The role of sodium fluorescein in pediatric supratentorial intra-axial tumor resection: new insights from a monocentric series of 33 consecutive patients

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Research Article

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Abstract

Surgical resection represents the mainstay of treatment, in pediatric central nervous system (CNS) tumors, and aggressive resection correlates with prognosis for several histotypes. Sodium fluorescein (SF), a green, water-soluble dye, is used as neurosurgical fluorescent tracer thanks to its property to accumulate in cerebral regions of blood-brain barrier disruption, acting as a valid tool to improve the extent of resection in tumors enhancing at preoperative MRI. Brain neoplasms represent a heterogeneous group of tumors in the pediatric age, constituting the most common solid cancers; they typically show a varying degree of contrast enhancement on MRI. In March 2016 the authors started a prospective, observational trial to evaluate intraoperative fluorescence's characteristics of CNS tumors, the percentage of extent of resection thanks to fluorescein aid and side effects related to fluorescein administration. This report is based on a retrospective analysis of a group of 33 consecutive pediatric patients harboring a supratentorial lesion. In 17 of 33 (51.5%) procedures fluorescence was reported as intense, in 14 of 33 (42.4%) moderate and in 2 of 33 (6.1%) slight. Intraoperative fluorescence corresponds to preoperative MRI documented contrast enhancement. In 28 of 33 (84.8%) surgical procedures SF was considered useful, in 2 of 33 (6.1%) partial useful, and in 3 of 33 (9.1%) not essential because the tumor was already recognizable. No adverse effect to SF administration was registered. Fluorescein-guided surgery with a dedicated filter on the microscope is a safe and effective technique to improve visualization and resection of different pediatric brain tumors.

Introduction

Central nervous system (CNS) tumors are the most common solid neoplasms in the pediatric population [1], with an incidence of 2.4 new cases for 100000 per year [2]. First, the WHO classification edited in 2016 [3] and, overall, the 2021 [4] update (CNS5) have introduced relevant innovations in pediatric tumors definition, underlining the preponderant role of genetic elements as diagnostic criteria if compared to morphological ones [5].

The most frequent intra-axial tumor types in pediatric population [6] are circumscribed astrocytic gliomas, ependymomas and embryonal tumors, such as medulloblastomas; other rarer entities, peculiar of youth age [7], are classified as pediatric-type high- or low-grade gliomas, lacking IDH mutations, driven by histone mutations or by mutations that result in MAPK pathway activation, respectively.

Pediatric brain tumors present specific localization: infratentorial neoplasms are more frequent in the first decade of life and most common histologies are medulloblastomas, pilocytic astrocytomas, posterior fossa ependymomas and diffuse brainstem gliomas; otherwise, supratentorial lesions constitute a rarer and more heterogeneous group with a bimodal age distribution at diagnosis, namely a peak of incidence under the age of two and another peak in the adolescence: principal tumors are circumscribed and pediatric-type astrocytomas, supratentorial ependymomas and optic gliomas [1, 2]. Regardless of tumor grade and location, vast majority of pediatric tumors present with varying degree of contrast enhancement (c.e.) on MRI due to damage in the blood-brain barrier (BBB) [8].
The mainstay of treatment is surgical resection [5, 9]: it plays a fundamental part as a cytoreductive surgery with the valuable role of providing enough biological material for histopathological and genetic analysis. Radical resection can be considered curative in mostly pediatric-type low-grade gliomas and in circumscribed astrocytic gliomas; by the way, surgical approach must be associated with adjuvant chemotherapy and radiotherapy in case of embryonal tumors or in pediatric-type high-grade gliomas, often with a poor prognosis. In all tumor entities, grade of resection correlates with patients’ prognosis.

In this perspective, the application of new technical tools aimed at improving the extent of resection (EOR) could be beneficial [10]; in this context, fluorophores have been studied and applied to improve the intraoperative visualizations of CNS neoplasms. 5-aminolevulinic acid (5-ALA), a biochemical precursor of hemoglobin that causes the synthesis and accumulation of fluorescent porphyrins in different lesions, has been applied but with inconsistent results in several malignant or benign pediatric tumors [11, 12]. Sodium fluorescein (SF) is a dye that, when intravenously injected, has the peculiar characteristic to accumulate in cerebral areas presenting a damage of the BBB [13]; the use of a dedicated filter in the surgical microscope, such as YELLOW 560 (Carl Zeiss Meditec, Oberkochen, Germany), with specific wavelength for fluorescein (540-690nm), allows to improve the tumor-brain discrimination intraoperatively, reducing also the dosage needed to obtain this effect [14, 15].

In recent years, also thanks to our preliminary studies and the approval, in July 2015 (determination 905/2015), of fluorescein molecule as a neuro-oncological tracer by the Italian Medicine Agency (AIFA), SF applications have increased exponentially. The basic functioning principle of being a vascular fluorophore provokes its accumulation in pathological areas of BBB disruption [13], as detectable with the preoperative contrast enhanced MRI.

According to AIFA determination, the intravenous (i.v.) injection of SF as a neurosurgical tracer during neuro-oncological procedures is approved and its cost is totally reimbursed by the Italian National Health System. A low dose (5mg/kg) of fluorescein is i.v. administrated at the end of patient intubation. In March 2016, the authors started a new prospective observational study, called FLUOCERTUM (FLUorescein in CERebral TUMors), regarding the use of SF as a fluorescent intraoperative tracer in patients with c.e. tumors of the CNS, both in adult and pediatric patients [14, 16].

Several reports regarding fluorescein-guided surgery have been published in recent years, even sparking a lively and controversial debate about a molecule that is now being accepted as self-evident [17]; after describing the management and results of our preliminary experience in a multicentric series of 24 pediatric patients in 2019 [16], we now retrospectively report on 33 children harboring a supratentorial tumor.

The purpose of the present study is to assess the contribution and the valuable role of fluorescein in the surgical resection and visualization of pediatric supratentorial intra-axial CNS tumors with a dedicated filter integrated in the surgical microscope.
Materials And Methods

Patients and Inclusion Criteria

In this study, we retrospectively reviewed the database of the prospective observational FLUOCERTUM study, started in March 2016 and approved by the Institutional Review Board, to identify the cohort of pediatric patients until August 2022. Written informed consent for the surgical procedure, including the use of fluorescein, as well as to participate in this observational study was obtained from the minors’ legal guardian/next of kin. The protocol and the retrospective case series revision have been approved by the Ethical Committee of the Fondazione IRCCS Istituto Neurologico Carlo Besta.

Clinical and Radiological Management

Preoperative assessment included physical and neurological examination (Lansky Play-Performance scale [LPS]), laboratory tests results and contrast-enhanced MRI for neuronavigation [18]. In preoperative MRI, patients were categorized based on preoperative contrast enhancement characteristics. To evaluate the EOR, a volumetric MRI examination was performed for each patient within 72 hours after surgery; in particular, to calculate the residual pathological volume, the hyperintense alterations in volumetric basal T1 acquisitions were subtracted from the volume of hyperintense tissue in post-contrast volumetric T1 images, to avoid the incidental inclusion of blood or blood product [19]. The EOR was calculated as a percentage of tumor resection based on early contrast-enhanced postoperative MRI. The postoperative clinical evaluation included a standard neurological examination as above as well as laboratory test (kidney function) and exclusion of occurrence of any side effect related to fluorescein injection. Clinical and neuroradiological long-term follow-up was performed for postoperative period as part of normal clinical practice, including parental telephonic interview.

Surgical Protocol

The standardized surgical protocol of fluorescein-guided technique, as already described in previous papers [20], is based on i.v. SF (Monico S.p.A., Venice, Italy) injection at standard dose of 5mg/kg, by a central or peripheral venous line, immediately upon completion of the induction of general anesthesia [21]. Surgery was performed with the aid of a surgical microscope equipped with an integrated fluorescent filter tailored to the excitation and emission wavelength of sodium fluorescein (YELLOW 560 – Pentero 900; Carl Zeiss Meditec, Oberkochen, Germany). During resection, the microscope could be switched alternatively from fluorescent to white-light illumination. In tumors located adjacent to eloquent areas, intraoperative neurophysiological monitoring was used. Tumors were removed in an inside-out fashion until all fluorescent tissue was removed, as considered feasible by the surgeon.

Intraoperative Fluorescence Characteristics and Side Effects

Fluorescence intensity was graded by the surgeon as high, moderate, faint or absent; surgeons were also asked to classify the use of SF per each procedure as useful, partial useful, useless or not essential to
achieve surgical aims. Furthermore, medical reports were evaluated for any possible adverse effect or allergic reaction to fluorescein administration.

**Histological Analysis**

Histopathological analysis was performed in each case; tumors were prospectively classified according to the 2016 or 2021 WHO classification of brain tumors by the neuro-pathology group of our Institute, with no additional costs respect to clinical practice [3, 4].

**Statistical Analysis**

The sample was described by means of the usual descriptive statistics: mean, median and standard deviation for continuous variables and proportions for categorical ones. ANOVA and Student’s *t*-tests were used to compare preoperative, immediate postoperative and long-term postoperative conditions; statistical significance was arbitrarily assigned with a P value < 0.05. PRISM software for Macintosh was used for the statistical analysis.

**Results**

**Patients Population**

A summary of the clinical condition at admission, discharge and follow-up, neuroradiological and surgical characteristics of the patients is presented in Table 1. Thirty-three patients were identified (15 males and 18 females; median age 10.4 years, ranging from 3 to 17 years) and considered for the analyses of the retrospective study.

Most patients underwent surgery until 2021 and tumors were classified according to 2016 WHO version of CNS classification. The following tumor subtypes were included: 9 (27.3%) pilocytic astrocytomas, 8 (24.2%) gangliogliomas, 1 (3.03%) ganglioglioma and subependymal giant cell astrocytoma, 3 (9.1%) dysembryoplastic neuroepithelial tumors (DNET), 2 (6.1%) pleomorphic xanthoastrocytomas (PXA) and 1 (3.03%) anaplastic pleomorphic xanthoastrocytoma, 2 (6.1%) ependymoblastomas, 1 (3.03%) angiocentric glioma, 1 (3.03%) choroid plexus papilloma, 1 (3.03%) extraventricular neurocytoma, 1 (3.03%) germ cell tumor, 1 (3.03%) H3K27M diffuse midline glioma, 1 (3.03%) papillary glioneuronal tumor, and 1 (3.03%) polymorphous low-grade neuroepithelial tumor of the young.

All the patients presented with variable contrast enhancement patterns in the preoperative MRI (Table 1). The pattern of c.e. was classified as follows: 10 cases (30.3%) of intense enhancement of the solid component with a large cyst, 9 patients (27.3%) with homogeneous and intense enhancement, 7 cases (21.2%) of heterogeneous and intense enhancement, 5 patients (15.2%) with heterogeneous and faint enhancement, and 2 cases (6.1%) of peripheral enhancement with central necrosis.

The median preoperative tumor volume was 43.38 cm$^3$, with a range from 0.05 cm$^3$ to 688.13 cm$^3$. 
Intraoperative fluorescence characteristics and surgeon’s opinion

No technical difficulties regarding the use of the microscope filter nor switching between white and yellow light were encountered during the surgical resections.

Homogeneously intense fluorescent staining was reported in 17/33 cases (51.5%); a moderate fluorescence was detected in 14/33 cases (42.4%) while in only 2 patients (6.1%) pathological tissue appeared slightly fluorescent (Table 1). In 5 multicystic tumors (15.1%), independently from the specific fluorescein enhancement, we observed a bright fluorescent cystic fluid.

In all cases but five, intraoperative fluorescence was deemed useful (84.8%) in achieving a complete resection or in confirming the accuracy of a biopsy sample, using a better delineation of the borders of the tumor tissue from the healthy parenchyma as compared with the conventional microsurgical technique using white-light illumination. In 2 cases (6.1%), SF was considered partially helpful in identifying the tumor (Table 1) due to the rare SF enhancement. Finally, in 3 patients (9.1%), the tumor was already recognizable under white light and fluorescent visualization was judged not essential in performing surgical resection.

Intraoperative fluorescence was detected in all cases out of 9 surgical procedures for pilocytic astrocytomas; the intensity of fluorescein enhancement was high in 4 cases (44.4%) and moderate in 5 patients (55.6%). Fluorescence was considered helpful for the identification of pathological tissue and surgical resection in 10 of 12 (83.3%) cases of pilocytic astrocytomas (Table 2).
Table 2
Intraoperative fluorescence characteristics and utility, based on tumor histology

<table>
<thead>
<tr>
<th>TUMOR TYPE</th>
<th>FLUORESCENCE</th>
<th>SURGEON’S OPINION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIGH</td>
<td>MODERATE</td>
</tr>
<tr>
<td>PILOCYTIC ASTROCYTOMAS</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>GANGLIOGLIOMAS + SEGA</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>DNET</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>PXA</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>EPENDYMOMBLASTOMAS</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>ANGIOCENTRIC GLIOMA</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>CHOROID PLEXUS PAPILLOMA</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>EXTRAVENTRICULAR NEUROCYTOMA</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>GERM CELL TUMOR</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>H3K27M DIFFUSE MIDLINE GLIOMA</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>PAPILLARY GLIONEURONAL TUMOR</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>POLYMORPHOUS LOW- GRADE NEUROEPITHELIAL TUMOR OF THE YOUNG</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Of the 9 surgeries for gangliogliomas, 6 cases (66.7%) showed intraoperative highly fluorescent staining, 2 (22.2%) moderate fluorescence and 1 (11.1%) faint enhancement. In this histotype, fluorescein was judged useful in almost all cases (7/9 – 77.8%), in 1 case (11.1%) partial useful and in the other one (11.1%) not essential because the tumor was already recognizable (Table 2).

Out of the three DNET, 2 patients (66.7%) showed an intense fluorescence whereas 1 (33.3%) a moderate fluorescence; in all procedure, the aid of SF was considered useful by the surgeon (Table 2).

During PXA surgery, SF was judged always useful in performing tumor resection: 2 cases (66.7%) presented a highly fluorescent staining and 1 case (33.3%) a moderate fluorescence (Table 2).

Bright intense fluorescence was reported in 2 ependymoblastomas, in 1 angiocentric glioma and in 1 papillary glioneuronal tumor; in all cases, SF was judged useful in performing surgery. Moderate fluorescence was detected in 1 choroid plexus papilloma, in 1 extraventricular neurocytoma, in 1 germ cell
tumor and in 1 H3K27M diffuse midline glioma: the aid of SF was judged not essential in choroid plexus papilloma and in extraventricular neurocytoma because these histotypes were already recognizable under white-light illumination whereas, for the other patient, the role of SF fluorescence was deemed useful. The polymorphous low-grade neuroepithelial tumor of the young showed faint intraoperative fluorescence and this tool was considered partial useul due to the poor enhancement (Table 2).

Significative intraoperative fluorescence was reported in 31 out of the 33 surgeries (93.9%) whose corresponding preoperative MRI documented contrast enhancement (Table 3). In case of intense c.e. MRI, a high fluorescence enhancement was reported in 10/16 cases (62.5%), moderate fluorescence in 5 cases (31.3%) and faint fluorescence in 1 case (6.3%). When preoperative MRI showed a characteristic c.e. pattern of intense enhancement of the solid component with a large cyst, fluorescence enhancement was predominantly intense (7/10) and predominant in 3 patients. In 5 patients, preoperative MRI presented a heterogeneous and faint pattern of c.e.: in this cohort of patients, fluorescent staining was moderate in 4 cases (80%) and tenuous in 1 case (20%). Two patients were characterized by a peripheral c.e. with central necrosis with a corresponding moderate fluorescence enhancement.

Table 3
Intraoperative pattern of fluorescence based on the pattern of enhancement at preoperative MRI

<table>
<thead>
<tr>
<th>Pattern of Enhancement</th>
<th>Overall</th>
<th>Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Homogeneous and Intense</td>
<td>9 (27.3%)</td>
<td>6</td>
</tr>
<tr>
<td>Heterogeneous and Intense</td>
<td>7 (21.2%)</td>
<td>4</td>
</tr>
<tr>
<td>Intense Enhancement of the solid component with a large cyst</td>
<td>10 (30.3%)</td>
<td>7</td>
</tr>
<tr>
<td>Heterogeneous and Faint</td>
<td>2 (6.1%)</td>
<td>0</td>
</tr>
<tr>
<td>Peripheral with Central Necrosis</td>
<td>5 (15.2%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Extent of resection

Gross total resection (GTR) was planned based on the preoperative MRI in 31 cases of the 33 (93.9%) surgical procedures; GTR was achieved in 22 patients (71%), in 1 case (3.2%) a minimal residual volume was highlighted by postoperative MRI despite the intraoperative subjective evaluation, both under white-light and YELLOW 560 filter visualization, of complete tumor removal. In the other 7 cases, the resection was subtotal with fluorescent residual spots in the context of eloquent tissue identified by means of intraoperative neurophysiological monitoring, to avoid neurological worsening (expected subtotal resection [STR] in 22.6%); in 1 patient (3.2%) with a huge and hemorrhagic lesion, resection was interrupted after having debulked a large component to relieve functional structures.

Regarding the STR group, these 8 patients had a median residual volume (RV) of 1.56cm$^3$ (0.05cm$^3$ – 2.51cm$^3$) or 4.3% (1.2% – 8.3%) of the initial tumor mass; 6 of 8 cases (75%) of subtotal tumor removal
had a > 95% EOR (Table 1).

Two frameless needle biopsies were performed in two patients harboring a deep located and diffuse tumor; the diagnostic accuracy of YELLOW 560 filter visualization of the samples was of 100% (Table 1).

**Side effects and outcome**

No adverse drug reaction related to SF injection was reported in this cohort of patients; the only remarkable and visible effect was the transient yellowish staining of urine, which disappeared in about 24 hours.

At baseline, 27/33 (81.8%) of patients had LPS of 80–100, indicating good clinical and neurological conditions. Surgical morbidity led to a postoperative decline in LPS in 2 patients at discharge; 27 patients were discharged with an unchanged LPS, whereas 4 patients presented with a clinical improvement using surgical treatment. At discharge, 27/33 (81.8%) of patients had LPS of 80–100 (Table 1).

Long-term follow-up data were available for all patients, all alive during this time (Table 1); the follow-up period ranged between 6 and 62 months, with a median follow-up of 33.5 months. Most patients (28/33–84.8%) were neuroradiologically stable (tumor free or stable remnant tumor); we did not find any statistically significant difference between preoperative, short- and long-term postoperative LPS. Three patients presented with tumor recurrency or progression disease, and two were slightly clinically worsened; two of them were scheduled for re-do surgery, whereas the other one was for adjuvant chemotherapy. Regarding the two patients of the biopsy group, one of them experienced tumor regression thanks to chemotherapeutic approach; otherwise, the other one presented tumor progression although adjuvant therapies, even including second line chemotherapy drugs.

**Discussion**

In our surgical series, fluorescein-guided technique is feasible and represents a useful adjunct in most of the pediatric intra-axial supratentorial tumors presenting with some degree of contrast enhancement at preoperative neuroimaging scans. Despite the heterogeneity of pediatric CNS tumors histotypes [1, 9], these neoplasms usually present with variable patterns of c.e. on their preoperative MRI [8]; the c.e. reflects the loss of endothelial cell-astrocyte foot process relationship in the altered BBB, revealing not only aggressive tumors but also such histotypes characterized by the same BBB damaging.

In particular, bright fluorescence was present in almost all cases of pilocytic astrocytomas, gangliogliomas, DNET and PXA, whereas it was less evident for choroid plexus papilloma and extraventricular neurocytoma and absent in the single reported case of polymorphous low-grade neuroepithelial tumor of the young. These findings are basically correlated to the characteristics enhancement of tumors at preoperative MRI; in particular, fluorescence was detectable in 93.9% contrasting-enhancing tumors. Moreover, the operating surgeon found SF to be useful in 84.8% of the cases for either achieving GTR or for confirming surgical fluorescent samples in tumor biopsy.
The standard of care for most pediatric tumors is surgical resection whereas adjuvant therapies are usually considered second-line treatments [5, 10]. The primary goal of resection is to maximize EOR while preserving the patient’s neurologic function and obtaining a histopathologic diagnosis. Innovative technical tools that can improve tumor visualization and borders discrimination between slight pathological peripheral neoplastic tissue and normal peritumoral brain parenchyma could have a positive influence in minimizing RV.

In this perspective, the application of new technical tools aimed at increasing the EOR could be beneficial in the management of pediatric brain tumors. Use of 5-ALA has been reported but with inconsistent results [22]: recent evidences regarding the advantages of 5-ALA in pediatric population, which is still off-label, suggest a valuable role in high-grade gliomas and in grade 3 ependymomas. Since these histotypes are more frequent in the supratentorial compartment, this last location is one of the most relevant predictors of usefulness of 5-ALA [23]; the group of Stummer, in a recent review, reported a judgement of helpfulness in less than 60% of supratentorial pediatric brain tumors scheduled for 5-ALA-guided surgery [11]. Even Preuß at al. reported a similar percentage of success of 5-ALA as a positive fluorescent dye in pediatric neuro-oncological surgery [24]. In the light of these results, many experienced surgeons in the 5-ALA field advise against the routine use of this fluorophore in pediatric brain surgery [12]. The principal drawback of 5-ALA-guided surgery is the lack of a strong predictor of usefulness in pediatric brain neoplasms: indeed, a strong fluorescence enhancement correlates neither with preoperative MRI c.e. nor with tumor aggressivity [9].

During the last years SF has emerged as intraoperative tracer able to improve brain-tumor visualization, due to its non-specific, vascular mechanism of action related to the accumulation in brain regions with BBB dysregulation, as it happens with MRI contrast enhancement [25, 26]. Additionally, the recent availability of an integrated and specific filters in the surgical microscope has contributed to the wide diffusion of fluorescein [14–16]. Previous experiences suggested that the use SF could be associated with a bright fluorescence of the tumor area in HGG [20], in metastases [27], in gangliogliomas [28], in pilocytic astrocytomas [29] and in spinal intramedullary lesions [30]. This was also associated with good results in term of extent of resection as well as progression free and overall survival.

For these reasons, we hypothesized that fluorescein could represent an ideal dye for the surgical management of CNS tumors in the pediatric population. In our preliminary analysis described in 2019 [16], we discussed that fluorescein plays a useful role during surgical resection of pilocytic astrocytomas, gangliogliomas and medulloblastomas by means of highlighting the bright fluorescent pathological tissue compared to the surrounding pinkish health brain parenchyma.

As the reimbursement of SF as a fluorescent tracer in neuro-oncology has been approved by the Italian Drug Agency in July 2015 (determination 905/2015, Gazette n.168, 22 July, 2015), and based on our extensive experience with HGG, we decided to start a prospective observational study (i.e., the FLUOCERTUM study) on the use of SF for the resection of aggressive tumors of the CNS, applying a standardized protocol independently from patient age and tumor location, with a dosage of 5mg/kg and
an i.v. injection immediately after patient intubation. From the prospective collected database, we were able to retrospectively select the pediatric population considered in this study.

Thirty patients enrolled in the study were scheduled for surgery with a previous planning of macroscopic resection but keeping in mind the philosophy of maximal safe resection: in this series, we obtained a high percentage of GTR (21/30, 70%). Minimal residual tumor (lower than 10% of preoperative tumor volume) at postoperative MRI was expected in 7 cases (23.3%), as it was involving eloquent areas and was therefore independent from the use of fluorescein-guided technique. Conversely, in only 1 case (3.3%), the residual tumor was an unexpected finding, based on the absence of clear residual intraoperative fluorescent tissue: this can be related, as for other fluorophores, to the fact that the residual tissue was not exposed during resection because hidden under the normal brain parenchyma. Finally, in the remaining other patient (3.3%), who presented a huge and hemorrhagic lesion, the surgical resection was only partial and interrupted after debulking of a large component to relieve functional structures in eloquent areas. Two patients were included in our protocol for fluorescein-guided needle biopsy; although the limitation of this case load, the diagnostic accuracy was of 100%.

We also found a good correspondence between pre-operative MRI c.e. and intraoperative fluorescence identification in several neoplastic histotypes: we hypothesized that pediatric tumors presenting a gadolinium uptake in T1 MRI sequences could be shown intraoperatively as a fluorescent mass, using SF with a dedicated filter in the surgical microscope; this consideration determined a frequent opinion of usefulness (28/33–84.8%). Intraoperative fluorescence was judged partial useful in 2 children (6.1%) and not essential in 3 patients (9.1%). Our considerations want to focus that the use of fluorescein remains questionable for tumors showing a minimal or a heterogeneous pattern of contrast medium uptake, since the high predictivity of SF role as an adjuvant tool in relation to preoperative MRI contrast enhancement. Despite the subjective intraoperative evaluation of fluorescein can constitute a major bias of our study, the clear and intense fluorescence delineating the tumor margin for such histologic entities, along with the GTR performed in almost all cases, apparently supports the great benefit of fluorescein utilization.

Nobody, between patients of our cohort, presented fluorescein-related side effects or adverse reactions to SF administration. The only visible manifestation of intraoperative fluorescein administration was the onset of transient and harmless yellowish stain of the urine, that rapidly disappears after 24 hours. We believe that the lack of any side effect, in particular any allergic reaction, was predominantly related to the low dosage used in this study, thanks to the use of a dedicated filter into the microscope, that allowed a more accurate identification of fluorescent tissue, as suggested firstly by our group [21].

Owing to the unspecied mechanism of action of SF, the use of this fluorescent tracer in pediatric neuro-oncology should be considered cautiously. However, these preliminary results seem to suggest that the properties of SF may represent a strength in this population, as most of the CNS pediatric tumors are characterized by a various degree of c.e. related to a damage in the BBB.

The main limitation of this study presented is represented by the heterogeneous histology of tumors included in this study; furthermore, the lack of a significant long-term follow-up prevents the elucidation
about overall survival: in fact, the authors considered only a surrogate indicator which is the EOR. Other known pitfalls of our research are the absence of the direct comparison of surgery with and without SF aid or between the use of SF and other available fluorophores, like 5-ALA. Otherwise, we must stress that, in most of the Countries, SF is still considered off label for neuro-oncological procedures. Thus, a widespread utilization of fluorescence-guided surgery will depend on the definitive approval by the competent authorities. Future prospective studies could better address these limitations by stratifying the cohorts according specific single tumor histology: larger and more homogeneous cohort of patients should be enrolled with a longer and more systematic period of follow-up.

**Conclusion**

On the basis of its unspecific mechanism of action, the use of SF as fluorescent tracer in pediatric neuro-oncology should be considered in several applications. Fluorescein-guided resection of CNS tumors is a feasible and safe technique, even in a pediatric population. In particular, fluorescein visualization by means of YELLOW 560 filter improved the intraoperative tumor visualization, especially of those lesions enhancing on the preoperative brain MRI. Future prospective studies with larger and more homogenous series should be performed to definitively assess the value of this intraoperative technique in increasing the extent of surgical resection and, therefore, in improving clinical outcome of this cohort of patients.

**Abbreviations List**

**AIFA** Agenzia Italiana del Farmaco (Italian Medicine Agency)

**BBB** Blood-Brain Barrier

**c.e.** contrast enhancement

**CNS** Central Nervous System

**DNET** Dysembryoplastic Neuroepithelial Tumor

**EOR** Extent of Resection

**GTR** Gross Total Resection

**i.v.** intravenous

**LPS** Lansky Play-Performance scale

**PXA** pleomorphic xanthoastrocytomas

**RV** Residual Volume

**SF** Sodium Fluorescein
Declarations

Ethics Statement and Consent: The study, involving human participants, was reviewed, and approved by the Ethical Committee of the Fondazione IRCCS Istituto Neurologico Carlo Besta. Written informed consent to participate in this study and for the publication of any potentially identifiable images or data included in this article was obtained from the individuals and provided by the minors' legal guardian/next of kin.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Conflict of Interest: Dr. Francesco Acerbi received honoraria from Carl Zeiss Meditec for lectures in International Meetings.

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Authors’ Contribution: Jacopo Falco and Francesco Acerbi: study concept and design; Jacopo Falco: writing - original draft preparation; Jacopo Falco and Francesco Acerbi: critical revision of the manuscript for intellectual content; All authors: acquisition of data, data analysis and interpretation; Francesco Acerbi: study supervision. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Acknowledgement: Not applicable.

References


Tables

Table 1 is available in the Supplementary Files section.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TABLE1.docx