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ORIGINAL ARTICLE

Sodium fluorescein in pediatric oncological neurosurgery: a pilot study on 50 children

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Abstract

Background Sodiumfluorescein (SF) is currently considered a valid intraoperative adjunct in the resection of high-grade brain lesions in adults. Experiences in pediatric groups and in low-grade gliomas and other low-grade lesions are still limited in literature, and subjective evaluation of fluorescence is still a limitation.

Material and methods This study retrospectively reviewed all patients with brain or spine lesions operated on from September 2021 to July 2022 in the Pediatric Neurosurgery Unit of Hôpital Femme Mère Enfant, Lyon, who had received 5 mg/kg of 10%. Surgery was performed using a YELLOW560 filter at crucial times. At the end of surgery, the first operator completed a questionnaire, including his opinion on whether SF had been useful in tumor resection, recorded as a binary variable. Post hoc, surgical images were reviewed using ImageJ, an open-source Java image processing platform. In order to compare independent discrete variables, we applied the Student's *t* test, and we applied the Chi-square or Fisher exact test for binary variables. A threshold of $p < 0.05$ was set for statistical significance.

Results We included 50 pediatric patients (0.2–17.6 years old). Forty/50 lesions showed SF uptake (80%). The differentiation between healthy and affected tissue, thanks to SF, subjectively evaluated by the surgeon, had as objective counterpart the statistically significant higher brightness of green in lesions, registered by the software ($p < 0.001$). SF overall allowed a good differentiation in 33/50 lesions, and overall utility of SF has been noted in 67% of them. When specifically considering gliomas, overall utility reached 75%.

Conclusion SF is a feasible, safe, and useful intraoperative adjunct in pediatric neurosurgery. In particular, it seems to have a promising role in some low-grade infiltrating glial tumors. The subjective evaluation of fluorescence seems to be reliable with respect to image analyses software.

Keywords Sodium fluorescein · Pediatric oncological neurosurgery · Brain lesions

Introduction

Tumors of the central nervous system are the most common solid neoplasms in children and adolescents [1], in particular low-grade gliomas (LGG) in slightly older patients and embryonal tumors such as medulloblastomas in the younger, which generally present a variable but remarkable degree of contrast gadolinium enhancement at preoperative magnetic resonance imaging (MRI) [1]. This is thought to be due to alteration in the blood brain barrier (BBB), which is altered in these types of tumors even in histologically low-grade lesions [2]. Moreover, pediatric neurosurgery also offers a wide range of other mass lesions requiring resection, such as inclusion cysts, lipomas of the conus, and epidural tumors [3, 4].

In the potentially evolutive oncological cases, it is known that maximal safe extent of resection (EOR) might play a major role on outcome and survival [5, 6], while in low-grade anomalies, maximal safe EOR could help in the balance between the risks of clinical deterioration and recurrence [3, 4].

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A certain number of intraoperative adjuncts have been proposed to improve the maximal safe EOR both in adult and pediatric oncological neurosurgery. In recent years, besides functional adjuvants as intraoperative neuromonitoring [7] or awake surgery [8, 9], intraoperative imaging has been developed from computed tomography to magnetic resonance [10] to ultrasound (with or without enhancement) [11]. The use of fluorophores has been introduced in this context.

Different centers around the world have progressively reported their experience with 5-aminolevulinic acid (5-ALA), a hemoglobin precursor which induces excess production of the endogenous protoporphyrin IX in tissues with a high cellular turnover rate, especially in adult high-grade gliomas, but also in some pediatric tumors [12].

More recently, some centers all around the world started to use sodium fluorescein (SF) as intraoperative adjunct. SF is a fluorescent dye, already used in neurosurgery since the 1940s [13], which accumulates in the extracellular space through diffusion, i.e., extravasation [14]. Its role in high-grade gliomas (HGG) has been repeatedly suggested [15], but some literature articles also reported its use in other malignant or benign conditions [16–21], as well as in exclusively pediatric populations [22, 23], generally without any type of adverse reaction. However, a definitive statement about feasibility and utility of SF in pediatrics has not been formulated yet. Moreover, the lack of objective evaluation of fluorescence still seems to be a limitation to compare reports and evaluate utility.

In our institution, we used SF-assisted surgery in children operated on for tumor surgery (including both potentially evolutive tumors and malformative masses such as dermoid cysts and lipomas). In order to evaluate the utility of this technique, we decided to report our experience over the last period.

Materials and methods

Data about children with brain and spine lesions operated on with SF from September 2021 to July 2022 in the Pediatric Neurosurgery Unit of Hôpital Femme Mère Enfant (Lyon) were retrieved for the study (this retrospective study was submitted to and approved by our institutional ethics committee, IRB de Neurochirurgie, no. IRB00011687, Collège de Neurochirurgie IRB #1: 2022/46). We searched for age at surgery, weight, SF administered dose, SF injection time, incision time, time on tumor, possible adverse reaction registered, tumor histology, tumor location, contrast enhancement, type of surgery planned and performed (biopsy, subtotal resection, or total resection), utility of SF in differentiating tumor and healthy tissue, and overall utility of SF.

Intraoperative data

The day of surgery, a dose of 5 mg/kg of SF (Fluorescéine sodique Faure® 10%, SERB SA, Bruxelles, Belgium) was administered intravenously at the end of anesthesia induction. Parental informed consent was obtained as already published elsewhere [24, 25].

Surgery was performed under a microscope equipped with a dedicated filter (KINEVO 900 with YELLOW 560 nm wavelength filter, Zeiss Meditec, Oberkochen, Germany), and the surgeon had to switch temporarily from white light to filter as soon as the tumor was visible, then at any significant moment during tumor resection.

Videos and photos were also regularly taken along the whole procedure.

A dedicated form was filled by the anesthesiologist (age at surgery, induction time, time at administration, time at incision, immediate adverse reactions, and late adverse reactions) and the surgeon (tumor location, contrast enhancement at MRI, planned EOR, intensity of fluorescence was recorded as a semi-quantitative, four-tier variable: +++ or bright, ++ or moderate, + or modest,—or absent, differentiation between lesion and healthy tissues, overall utility).

Image analysis

Videos and photos taken from the surgical microscope were retrospectively reviewed and analyzed with an open-source Java image processing platform, ImageJ® (Version 1.53, National Institute of Health, Bethesda, Maryland, USA) as already suggested elsewhere [26].

A region of interest (ROI) was selected on an area of the lesion and on a zone of healthy tissue in proximity. For a ROI, the platform can return a color analysis quantifying the content of each color (frequency of every shade of a color, marked with a number from 0, the darkest, to 255, the brightest). We registered red and green quantities for each selected ROI (Fig. 1).

Statistical analysis

For statistical analyses, we used an online open-source software, jamovi® (www.jamovi.org) [27].

We calculated frequencies, mean values, and dispersion measures. In order to compare independent discrete variables, we applied the Student's *t* test, and we applied the Chi-square or Fisher exact test for binary variables. A threshold of $p < 0.05$ was set for statistical significance.

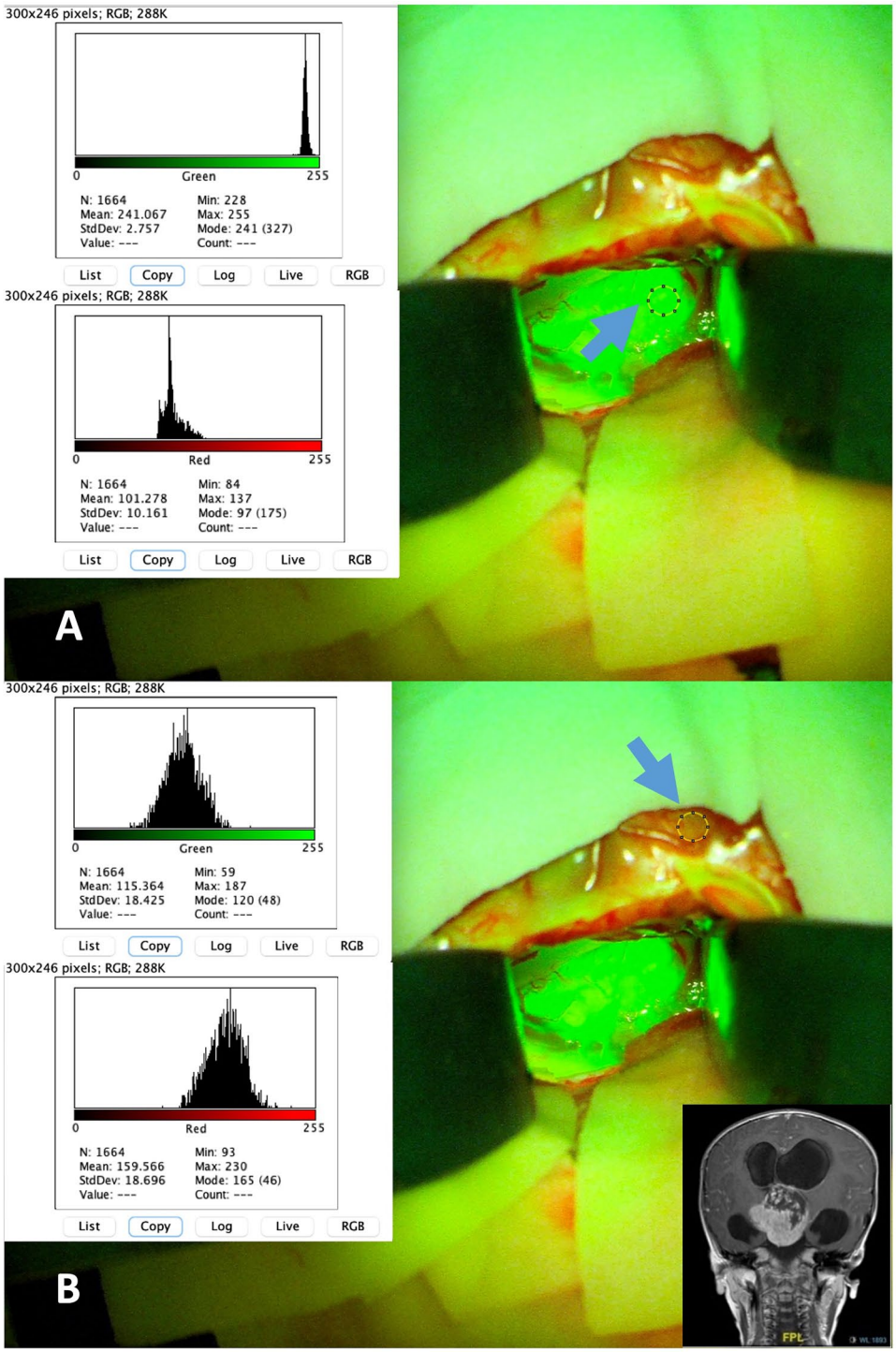

Fig. 1 Example of utilization of ImageJ®. A region of interest (ROI) was selected on an area of the lesion (blue arrow in **A**) and on a zone of healthy tissue in proximity (blue arrow in **B**). For each ROI, the platform can return a color analysis quantifying the content of each color, expressed both numerically and with a histogram (on the left of both **A** and **B**). We registered both green quantity for lesion and healthy tissue, and red quantity for lesion and healthy tissue. The bottom right square represents the preoperative MRI post-contrast T1 image of the lesion intraoperatively photographed

Results

General findings

We retrospectively included 50 pediatric patients, aged from 3 months to 17.6 years old and with a weight ranging from 8 to 68 kg (table in the Supplementary material).

Location was supratentorial in 27 cases, infratentorial in 15, and spinal in 8 (intradural in 6 and epidural in 2).

Gross total resection (GTR) was the planned aim of surgery in 34/50 interventions, and this goal was reached in every one of them. In 12/50 cases, a partial/subtotal resection (STR) was planned and reached, while in the remaining 4/50, a biopsy was realized.

The pathological analysis showed low-grade lesions in 34 cases (28 WHO grade I tumors, 1 WHO grade II neoplasm, and 5 benign mesenchymal lesions), while high-grade lesions amounted to 15, with 2 WHO grade III tumors, 10 WHO grade IV neoplasms, and 3 other mesenchymal malign lesions. A single lesion remained histologically unclassifiable.

Association between intensity of fluorescence and pathology is summarized in Fig. 2.

All patients received a dose of 5 mg/kg of 10% SF. In 5/50 cases, data about injection time were incomplete and were therefore excluded from this analysis. Time of injection preceded the incision in 41/45 patients (91%). However, it was not always at the end of induction. Overall, we had data about injection time, time of incision, and time on tumor, and we noted some degree of intraoperative fluorescence in the 35/45 cases, while in 10/45 cases, the lesion was non fluorescent.

When evaluating the delay between injection time and incision, in the majority of cases, the time interval ranged from 31′ to 1 h (Fig. 3A), and with this protocol, we also observed the highest percentage of good differentiation between healthy and tumor tissue (Fig. 3A).

On the other hand, when evaluating the period of time between injection and tumor exposition, it ranged from 1 h 31′ and 2 h in almost one-third of cases (13/45, Fig. 3B). We observed a good differentiation of the interface healthy tumor in 77% of these cases (10/13) and in 100% of cases when 2 h–2 h 30 had passed (4/4 — Fig. 3B).

We noted no adverse effect after the injection of SF. A case of late hypotension was noted, but causality with SF could not be definitively determined (we could neither exclude a link with SF injection, nor with tumor removal itself). No sequelae were registered in this case. In every other case, neither immediate nor late adverse events were described. Each patient had a self-limiting yellowish discoloration of urine in the 24 h following surgery.

Enhancement: "quality" of fluorescence and comparison with gadolinium enhancement at MRI

Contrast enhancement at preoperative MRI (table in the supplementary material) could not be determined in 4/50 cases (2 dermoid cysts and 2 lipomas). In the remaining 46 cases, a variable amount of gadolinium enhancement was registered in 35/46 cases (modest in 4, moderate in 15, intense/bright in 17), whereas no uptake was evident in 11/46 cases.

As far as it concerns intraoperative fluorescence (Table 1), we equally divided the cases in two groups: enhancing and not enhancing. SF enhancement was globally found in 40/50 cases (80%).

We then compared MRI enhancement and intraoperative SF enhancement (Table 1).

Overall, 34 out of 35 lesions showing preoperative gadolinium enhancement showed intraoperative fluorescence (97%).

Among 11 lesions not enhancing on preoperative MRI, 1 showed a bright SF uptake and 1 a moderate one, while 3 had a modest fluorescence and 6 no fluorescence at all.

When looking at the biunivocal associations (grey shaded cells in Table 1), pathologies are as follows:

- Among the 10 lesions that were bright (+++) both at preoperative gadolinium MRI and intraoperative fluorescence, we found 6 pilocytic astrocytomas, 1 gangliogliomas, 1 meningioma, 1 craniopharyngioma, and 1 atypical choroid plexus papilloma.
- Among the 6 lesions showing moderate gadolinium enhancement and SF uptake (++), there were 3 medulloblastomas, 2 pilocytic astrocytomas, and 1 craniopharyngioma.
- Among the 6 lesions showing neither gadolinium enhancement nor intraoperative fluorescence, we counted 2 low-grade gliomas (1 pilocytic astrocytoma and 1 epileptogenic oligoid tumor CD34 +), 1 anaplastic glioma G34 mutated, 1 dysembryoplastic neuroepithelial tumor (DNET), 1 metastasis from alveolar rhabdomyosarcoma, and 1 lesion not otherwise specified.

Image analysis: "quantity" of fluorescence

We retrospectively analyzed the images taken during surgery in order to evaluate the possibility of differentiating healthy vs. altered tissue using a software and to determine whether the subjective perception of the surgeon may have an objective, numerical counterpart.

In 1/50 case, we had no available images, while in 5/50 cases, we had only images and the end of surgery, when no tumor was visible anymore. We had therefore 44 suitable cases for our analysis.

We subdivided the cases as follows (Fig. 4):

– Group A, with intraoperative SF uptake:

  - Subgroup A1, group in which the surgeon reported good differentiation between healthy and altered tissue (28 cases, Fig. 4A)
  - Subgroup A2, group in which the surgeon reported no differentiation between healthy and altered tissue (7 cases, Fig. 4B)

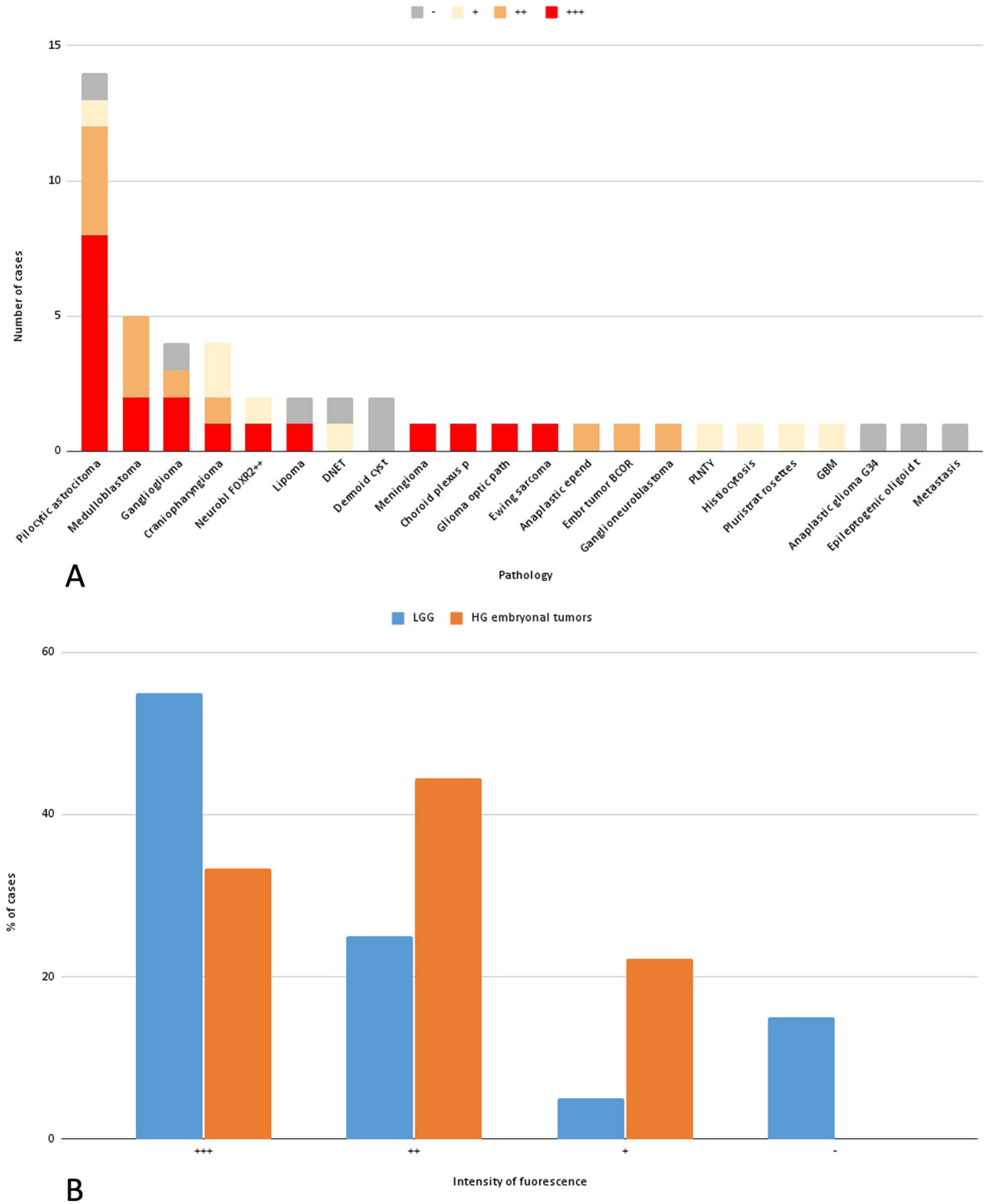

Fig. 2 Association between intensity of fluorescence and pathology. **A** shows how many cases for each tumor showed a certain degree of fluorescence. **B** analyzes percentages of degree of fluorescence for histological groups: low-grade gliomas (LGG, 20 cases) and high-grade (HG) embryonal tumors (9 cases). DNET, dysembryoplastic neuroepithelial tumor; embr tumor BCOR, embryonal tumor subtype BCOR-ITD; GBM, glioblastoma; Neurobl, neuroblastoma; p, papilloma; path, pathways; PLNTY, polymorphous low-grade neuroepithelial tumor of the young; pluristratified rosettes, embryonal tumor with pluristratified rosettes; t, tumor. Medulloblastomas were considered as a single group independently from their molecular subtype

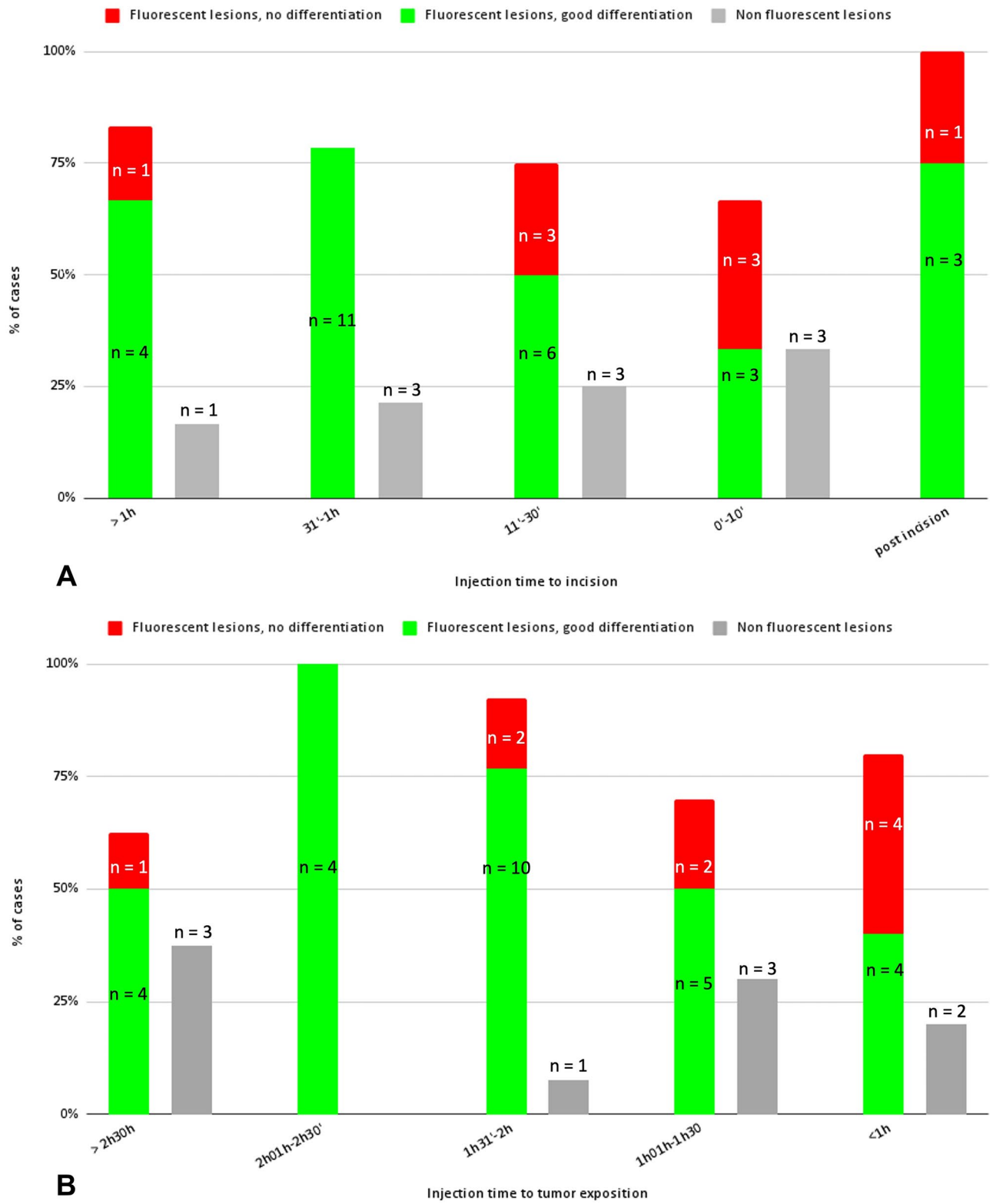

Fig. 3 Injection time and intensity of intraoperative fluorescence. **A** Time interval between injection and incision. **B** Time interval between injection and tumor exposition

Table 1 Enhancement at preoperative MRI vs SF intraoperative uptake

MRI\SF	+++	++	+	-
+++	10	3	3	0
++	6	6	3	0
+	1	2	0	1
-	1	1	3	6

Shaded cells represent the perfect correspondance between intensity of gadolinium enhancement at the preoperative MRI and intensity of intraoperative fluorescence from sodium fluorescein

MRI magnetic resonance imaging, *SF* sodium fluorescein, +++ bright, ++ moderate, + modest, – absent

– Group B, without intraoperative SF uptake (9 cases)

For every group, we calculated for both altered tissue and healthy tissue: the mean of all values of $mean_{green}$ and $mean_{red} \pm$ the standard deviation, the median of all values of $mean_{green}$ and $mean_{red}$, the min and the max registered $mean_{green}$ and $mean_{red}$. We then compared green in healthy vs. affected tissue and red in healthy vs. affected tissue.

In group A1, tissue differentiation was statistically significant for the color green, while it was not for the color red (Table 2).

For this group, we did the same analysis under white light, to evaluate if SF can differentiate as well under this condition, even if the human eye cannot perceive it. In this case, tissue differentiation was not statistically significant for the color green, but it was for the color red, i.e., the altered tissue was less red than the healthy tissue (Table 2).

Group A2 included cases in which the surgeon reported impossibility of tissue differentiation, although the tumor

Table 2 Comparison of green and red quantities in healthy tissue vs. affected tissue

Group A, subgroup A1			
Mean ± SD (median), 95% CI, **YELLOW 560 nm**			
Complementary colors	Healthy tissue ($n=28$)	Affected tissue ($n=28$)	Student's t p-value
Green mean value	132.29 ± 42.77 (127.17) 95% CI [116.45, 148.14]	226.31 ± 36.92 (241.53) 95% CI [212.63, 239.98]	**< .001**
Red mean value	170.07 ± 47.44 (171.75) 95% CI [152.50, 187.65]	153.06 ± 47.08 (167.23) 95% CI [135.62, 170.50]	0.184
Mean ± SD (median), 95% CI, **White Light**			
Complementary colors	Healthy tissue ($n=27$*)	Affected tissue ($n=27$*)	Student's t p-value
Green mean value	115.70 ± 30.32 (116.91) 95% CI [104.27, 127.14]	126.92 ± 58.39 (123.93) 95% CI [104.89, 148.94]	0.380
Red mean value	229.97 ± 25.54 (237.65) 95% CI [220.33, 239.60]	206.95 ± 47.50 (223.41) 95% CI [189.04, 224.87]	**0.031**
Group A, subgroup A2			
Mean ± SD (median), 95% CI, **YELLOW 560 nm**			
Complementary colors	Healthy tissue ($n=7$)	Affected tissue ($n=7$)	Student's t p-value
Green mean value	162.51 ± 55.83 (151.97) 95% CI [121.15, 203.86]	184.65 ± 47.55 (192.68) 95% CI [149.43, 219.87]	0.440
Red mean value	179.71 ± 31.00 (169.40) 95% CI [156.75, 202.67]	152.24 ± 15.08 (151.65) 95% CI [141.07, 163.41]	0.057
Group B			
Mean ± SD (Median), 95% CI, **YELLOW 560 nm — 1st subanalysis**			
Complementary colors	Healthy tissue ($n=9$)	Affected tissue ($n=9$)	Student's t p-value
Green mean value	204.62 ± 35.12 (199.49) 95% CI [181.68, 227.57]	154.20 ± 47.68 (141.24) 95% CI [123.05, 185.35]	**0.021**
Red mean value	195.99 ± 34.32 (193.58) 95% CI [173.57, 218.41]	170.37 ± 53.17 (167.78) 95% CI [135.63, 205.11]	0.242
Mean ± SD (Median), 95% CI, **YELLOW 560 nm — 2nd subanalysis**			
Complementary colors	Healthy tissue ($n=7$)	Affected tissue ($n=7$)	Student's t p-value
Green mean value	193.83 ± 32.06 (193.83) 95% CI [170.08, 217.57]	161.72 ± 51.72 (168.84) 95% CI [123.41, 200.03]	0.188
Red mean value	205.55 ± 28.43 (208.25) 95% CI [184.48, 226.61]	176.81 ± 34.24 (167.78) 95% CI [151.44, 202.17]	0.113

Every value is expressed in pixel. Statistically significant p-value is set at $p < 0.05$
Statistically significant *p-values* are enhanced in bold
*For 1 patient, no images under white light were available

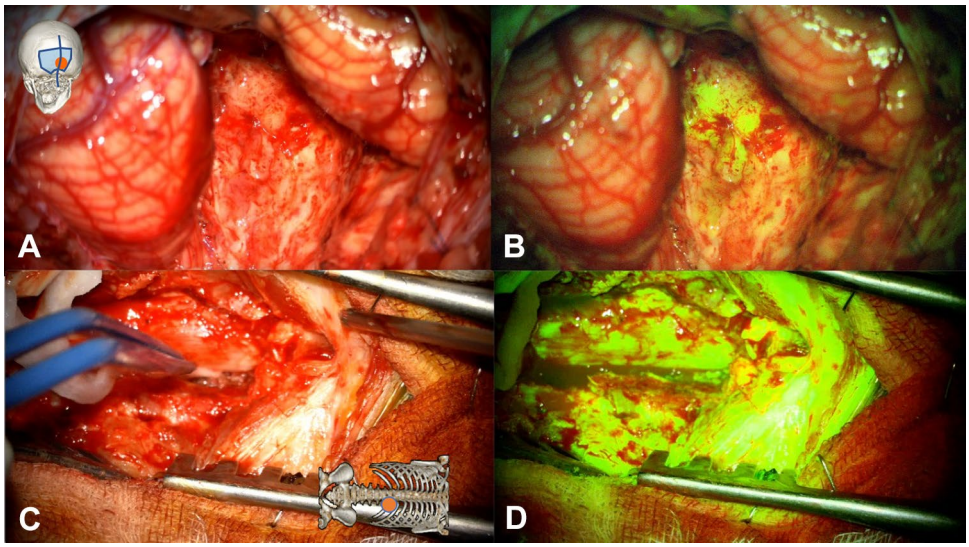

Fig. 4 An example for good/no differentiation and explication of "utility". **A**, **B** show a pilocytic astrocytoma in the group 1, with intraoperative SF uptake and good differentiation between healthy and altered tissue, not well visible under white light, therefore being considered useful by the surgeon. **C**, **D** represent a spinal epidural Ewing's sarcoma in group 2, with intraoperative SF uptake but no differentiation between healthy and altered tissue (both lesion and healthy tissue showed fluorescence); it was not helpful for the surgeon, who preferred operating under white light since the anatomical limits of the epidural tumor were clearly demarcated

showed SF uptake. That is because the surrounding healthy tissue showed excessive SF uptake too. In this case, no statistically significant tissue differentiation was present either for the color green or for the color red (Table 2).

In group B, lesions reported as "not intraoperatively enhancing" were gathered. Interestingly, the intensity of color green was significantly different regarding tumor differentiation, with higher values of green found in the healthy tissue (Table 2). We went back to raw data and analyzed them. We found 7/9 cases in which the surgeon had reported "no good differentiation of healthy vs. affected," but also 2/9 cases in which the surgeon had reported a "good differentiation" between healthy and affected tissue, with the healthy tissue being fluorescent while the affected tissue not.

Therefore, we repeated the statistical test excluding these two outliers (Table 2), and effectively, tumor differentiation was neither statistically significant for red nor for green, as reported by the surgeon.

Utility

SF allowed a good differentiation between lesion and healthy tissue and was then considered "useful for differentiation" by the surgeon, in 33/50 cases (66%, Fig. 4A and B). In 7/50 cases, the tumor showed SF uptake, but the adjacent healthy tissue too (14%, Fig. 4C and D), while in 10/50 (20%), no intraoperative fluorescence was registered.

The Chi-square test evaluating utility of SF in low-grade gliomas (20 cases) vs other lesions (30 cases) failed to reach significance.

Then, we selected lesions showing SF uptake (40 cases); the Chi-square test found marginal significance ($p < 0.049$), which after Bonferroni's correction was not significant.

Finally, we evaluated only the lesions showing good differentiation between healthy and tumoral tissue (33 cases). The Chi-square test was not statistically significant.

Among the 33 cases in which it allowed differentiation, SF was considered overall useful in 22/33 (67%). In fact, in the remaining 11 cases, although a good differentiation was visible with SF, it did not add any further information to the standard white-light vision (not essential, 33%).

Interestingly, when focusing on the low-grade glioma group (20 cases), we observed SF uptake in 17/20 (85%) and utility in differentiation between lesion and healthy tissue in the majority of them, 94% (16/17). Analyzing the overall utility in this subgroup, SF was considered not essential in 25% and very useful in 75% of them (4/16 vs 12/16).

Discussion

SF appears to be a feasible and safe intraoperative adjunct in pediatric neurosurgery, promising to be useful in particularly selected cases, such as benign though infiltrating lesions like some low-grade gliomas.

As for our knowledge, this is the largest consecutive pediatric series to date about intraoperative use of SF, with research conducted in an exclusively pediatric neurosurgery

unit. Moreover, for some kinds of pathologies, SF uptake had never been described before.

SF injection: safety, dose, and time

SF showed a user-friendly profile and a generalized safety in our series. SF was easy and rapid to prepare, only requiring an easy dilution calculating a dose of 5 mg/kg or 0.05 ml/kg, as for the majority of literature reports [16, 28, 29], and injection through a venous line. A single case of a late hypotensive episode has been reported by the anesthesiologist, but finally causality could not be definitively determined, and hypotension had no consequences. We hypothesize that hypotension could be linked to the surgical procedure itself, rather than to SF. In every other case, no adverse events were described; in particular, patients showed stability of vital parameters, no variation in renal functionality, and no allergic reactions, confirming what has already been reported in the literature both in adults [16, 17, 28] and in children [22, 23, 30, 31]. Adverse events following SF utilization have occasionally been reported with high dosages [32] and through intrathecal administration [33–35] rather than parenteral injection.

All patients showed a self-limiting yellowish discoloration of urine for about 24 h following surgery, as already reported [19]. This is an expected event directly related to SF metabolism, which generally excreted in part through the enterohepatic circulation but mostly through urines [36].

Although the dose was standard, time of injection was not always at the end of induction as generally suggested [37, 38], in some cases because it was calculated that it would not have been 1 h before dural opening/exposition of the tumor, in some other cases due to technical difficulties.

Interestingly, we did not find any association between injection time and SF uptake when considering incision time (Fig. 3). On the one hand, in 75% of the cases in which SF was injected after incision, we could nevertheless detect a good differentiation between healthy and altered tissue. On the other hand, even when SF was injected more than 1 h prior to skin incision, we registered a good discrimination ability in the majority of lesions.

The interval between SF injection and tumor exposition may be a more useful variable. The higher rates of good differentiation were found when SF was injected between 1 h 31 and 2 h 30 prior to tumor exposition, with progressively higher proportions of inadequate differentiation the earlier, leading to the hypothesis that insufficient wash-out time was given to healthy tissue [38]. No trend can be observed for non-fluorescent lesions, although a slightly higher number of tumors without SF uptake were seen when the dye was injected more than 2 h 30 prior to tumor exposition probably because of the prolonged wash-out.

This series includes a limited number of patients, but our results may suggest that the intensity of fluorescence and its variability are influenced by injection time and wash-out. However, it is likely that other factors may intervene, linked to the patient and to the histopathology, as previously suggested [39]. In particular, histopathology could play a role both by altering at different degrees the BBB and in increasing or decreasing SF wash-out. Carefully calibrated protocols should be applied in the future to determine the influence of proper injection timing in fluorescence intensity variability.

Gadolinium enhancement at preoperative MRI vs. intraoperative fluorescence

When analyzing the association of fluorescence perception with respect to gadolinium enhancement at the preoperative MRI (Table 1), 97% of cases with preoperative gadolinium enhancement showed some degree of intraoperative fluorescence.

In the 16 cases with intense/bright enhancement at preoperative MRI, SF uptake was found in every case (100%) and was bright in 62.5%. In the 15 cases with moderate gadolinium uptake, again, every case showed fluorescence (100%), which was moderate in 40%, and bright in another 40%.

Interestingly, in the 4 cases of modest preoperative gadolinium enhancement, the lesion showed a bright or moderate fluorescence in 3, while in 11 lesions not enhancing at the preoperative MRI, 45% showed some degree of fluorescence.

These findings may suggest an association between preoperative MRI enhancement and intraoperative SF uptake. For the most represented pathologies, we further analyzed it. In pilocytic astrocytomas, intraoperative fluorescence precisely reproduced gadolinium enhancement in 64% (9/14 cases), while in 29% (4/14) fluorescence was a bit more intense than gadolinium. In medulloblastomas, intraoperative fluorescence precisely reproduced gadolinium enhancement in 60% (3/5), and in the remaining 40% (2/5), SF was considered a step brighter than gadolinium enhancement.

In general, it is to be noted that when considering all the moderate (++) gadolinium uptake cases, fluorescence was brighter than MRI enhancement in 40%; also, in 75% of cases with a modest (+) preoperative enhancement, SF uptake was bright or moderate; and in 40% of not-gadolinium uptake cases, fluorescein uptake could be registered. In other words, fluorescence may often seem to appear slightly "brighter" than MRI enhancement. A possible explanation may lay in the characteristics of SF. In the CNS, it generally extravasates in the extracellular space when the BBB is disrupted, but some experiences of staining beyond a contrast-enhancing region have been reported [40, 41], hypothesizing a possible difference in vascular permeability to SF and gadolinium.

This may result from the smaller molecular size of SF, making it able to permeate larger areas, through lesser disruptions of the BBB.

Of course, some other causal factors may contribute, such as the subjectivity of perception and the variability of MRI acquisition time after gadolinium injection, as well as the anatomopathological characteristics of the lesion itself, which should be evaluated in future studies.

Can surgeon's perception be objectivated?

In order to evaluate the reliability of human eye in fluorescence perception, and verify if it may have an objective, numerical counterpart, we conducted a retrospective image analysis as already proposed elsewhere [26] (Table 2).

In the group in which the surgeon evaluated that differentiation was good (33/50 cases, 66%), we found a statistically significant difference in green brightness regarding the tumor, and only under the specific filter ($p < 0.001$). We also found a less strong statistically significant difference for the color red under white light ($p = 0.031$). This seems intuitive, as a certain number of tumors show a slightly different greyish discoloration when compared to healthy tissue.

On the other hand, when the surgeon found no sufficient differentiation (7/50, 14%), measurements of color brightness found no statistically significant difference between altered or healthy tissue. A previous study suggested that red brightness could also allow a good distinction between tissue and tumor, too [26]. However, that study included peripheral nerve tumors, with healthy tissue being a healthy segment of the nerve. These may of course present different color saturations with respect to CNS tumors, brain/spine parenchyma, and extradural tissues.

Moreover, a specific filter seems to be necessary in order to obtain a good differentiation, since in our series, no statistically significant differences were shown with low-dose SF, neither by the human eye nor by image analysis. This could appear as a major limitation to centers without a specifically equipped microscope, but it is not the case. In some reports in the literature, other filters have been used [42], or in some cases even home-made ones which were apparently good surrogates [43].

Interestingly, in the group without SF uptake (10/50, 20%), we initially saw a statistically significant difference in green brightness, with healthy tissue being brighter. We compared this with the surgeon's opinion in tissue distinction, and in 2 cases, it was marked as positive, the healthy tissue being brightly fluorescent, while tumor was not at all. This "negative fluorescence" should be carefully evaluated to determine in which specific cases it occurs, and if it can be counted as useful.

Overall, these software-based findings generally confirmed the surgeon's perception that, although still subjective, SF seems to turn out as quite reliable.

Sodium fluorescein utility in pediatric neuro-oncology

Coming to overall utility in surgery, in our series, the goal of surgery was reached in every case, i.e., GTR was achieved in every case for which it was planned, as well as STR or biopsy. We assume that SF probably played a role in achieving these goals in some cases.

Among the 33 cases in which it allowed tissue differentiation, SF was considered overall useful in a vast majority of cases (67%), especially in identifying infiltrative borders, verifying tumor bed at the end of surgery or localize remnants (Fig. 4A, B). In the remaining 11 cases, although a good differentiation was visible with the dye, it did not add any further information to the standard white-light vision, and therefore, the surgeon evaluated it as not essential (33%).

When repeating this analysis on the subgroup of low-grade gliomas, good distinction was found in 94% of staining tumors, and overall utility reached the 75%. This may be explained by the infiltrative nature of these pathologies, in which an adjunct may reveal extremely useful to assist the surgeon at boundaries towards unaffected tissues (Fig. 4). The role of SF in low-grade tumors is still debated in the literature, although a progressive number of series are reporting positive results in these groups [19, 21]. SF appears as one among several new tools, like intraoperative imaging and monitoring, helping to enhance the accuracy of tumor resection. In spite of the scientific boom in molecular biology, the diffuse or circumscribed nature of LGG is still a key feature for decision-making. Maximizing the information on macroscopic histopathology thus remains a major goal of pediatric neurooncological management.

Limitations and future perspectives

This study has some limitations, including its retrospective nature, the limited number of and the wide range of pathologies involved. Large, histologically homogeneous and prospective studies are required to evaluate whether our results are reproducible. Secondly, although we paid attention to maintain the same environmental light conditions, global brightness of the images may vary because of external factors. Third, although a tentative to standardize subjective evaluation in this research work, SF rating is still dependent on subjective perception. For these reasons, studies using objective measurement of brightness should be attempted in the future, not only for intraoperative fluorescence, but maybe also for evaluating the intensity of gadolinium enhancement at the preoperative MRI.

Conclusion

SF shows as a feasible and safe intraoperative adjunct in pediatric neurosurgery, being moreover user-friendly and cheap. Fluorescence uptake appears associated with gadolinium enhancement at the preoperative MRI, and its evaluation by human eye appears reliable when compared to an objective image analysis made by a software.

SF may have an especially promising role in LGG, but more generally, we consider that it should be integrated in the surgical routine of tumor resection in children.

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Author contribution Federico Di Rocco and Camilla de Laurentis contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Camilla de Laurentis; Federico Di Rocco, Matthieu Vinchon, and Alexandru Szathmari supervised the process. The first draft of the manuscript was written by Camilla de Laurentis and Federico Di Rocco, and all the authors (Fred Bteich, Pierre Aurélien Beuriat, Laryssa Crystinne Azevedo Almeida, Sylvie Combet, Carmine Mottolese, Matthieu Vinchon, Alexandru Szathmari) commented on previous versions of the manuscript. All the authors (Camilla de Laurentis, Fred Bteich, Pierre Aurélien Beuriat, Laryssa Crystinne Azevedo Almeida, Sylvie Combet, Carmine Mottolese, Matthieu Vinchon, Alexandru Szathmari, Federico Di Rocco) read and approved the final manuscript.

Availability of data and materials We the authors commit ourselves to provide all data upon request.

Declarations

Ethics approval and consent to participate This retrospective study was submitted to and approved by our institutional ethics committee, IRB de Neurochirurgie, no. IRB00011687, Collège de Neurochirurgie IRB #1: 2022/46. Parental informed consent was obtained.

Consent for publication Not applicable (anonymization of data).

Conflict of interest We the authors declare having no conflict of interest.

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