Pediatric Neurosurgery

Research Article

Pediatr Neurosurg 2020;55:268–279
DOI: 10.1159/000511289

Received: November 7, 2019
Accepted: August 29, 2020
Published online: November 18, 2020

The Use of 5-Aminolevulinic Acid to Assist Gross Total Resection of Paediatric Posterior Fossa Tumours

Jason John Labuschagne[a, b, c]

[a]Department of Neurosurgery, University of the Witwatersrand, Johannesburg, South Africa; [b]Department of Paediatric Neurosurgery, Nelson Mandela Children's Hospital, Johannesburg, South Africa; [c]Netcare Unitas Hospital, Centurion, South Africa

Keywords
5-aminolevulinic · 5-aminolevulinic acid · Fluorescence · Brain tumour

Abstract
Background: 5-aminolevulinic acid (5-ALA) use is well established in the resection of adult high-grade gliomas. There is growing interest in its usefulness in the paediatric population. The potential benefit of 5-ALA-guided resection motivated our unit to offer the established adult protocol as off-label use. ***Objective:*** to determine if 5-ALA guided resection was routinely useful and offered increased gross total resection (GTR) results. ***Methods:*** Nineteen patients harbouring a posterior fossa tumour suggestive of either an ependymoma or medulloblastoma (MB) underwent surgery between January 2018 and October 2019. The mean age was 5 years (range 2–12 years). A dose of 20 mg/kg of 5-ALA (Gliolan®) was given 4 h preoperatively. Intraoperatively, the tumours were viewed under violet-blue light and the presence of fluorescence was recorded. Fluorescence status was compared with histopathological classification and grade, Ki-67 index, GTR rate, and a subjective determination of "usefulness" was determined. ***Results:*** The case series included ependymoma grade II ($n = 6$), ependymoma grade III ($n = 4$), and MB grade IV ($n = 9$). For the combined cohort, the strong fluorescence rate was 68% ($n = 13$), the heterogenous fluorescence rate was 26% ($n = 5$), and the completely negative fluorescence rate was 5% ($n = 1$). The strong fluorescence rate of 90% found in the combined ependymoma group compared to the 45% strong fluorescence rate in the MB group was statistically significant ($p = 0.05$). Within the MB group the Ki-67 index was found to be significantly higher in the strongly fluorescent group as opposed to the patchy or non-fluorescent group (77.5 vs. 40%, $p = 0.016$). Fluorescence was determined to be useful in 63% of all cases. There was no significant relationship between fluorescence and GTR. The relationship between perceived usefulness and resection was not statistically significant. No adverse drug reactions were recorded. ***Conclusion:*** This case series adds to the growing body of evidence demonstrating the safety of 5-ALA in the paediatric population. 5-ALA guided resection was found to be useful in the majority of cases but this did not correlate with GTR status. Ependymomas reliably fluoresce in 90% of cases, and 5-ALA-guided resection should be considered when a preoperative diagnosis of ependymoma is suspected.

karger@karger.com
www.karger.com/pne

Jason John Labuschagne
Paediatric Neurosurgery, Nelson Mandela Children's Hospital
1 Jubilee Road
Johannesburg 2193 (South Africa)
jason.labuschagne@icloud.com

Introduction

5-Aminolevulinic acid (5-ALA) is a precursor molecule in the heme biosynthetic pathway. Its metabolite protoporphyrin (PPIX), produced in the mitochondria, accumulates in certain tumour cells, most likely due to a deficiency of ferrocheletase in these cells. PPIX demonstrates red fluorescence when excited by violet-blue light [1]. Multiple previous studies have demonstrated that certain central nervous system (CNS) neoplasms preferentially take up 5-ALA and convert it to PPIX, allowing the differentiation of neoplastic and normal brain tissues during tumour resection [2, 3]. A randomized, controlled multicentre phase III trial in adult glioblastoma and subsequent publications in adult patients [4–6] demonstrated an increase in the rate of gross total resection (GTR) and an improvement in survival with 5-ALA-guided resection.

To our knowledge, no reports of randomized, controlled trials of fluorescence-guided surgery for paediatric brain tumours have been published. The first successful use of 5-ALA in a child was published in 2009 [7] and subsequently several case reports and case series [8–12] have been published, reporting the use of 5-ALA in this population group across a variety of CNS neoplasms including pilocytic astrocytomas, medulloblastomas (MBs), ependymomas, malignant gliomas and others.

The potential benefits of an intraoperative tumour specific marker for paediatric brain tumour surgery, and the assumed benefit of 5-ALA-guided resection, motivated our unit to offer the established adult protocol to parents as an individual treatment attempt. In this manuscript, we present our experience regarding the safety and "usefulness" of fluorescence-guided surgery with 5-ALA for the resection of posterior fossa tumours (specifically, ependymomas and MBs) in children. To our knowledge this is the largest single-centre series of 5-ALA-guided resection in paediatric posterior fossa tumour resection.

Material and Methods

Patients

Patients aged <16 years with the diagnosis of a posterior fossa brain tumour suggestive of either an ependymoma or MB on preoperative MRI were considered suitable for treatment with 5-ALA-assisted surgery. Patients in whom MRI features were suggestive of a localized, low-grade lesion, typically lesions thought to be a pilocytic astrocytoma, were excluded. Primary brainstem lesions were also excluded from this series. Exclusion criteria included any pre-existing hepatic or renal disease, abnormal renal or hepatic function, any known cutaneous hypersensitivity, or a first-degree relative with porphyria. Parents or guardians after being informed about the potential benefit and risks derived from the existing adult data were offered the treatment as an off-label use. Following an explanation regarding the lack of safety and efficacy data from clinical trials in the paediatric population and the character of an individual treatment attempt, written informed consent was obtained on behalf of the children from their parents or guardians. The Human Research Ethics Committee of the medical faculty of the University of the Witwatersrand approved the scientific analyses of these cases.

Study Protocol

Patients were treated according to the previously reported adult protocol of Stummer et al. [6]. All patients received intravenous Dexamethasone 0.25 mg/kg (body weight)/day in 4 divided doses for 2 days prior to the surgery. A single dose of 5-ALA (Gliolan®) 20 mg/kg (body weight) suspended in 50 mL of tap water was administered orally 4 h prior to the predicted "cutting time" in the presence of medical personnel. At surgery, following tumour exposure, a microscope equipped with a violet-blue light source (Zeiss, Kinevo 900, Carl Zeiss AG, Oberkochen, Germany) was used to evaluate for fluorescence. Tumour resection was performed using microsurgical instruments as well as an ultrasound surgical aspirator (CUSA: Integra Lifesciences Corporation, Princeton, NJ, USA) under white microscope light, switching repeatedly to violet-blue illumination mode to visualize red fluorescence in the operative field. Neuronavigation (STEALTH, Medtronic, Minneapolis, MN, USA), intraoperative monitoring, and intraoperative ultrasound were used as required. Routine prophylactic measures were employed to diminish patient light exposure before, during, and for 48 h after surgery.

Assessment of Parameters

Patients were clinically examined during their post-operative course to assess for new neurological deficits or surgical complications. Patients were clinically assessed for signs of adverse drug reactions, such as erythema. Extent of resection was assessed on post-operative gadolinium-enhanced MRI obtained within 48 h of surgery, and graded as either GTR, that is, no residual tumour enhancement, near total resection (NTR), that is, a thin rim of gadolinium enhancement of the tumour cavity/wall, or subtotal resection, that is, residual nodular enhancement. Neuropathological diagnosis was obtained and classified according to the WHO classification of tumours of the CNS [13]. The MB group was classified according to the WHO histological classification and not WHO genetic classification as our unit did not have genetic subtyping available at the time.

Statistical Analysis

Averages were expressed as means. Due to the small sample size, the relationships between categorical variables were investigated by means of Fisher's exact test. The Mann-Whitney U test was used to compare the median scores of 2 groups. All statistics were performed using SSPS Version 25.0 for Windows (SPSS Inc., Chicago, IL, USA). A p value <0.05 was considered statistically significant.

Table 1. Patients' characteristics and outcomes

Case number	Tumour histology (WHO grade)	Age, years	Gender	Presentation	Quality of fluorescence	Extent of resection	Usefulness of fluorescence	Ki-67	Adverse events
1	MB (IV)	12	F	Vomiting, headache, ataxia	Strong	GTR	Yes	70	Significant posterior fossa syndrome
2	MB (IV)	12	F	Vomiting, headache, ataxia	Strong	GTR	Yes	80	Nil
3	MB (IV)	5	M	Vomiting, headache, truncal ataxia	Vague/heterogeneous	GTR	Yes	45	Nil
4	MB (IV)	2	F	Anorexia, emaciation, vomiting, depressed LOC	Vague/heterogeneous	GTR	Yes	40	Nil
5	MB (IV)	5	M	Torticollis, truncal ataxia, regression in milestones	Vague/heterogeneous	GTR	Yes	40	Nil
6	MB (IV)	4	M	Headache	None	GTR	No	30	Nil
7	MB (IV)	3	M	Abducent nerve palsy, delayed milestones, vomiting	Strong	GTR	No	80	Nil
8	MB (IV)	10	F	Rapidly declining LOC	Strong	NTR	Yes	80	Nil
9	MB (IV)	4	M	Ataxia, abducent nerve palsy	Vague/heterogeneous	GTR	No	20	Nil
10	EP (II)	2	M	Vomiting failure to thrive	Strong	NTR	Yes	3	Nil
11	EP (II)	2	F	Vomiting, delayed milestones, ataxia	Strong	GTR	Yes	2	Nil
12	EP (II)	4	F	Vomiting, headaches, ataxia	Strong	GTR	No	7	Nil
13	EP (II)	3	M	Vomiting hemiplegia and depressed LOC	Vague/heterogeneous	GTR	Yes	20	Transient CN VI palsy
14	EP (II)	7	M	Headaches	Strong	GTR	No	10	Nil
15	EP (III)	5	M	Vomiting, headache ataxia	Strong	GTR	No	7	Nil
16	EP (III)	4	F	Vomiting, headache, ataxia	Strong	GTR	Yes	10	Nil
17	EP (III)	4	M	Vomiting torticollis, ataxia	Strong	GTR	Yes	20	Transient worsening of ataxia
18	EP (III)	2	F	Vomiting, abducens nerve palsy	Strong	NTR	Yes	50	Nil
19	EP (III)	8	M	Headache, declining vision	Strong	NTR	No	20	Nil

MB, medulloblastoma; GTR, gross total resection; NTR, near total resection.

Results

Nineteen patients (11 male, 8 female) underwent fluorescence-guided surgery for brain tumour resection between January 2018 and October 2019. The mean age was 5 years (range 2–12 years). Pathological diagnosis according to WHO classification was ependymoma grade II ($n = 6$), ependymoma grade III ($n = 4$), and MB grade IV ($n = 9$). Histological subtyping of the MBs identified 7 classic MBs, 1 desmoplastic/nodular MB, and 1 MB with extensive nodularity. Table 1 summarizes the patients' characteristics and outcomes.

Intraoperative Observation of 5-ALA Fluorescence

In the MB group, we found strong fluorescence in 45% ($n = 4$) of patients, and a combined heterogenous or no fluorescence rate of 55% ($n = 5$). In the ependymoma grade II group, we had a strong fluorescence rate of 83% ($n = 5$), a heterogenous fluorescence rate of 17% ($n = 1$), and no patients with completely negative fluorescence. In

the ependymoma grade III group, we had a 100% ($n = 4$) strong fluorescence rate. For the combined cohort, the strong fluorescence rate was 68% ($n = 13$), the heterogenous fluorescence rate was 26% ($n = 5$), and the completely negative fluorescence rate was 5% ($n = 1$). No statistical relationship between tumour type and fluorescence was found ($p = 0.163$) when the ependymoma groups were classified separately according to WHO grading into ependymoma and anaplastic ependymoma. If however the ependymoma subtypes were combined, as is the case clinically given the current molecular subtyping rather than histopathological typing of ependymoma [13], the strong fluorescence rate of 90% found in ependymoma compared to the 45% strong fluorescence rate in the MB group resulted in a statistically significant difference ($p = 0.05$) for tumour subtype and fluorescence rate.

Degree of Resection and Usefulness of Fluorescence

We attained post-operative MRI confirmed GTR in 73% of all the tumours and a 17% NTR in the remainder. Fluorescence was determined to be useful in 63% of cases and to be non-useful in 36% of cases. The relationship between fluorescence and GTR was non-significant ($p = 1.00$). The relationship between perceived usefulness and resection was not statistically significant ($p = 0.603$). At the end of surgery, no patients had solid fluorescent tissue still visible in the surgical field.

Tumour Grade and Ki-67 Characteristics and Fluorescence Rate

When correlating Ki-67 with WHO grade, we found that our WHO grade II tumours had an average Ki-67 index of 8.1, the grade III average was 25.0, and grade IV average was 57.2. Within the MB group, the Ki-67 index was found to be significantly higher in the strongly fluorescent group as opposed to the patchy or non-fluorescent group (77.5 vs. 40%, $p = 0.016$).

Gadolinium Enhancement and Fluorescence Rate

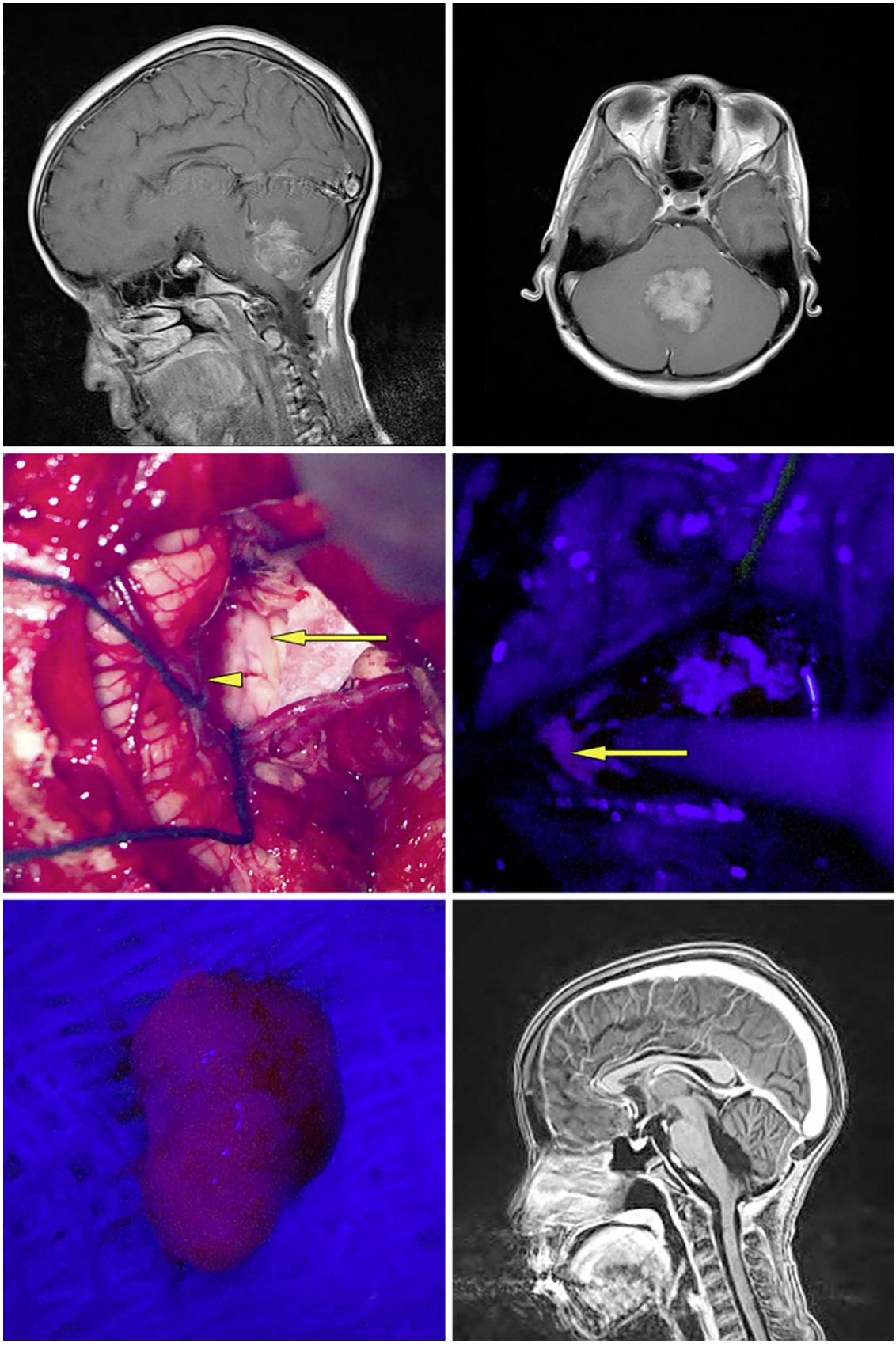
All 6 of the ependymoma grade II tumours demonstrated avid gadolinium contrast enhancement as did all 4 of the ependymoma grade III tumours. Only 2 of the 9 (22%) MBs failed to enhance post gadolinium administration, the other 7 (78%) demonstrating avid gadolinium contrast enhancement. The 2 non-enhancing MBs both demonstrated strong fluorescence. Figure 1 demonstrates a classic MB, with avid post gadolinium enhancement that intraoperatively was found to have vague 5-ALA fluorescence. Figure 2 demonstrates a classic MB, with very poor gadolinium enhancement demonstrating strong intraoperative 5-ALA fluorescence. Figure 3 demonstrates the typical strong fluorescence found within the ependymoma group.

Complications

Three patients developed complications. One patient developed a transient cranial nerve VI palsy from cerebrospinal fluid over-drainage. The patient had hydrocephalus on preoperative scanning, and an EVD was left in situ post-operatively. Following a period of inadvertent over-drainage of CSF, he developed a left-sided CN VI palsy, that resolved rapidly with clamping of the drain followed by drain removal. He had a MRI performed within 48 of surgery revealing GTR. The second complication was in a 12-year-old female patient with a MB. The patient had a strongly fluorescent MB resected, with tumour diffusely infiltrating the vermis. Fluorescence was essential in guiding a complete resection. The patient developed cerebellar mutism and emotional lability, characteristic of posterior fossa syndrome [14, 15]. The additional extent of resection, "encouraged" by the positive fluorescence within the cerebellum, was felt to have contributed to the posterior fossa syndrome symptoms. This had partially resolved by her last clinical visit. The third complication was significantly worsening of ataxia in a 4-year-old male patient with a grade III ependymoma. This recovered within 6 weeks. There were no complications directly attributable to the administration of the 5-ALA such as liver enzyme derangement or skin sensitivity.

Discussion

The prognostic significance of GTR for paediatric brain tumours is well documented. In children, MB is the most frequent malignant tumour of the CNS accounting for 15–20% of paediatric CNS tumours [16]. The extent of surgical resection in these tumours is a strong prognostic factor. Patients with <1.5 cm^2 post-operative residual having a superior overall survival [17, 18]. Likewise, complete resection remains the most important prognostic factor for children with ependymoma [19, 20]. However, GTR is a challenge as MBs frequently infiltrate adjacent vulnerable tissue, including the brainstem [21, 22], making clear intraoperative identification of the tumour difficult. Likewise, ependymomas, in particular the lateral group [23–28], have been shown to invade the brainstem, cerebellar peduncle and cerebellum, making clear intraoperative identification of the tumour imperative. Intraoperative visualization of these tumours may thus con-



1

(For legend see next page.)

tribute to improved GTR rates and lower surgical morbidity.

Despite the good in vitro evidence of MB cells to accumulate PPIX [21, 29–32], the clinical reports of MB fluorescence are conflicting and ambiguous [33–35]. Our result of a strong fluorescence rate of 44% is similar to the existing literature [2, 33–35]. Our results of the ependymoma grade II group having a strong fluorescence rate of 83% with the grade III group having a 100% strong fluorescence rate is in line with the rate and extent of fluores-

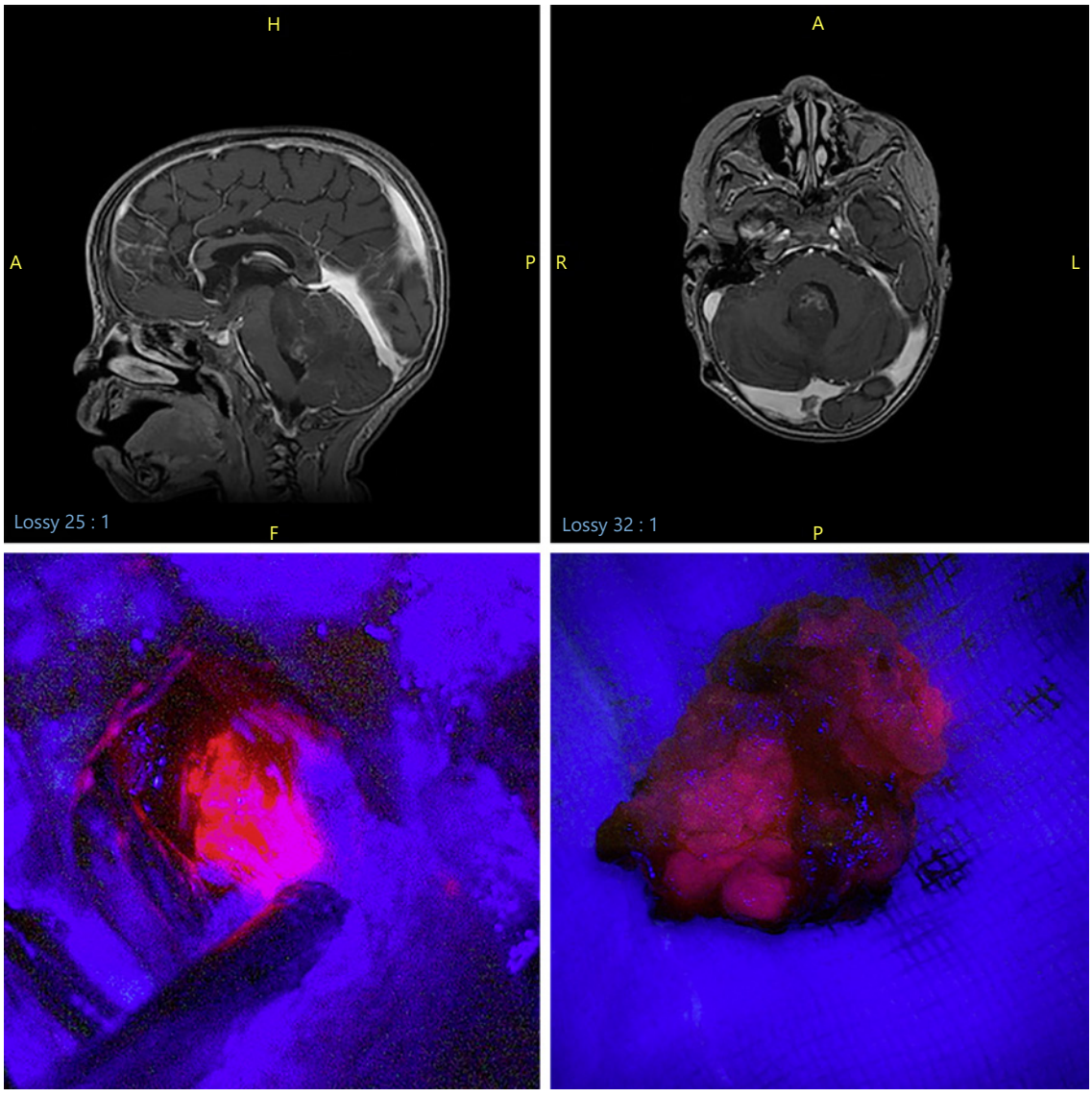
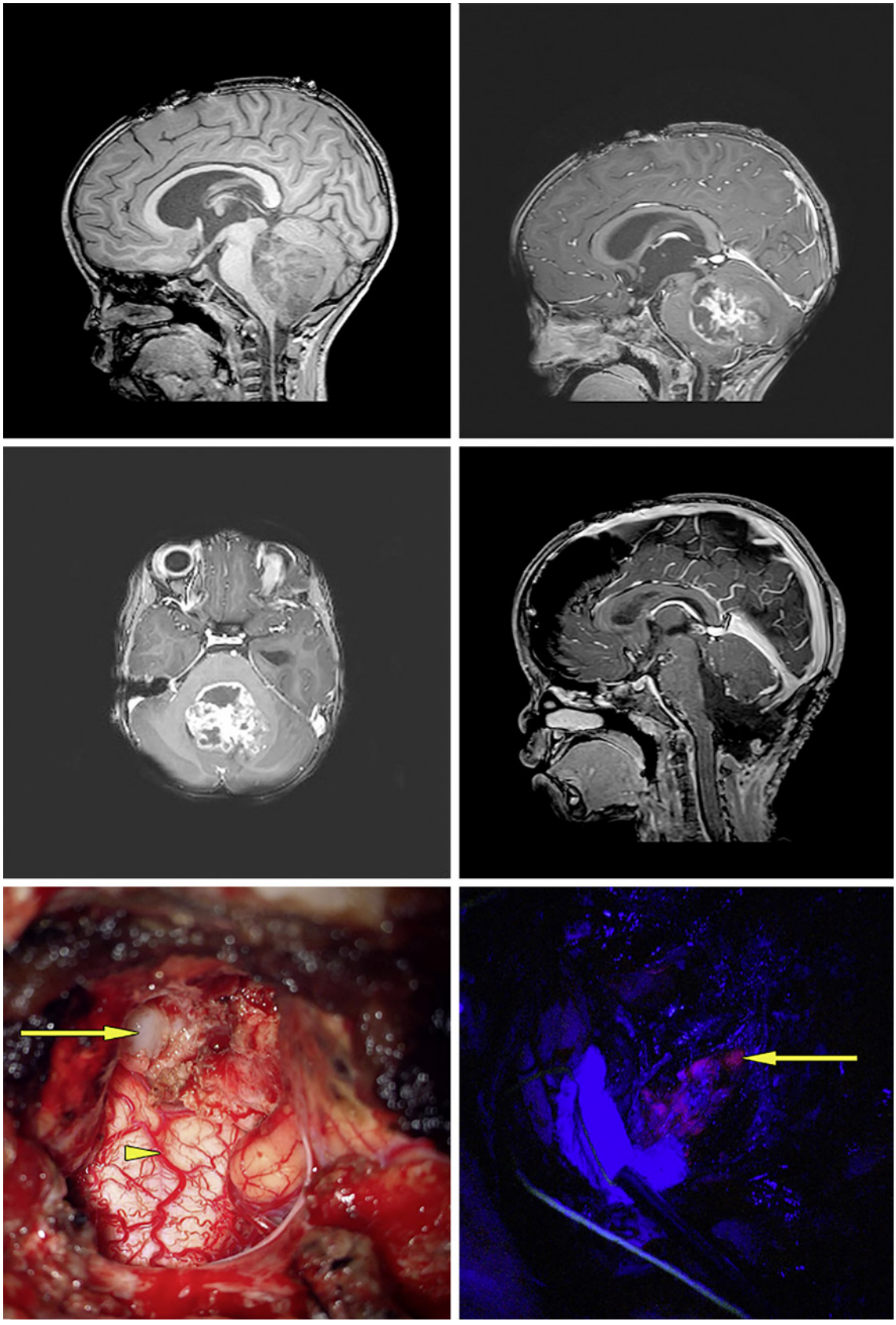


Fig. 2. MRI and intraoperative images: Classic MB. Top row: Post-contrast sagittal and axial MRI of non-enhancing classic MB. Bottom left: Bright fluorescence of tumour margin at cerebellar-tumour interface, deemed useful intraoperatively. Bottom right: Ex vivo, intense/bright fluorescence of tumour bulk. MB, medulloblastoma.

Fig. 1. MRI and intraoperative images: Classic MB. Top row: Post-contrast sagittal and axial MRI of an avidly enhancing classic MB. Middle left: White-light microscopic view, reveals complete resection from 4th ventricle, with median sulcus (yellow arrow) visible, thin layer of neoplastic tissue in lateral recess of 4th ventricle. Right middle: Vague but useful fluorescence of abnormal tissue in lateral recess. Bottom left: Ex vivo, low intensity but still clearly visible fluorescence of tumour bulk. Bottom right: GTR confirmed on post-operative MRI. MB, medulloblastoma; GTR, gross total resection.



3

(For legend see next page.)

cence in clinical studies of both infratentorial [35] and spinal ependymomas [36], approaching 90 and 100% positive strong fluorescence for ependymoma grade II and grade III respectively [35, 37, 38].

Contrast enhancement was almost uniform across the entire cohort of tumours, only 2 (10%) tumours (a classic MB arising in the left lateral cerebellar hemisphere, and a classic MB arising in the midline from the roof of the fourth ventricle) failed to enhance post gadolinium administration. Both these tumours demonstrated strong intraoperative fluorescence. Contrast enhancement was therefore not a reliable predictor of positive intraoperative fluorescence or lack thereof. Likewise, although none of our patients had visible fluorescence of the tumour bed at the end of the tumour resection, 17% did display some degree of tumour bed enhancement on post-operative gadolinium-enhanced MRI. The accumulation of gadolinium is mainly based on blood-brain barrier (BBB) disruption, while the accumulation of 5-ALA requires metabolic conversion into fluorescing PPIX in cells [39]. Interestingly, Stummer et al. [40] found that in glioma surgery some patients with residual intraoperative fluorescence showed no residual enhancement on post-operative MRI. Likewise, Widhalm et al. [41] found intraoperative 5-ALA fluorescence to be an accurate marker for identification of anaplastic foci in gliomas with non-significant contrast enhancement. Skjøth-Rasmussen et al. [42] in a case report described the use of 5-ALA to assist in the complete removal of a residual non-enhancing part of a MB. At relook surgery, they found low intensity but still valuable fluorescence of an infiltrative MB that failed to enhance on post-contrast MRI, and reported that the 5-ALA was essential in obtaining GTR. Stummer et al. [35], in a European survey of 78 paediatric patients, found that contrast enhancement by itself did not reliably predict "useful" fluorescence.

As yet the mechanism behind the heterogeneity of fluorescence in MB remains unclear. In vitro studies have demonstrated differences in the kinetics of PPIX accumulation in different cell lines [30, 31], and theoretically, the different MB molecular subgroups [13] could have differing molecular metabolism [31, 32] which could account for the mixed fluorescence rates for MB. Additionally, in vitro studies by Puppa et al. [43] have shown that different cellular subsets of MB may differ in PPIX accumulation. Likewise, Briel-Pump et al. [21] demonstrated that in MB cell lines, there is always a substantial number of cells that remained negative, in comparison to glioblastoma cell lines in which nearly all cells became positive for PPIX fluorescence. Whether such accumulating and non-accumulating cells represent distinct subsets and whether they contribute to the heterogenous appearance of MB fluorescence remains to be determined. In our series, 4 of the classic MBs demonstrated strong fluorescence whereas 2 demonstrated heterogenous fluorescence and 1 had no intraoperative fluorescence. Both the desmoplastic/nodular MB and the MB with extensive nodularity had heterogenous fluorescence only. These results are similar across the literature in which the authors report the histological subtype of MBs with approximately 25–50% of classic MBs displaying meaningful fluorescence [11, 35, 42, 44, 45]. The literature on the other subtypes of MBs is scarce. Barbagallo et al. [46] reported minimal fluorescence of a desmoplastic MB, whereas we found heterogenous but "useful" fluorescence in our single case of desmoplastic MB. Burford et al. [47] found no fluorescence in the single case of anaplastic MB that they reported on. The difference in BBB permeability might in part explain the difference in tumour fluorescence [48–50] amongst different histology types; however, differing molecular metabolism may prove to be more important. Saito et al. [51] reported that in glioma cases multivariate analysis showed that only isocitrate dehydrogenase 1 status predicted 5-ALA fluorescence, whereas contrast enhancement did not.

The immunohistochemical expression of protein Ki-67 is strongly associated with cellular proliferation and is widely used to evaluate the mitotic index in tumours. MBs are rapidly proliferating tumours, reflected typically by a very high Ki-67 expression [52–54]. In our study, the mean number of Ki-67 positive cells in the MB group was 57.2 (range 20–80). This is higher than the reviewed literature [52–60], but this high variability has been reported in other studies [55, 56, 58]. Although the Ki-67 proliferative index has been associated with aggressiveness and patient survival in glioma patients, its prognostic sig-

Fig. 3. MRI and intraoperative images: Ependymoma. Top row: Sagittal pre- and post-contrast MRI of ependymoma demonstrating significant contrast enhancement. Middle left: Axial post-contrast MRI revealing avid enhancement. Middle right: Post-operative MRI demonstrating GTR. Bottom left: White-light microscopic view, demonstrates posterior aspect of cervico-medullary junction (arrowhead) with ependymoma (arrow) protruding through the 4th ventricle outlet. Bottom right: Heterogenous fluorescence, with bright enhancement of tumour capsule as it protrudes through 4th ventricle outlet. The tumour bulk and margin demonstrated bright enhancement and was deemed "useful." GTR, gross total resection.

nificance in MB is controversial [61, 62]. Ertan et al. [55], in a report of 42 MBs, reported that Ki-67 had no impact on prognosis, with similar findings being reported by Miralbell et al. [58] and Meurer et al. [54]. Conversely, Nam et al. [63] found a high Ki-67 index to be associated with a poorer outcome. Different groups, by stratifying their results with a Ki-67 proliferation index above 20% [64], 30% [56], 40% [57], or 50% [59, 65] were able to show a worse overall survival in the higher Ki-67 cohort. In our study, the mean number of Ki-67 for ependymomas grade II was 13 (range 2–20) and for ependymoma grade III was 25 (range 10–50). Several studies have found that Ki-67 correlated well with WHO tumour grade, tumour cellularity, microvascular proliferation, and mitotic activity [53, 66–69], with several studies also finding a significant correlation between Ki-67 and survival/prognosis in the ependymoma group [70–73].

Interestingly, in both ependymomas [38] and other non-glioma CNS tumours [74, 75], the degree of fluorescence and proliferative index are not correlated, whereas in gliomas fluorescence increases proportionally with tumour grade and MIB-1 proliferative index [39, 76, 77]. Moschovi et al. [59] and Bennetto et al. [70] found a strong linear correlation between Ki-67 index and the density of new blood vessel formation in children with MB and posterior fossa ependymomas respectively. Despite this angiogenesis, it appears that to a large extent an intact BBB is efficient at preventing 5-ALA from entering the brain [78], explaining the linear but not identical [39] correlation between gadolinium-enhanced MRI and PPIX florescence [79].

The "usefulness" of fluorescence-guided resection of the common posterior fossa paediatric brain tumour defined as "a change in surgical strategy or identification of residual tumour based on 5-ALA fluorescence" [35], has been found to be variable and not routinely useful to the surgeon in a number of surgical series [11, 30, 42, 44, 46, 80, 81]. Stummer et al. [35] in a multicentre retrospective survey combining 78 cases found that in supratentorial, strongly enhancing tumours, fluorescence was frequently "useful," whereas in infratentorial tumours fluorescence was less likely to be useful. In a systemic review of 175 published cases, Schwake et al. [34] demonstrated that 5-ALA-guided surgery had an influence on the grade of resection ($p < 0.001$). We attained post-operative MRI confirmed GTR in 73% of all the tumours and a 17% NTR in the remainder. Fluorescence was determined to be useful in 63% of cases and to be non-useful in 36% of cases. Neither strong fluorescence nor subjective usefulness was statistically significant in predicting GTR. Interestingly, in 3 cases in which the fluorescence was heterogenous, fluorescence was still regarded as useful as it allowed identification of infiltrative tumour within the cerebellar hemispheric which otherwise looked normal under white-light microscopy alone.

Pharmacokinetic data for 5-ALA are not available for paediatric subjects [82, 83]. However, according to the general literature regarding the pharmacodynamics in children [84], by 1 year of age the gastrointestinal absorption, hepatic, and renal clearance mechanisms approach that of adult levels. Based on these data, significant pharmacokinetic differences between children over the age of 1 year and adults are not expected [44]. For this reason, with respect to dosing we chose to adhere to the adult protocol of 20 mg/kg 5-ALA (Gliolan®). The 4 largest series [11, 35, 44, 85] looking at the use of 5-ALA in the paediatric group have also used this dosing regimen. Given the extremely low rate of side effects [11, 33–35, 44, 85] reported with this dose, it appears to be appropriate for children aged over 1 year of age. Very few data are available for children under the age of 1 year. The kinetics of porphyrin synthesis however appears to be different in paediatric CNS tumours, in particular MBs, as opposed to adult gliomas, in that in vitro MB cell lines appear to achieve their peak fluorescence at 6 h as opposed to at 3 h for glioma cell lines [31]. For this reason, we chose to administer the 5-ALA slightly earlier than in adult practice, 4 h prior to predicted cutting time, such that we could anticipate reaching peak fluorescence levels during the predicted "critical" time of tumour margin resection.

Limitations

The most significant limitation to our study was the unavailability of molecular subtyping of MBs and ependymomas at the time of the study. Molecular subtyping is more likely to be predictive of fluorescence than any other single variable [51]. Additionally, quantifying fluorescence levels is subjective and suffers from intra-observer and inter-observer variability [33]. Multiple classification systems of 5-ALA fluorescence exist [86], and there is no standard of reporting on diagnostic accuracy and clinical utility [87]. A consensus on reporting standard is needed and the addition of quantitative spectrometric analysis or high-resolution microscopy should be considered [88, 89].

Conclusions

We think that 5-ALA can be safely administered to paediatric patients. We found strong fluorescence in 68% of all posterior fossa tumours, and fluorescence was found to be useful in 63% of all patients. Statistically however this did not influence the extent of resection. The rate of fluorescence in the ependymoma group was significantly higher than in the MB group. Our paper supports the view that the routine use of 5-ALA might have a use in the resection of CNS tumour in children, especially in children with suspected or confirmed ependymoma; however, its routine use is not reliably useful and does not alter resection status and thus likely outcomes.

Acknowledgements

The author would like to thank Dr. S. Taylor and Dr. C. Lee for the help with the preparation of this manuscript.

Statement of Ethics

Written informed consent was obtained on behalf of the children from their parents or guardians. The Human Research Ethics Committee of the medical faculty of the University of the Witwatersrand approved the scientific analyses of these cases, reference number M200151.

Conflict of Interest Statement

The author reports no declarations of interest. The author alone is responsible for the content and writing of the paper.

Funding Sources

The author declares that no funding has been received in conjunction with the generation of this manuscript.

Author Contributions

Dr. Labuschagne was responsible for the conception and design of the manuscript along with the acquisition, analysis, and interpretation of the data. Dr. Labuschagne was responsible for the final approval of the version to be published.

References

1 Stepp H, Beck T, Pongratz T, Meinel T, Kreth FW, Tonn JC, et al. ALA and malignant glioma: fluorescence-guided resection and photodynamic treatment. J Environ Pathol Toxicol Oncol. 2007;26(2):157–64.
2 Ferraro N, Barbarite E, Albert T, Berchman E, Shah A, Bregy A, et al. The role of 5 aminolaevulinic acid in brain tumor surgery: a systemic review. Neurosurg Rev. 2016;39(4):545–55.
3 Stummer W, Stocker S, Wagner S, Stepp H, Fritsch C, Goetz C, et al. Intraoperative detection of malignant gliomas by 5-aminolevulinic acid-induced porphyrin fluorescence. Neurosurgery. 1998;42(3):518–25.
4 Pichlmeier U, Bink A, Schackert G, Stummer W. Resection and survival of glioblastoma multiforme: an RTOG recursive partitioning analysis of ALA study patients. Neuro Oncol. 2008;10(6):1025–34.
5 Schucht P, Beck J, Abu-Isa J, Andereggen L, Murek M, Seidel K, et al. Gross total resection rate in contemporary glioblastoma surgery: results of an institutional protocol combining 5-aminolevulinic acid intraoperative fluorescence imaging and brain mapping. Neurosurgery. 2012;71(5):927–35.
6 Stummer W, Pichlmeier U, Meinel O, Zanella F, Reulen HJ. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma, a randomized controlled multicenter phase lll trial. Lancet Oncol. 2006;7(5):359–60.
7 Ruge JR, Liu J. Use of 5-aminolevulinic acid for visualization and resection of a benign pediatric brain tumor. J Neurosurg Pediatr. 2009;4(5):484–6.
8 Agawa Y, Wataya T. The use of 5-aminolevulinic acid to assist gross total resection of pediatric astroblastoma. Childs Nerv Syst. 2018;34(5):971–5.
9 García L, Artero J, Sánchez ZM. Macías: fluorescence-guided resection with 5-aminolevulinic acid of meningeal sarcoma in a child. Childs Nerv Syst. 2015;33(5):1177–80.
10 Kim AV, Khachatryan VA. [Intraoperative fluorescence diagnosis using 5-aminolevulinic acid in surgical treatment of children with recurrent neuroepithelial tumors]. Zh Vopr Neirokhir Im N N Burdenko. 2017;81(1):51–7.
11 Preuß M, Renner C, Krupp W, Christiansen H, Fischer L, Merkenschlager A. The use of 5-aminolevulinic acid fluorescence guidance in resection of pediatric brain tumors. Childs Nerv Syst. 2013;29:1263–7.
12 Suzuki T, Scoichiro I, Kohei F, Tomoyuki K, Mitsuaki S, Adachi J, et al. Neuroendoscopic photodynamic diagnosis and biopsy of intraventricular germinomas using 5-aminolevulinic acid. Childs Nerv Syst. 2012;28:1589–669.
13 Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, et al. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. Acta Neuropathol. 2016;131(6):803–20.
14 Avula S, Mallucci C, Kumar R, Pizer B. Posterior fossa syndrome following brain tumor resection: review of pathophysiology and a new hypothesis on its pathogenesis. Childs Nerv Syst. 2015;31:1859–67.
15 Gudrunardottir T, Sehested A, Juhler M, Grill J, Schmiegelow K. Cerebellar mutism: definitions, classification and grading of symptoms. Childs Nerv Syst. 2011;27(9):1361–3.
16 Massimino M, Biassoni V, Gandola L, Garré ML, Gatta G, Giangaspero F, et al. Childhood medulloblastoma. Crit Rev Oncol Hematol. 2019;135:1–134.
17 Albright A, Wissoff J, Zeltzer P, Boyett J, Rorke L, et al. Effects of medulloblastoma resections on outcome in children: a report from the children's cancer group. Neurosurgery. 1996;38(2):265–71.

18 Zeltzer, Boyett J, Finlay J, Albright A, Rorke L, Milstein J, et al. Metastasis stage, adjuvant treatment, and residual tumor are prognostic factors for medulloblastoma in children: conclusion from the children's cancer group 921 randomized phase III study. J Clin Oncol. 1999;17(3):832–45.
19 Massimino M, Solero CL, Garrè ML, Biassoni V, Cama A, Genitori L, et al. Second-look surgery for ependymoma: the Italian experience. J Neurosurg Pediatr. 2011;8(3):246–50.
20 Sandford RA, Merchant TE, Zwienenberg-Lee M, Kun LE, Boop FA. Advances in surgical techniques for resection of childhood cerebellopontine angle ependymomas are key to survival. Childs Nerv Syst. 2009;25:1229–40.
21 Briel-Pump A, Beez T, Ebbert L, Remke M, Weinhold S, Sabel MC, et al. Accumulation of protoporphyrin IX in medulloblastoma cell lines and sensitivity to subsequent photodynamic treatment. J Photochem Photobiol B. 2018;189:298–305.
22 Meyers SP, Kemp SS, Tarr RW. MR imaging features of medulloblastomas. AJR Am J Roentgenol. 1992;158(4):859–65.
23 Ferguson SD, Levine NBL, Suki D, Tsung AJ, Lang FF, Sawaya R, et al. The surgical treatment of tumors of the fourth ventricle: a single-institution experience. Int J Surg Case Rep. 2013;4(10):842–5.
24 Ikezaki K, Matsushima T, Inoue T, Yokoyama N, Kaneko Y, Fukui M, et al. Correlation of microanatomical localization with postoperative survival in posterior fossa ependymomas. Neurosurgery. 1993;32(1):38–44.
25 Nazar GB, Hoffman HJ, Becker LE, Jenkin D, Humphreys RP, Hendrik EB. Infratentorial ependymomas in childhood: prognostic factors and treatment. J Neurosurg. 1990;72:408–17.
26 Pierre-Kahn A, Hirsch JF, Roux FX, Renier D, Sainte-Rose C. Intracranial ependymomas in children. Survival and functional results of 47 cases. Childs Brain. 1983;10(3):145–56.
27 Qui B, Wang Y, Wand W, Wang C, Wu P, Bao Y, et al. Microsurgical management of pediatric ependymomas of the fourth ventricle via the transcerebellomedullary fissure approach: a review of 26 cases. Oncol Lett. 2016;11(6):4099–106.
28 Spagnoli D, Tomei G, Ceccarelli G, Grimoldi N, Lanterna A, Bello L, et al. Combined treatment of fourth ventricle ependymomas: report of 26 cases. Surg Neurol. 2000;54(1):19–26.
29 Chu ES, Wong TK, Yow CM. Photodynamic effect in medulloblastoma: downregulation of matrix metalloproteinases and human telomerase reverse transcriptase expressions. Photochem Photobiol Sci. 2008;7(1):76–83.
30 Ritz R, Scheidle C, Noell S, Roser F, Schenk M, Dietz K, et al. In vitro comparison of hypericin and 5-aminolevulinic acid-derived protoporphyrin IX for photodynamic inactivation of medulloblastoma cells. PLoS One. 2012;7(12):e51974–10.
31 Schwake M, Günes D, Köchling M, Brentrup A, Schroeteler J, Hotfilder M, et al. Kinetics of porphyrin fluorescence accumulation in pediatric brain tumor cells incubated in 5-aminolevulinic acid. Acta Neurochir. 2014;156(6):1077–84.
32 Schwake M, Nemes A, Dondrop J, Schroeteler J, Schipmann S, Senner V, et al. In-vitro use of 5-ALA for photodynamic therapy in pediatric brain tumors. Neurosurgery. 2018;83(6):1328–37.
33 Roth J, Constantini S. 5ALA in pediatric brain tumors is not routinely beneficial. Childs Nerv Syst. 2017;33(5):787–92.
34 Schwake M, Schipmann S, Müther M, Köchling M, Brentrup A, Stummer W. 5-ALA fluorescence-guided surgery in pediatric brain tumors-a systematic review. Acta Neurochir. 2019;161(6):1099–108.
35 Stummer W, Rodriques F, Schucht P, Preuss M, Wiewrodt D, Nestler U, et al. Predicting the "usefulness" of 5-ALA- derived tumor fluorescence for fluorescence-guided resections in pediatric brain tumors: a European survey. Acta Neurosurg. 2014;156:2315–24.
36 Wainwright JV, Endo T, Cooper JB, Tominaga T, Schmidt MH. The role of 5-aminolevulinic acid in spinal tumor surgery: a review. J Neurooncol. 2019;141(3):575–84.
37 Millesi M, Kiesel B, Woehrer A, Hainfellner JA, Novak K, Martínez-Moreno M, et al. Analysis of 5-aminolevulinic acid-induced fluorescence in 55 different spinal tumors. Neurosurg Focus. 2014;36(2):E11–9.
38 Moreno RG, García LMB, Bastidas HI, Tirado CAM, Flores AM, Cabezas JPS, et al. Fluorescence guided surgery with 5-aminolevulinic acid for resection of spinal cord ependymomas. Asian Spine J. 2019;13(1):119–25.
39 Floeth FW, Sabel M, Ewelt C, Stummer W, Felsberg J, Reifenberger G, et al. Comparison of (18)F-FET PET and 5-ALA fluorescence in cerebral gliomas. Eur J Nucl Med Mol Imaging. 2011;38(4):731–41.
40 Stummer W, Novotny A, Stepp H, Goetz C, Bise K, Reulen HJ. Fluorescence-Guided resection of glioblastoma multiforme by using 5-aminolevulinic acid-induced porphyrins: a prospective study in 52 consecutive patients. J Neurosurg. 2000;93(6):1003–13.
41 Widhalm G, Kiesel B, Woehrer A, Traub-Weidinger T, Preusser M, Marosi C, et al. 5-aminolevulinic acid induced fluorescence is a powerful intraoperative marker for precise histopathological grading of gliomas with non-significant contrast-enhancement. PLoS One. 2013;8(10):e76988–8.
42 Skjøth-Rasmussen J, Bøgeskov L, Sehested A, Klausen C, Broholm H, Nysom K. The use of 5-ALA to assist complete removal of residual non-enhancing part of childhood medulloblastoma: a case report. Childs Nerv Syst. 2015;31(11):2173–7.
43 Puppa AD, Gioffré G, Gardiman MP, Frasson C, Cecchin D, Scienza R, et al. Intra-operative 5-aminolevulinic acid (ALA)-induced fluorescence of medulloblastoma: phenotypic variability and CD133+ expression according to different fluorescence patterns. Neurol Sci. 2014;35:99–102.
44 Beez T, Sarikaya-Seiwert S, Steiger HJ, Hänggi D. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of brain tumors in children – a technical report. Acta Neurochir. 2014;156(3):597–604.
45 Eicker S, Sarikaya-Seiwert S, Borkhardt A, Gierga K, Turowski B, Heiroth HJ, et al. ALA-induced porphyrin accumulation in medulloblastoma and its use for fluorescence-guided surgery. Cent Eur Neurosurg. 2011;72(2):101–3.
46 Barbagallo GM, Certo F, Heiss K, Albanese V. 5-ALA fluorescence-assisted surgery in pediatric brain tumors: report of three cases and review of the literature. BR J Neurosurg. 2014;28(6):750–4.
47 Burford C, Kayal N, Pandit A, Tailor J, Lavrador J, Bravo A. 5-aminolevulinic acid aided resection of pediatric brain tumours: the UK's first case series. Neuro Oncol. 2017 Jan;19(Suppl 1):i11.
48 Zhang C, Boop FA, Ruge J. The use of 5-aminolevulinic acid in resection of pediatric brain tumors: a critical review. J Neurooncol. 2019;141(3):567–73.
49 Wolburg H, Noell S, Fallier-Becker P, Mack AF, Wolburg-Buchholz K. The disturbed blood-brain barrier in human glioblastoma. Mol Aspects Med. 2012;33(5–6):579–89.
50 Warnke PC, Kopitzki K, Timmer J, Ostertag CB. Capillary physiology of human medulloblastoma: impact on chemotherapy. Cancer. 2006;107(9):2223–7.
51 Saito K, Hirai T, Takeshima H, Kadota Y, Yamashita S, Ivanova A, et al. Genetic factors affecting intraoperative 5-aminolevulinic acid-induced fluorescence of diffuse gliomas. Radiol Oncol. 2017;51(2):142–50.
52 Grotzer MA, Geoerger B, Janss AJ, Zhao H, Rorke LB, Phillips PC. Prognostic significance of Ki-67 (MIB-1) proliferation index in childhood primitive neuroectodermal tumors of the central nervous system. Med Pediatr Oncol. 2001;36(2):268–73.
53 Kayaselçuk F, Zorludemir S, Gümürdühü D, Zeren H, Erman T. PCNA and Ki-67 in central nervous system tumors: correlation with the histological type and grade. J Neurooncol. 2002;57(2):115–21.
54 Meurer RT, Martins DT, Hilbig A, Ribeiro MC, Roehe AV, Barbosa-Coutinho LM, et al. Immunohistochemical expression of markers Ki-67, NeuN, synaptophysin, P53 and Her2 in medulloblastoma and its correlation with clinicopathological parameters. Arq Neuropsiquiatr. 2008;66(2B):385–90.
55 Ertan Y, Sezak M, Demirağ B, Kantar M, Cetingül N, Turhan T, et al. Medulloblastoma: clinicopathologic evaluation of 42 pediatric cases. Childs Nerv Syst. 2009;25(3):353–6.

56 Ferrari AF, Araújo MBM, Aquiar PH. Please JPP: medulloblastoma. Arq Neuropsiquiatr. 2003;61(3-A):547–51.
57 Jadali F, Amini E, Esfahani M, Alavi S. Pediatric medulloblastoma and the prognostic value of MIB-1 proliferative factor. IJBC. 2009;5:7–10.
58 Miralbell R, Tolnay M, Bieri S, Probst A, Sappino A, Berchtold W, et al. Pediatric medulloblastoma: prognostic value of p53, bcl-2, Mib-1 and microvessel density. J Neurooncol. 1999;45(2):103–10.
59 Moschovi M, Koultouki E, Stefanaki K, Sfakianos G, Tourkantoni N, Prodromou N, et al. Prognostic significance of angiogenesis in relation to Ki-67, p-53, p-27, and bcl-2 expression in embryonal tumors. Pediatr Neurosurg. 2011;47(4):241–7.
60 Vasugi GA, Sundaram S, D'Cruze L, Rajendrau A, Scott JX. Comparative immunohistochemical analysis of Ki-67 in a spectrum of pediatric solid tumors. Asian J Neurosurg. 2018;13(4):1026–32.
61 Ming-Tak Ho D, Hsu C, Wong T, Ting L, Chiang H. Atypical teratoid/rhabdoid tumor of the central nervous system: a comparative study with primitive neuroectodermal tumor/medulloblastoma. Acta Neuropathol. 2000;99:482–8.
62 Quiñones-Hinojosa A, Sanai N, Smith JS, McDermott MW. Techniques to assess the proliferative potential of brain tumors. J Neurooncol. 2005;74(1):19–30.
63 Nam DH, Wang KC, Kim YM, Chi JG, Kim SK, Cho BK. The effect of isochromosome 17q presence, proliferative and apoptotic indices, expression of c-erbB-2, bcl-2 and p53 proteins on the prognosis of medulloblastoma. J Korean Med Sci. 2000;15(4):452–6.
64 Ito S, Hoshino T, Prados MD, Edwards MSB. Cell kinetics of medulloblastomas. Cancer. 1992;70(3):672–8.
65 Patereli A, Alexiou GA, Stefanaki K, Moschovi M, Doussis-Anagnostopoulou I, Prodromou N, et al. Expression of epidermal growth factor receptor and HER-2 in pediatric embryonal brain tumors. Pediatr Neurosurg. 2010;46(3):188–92.
66 Sharma V, Shoaib Y, Gupta LN, Dagar A. P53 and Ki-67 expression in primary pediatric brain tumors: does it correlate with presentation, histological grade, and outcome. Asian J Neurosurg. 2018;13(4):1026–32.
67 Suzuki S, Oka H, Kawano N, Tanaka S, Utsuki S, Fujii K. Prognostic value of Ki-67 (MIB-1) and p53 in ependymomas. Brain Tumor Pathol. 2001;18(2):151–4.
68 Vaishali SS, Tatke M, Singh D, Sharam A. Histological spectrum of ependymomas and correlation of p53 and Ki-67 expression with ependymoma grade and subtype. Indian J Cancer. 2004;41(2):66–71.
69 Valshall SS, Tatke M, Singh D, Sharma A. Histological spectrum of ependymomas and correlation of p53 and Ki-67 expression with ependymoma grade and subtype. Indian J Cancer. 2004;41(2):66–71.
70 Bennetto L, Foreman N, Harding B, Hayward R, Ironside J, Love S, et al. Ki-67 immunolabelling index is a prognostic indicator in childhood posterior fossa ependymomas. Neuropathol Appl Neurobiol. 1998;24(6):434–40.
71 Korshunov A, Golanov A, Timirgaz V. Immunohistochemical markers for intracranial ependymoma recurrence. An analysis of 88 cases. J Neurol Sci. 2000;177(1):72–82.
72 Verstegen MJT, Troost D, Leenstra S, IJlst-Keizers H, Bosch DA. Proliferation- and apoptosis-related proteins in intracranial ependymomas: an immunohistochemical analysis. J Neurooncol. 2002;56(1):21–8.
73 Zamecnik J, Snuderl M, Eckschlager T, Chanova M, Hladikova M, Tichy M, et al. Pediatric intracranial ependymomas: prognostic relevance of histological, immunohistochemical, and flow cytometric factors. Mod Pathol. 2003;16(10):980–91.
74 Motekallemi A, Jeltema HR, Metzemaekers JD, Van Dam GM, Crane LM, Groen RJ. The current status of 5-ALA fluorescence-guided resection of intracranial meningiomas – a critical review. Neurosurg Rev. 2015;38(4):619–28.
75 Puppa AD, Rustemi OR, Gioffré G, Troncon I, Lombardi G, Rolma G, et al. Predictive value of intraoperative 5-aminolevulinic acid-induced fluorescence for detecting bone invasion in meningioma surgery. J Neurosurg. 2014;120:840–5.
76 Ishihara R, Katayama Y, Watanabe T, Yoshino A, Fukushima T, Sakatani K. Quantitative spectroscopic analysis of 5-aminilevulinic acid-induced protoporphyrin IX fluorescence intensity in diffusely infiltrating astrocytomas. Neurol Med Chir (Tokyo). 2007;47(2):53–7; discussion 57.
77 Sanai N, Snyder LA, Honea NJ, Coons SW, Eschbacher JM, Smith KA, et al. Intraoperative confocal microscopy in the visualization of 5-aminolevulinic acid fluorescence in low-grade gliomas. J Neurosurg. 2011;115(4):740–8.
78 Ennis SR, Novotny A, Xiang J, Shakui P, Masada T, Stummer W, et al. Transport of 5-aminolevulinic acid between blood and brain. Brain Res. 2003;959(2):226–34.
79 Samkoe KS, Gibbs-Strauss SL, Yang HH, Khan Hekmatyar S, Jack Hoopes P, O'Hara JA, et al. Protoporphyrin IX fluorescence contrast in invasive glioblastomas is linearly correlated with Gd enhanced magnetic resonance image contrast but has higher diagnostic accuracy. J Biomed Opt. 2011;16(9):096008.
80 Wataya T. Surg-34. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of pediatric brain tumors. Neuro Oncol. 2017 Nov;19(Suppl 6):vi242.
81 Schwake M, Kaneko S, Suero Molina E, Müther M, Schipmann S, Köchling M, et al. Spectroscopic measurement of 5-ALA-induced intracellular protoporphyrin IX in pediatric brain tumors. Acta Neurochir. 2019;161(10):2099–105.
82 Eljamel MS, Goodman C, Moseley H. ALA and photofrin fluorescence-guided resection and repetitive PDT in glioblastoma multiforme. A single centre phase III randomised controlled trial. Lasers Med Sci. 2008;23(4):361–7.
83 EMEA. 2007. Gliolan: EPAR – scientific discussion. www.ema.europa.eu/docs/en_GB/document_library/EPAR_Scientific_Discussion/human/000744/WC500021788.pdf.
84 Alcorn J, McNamara PJ. Pharmacokinetics in the newborn. Adv Drug Deliv Rev. 2003;55(5):667–86.
85 Goryaynov SA, Okhlopkov VA, Golbin DA, Chernyshov KA, Svistov DV, Martynov BV, et al. Fluorescence diagnosis in neurooncology: retrospective analysis of 653 cases. Front Oncol. 2019;9(830):830–8.
86 Kamp MA, Molle ZK, Munoz-Bendix C, Rapp M, Sabel M, Steiger H-J, et al. Various shades of red–a systematic analysis of qualitative estimation of ALA-derived fluorescence in neurosurgery. Neurosurg Rev. 2018;41(1):3–18.
87 Stummer W, Koch R, Valle RD, Roberts DW, Sanai N, Kalkanis S, et al. Intraoperative fluorescence diagnosis in the brain: a systematic review and suggestions for future standards on reporting diagnostic accuracy and clinical utility. Acta Neurochir. 2019;161(10):2083–98.
88 Khurana AKM, Moriyama Y, Wilson BC. Quantification of in vivo fluorescence decoupled from the effects of tissue optical properties using fiber-optic spectroscopy measurements. J Biomed Opt. 2010;15(6):1–12.
89 Wei L, Fujita Y, Sanai N, Liu JTC. Toward quantitative neurosurgical guidance with high-resolution microscopy of 5-aminolevulinic acid-induced protoporphyrin IX. Front Oncol. 2019;9(592):1–7.