Where and When to Cut? Fluorescein Guidance for Brain Stem and Spinal Cord Tumor Surgery—Technical Note

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BACKGROUND: Spinal cord and brain stem lesions require a judicious approach with an optimized trajectory due to a clustering of functions on their surfaces. Intraoperative mapping helps locate function. To confidently locate such lesions, neuronavigation alone lacks the desired accuracy and is of limited use in the spinal cord.

OBJECTIVE: To evaluate the clinical value of fluoresceins for initial delineation of such critically located lesions.

METHODS: We evaluated fluorescein guidance in the surgical resection of lesions with blood-brain barrier disruption demonstrating contrast enhancement in magnet resonance imaging in the spinal cord and in the brain stem in 3 different patients. Two patients harbored a diffuse cervical and thoracic spinal cord lesion, respectively. Another patient suffered metastatic lesions in the brain stem and at the floor of the fourth ventricle. Low-dose fluorescein (4 mg/kg body weight) was applied after anesthesia induction and visualized using the Zeiss Pentero 900 Yellow560 filter (Carl Zeiss, Oberkochen, Germany).

RESULTS: Fluorescein was helpful for locating lesions and for defining the best possible trajectory. During resection, however, we found unspecific propagation of fluorescein within the brain stem up to 6 mm within 3 h after application. As these lesions were otherwise distinguishable from surrounding tissue, monitoring resection was not an issue.

CONCLUSION: Fluorescein guidance is a feasible tool for defining surgical entry zones when aiming for surgical removal of spinal cord and brain stem lesions. Unselective fluorescein extravasation cautions against using such methodology for monitoring completeness of resection. Providing the right timing, a window of pseudoselectivity could increase fluoresceins' clinical value in these cases.

KEY WORDS: Fluorescein, Brain stem, Spinal cord, Metastasis, Glioma

Intramedullary lesions are extremely rare and the available literature data are scarce.[1] Predominantly, spinal cord tumor lesions involve ependymomas or low-grade astrocytomas.[2-5] Surgically, spinal cord lesions and brain stem tumors are challenging to treat. To date, intraoperative neurophysiological monitoring and other intraoperative tools, eg, ultrasound, help assist resection. However, spinal cord and brain stem tissue are vulnerable, and the myelotomy zone as well as intramedullary exploration should be reduced to a minimum. Surgery often remains the last step in treatment, since it often involves risks for neurological deficits.[6]

Over the past 20 yr, fluorescence guidance with 5-aminolevulinic acid (5-ALA) improved gross-total resection of high-grade glioma.[7] Recent reviews state fluorescence-guided resection with 5-ALA as one of the best intraoperative tools to improve resection.[8,9] Inspired by the breakthrough of this technique, other fluorophores have been exploited and analyzed to evaluate their clinical use. After introduction of a new filter system from Zeiss (Yellow 560,

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ABBREVIATIONS: 5-ALA, 5-aminolevulinic acid; **EMG,** electromyography; **MEP,** motor evoked potential; **MRI,** magnetic resonance imaging; **SSEP,** somatosensory evoked potentials

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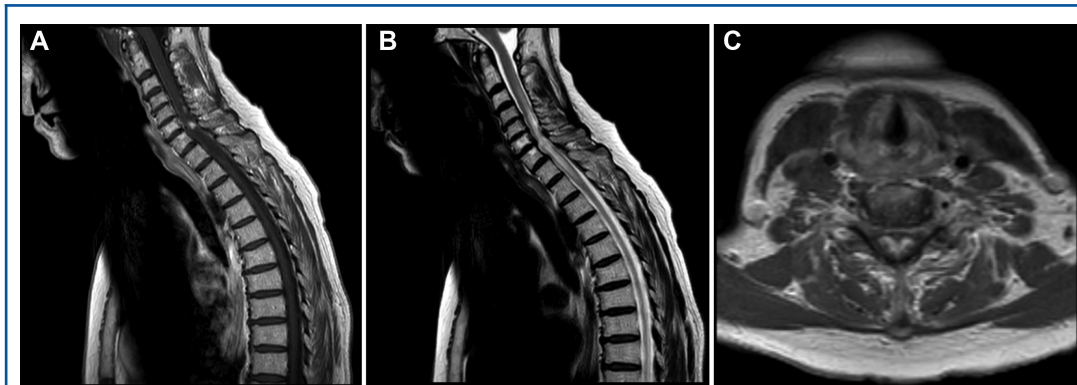
FIGURE 1. *Patient harboring a lesion in the cervical spinal cord. Patient no. 1: MRI gadolinium contrast-enhanced T1-weighted imaging in* **A**, *sagittal and* **C**, *axial cuts, and* **B**, *T2-weighted imaging of a spinal cord lesion at the height at the C6 and C7 level.*

Carl Zeiss, Oberkochen, Germany), fluorescein has gained recent attention in the field of neuro-oncology.[10-14] Fluorescein provides the advantage of possessing strong fluorescence. It has been used in ophthalmology for retinal angiography for decades.[15,16] As an exogenous fluorescent agent, fluorescein marks regions of blood-brain barrier disruption.[12] This characteristic can be both an advantage or disadvantage, since extravasation occurs at resection margins during surgery,[17] and in the peritumoral edema.[13,14] However, since gadolinium-contrast enhancement in magnetic resonance imaging (MRI) represent regions of blood-brain barrier disruption, we aimed to evaluate fluorescein as a possible fluorophore for fluorescence guidance. For this purpose, we critically evaluated the value of fluorescein in 3 surgical cases of spinal cord and brain stem tumor lesions.

METHODS

Three patients (n = 3) harboring spinal cord and brain stem lesions as described below were operated in the Department of Neurosurgery, University Hospital of Münster, in the year of 2017, and included in this study. After consultation with the local ethical committee of our medical faculty concerning compassionate use of a drug in an off-label setting, written informed consent was obtained from all subjects included for this application. These patients underwent neurosurgical intervention with intraoperative neurophysiologic monitoring involving motor evoked potential (MEP) and somatosensory evoked potential (SSEP) and electromyography (EMG). We used fluorescein guidance in all patients (n = 3). 5-ALA was additionally administered in the first patient, since glioma as an entity was suspected.

We administered low-dose fluorescein (4 mg/kg body weight, Fluorescein Alcon® 10%, Alcon Pharma GmbH, Freiburg, Germany) intravenously after anesthesia induction, as previously described.[18] For visualization of fluorescein fluorescence, the Zeiss Pentero 900 Yellow 560 filter (Carl Zeiss) was implemented. 5-ALA (20 mg/kg body weight, Gliolan®, Medac, Wedel, Germany) was administered as previously described[19] and the Zeiss Pentero Blue 400 filter was used for its visualization. Each surgery was performed by a senior neurosurgeon.

Patient No. 1

A 56-yr-old female patient presented to our department due to development of an ataxic gait and dysesthesia, as well as hyperalgesia of both upper and lower limbs. MRI revealed an intramedullary contrast-enhancing lesion in the T1-weighted imaging at the height of C6/C7 with relevant and extended perifocal edema (Figure 1). A thorough neurological examination and diagnostic evaluation, including cerebral spinal fluid analysis, did not deliver a diagnosis for the demonstrated lesion. For this reason, neurosurgical intervention was indicated. The aim, due to the localization of the lesion, was to perform an open biopsy and, if resectable, a gross-total resection.

Patient No. 2

This 48-yr-old female patient with a 5-yr history of metastatic breast carcinoma presented to our department with progressive unsteady gait and vegetative circulation disorders. Furthermore, the patient demonstrated deterioration of a known nerve III palsy. The patient underwent neurosurgical intervention in the year 2013 because of a cerebellar metastasis. Two other lesions along the third and fifth cranial nerve were known since the year 2015. An MRI demonstrated 2 new ring contrast-enhancing lesions in the gadolinium T1-weighted imaging in the brain stem and at the floor of the fourth ventricle (Figure 2). Because of the oncological history, radiation therapy was not possible. After interdisciplinary discussion within the tumor board, and extensive discussion with the patient, neurosurgical removal of both lesions was planned.

Patient No. 3

A numbness of the sole of the feet, which existed for around 2 yr prior neurosurgical presentation, together with paresthesia of the left thigh and slight bladder disorders led to radiological imaging by this 42-yr-old male patient. Neurological examination further demonstrated a paraspasticity with increased muscle tone and reflexes. MRI revealed a central intramedullary weak ring-enhancing lesion at the height of Th4 and Th5. After discussion within the interdisciplinary tumor board, neurosurgical intervention was indicated.
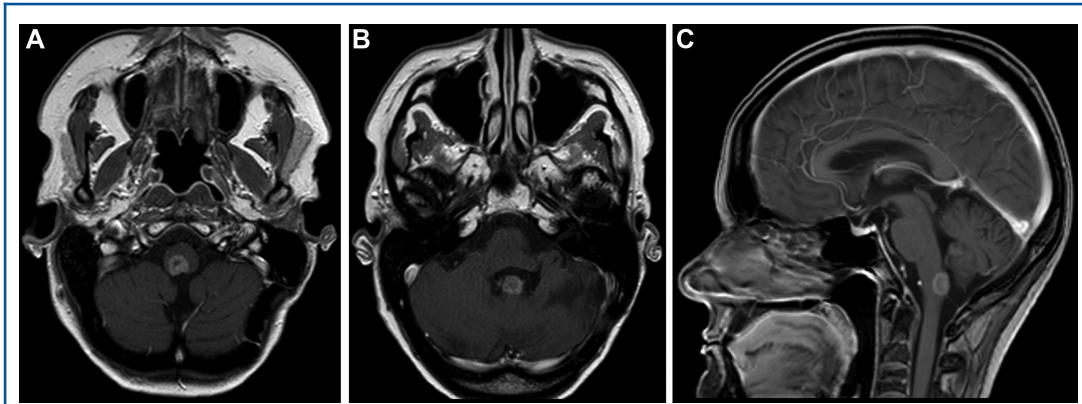
FIGURE 2. *Female patient with a metastatic lesion in the brain stem and at the floor of the forth ventricle. Patient no. 2: MRI gadolinium contrast-enhanced T1-weighted imaging in axial cuts demonstrating* **A***, brain stem lesion and* **B***, tumor at the floor of the fourth ventricle, as well as* **C***, sagittal planes. Both tumors revealed ring gadolinium enhancement.*
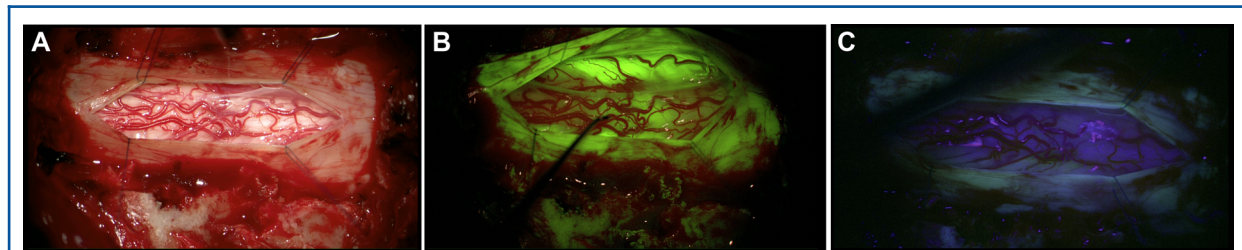

FIGURE 3. *Intraoperative view under white-light microscopy and different fluorescence filters of Patient no. 1. Intraoperative visualization of the spinal cord at the height of the C6 and 7 level with* **A***, white-light microscopy,* **B***, the Yellow 560 Zeiss filter after low-dose fluorescein application (Alcon, 10% solution, 4 mg/kg body weight) and* **C***, the Blue 400 Zeiss filter for protoporphyrin IX (PPIX)-fluorescence demonstration. Fluorescein fluorescence can be seen in the midline of the spinal cord. The nerve root, since it does not possess a blood-brain barrier, demonstrates strong fluorescein fluorescence. PPIX-fluorescence was not observed.*
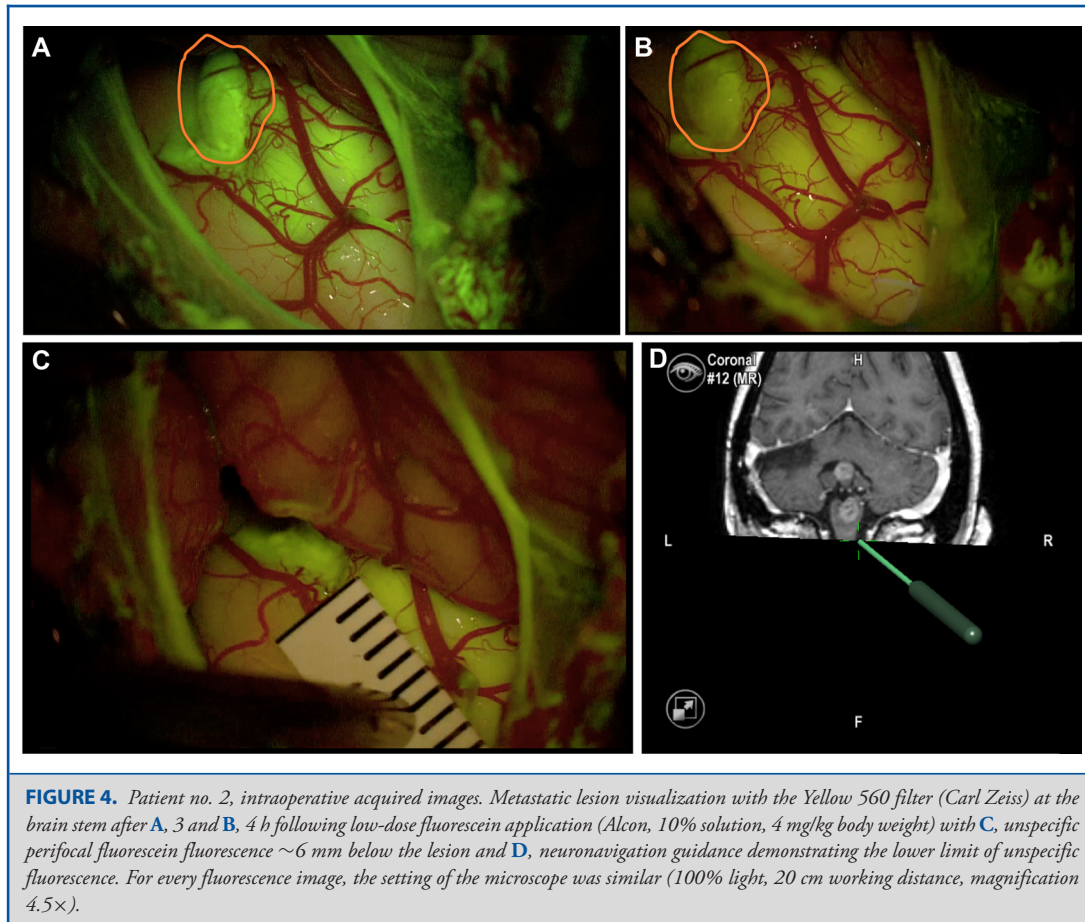
RESULTS

Patient No. 1

The spinal cord was approach through C6 and C7 lamnio-plastia under SSEP, EMG tibial, and median nerve SSEP monitoring. The dura was opened in the midline. Intraopera-tively, fluorescein assisted in finding the zone for myelotomy, since fluorescein fluorescence could be observed at the superficial spinal cord. The region matched anatomically with the superficial gadolinium-contrast enhancement in MRI. Furthermore, intraoperative ultrasound examination was performed as control. During intramedullary exploration, fluorescence guidance was not helpful in distinguishing between pathological and normal spinal cord tissue. Since spinal nerves do not have a blood-brain barrier, fluorescein fluorescence could be strongly demonstrated here. ALA fluorescence was not clearly observed (Figure 3). An intraoperative frozen section was performed, revealing low grade, diffuse astrocytoma, as later confirmed by final neuropathological assessment (IDH1 wildtype).

Patient No. 2

An infracerebellar midline approach with widening of the foramen magnum in park-bench positioning was used to reach the brain stem and the forth ventricle. Monitoring included EMGs of the seventh, ninth, 10th, 11th, and 12th nerves, the 10th nerve via a laryngeal tube electrode, acoustic evoked potentials, MEP, and SSEP (tibial and median nerves). First, the lesion at the roof of the fourth ventricle at the end of the cerebellum was approached via a narrow corridor, its location verified by blood-brain barrier disruption and ensuing visible fluorescein fluorescence. Fluorescein fluorescence was demonstrated to be the strongest at the brain stem lesion, which was approached after removal of the lesion in the roof of the fourth ventricle. However, approximately 3 to 4 h after application, fluorescein fluorescence was darker in parts of the lesion covered by the medulla. Conversely, fluorescein had appeared in the peritumoral zone, as far as 6 mm inferior to the lesion about 3 h after application, as confirmed by direct measurements and neuronavigation (Figure 4; **Video**, **Supplemental Digital Content**). The

FIGURE 4. *Patient no. 2, intraoperative acquired images. Metastatic lesion visualization with the Yellow 560 filter (Carl Zeiss) at the brain stem after **A**, 3 and **B**, 4 h following low-dose fluorescein application (Alcon, 10% solution, 4 mg/kg body weight) with **C**, unspecific perifocal fluorescein fluorescence ~6 mm below the lesion and **D**, neuronavigation guidance demonstrating the lower limit of unspecific fluorescence. For every fluorescence image, the setting of the microscope was similar (100% light, 20 cm working distance, magnification 4.5×).*

nerve rootlets showed strong fluorescence as did the dura and the arachnoid membrane. At the end of resection (approximately 5 h after application), fluorescence had diminished remarkably in a diffuse manner surrounding the lesion (Figure 4; **Video, Supplemental Digital Content**). Histopathological evaluation of tumor tissue demonstrated a metastatic lesion from an adenocarcinoma.

Patient No. 3

A Th3, Th4, and Th5 laminoplastia was performed to reach the thoracic spine. Fluorescein alone was administered prior to surgery. Intraoperative neurophysiologic monitoring including SSEP monitoring of the tibial nerve was employed. After reaching the thoracic spinal cord, fluorescein fluorescence could not be observed, neither at the surface nor during resection was fluorescence visualized. Once again, the spinal nerves and the dura demonstrated strong fluorescein fluorescence. Neuropathologic examination of tumor tissue demonstrated an ependymoma grade II. Fluorescein fluorescence was again demonstrated in the dura and at the end of resection leaking into the tumor cavity.

DISCUSSION

Neoplastic spinal cord lesions are not only extremely rare but are also correlated with a poor prognosis.[6] Surgery is not always feasible, but is sometimes necessary, since intraoperatively acquired tumor tissue and neuropathological examination can provide a working diagnosis.[3] Even though prognosis and neurological outcome have been improved by means of surgical and technical advances, e.g., ultrasound aspirator, as well as by development of radiological imaging, neurological outcome remains poor after surgery of malignant tumors, decreasing with a rising grade of malignancy.[5,20] Therefore, every tool that can increase patient safety should be evaluated.

Historically, fluorescein was the first fluorophore to be applied for brain tumor surgery. One of its drawbacks, however, is its extravasation into perifocal edematous brain fluorescence that was already noted by Moore in 1948.[21]

With novel filter systems for commercially available microscopes fluorescein has again attracted attention.[12] In a recent publication, our group discussed the window of what we called "pseudoselectivity" for marking tumor tissue after fluorescein
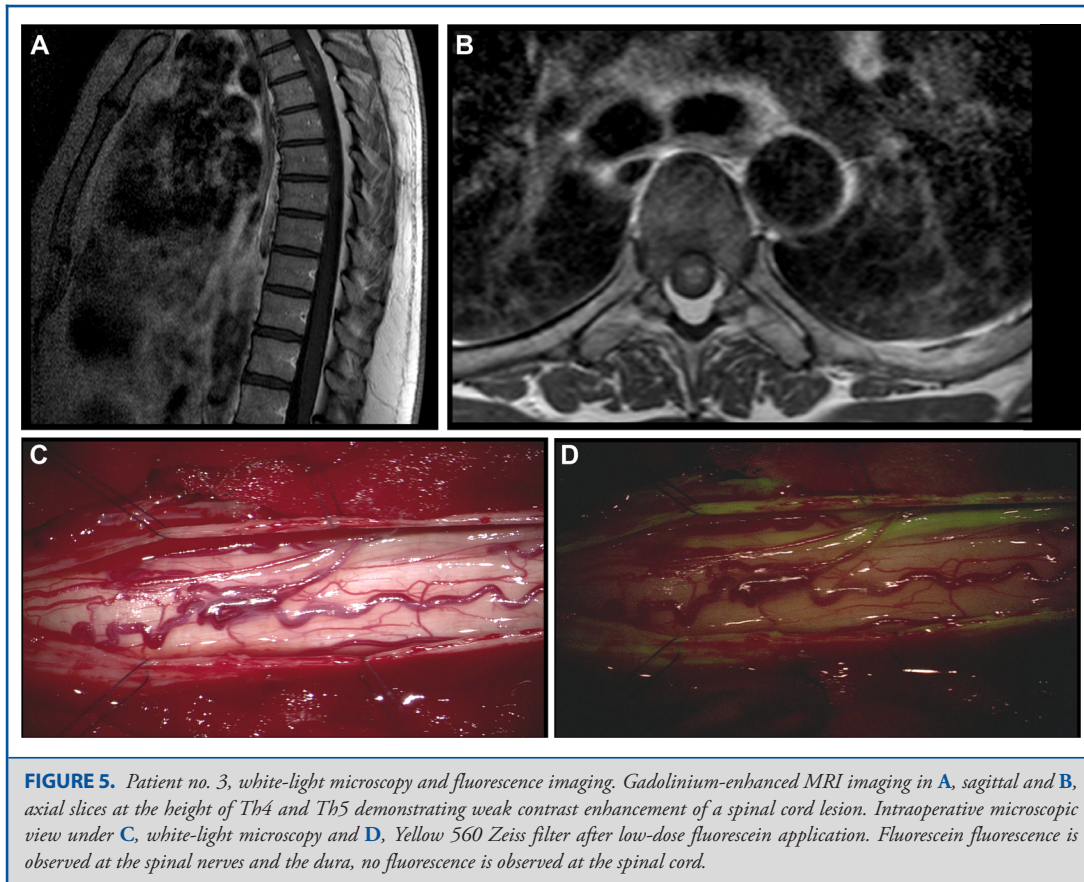
FIGURE 5. *Patient no. 3, white-light microscopy and fluorescence imaging. Gadolinium-enhanced MRI imaging in* **A***, sagittal and* **B***, axial slices at the height of Th4 and Th5 demonstrating weak contrast enhancement of a spinal cord lesion. Intraoperative microscopic view under* **C***, white-light microscopy and* **D***, Yellow 560 Zeiss filter after low-dose fluorescein application. Fluorescein fluorescence is observed at the spinal nerves and the dura, no fluorescence is observed at the spinal cord.*

injection (Figure 6).[13] Fluorescein will not only be in edema, however. All viable tissues, which are perfused by fluorescein will fluoresce more or less strongly, an effect which we are using for creating more background detail during a dual-labeling approach concomitantly to 5-ALA-induced tumor fluorescence, as recently published.[13]

Nevertheless, lesions with extravasation of fluorescein due to blood-brain barrier disruption will at first be visible to the surgeon, despite the limitations and confounders involved in using fluorescence at later stages of surgery for monitoring completeness of resection.[14] We thus hypothesized that we might utilize this characteristic for locating small lesions in critical regions such as the brain stem or the spinal cord. Such lesions are typically small, and choosing the correct approach based on mapping and structural information is crucial. Our experience showed this approach to be feasible and of value.

On the other hand, our findings directly illustrate how timing is extremely important when working with fluorescein fluorescence and to what rate fluorescein propagates with edema. In our brain stem lesion, we were able to directly visualize unspecific fluorescence up to 6 mm away from the original lesion (Figure 4). Fluorescein will initially accumulate in the regions of blood-brain barrier disruption but will shortly thereafter propagate beyond tumor margins with perifocal edema, due to the lack of any specific affinity to tumor cells.[22] Gradually moving at a speed of around 2 mm per hour in humans, fluorescein will spread with the edema in the peritumoral zone.[23] This raises the biggest challenge when working with this fluorophore regarding timing and interpretation of the signal in tissue.

We can generally say that from our experience, albeit limited, somewhere between 2 and 4 h after low-dose application would be the window of what we call "pseudoselectivity" for enhancement in these lesions. After this time, unspecific fluorophore in the tissue has waned far enough to observe a meaningful difference between the region of blood-brain barrier breakdown and unspecific blood-borne fluorophore. We believe more that further studies aiming for timing description of the pharmacokinetics of this fluorophore are needed.

Fluorescein guidance was not useful in all studied tumor entities. While fluorescein fluorescence was demonstrated in glioma und metastatic tissue demonstrating strong contrast enhancement in MRI and reaching the surface of the spinal cord and brain stem, we did not observe the value of this method in a central lesion with weak contrast enhancement, which was later demonstrated as an ependymoma (grade II). Hence, fluorescein appears to be useful in the context of strongly contrast-enhancing
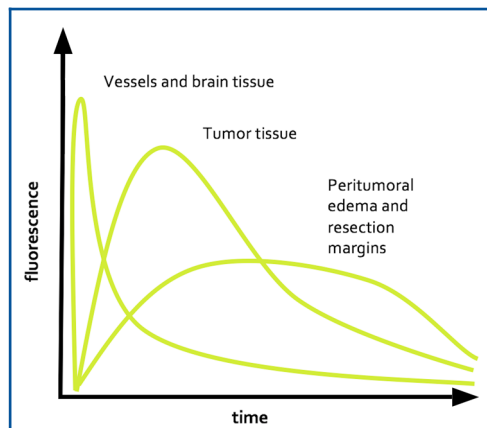
FIGURE 6. *Fluorescein pharmacokinetics scheme. Scheme demonstrating pharmacokinetics of fluorescein. Fluorescein will first concentrate in vessels and in perfused tissues. Afterwards, it will highlight regions of blood-barrier breakdown and slowly extravasate from the tumor within the peritumoral zone at a speed of approximately 2 mm/h. The first window of extravasation within the tumor before spreading into perifocal edema explains the pseudoselectivity of the fluorophore for these lesions. Hence, timing of application and visualization is therefore essential.*

lesions in MRI. Further stratification is needed to identify suitable tumor tissue for this approach.

Fluorescein provides the advantage of highlighting different lesions with blood-brain barrier breakdown, regardless of the entity. In a recent report, fluorescein was studied for feasibility when biopsying lesions presenting with a disrupted blood-brain barrier within the ventricular system. In this series from 9 patients, fluorescein was helpful in enhancing different lesions, ie, histiocytosis, astrocytomas, germinomas, lymphomas, and chronic granulomatosis.[25]

It is important to know the limits of this fluorophore, though. We did not find fluorescein reliable for resection guidance. Nevertheless, if this fluorophore is shown to be as low risk and easily applicable in neurosurgery as described,[10,11] it can add to the surgical algorithm of lesions located in the vulnerable spinal cord or brain stem. Nevertheless, life-threatening reactions, i.e., anaphylactic reactions after application, have been discussed.[24] Furthermore, this drug is still off-label for its application in neurosurgery and each surgeon should comply with their own country's regulations before using this technique.

In our opinion, a combination of several tools could provide optimal guidance for each step of surgery. Based on our previous experience, we additionally find intraoperative ultrasound in combination with intraoperative neurophysiologic monitoring as a reliable intraoperative guidance for intramedullary exploration and tumor resection. Ultrasound assists in finding the right level of the lesion within the spinal cord and provides real-time information during resection, whereas fluorescein could assist in finding the exact entry point for myelotomy.

Limitations

We are aware of the limits of this study, especially regarding the number of patients presented here. Nevertheless, these lesions are extremely rare. On the other hand, fluorescein is used on an off-label setting since it is not approved for fluorescence-guided resection. Subsequently, our findings, as a feasibility study, provide the proof of a principle.

CONCLUSION

Fluorescein guidance appears to be a feasible tool for finding entry zones when aiming for surgical removal of spinal cord and brain stem lesions demonstrating blood-brain barrier breakdown in MRI. However, fluorescein propagates unspecifically into the peritumoral zone, reducing its value for assistance throughout the resection. Furthermore, additional studies are needed for finding the optimal timing for fluorescein visualization. Providing the right timing, a small window of pseudoselectivity could increase fluoresceins' clinical value in this type of surgery.

Disclosures

Prof Dr Med Stummer has received speaker's fees by Medac, Zeiss, and Leica. Dr Med Molina has no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

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COMMENTS

Fluorescence guided surgery using fluorescein is becoming more widespread in neurosurgery. This is an interesting and timely paper as it expands the possible uses of the technique, while, at the same time, detailing the limitations and warnings of the use. Use of a non-specific dye like this requires a precise understanding of the mechanisms of work, which the authors explain carefully.

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This review of 3 patients with brainstem and spinal cord intramedullary lesions resected with the assistance of fluorescence is an extremely abbreviated series but does highlight the limitation of this modality for such surgery. The authors are to be congratulated on their judicious use of the technique and recognizing the need to supplement it with additional methods for safe optimal resection.

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