell heterogeneity of porphyrin IX fluorescence.

Conclusion: 5-ALA-induced PIX fluorescence improves the results in high-grade glioma surgery for gross total resection. Specificity and sensitivity in regions of solid fluorescence are very high. Quantitative analysis of fluorescence intensity corrects the reduced reliability of the method in areas of vague fluorescence and renders gross total resection more feasible without additional risk to the patient. PIX fluorescence is easy to implement in daily neurosurgical practice and side effects are very few. Heterogeneous tumours with lower grade elements and satellite lesions cannot be reliably resected using fluorescence-assisted surgery alone. In these cases the additional use of intraoperatively updated imaging-based neuronavigational methods (MR, ultrasound) is needed.

Key words: 5-aminolaevulinic acid; glioma; fluorescence analysis

Introduction

Despite improvements in local tumour control, the prognosis for high-grade glioma patients remains dismal. The biological characteristics of high-grade glioma cells, such as their marked invasive tendency, very high proliferation rate and genetic variability, combined with the obstacle of the blood-brain barrier and lack of redundancy for brain structures, leave few therapeutic options.

As far as surgery is concerned, resection of the main tumour mass to achieve debulking in symp-

tomatic patients is a widely accepted strategy, as is biopsy for diagnostic procedures. The actual benefit of aggressive tumour resection for patient survival in high-grade gliomas, however, remains controversial. Recent publications based on pre- and postoperative volumetric MR imaging data redefine the relevance of extensive cytoreduction, with increasing evidence in favour of surgical resection as a paramount prognostic factor for patient survival [1]. However, for the benefit to be statistically significant more than 98% of the ini-

tial tumour mass must be removed. Since intrinsic tumours such as gliomas do not have a distinct margin between the tumour mass and the surrounding brain, achieving such substantial tumour resection represents a major challenge to the neurosurgeon and, according to historical data, is feasible only in about 20% of high-grade glioma cases. Residual contrast enhancement in postoperative MR images as a sign of subtotal resection is regularly detected at the resection margins, reflecting the difficulty of accurately distinguishing intraoperatively between areas of infiltration and oedematous brain [2].

Neoplastic cells synthesise abundant intracellular protoporphyrin IX (PIX) after systemic or topical administration of 5-aminolaevulinic acid (5-ALA). Illumination of PIX at an appropriate

ultraviolet wavelength (440 nm) induces a visible (635 nm) red fluorescence which has been used for years in ophthalmology, urology, dermatology and other clinical settings to improve diagnosis and facilitate treatment. The use of 5-ALA to aid in the intraoperative identification of malignant gliomas is considerably newer [3] and was recently evaluated in a multicentre study, showing better cytoreduction and marked benefit for the patients [4].

Over the last 12 months we have used 5-ALA induced PIX fluorescence to assist in the resection of high-grade gliomas in 74 cases. We report on the feasibility of the method in daily clinical practice, on the benefits for patient and surgeon, the technical limitations and ways of overcoming those limitations.

Figure 1a

Intraoperative microscopic image of a glioblastoma multiforme as seen under 440 nm UV light (left, tumour is red) and corresponding pseudocolour enhanced intensity quantification (right). The quantification of PIX fluorescence helps to define the resection margins more precisely.

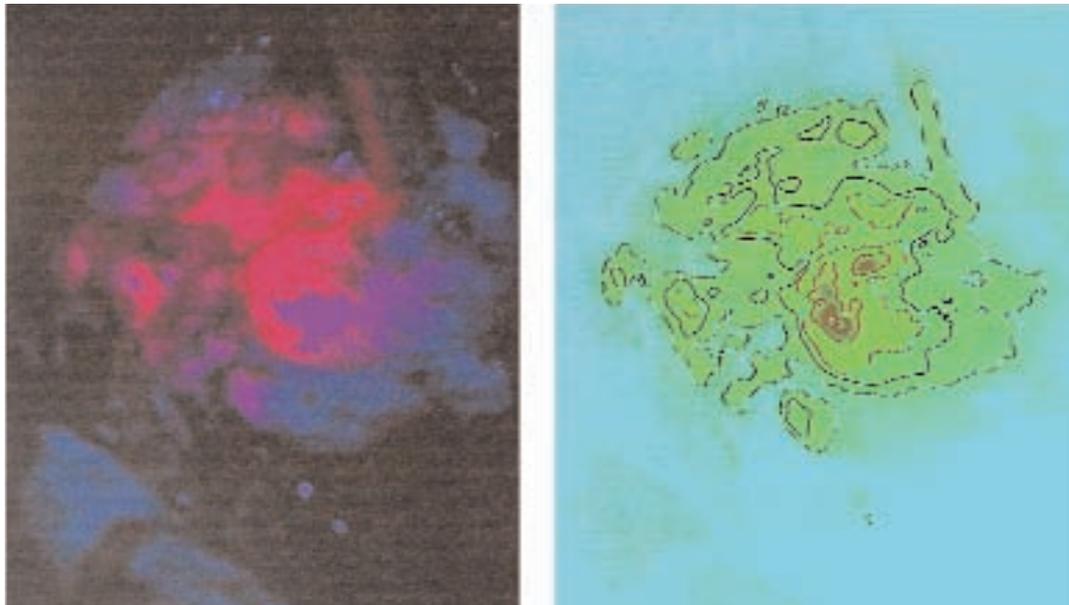


Figure 1b

Tumour samples are taken with a biopsy needle to ensure consistent sample size and are analysed by a photomultiplier to evaluate the virtual fluorescence intensity map created from the microscope's video signal. Specimen location (bottom left) and corresponding colour-coded graph (right). The white sample represents background noise (measured in a Petri dish). The corresponding histologies are shown under the graph.

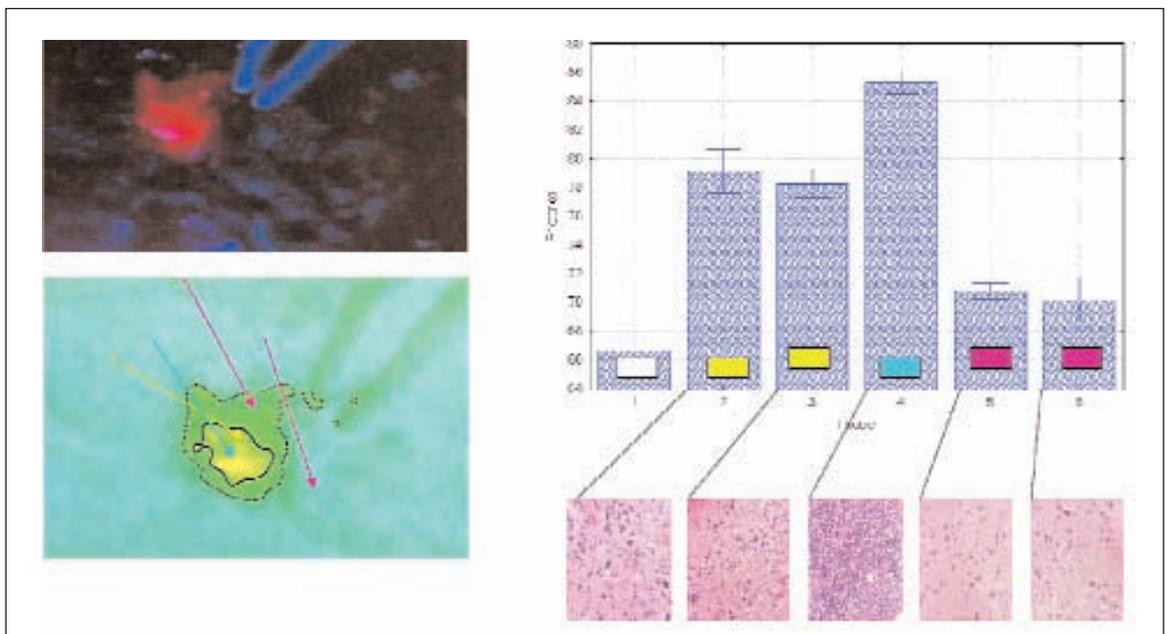
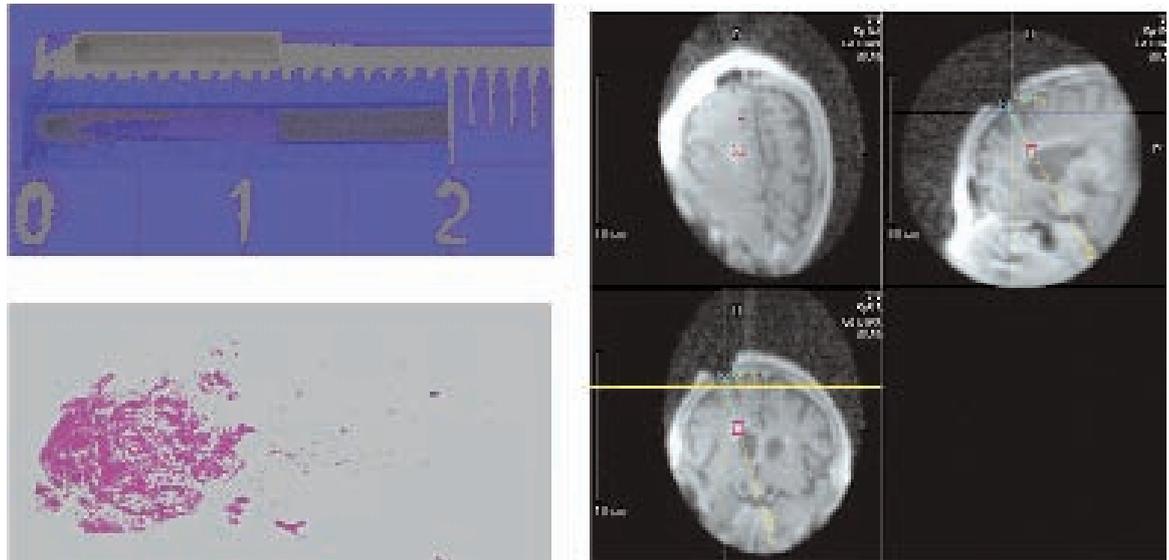


Figure 2

Top left: detail of the tip of the brain biopsy needle used. Inset: same biopsy needle with tissue cylinder still in place as seen under 440 nm UV light. Bottom left: histology (PAS) of the same tissue sample as above, showing the tumour demarcation line, matching what was seen under UV light. Right: the intraoperative MR images (axial, sagittal and coronal gadolinium-enhanced T1 sequences) demonstrate a 4 mm periventricular lesion and the biopsy needle in place.



Methods

Patients with suspected malignant glioma received 20 mg/kg of 5-ALA (medac GmbH, 22880 Wedel, Germany) 5 to 6 hours preoperatively. Surgery was then performed under standard conditions, with no specific modifications in the operating room setting. Patients were kept indoors for 48h after drug intake, away from direct sunlight exposure.

The 440 nm ultraviolet (UV) light source, an optional component of the OPMI Pentero microscope (Carl Zeiss AG, 73447 Oberkochen, Germany), was operated by the surgeon with a switch on the microscope handle during surgery. All operative cases were assessed for fluorescence and histology. Further resections under fluorescent light after the surgeon's initial evaluation of "gross total resection" under white light were specifically recorded.

Tissue samples were labelled according to the surgeon's subjective impression under white and UV light. The exact location of these samples was noted on the preoperative MR images and, if available, on the FET-PET scans. Correlations between the tissue samples' histology, their degree of fluorescence (vague +, solid ++), their appearance under white light (no tumour, possible

tumour, tumour, necrosis), and their MR presentation resulted in an estimated specificity and sensitivity of those modalities

Using a computer program developed in-house the 550–740 nm wavelength video signal from the microscope was analysed and the virtual intensity of the fluorescence defined relative to the patient's individual maximum fluorescent region. Specimens taken from corresponding areas were also analysed by photomultiplier and the same specimens then sent for histology (Figure 1a, 1b).

Frameless stereotactic biopsies were performed with the aid of the Treon neuronavigation system (Medtronic Navigation, Louisville, CO 80027, USA). Magnetic resonance images were taken using a semiportable intraoperative scanner (PoleStar N20, Medtronic Navigation, Louisville, CO 80027 USA) with the biopsy needle still in place to verify the exact placement and provide an objective reference. Identical protocols for administration of 5-ALA in patients for gross total resection and biopsy were used. In the case of biopsies, tissue specimens were placed under the Pentero microscope and inspected under UV light (Figure 2).

Results

Between May 2006 and May 2007, "compassionate use" of 5-ALA was obtained from Swissmedic for 75 patients, based on the suspected diagnosis of malignant glioma on preoperative imaging. Four of these patients never received 5-ALA (early emergency operation in two, elevated liver enzymes in one and missed drug administration in one); three patients were operated on twice using PIX fluorescence within the above time frame. Thus altogether 74 fluorescence assisted surgical interventions for brain tumours were performed over a one-year period.

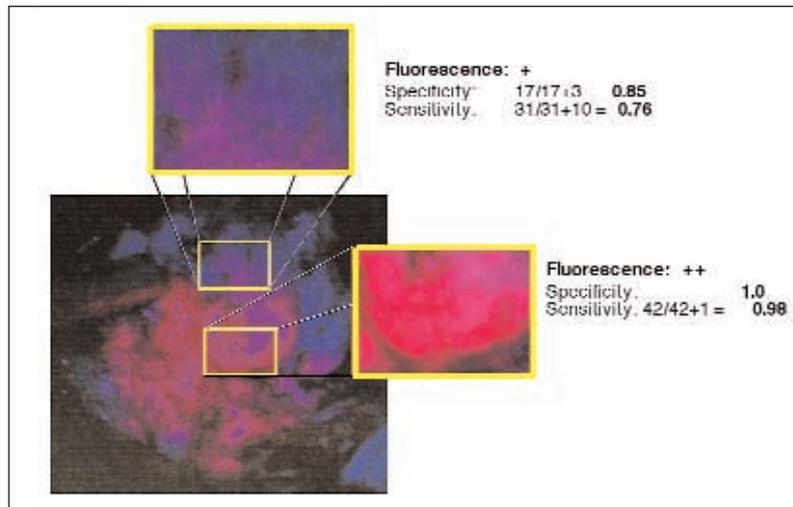
Adverse effects were observed in two patients: one patient showed slight reddening of the face

and arms approximately 11 hours after oral intake of 5-ALA (20 mg/kg), and persisting for 2 days; the other patient developed generalised oedema including mucosal surfaces and required 4 days' monitoring in the intensive care unit. Both patients made a complete recovery.

Of the 71 patients who received 5-ALA, 57 were diagnosed with a glioma (47 glioblastoma multiforme [GBM], five anaplastic astrocytomas, four fibrillary astrocytomas and one anaplastic ependymoma); the remaining 14 patients presented tumours of other histological origin. Except in one patient with GBM, all malignant gliomas showed solid fluorescence; in none of the

Figure 3

Intraoperative microscopic image of a glioblastoma multiforme as seen under 440 nm UV light (tumour is red fluorescence). Enlarged are shown tumour regions with “solid” (++) and “vague” (+) fluorescence; sensitivity and specificity for PIX fluorescence are strongly influenced by the intensity of the fluorescence.



low-grade gliomas was fluorescence visible. Seven non-glial pathologies also presented positive fluorescence (one anaplastic meningioma, two lymphomas, two metastases of colon carcinoma and two cases of vasculitis).

Residual positive fluorescence was visible and required further resection in 38 out of 42 cases with intended gross total removal. Non-glial neoplasias showed residual tumour tissue with positive fluorescence in only one case. The calculated specificity for “solid” fluorescence was 100% and the calculated sensitivity 98%. In regions of

“vague” fluorescence resection was more prone to error, but with a calculated sensitivity of 76% and a calculated specificity of 85% fluorescence-guided surgery proved still better than the surgeon’s estimate alone under white light (specificity 68%, sensitivity 66%) (Figure 3). Preliminary results for optical quantification of fluorescence intensity show equally high numbers for specificity and sensitivity of “solid” fluorescence down to 30–20% of the initial intensity. No solid tumour was found which had less than 15% of initial fluorescence intensity (Figure 1b).

Discussion

Applicability and complications

Considering that only four patients out of 71 (5.3%) dropped out, the use of 5-ALA in daily clinical routine is straightforward. In emergency cases the enzyme kinetics-dependent time lapse of 5–6 hours until maximal fluorescence intensity is reached is impossible to achieve; accordingly, some of our “missed 5-ALA administrations” were rapidly deteriorating patients in need of emergency surgery. Although direct sunlight exposure was avoided, we encountered one minor and one serious adverse effect after oral intake of 5-ALA (complication rate 2.7%). In neither of these two patients was metabolic or liver disease known or identified afterwards. Further indicators to define patients with a potentially higher risk of complications after intake of 5-ALA are therefore warranted.

Gross total resection

All but one patient with malignant glioma showed positive fluorescence. Residual tumour with positive fluorescence was detected and removed in over 90% of all intended gross total resections. The high specificity and sensitivity of solid fluorescence ensure more radical tumour removal, since functional neuronal remnants are

virtually nonexistent in these regions [5]. Tracking an intensely fluorescent tumour renders resection more secure and rapid, the clearcut distinction between neoplasia and brain being reassuring for the surgeon during and after tumour removal. The situation is however very different in regions of vague fluorescence. Specificity is lower and interpretation of the residual “red” of fluorescence is surgeon-dependent. Within these infiltrative zones intact neuronal structures and axonal connections are to be expected [5], and damage to these may result in serious postoperative neurological deficits. To avoid the imprecision of the terms “solid” and “vague”, and to reduce the subjective interpretation range of fluorescence (thereby improving the specificity and sensitivity of the method within areas of “vague” fluorescence), a system of optical quantification and false colour reproduction of PIX fluorescence intensity has been developed (Figure 1a). With this system the intensity of the patient’s individual PIX fluorescence in relation to the tumour’s maximum can be correctly quantified and vague fluorescence can now be defined as 15% to 30% of the initial tumour’s maximum value. The histological specifications of the tumour and infiltrated brain in relation to the analysed fluorescence intensity

may in the near future help to define more clearly the borders between brain and tumour.

The PIX fluorescence technique is a helpful addition to the tools available for gross total resection of malignant gliomas. The goal of at least 98% volume reduction can be made easier and at the same time safer by optimising the method through implementation of false colour reproduction of fluorescence intensity.

Gross total resection in non-glial tumours

Although some non-glial intracranial tumours, such as metastases of colon carcinoma or one case of anaplastic meningioma, also showed positive fluorescence, this technique did not alter or facilitate surgical strategies in these instances, and we saw no gain with regard to resection volume in these tumours. In all but one of these cases the tumour could be removed completely under conventional white light, without residual solid fluorescent tissue remaining. Even more disturbingly, metastases often showed a vague fluorescent peripheral rim within adjacent brain which proved histologically negative. This false-positivity may be due to a blood-brain barrier disruption and/or a consequence of proliferative microglia in the tumour's vicinity. This vague fluorescence potentially leads to erroneous judgments and resection of non-neoplastic brain structures. On the basis of these findings we consider 5-ALA-induced fluorescence unsuitable for gross total resection of non-glial intracranial tumours.

Biopsy

The high specificity and sensitivity of solid fluorescence in high-grade gliomas allow immediate and reliable identification of these tumours. We therefore used 5-ALA fluorescence even in tumour cases that were considered only for partial resection or even biopsy. When performing partial resections or open biopsies with this technique we expect to achieve a more accurate and more sensitive diagnosis by sampling regions of solid fluorescence for histology. Stereotactic needle biopsies, mainly used in tumours that are deep-seated or located in the brain stem, need no further samples if the first tissue fragment shows intense solid fluorescence. In the last 12 months 15 patients underwent fluorescence-controlled biopsies. In the 10 cases that were diagnosed with malignant glioma, all already showed solid fluorescence in their first sample and all histologies were conclusive. As a proof of concept, the accuracy of the biopsy needle placement was verified by intraoperative MRI in three cases. The corre-

lation of the tumour's location, the position of the biopsy needle and the detectable fluorescence were determined (Figure 2). In all three cases an exact match could be shown: the demarcation line of the positive fluorescence matches the intraoperative imaging and the histological tumour border. We therefore take the view that in cases of initial solid fluorescence the need to take multiple samples to reduce sampling error is no longer necessary. In these cases even frozen section verification can be omitted and positive fluorescence alone relied on. This makes for a significantly shorter operation and a less invasive procedure, further reducing the morbidity of diagnostic brain biopsies.

Limitations

High-grade gliomas are very often heterogeneous tumours with areas of low-grade differentiation, highly proliferative regions and necrosis. Areas of necrosis and regions consisting of low-grade elements do not show PIX fluorescence. An exclusively fluorescence-controlled resection would therefore fail to meet the goal of gross total removal in a fair number of cases.

Gliomas are notoriously invasive tumours and their migratory behaviour along axonal and basal membrane-like structures [6–8] often leads to satellite lesions far away from the main tumour mass. UV light with a wavelength of 440 nm has a penetration power of only about 0.5 mm from the tissue surface; satellites therefore, even in very close proximity to the main mass, will not be detected. These limitations of 5-ALA-induced fluorescence can easily be overcome using an intraoperatively updated neuronavigational system (MR, ultrasound).

A shortcoming that cannot be easily overcome is the inconsistent ability of different high-grade glioma cells to produce PIX after administration of 5-ALA [9]. Among our own 74 fluorescence-guided cases we found one patient with a malignant glioma not showing active fluorescence. That particular patient had had surgery before and been treated with radiotherapy and concomitant chemotherapy (TMZ). Although intraoperatively the recurrent tumour proved to be of a very fibrous and hard consistency, the histological features were those of a typical glioblastoma multiforme. In what ways and to what extent chemotherapeutic agents, antiepileptic drugs and steroids or ionising radiation can influence the ability of malignant glioma cells to produce PIX is currently under further investigation in our laboratory.

Conclusions

5-ALA induced PIX fluorescence improves the results in high-grade glioma surgery when the goal is gross total resection. Its specificity and sensitivity in regions of solid fluorescence are very high. Quantitative analysis of fluorescence intensity corrects the reduced reliability of the method in areas of vague fluorescence and renders gross total resection more feasible without additional risk to the patient. PIX fluorescence is easy to implement in daily neurosurgical practice and side effects are very few. Non-glial tumours do not benefit to the same extent when operated on with fluorescence-assisted surgery, since complete re-

section of such tumours is often not hindered by visual discrimination errors.

Since fluorescence can define the region of tissue sampling more accurately, multiple samples are often not necessary in biopsies using 5-ALA and UV light.

Heterogeneous tumours with lower grade elements and satellite lesions cannot be reliably resected by fluorescence-assisted surgery alone. In these cases the additional use of intraoperatively updated imaging-based neuronavigational methods (MR, ultrasound) is required.

References

- 1 Lacroix M, bi-Said D, Fourney DR, Gokaslan ZL, Shi W, DeMonte F, et al. A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. *J Neurosurg.* 2001;95:190-8
- 2 Kowalczyk A, Macdonald RL, Amidei C, Dohrmann G, III, Erickson RK, Hekmatpanah J, et al. Quantitative imaging study of extent of surgical resection and prognosis of malignant astrocytomas. *Neurosurgery.* 1997;41:1028-36.
- 3 Stummer W, Stepp H, Moller G, Ehrhardt A, Leonhard M, Reulen HJ. Technical principles for protoporphyrin-IX-fluorescence guided microsurgical resection of malignant glioma tissue. *Acta Neurochir. (Wien)* 1998;140:995-1000.
- 4 Stummer W, Pichlmeier U, Meinel T, Wiestler OD, Zanella F, Reulen HJ. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. *Lancet Oncol.* 2006;7:392-401.
- 5 Yoshinaga Kajimoto, Hidekazu Tanaka, Minoru Myiashita, Shigemichi Ichioka, Jun Aoki, Shin-Ichi Myiatake, Toshihiko Kuroiwa. The relationship between histopathological findings and 5-aminolevulinic acid induced fluorescence in glioma surgery. 2005. 6th Annual Symposium of Japanese 5-ALA Association.
- 6 Berens ME, Giese A: "...those left behind." *Biology and oncology of invasive glioma cells. Neoplasia.* 1999;1:208-19.
- 7 Giese A, Westphal M. Glioma invasion in the central nervous system. *Neurosurgery.* 1996;39:235-50.
- 8 Scherer HJ. The forms of growth in gliomas and their practical significance. *Brain.* 1940;63:1-35.
- 9 Eleouet S, Rousset N, Carre J, Vonarx V, Vilatte C, Louet C, et al. Heterogeneity of delta-aminolevulinic acid-induced protoporphyrin IX fluorescence in human glioma cells and leukemic lymphocytes. *Neurol Res.* 2000;22:361-8.

SMW

Established in 1871
Formerly: Schweizerische Medizinische Wochenschrift
Swiss Medical Weekly

The European Journal of Medical Sciences

The many reasons why you should choose SMW to publish your research

What Swiss Medical Weekly has to offer:

- SMW's impact factor has been steadily rising. The 2006 impact factor is 1.346.
- Open access to the publication via the Internet, therefore wide audience and impact
- Rapid listing in Medline
- LinkOut-button from PubMed with link to the full text website <http://www.smw.ch> (direct link from each SMW record in PubMed)
- No-nonsense submission – you submit a single copy of your manuscript by e-mail attachment
- Peer review based on a broad spectrum of international academic referees
- Assistance of professional statisticians for every article with statistical analyses
- Fast peer review, by e-mail exchange with the referees
- Prompt decisions based on weekly conferences of the Editorial Board
- Prompt notification on the status of your manuscript by e-mail
- Professional English copy editing

Editorial Board

Prof. Jean-Michel Dayer, Geneva
Prof Paul Erne, Lucerne
Prof. Peter Gehr, Berne
Prof. André P. Perruchoud, Basel
Prof. Andreas Schaffner, Zurich
(editor in chief)
Prof. Werner Straub, Berne (senior editor)
Prof. Ludwig von Segesser, Lausanne

International Advisory Committee

Prof. K. E. Juhani Airaksinen, Turku, Finland
Prof. Anthony Bayes de Luna, Barcelona, Spain
Prof. Hubert E. Blum, Freiburg, Germany
Prof. Walter E. Haefeli, Heidelberg, Germany
Prof. Nino Kuenzli, Los Angeles, USA
Prof. René Lutter, Amsterdam, The Netherlands
Prof. Claude Martin, Marseille, France
Prof. Josef Patsch, Innsbruck, Austria
Prof. Luigi Tavazzi, Pavia, Italy

We evaluate manuscripts of broad clinical interest from all specialities, including experimental medicine and clinical investigation.

We look forward to receiving your paper!

Guidelines for authors:

http://www.smw.ch/set_authors.html

All manuscripts should be sent in electronic form, to:

EMH Swiss Medical Publishers Ltd.
SMW Editorial Secretariat
Farnsburgerstrasse 8
CH-4132 Muttenz

Manuscripts: submission@smw.ch
Letters to the editor: letters@smw.ch
Editorial Board: red@smw.ch
Internet: <http://www.smw.ch>