Intraoperative imaging of brain tumors with fluorescein: confocal laser endomicroscopy in neurosurgery. Clinical and user experience

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OBJECTIVE Confocal laser endomicroscopy (CLE) is an established tool in basic research for tissue imaging at the level of microstructures. Miniaturization and refinement of the technology have made this modality available for operative imaging with a handheld device. Sufficient image contrast is provided by the preoperative application of fluorescein sodium. The authors report their first experiences in a clinical case series using the new confocal laser endomicroscope.

METHODS Handling, operative workflow, and visualization of the CLE were critically evaluated in 12 cases of different CNS tumors. Three different imaging positions in relation to the tumor were chosen: the tumor border (I), tumor center (II), and perilesional zone (III). Respective diagnostic sampling with H & E staining and matching intraoperative neuro-navigation and microscope images are provided.

RESULTS CLE was found to be beneficial in terms of high-quality visualization of fine structures and for displaying hidden anatomical details. The handling of the device was good, and the workflow was easy.

CONCLUSIONS Handling ergonomics and image acquisition are intuitive. The endomicroscope allows excellent additional visualization of microstructures in the surgical field with a minimally invasive technique and could improve safety and clinical outcomes. The new confocal laser endomicroscope is an advanced tool with the potential to change intracranial tumor surgery. Imaging of these microstructures is novel, and research with comparative validation with traditional neuropathological assessments is needed.

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KEYWORDS CONVIVO; confocal endomicroscopy; laser; fluorescein sodium; fluorescence-guided surgery; neurosurgery

THE concept of intraoperative labeling of tumorous tissue or vascular structures has intrigued surgeons for decades. The emergence of fluorescence-based imaging techniques highlighting tumors or vascular structures during surgery has improved intraoperative decision-making. In principle, a clear distinction is provided to discern the lesion to be resected from surrounding viable tissue, which can guide through the most delicate process of tumor resection. This holds particularly true for neurosurgery, where manipulation or resection of tissue is done at a microsurgical level. Currently, the methods employed often rely on surgical microscopes equipped with appropriate light sources and filters to visualize the fluorophore.

To visualize microstructures at high magnification, this principle has been refined even further, resulting in the development of the confocal laser endomicroscope. In essence, this technique can deliver in vivo images in real time without the need for tissue extraction. Based on the application of a fluorescent agent, a scanner probe through gentle contact with the tissue surface reveals optical details at the subsurface level. The device emits focused laser light via a probe into the tissue and recaptures emanating light from the focal plane while rejecting light from outside the focused spot.¹ This enables visualization of an area of interest on a specific focal plane in a tissue, hence “confocal.” Images can be obtained not only in the x- and

ABBREVIATIONS CLE = confocal laser endomicroscopy; 5-ALA = 5-aminolevulinic acid.
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FIG. 1. Intraoperative probe view under white light (left) and YE560 filter view (right).

y-axes but also in the z-axis as z-stacks (an automated recording of a user-defined range of images relative to the current focal plane).

The miniaturization and technical refinement of this technique have resulted in a device readily available for intraoperative use in neurosurgery (CONVIVO, Carl Zeiss AG). The technical evolution of fluorescence-guided surgery in neurosurgery has produced crucial innovations in the recent decade.² Among the popular agents, 5-aminolevulinic acid (5-ALA) was clinically validated in a pivotal study by Stummer et al.³ Its approval, however, is limited for use in patients with high-grade gliomas. In other entities such as brain metastases, the results have varied.⁴ Fluorescein sodium (FNa) has recently reemerged as a promising fluorophore, with retrospective and prospective data suggesting that FNa improves visualization and resection of many CNS tumors.^{5–11} A prerequisite for staining of CNS lesions is a pathological alteration of the blood-brain barrier, which equally applies for 5-ALA and FNa. In the case of FNa, the presumed mechanism of action is passive staining of the extracellular space in areas with a blood-brain barrier breakdown, corresponding to gadolinium uptake on MRI.¹² The safety of FNa has been demonstrated through decades of experience in ophthalmology, where its properties as a vascular dye have been exploited.¹³

Intraoperative diagnosis in neurosurgery is traditionally provided by histopathological frozen section analysis to instantly assess the tumor entity and monitor surrounding tissue for tumor-free margins as the gold standard. This technique relies on the examination of extracted tissue samples with different staining methods using benchtop microscopes. The diagnostic yield is dependent on the technique used, and, through sampling errors and artifacts, the accuracy can be as low as 50%.^{14,15} Furthermore, waiting times for the results prolong the time to surgery. Confocal laser endomicroscopy (CLE) offers the possibility for fluorophore-dependent in vivo microscopy to neuropathologists, potentially ameliorating these shortcomings. Thus far, there is a scarcity of scientific evidence for CLE in neurosurgery, with only a few studies exploring its feasibility and safety.^{16,17} As with conventional neuropathological assessment, in CLE, staining with a fluorescent dye is needed for sufficient image contrast, which is provided by FNa in the case of CONVIVO (Fig. 1).

CLE with FNa has provided a real-time intraoperative assessment of suspected tumor and healthy tissue (digital

biopsies),¹⁸ omitting the need for time-consuming tissue transfer, fixation, and interpretation to improve the diagnostic yield and shorten sampling times.^{16,19} All in all, through real-time tissue evaluation, this technique has a true potential to further refine surgical decision-making and strategy.²⁰ As the CONVIVO device has just recently been introduced, at this time the existing data are not sufficient to compare the quality of frozen section analysis with CLE findings.¹⁸

Here, we present our first experience with FNa and CLE in different CNS tumors. We provide intraoperatively acquired CLE images with matching frozen section analysis, microscope photographs, and intraoperative navigation. This user report offers information on the feasibility, safety, and potential applications of this technique.

Methods

Patients and Radiological Evaluation

In this study, we included 12 nonconsecutive patients who were operated on at our institution in July 2020. Inclusion criteria were suspected intracranial tumor confirmed by gadolinium-enhanced MRI, informed consent about the off-label use of FNa, and no known allergy to FNa. All patients underwent preoperative and prompt postoperative (within 72 hours after surgery) contrast-enhanced MRI. A review of preoperative imaging–determined localization and contrast-enhancing behavior of the tumor was conducted. The retrospective study was approved by our institutional review board.

Surgical Protocol

Fluorescein Sodium

FNa (Alcon) 10% injection solution is frequently utilized in ophthalmology and optometry for the diagnosis of corneal abrasions, corneal ulcers, herpetic corneal infections, and retinal angiography.²¹ Moreover, since its discovery, FNa has been found to be useful in a myriad of scientific and civil areas and is listed in the *WHO List of Essential Medicines*.²² Adverse reactions are rare and have been extensively studied and are therefore well known. The administration of FNa for the purpose of tumor visualization is currently an off-label use. Following earlier reports,^{6,18,23} a weight-adjusted dose (5 mg/kg body weight) of 10% FNa was injected intraoperatively via a central venous line. To observe changes in enhancement, the time of injection varied (Table 1).

TABLE 1. Patient characteristics

Case No.	Diagnosis	Time Btwn FNa & Imaging (mins)	Tumor Contrast Enhancement on MRI	Imaging Position & FNa Fluorescence		
				I	II	III
1	Lung cancer metastasis	60	Homogeneous	+	+	+
2	Glioblastoma WHO grade IV	30	Homogeneous	+	+	+
3	Oligodendroglioma WHO grade II	120	Inhomogeneous	+	+	–
4	Glioblastoma WHO grade IV	30	Homogeneous	+	+	+
5	Neurocytoma WHO grade II	60	Inhomogeneous	+	+	–
6	Melanoma brain metastasis	30	Rim-like	+	+	+
7	Brain metastasis of a carcinoma, not otherwise specified	60	Rim-like	+	+	+
8	Gliosis/recurrent hemangiopericytoma	45	Homogeneous	+	+	–
9	Colon cancer metastasis	10	Rim-like	+	+	–
10	Glioblastoma WHO grade IV	20	Rim-like	+	+	+
11	Glioblastoma WHO grade IV	45	Rim-like	+	–	+
12	Melanoma brain metastasis	65	Rim-like	+	+	–

+ = positive; – = negative.

Intraoperative CLE Imaging and Diagnostic Sampling

After craniotomy and durotomy, on contact with the suspected tumor, CLE imaging commenced, after which a snapshot of the position according to neuronavigation findings was obtained (still picture acquisition through the microscope), and the selected tissue was removed. Before imaging, the CLE microscope probe is dressed in a sterile drape and is then ready to be inserted into the resection cavity. During routine tumor microsurgery, these steps were conducted at different locations: at the border or capsule of the tumor, referred to as the tumor capsule (I) (Fig. 2); at the tumor center (II) (Fig. 3); and at the potential infiltration or edema zone where the fluorescence signal started to become faint, referred to as the perilesional zone (III) (Fig. 4).

Tumor Resection

In between these steps, tumorous tissue was removed with the aid of FNa-induced fluorescence visualized with an additional filter on the operating microscope (KINEVO 900 or PENTERO 900, ZEISS Meditec). This filter (YEL-LOW 560) is tailored to the excitation and emission wavelength of FNa.²⁴ When applicable, the Cavitron Ultrasonic

Surgical Aspirator (Integra), intraoperative monitoring, and intraoperative ultrasound were used. Typically, the lesions exhibit bright yellow staining through the microscope filter when approached (Fig. 1 right). Resection was stopped when the yellow-green staining of the enhancing tissue became faint and pinkish nonenhancing tissue appeared at the circumference of the tumor. Unless continuation of surgery was deemed unsafe, for instance, because of venturing into eloquent areas, surgical intervention was finished after removal of all fluorescing tissue as confirmed by the filter view. The addition of CLE imaging to the surgical workflow did not change the resection in any case.

Intraoperative Fluorescence Characteristics and Side Effects

Surgical reports were screened for subjective evaluation of the grade of fluorescent staining of the targeted lesion. The screening was conducted for any reference to the degree of fluorescent staining: bright versus medium and heterogeneous versus effectively no fluorescence. Fur-

FIG. 2. Case 4. H & E–stained slide (left; original magnification $\times 200$) and CLE image (right) obtained during biopsy of a glioblastoma, imaging position I (tumor border). Microscopic examination revealed a highly cellular, diffuse infiltrating glioma with small, hyperchromatic nuclei and hemorrhages.

FIG. 3. Case 6. H & E–stained slide (left; original magnification $\times 200$) and CLE image (right) obtained during biopsy of a metastatic malignant melanoma, imaging position II (tumor center). The malignant tumor is characterized by densely packed epithelioid cells with a high nuclear-to-cytoplasmic rate, round nuclei, and incidental large nucleoli. The blood vessels captured in this image enclosed activated, prominent endothelial cells.

FIG. 4. Case 4. H & E–stained slide (**left**; original magnification $\times 200$) and CLE image (**right**) obtained during biopsy of a glioblastoma, imaging position III (perilesional zone). The infiltration zone is characterized by an increased cell density of gray and subcortical white matter. In the cortical areas, perineuronal satellitosis is evident and signals the formation of secondary structures.

thermore, medical reports were evaluated for any possible adverse effect or allergic reaction to FNa.

Results

Fluorescence

Homogeneous or heterogeneous yellow-green fluorescent macroscopic staining of the tumor tissue was observed in all patients ($n = 12$; 100%). According to the surgical report, the staining was considered helpful guidance in all cases. Data on demographic patient characteristics, histopathological classification of the disease, timing, and dosing for the injection of FNa were available for all patients (Table 1).

CLE Imaging

Tumor surgery was performed using standard microsurgical techniques depending on the tumor location and type of tumor expected and adapted to the intraoperative situation. CLE could be safely performed, as the small and versatile probe mirrors any other microsurgical instrument that is held in one hand and inserted into the cavity without traumatizing healthy tissue (Fig. 1).^{18,20,25} A movable monitor mounted on top of the device serves as a viewer and as a user interface via touchscreen capabilities. Furthermore, a foot control panel can be connected and allows surgeon-controlled image acquisition.

Adjustable parameters, which are set first, are a choice of filters. The best image quality is obtained with the green filters, of which the bandpass filter produced better interpretable images than the longpass filter. Another baseline adjustment is gain, which was set at 2400 with good results. Laser power and brightness can be adjusted on the fly on a scale from 0% to 100%; ideally, the laser was set at 50% or below and the brightness adjusted during imaging. Additionally, an autobrightness mode can be activated and speed up the process of image acquisition. After sterile draping of the probe, the 0 position of the window has to be determined to find the optimal depth for initial imaging. In the viewing mode, there are two imaging speeds, fast (0.44 sec/frame) and lower resolution or slow (1.29 sec/frame) and higher resolution (1920×1080 pixel). Images can be recorded continuously as a time series or sequentially as single frames. Another feature is a z-stack option, imaging preset layers sequentially along the z-axis to a depth of up to 30 μm below the window of

the sterile cover. There are 3 zoom options: $\times 1$, $\times 1.4$, and $\times 2$. We found the $\times 1$ zoom the clearest option with a field of view of $475 \times 267 \mu\text{m}$ (width by height).

CLE was integrated seamlessly in the surgical flow. Representative images of different tumor entities and different intraoperative areas are depicted in Figs. 2–4. As the interpretation of CLE images needs further systematic evaluation, CLE images in this case series did not alter surgical tactics or the procedure.

Timing of FNa Administration

In all patients, a weight-adapted dose of 5 mg/kg FNa was administered intravenously prior to imaging. The timing varied. In essence, a shorter elapsed time resulted in more assessable images.

Adverse Events

We did not encounter any morbidity or mortality attributable to the use of FNa. Furthermore, no major side effects related to fluorescein throughout the observation period, apart from yellow-colored urine and, in some patients, slight yellow discoloration of the skin, were documented.

Discussion

Most surgeries of different lesions of the CNS still solely rely on tactile differentiation and visual cues to guide tumor resection. Technical advances have aimed at bridging that gap. In the past decades, efforts to safely aid maximal extent of resection have added the operating microscope, intraoperative MRI, ultrasound, and neuro-navigation to the neurosurgeon's armamentarium. Fluorescence-guided surgery has been part of this evolution. In that respect, FNa has regained interest among the neurosurgical community. In numerous tumors of the CNS, including high-grade gliomas, metastases, and hemangioblastomas, FNa has been shown to safely guide tumor resection.^{5–9,26} While FNa fluorescence can be observed by the naked human eye at high doses,²⁷ the addition of a specific filter allows for a significant dose reduction, lowering the odds for dose-related complications. Furthermore, good image quality that depicts anatomy in high detail allows for continued microsurgical work in filter mode, an advantage not seen with other fluorescent agents such as indocyanine green or 5-ALA.

Here, we report our experience with CLE in 12 patients in whom contrast-enhancing lesions were removed with FNa guidance. We did not see any major differences in terms of image quality with respect to the timing of FNa injection. Data by Folaron et al. have suggested that peak fluorescence is induced by FNa at 15–30 minutes, and the best viewing opportunities occur within 90 minutes of injection.²⁸ In our series, most CLE images were obtained within that time frame.

Several CLE systems are being utilized in medicine, mainly in gastrointestinal interventions and research settings, such as Cellvizio (Mauna Kea Technologies), EC3870 K (Pentax), or the CONVIVO. During gastrointestinal interventions such as colonoscopy, CLE can guide diagnosis and polypectomy.²⁹

The emergence of a handheld probe-based CLE system has facilitated proof-of-concept studies in animals. Belykh et al. found high diagnostic accuracy in a mouse glioma model, with a specificity of 86% and a sensitivity of 96% when neuropathologists had to differentiate between unaffected and tumorous tissue based on CLE-generated images.³⁰ These promising findings extended into ex vivo and in vivo human studies. Snuderl et al. obtained images of different tumor tissues and normal brain ex vivo in a multimodal fashion with CLE and were able to render diagnoses in a comparable fashion to those rendered by H & E slides.³¹ Sanai et al. utilized an earlier version of the CONVIVO in 31 patients for FNa-based in vivo CLE, obtaining images of different brain tumors, and found a high concordance with routine diagnostics.³² More recently, in a prospective study with 74 consecutive patients, Martirosyan et al. showed sensitivities and specificities, respectively, of 94% and 91% in gliomas and 93% and 97% in meningiomas when in vivo and ex vivo CLE images were compared with a standard frozen section for diagnosis.³³ Therefore, CLE with FNa could improve intraoperative decision-making and safety in neurosurgery.³⁴ The potential applications of CLE in neurosurgery are manifold. Potentially, it could improve the extent of resection, equip the surgeon with a tool to precisely define necessary resection margins, shorten the surgery, and provide rapid intraoperative diagnosis, which instantly benefits patients. In the future, with the establishment of a large CLE image data collection, deep learning could offer automated classification.³⁵

Thus far, comprehensive evaluation of CLE for neurosurgical purposes is pending. FNa applied for the purpose of tumor visualization is still an off-label use. Since CLE offers an insight into a living system, we will have to learn what we see. The optimal application of CLE remains unclear as well as the timing and dose of the FNa contrast medium.

In our study, FNa was utilized to help with visualization and maximize resection. Our results were obtained using standard image guidance and microscopy with FNa fluorescence. We did not encounter any side effects attributable to the use of FNa intraoperatively or during the hospital stay. Neither did we encounter any adversities to the surgical workflow or ergonomics.

Conclusions

FNa and CONVIVO CLE are safe and feasible tools. Further prospective evaluations are needed to help neurosurgeons determine the validity of CLE and to confirm its promising potential.

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Conception and design: Höhne, Riemenschneider, Schmidt. Acquisition of data: Höhne, Schebesch, Zoubaa, Riemenschneider. Analysis and interpretation of data: Höhne, Zoubaa, Schmidt. Drafting the article: Höhne. Critically revising the article: Schebesch, Proescholdt. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Höhne. Statistical analysis: Riemenschneider, Schmidt.

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