

UNIVERSITA' DEGLI STUDI DI PAVIA

Corso di Dottorato in Medicina Sperimentale

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Fluorescein-aided Neurosurgery

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1- Introduction

As neurosurgeons, during our consultations we are often confronted with scared patients who have recently been diagnosed a certain, obscure pathology. Despite our best endeavors to inform them about their condition and be as reassuring as possible, their attentions, fears and hopes boil down to one and one only major issue: can you cure it, doctor? Can you take it away?

Unfortunately, it is not always so.

In their frightened minds, the expectable second question is therefore: why? Why can't you just crack my head open, put some stitches here and there, take out the garbage and close it back up, as if nothing happened?

In this era of virtual reality and self-driving cars, it is actually difficult to face their disappointment as we try to explain them that modern Neurosurgery is not that easy yet.

We made enormous advances, in particular in the last century. Starting from a meticulous anatomical knowledge of the cerebral structures and passing through the “microscope revolution” we arrived in the modern neuronavigation era in which we can render and correlate the preoperative imaging to the patient in real time.

Real life is far too different from simulation and technological promises, though. Some tumors are often almost undistinguishable from the brain in normal vision and our individual estimate of vessel patency may be proved completely wrong at the ischemic post-operative imaging. Sometimes, even with all the best resources, we are blindly performing life-saving procedures.

Basically, this PhD thesis is the synthesis of a personal research journey through the darkness of some of the most frightful, neurosurgical pathologies enlightened by intraoperative Fluorescence.

I am going to describe the optical and pharmaceutical properties of Fluorescein and explain how I assembled a low-cost fluorescence detection system. I will then go through all the fields of Neurosurgery to which I applied this technology from high-grade tumors to vascular and minimally invasive surgery.

2- Fluorescein, a multipurpose drug

2.1 – History of Fluorescence use in Neurosurgery

Intraoperative fluorescein detection has been used in Neurosurgery for multiple purposes: from the pioneering work by Moore in 1948, many papers investigated its use mainly in the fields of vascular and oncological surgery (Moore). In particular, the rationale of the use of fluorescein was to better outline the vessel and / or borders of the treated tumours. Even if it has been recently employed also at high doses without filters (Okuda, Shinoda, Uzuka), many authors tried some modification of the optics in order to enhance the fluorescence under microscopic view at low doses, too. The first surgeons who pioneered the use of filter systems were Feindel and colleagues in 1967. Still, the first modification of the microscope optics was carried out by Wrobel et al in 1994 who applied a filter system tailored on the fluorescence spectrum of fluorescein. In particular, they constructed an apparatus consisting of a cyan-violet light source shedding light to the surgical field via a fiber optic system and a yellow filter placed on the microscope oculars or on plastic goggles worn by the surgeon. Both color modifications were performed with the aid of photographic gelatin filters. Although they concluded that there was a general usefulness in this system, they complained that "... the incident illumination provided by the fiber optic system is not sufficient to permit safe manipulation..." as well as that operations were generally to be carried out in "low-light conditions". These limitations were partially overcome in two 1998 publications: Kuroiwa et al. and Stummer et al. reported the use of glass interference filters tailored on the fluorescence spectrum of fluorescein and porphyrins, respectively. Similarly, in 2003 new researches were published on the use of Indocyanine Green fluorescence (ICG) in Neurosurgery (Raabe).

The development of these fluorescence systems was empowered by industries: the first articles in the field of neurosurgery describing the use of commercial microscopes equipped with fluorescence modules date back to 2005 for ICG, 2006 for *5-aminolevulinic acid* (5-ALA) and 2013 for fluorescein (Raabe, Stummer, Acerbi).

2.2 – Fluorophores: pharmaco-optical properties

Several fluorophores have been studied as real-time contrast enhancers, but only ICG, 5-ALA and Fluorescein have been repeatedly employed. Their optic properties, action, pharmacodynamic and pharmacokinetic characteristics are different. In an endeavor to schematize their function, we can define 5-ALA as a lesion-specific fluorophore. As a matter of fact, 5-ALA is a natural precursor for heme synthesis and each cell metabolizes it by producing protoporphyrin-IX (PpIX). Still, only in tumor cells there is a significant accumulation of PpIX, which is a photoactive compound that absorbs violet-blue light and transmits it back to the red spectrum (Eljamel). Due to its emission spectrum, it is usually impossible to actively operate, once its detection filtering system is set in place. On the contrary ICG is a vascular-specific contrast enhancer: once injected into the human blood stream, it becomes fluorescent and detectable by dedicated cameras (no real-time visualization is available, though) when excited with specific wavelength light in the near infra-red (NIR) spectrum (Raabe). This is reflected by their administration pattern: 5-ALA must be administered roughly 3 hours prior its detection in the living tissue for its metabolization to take place, whereas ICG could be administered repeatedly on-time as a bolus. Fluorescein has somewhat mixed characteristics: it could be administered on time as a bolus depicting the vessels and the perfusion but at the same time it could also be identified as a deposit in the interstitial space of living tissues.

Fluorescein is an oral or, most frequently, endovenous tracer. Once in the blood stream it is metabolized to fluorescein monoglucuronide and is excreted by the kidney and conjugated in the liver (Grotte). Its peak excitation occurs at 494 nm and its peak emission at 521 nm. It can also distribute well in the interstitial space, as demonstrated by its Volume of Distribution (Vd) which is around 0.5 L/Kg (Grotte). As a comparison, ICG Vd is 0.07 L/Kg (Niemann), whereas the Vd of Gadolinium contrast-enhancing agents is around 0.25 L/Kg

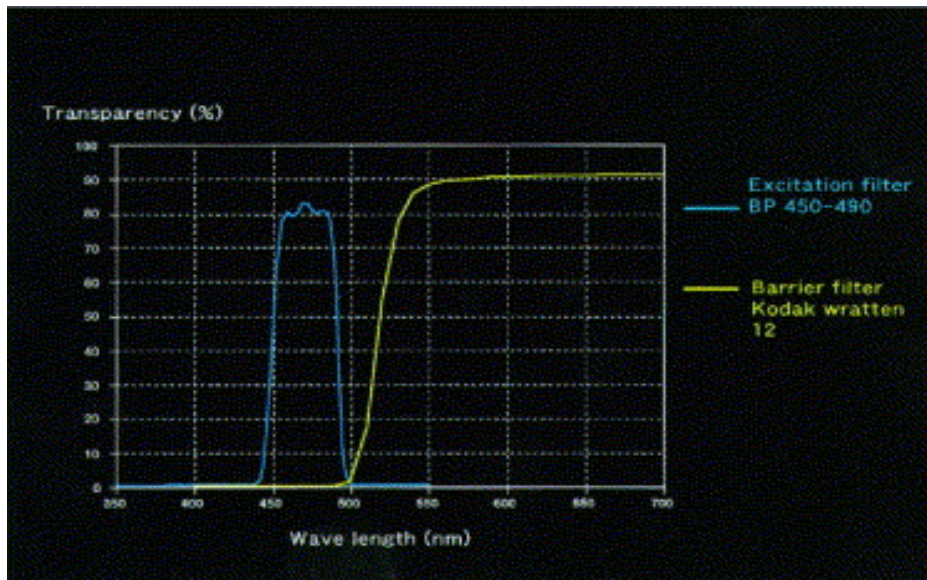
(Aime), a rather similar interstitial space-permeation value. Thanks to its extensive use in the ophthalmology field, fluorescein showed a consolidated favorable safety profile at low doses (<10 mg/Kg) (Placantonakis) but there have been some reports of major adverse reactions as the dose increases (Dilek, Moonsbrugger). As for the legal implications of the use of fluorophores, on the basis of the recent reports on safety and effectiveness of sodium fluorescein in glioma surgery, the Italian Medicine Agency (AIFA) approved its use at a dose of 5 mg/Kg (determination 905/2015, Italian Government Gazette n.168, 22 July 2015). On the contrary, in many countries the use of ICG is still off-label, while 5-ALA is still lacking FDA approval.

The most relevant characteristics of these three fluorophores are summarized in the table below.

	FDA	REAL TIME VISUALIZATION	SPECIFICITY	ADMINISTRATION TIMING	COST
FLUORESCEIN	YES	+++	+	++	5 EUR
ICG	YES	-	-	+++	150 EUR
5-ALA	NO	+	+++	-	900 EUR

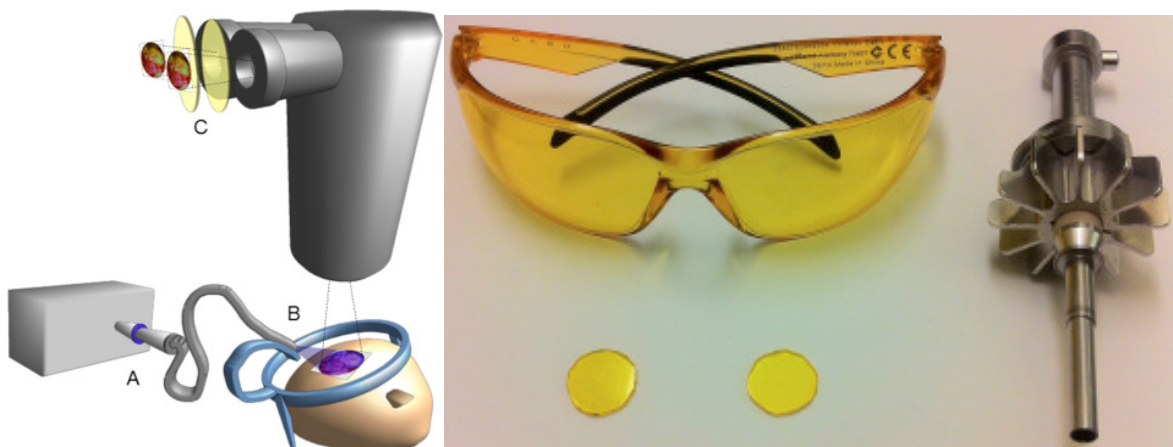
2.3 – Optic apparatus for Fluorescein detection

In the spending-review era of medical expenses, the Department I was working in was not equipped with the more up-to-date fluorescence-ready microscopes. Although the industries are to be commended for their efforts, these systems need a cutting-edge microscope and accessory modules for all the different fluorescent dyes, thus implying that the encompassing total cost of the instrumentation technology would easily amount to a few hundred thousand euros. After few congress reports on this new technology, though, we too believed that the possibility to detect fluorescein as a contrast agent during neurosurgical operation could become a game-changer. In reviewing literature, I found that the prototypical apparatuses were definitely at a low cost but lacked reliability, reproducibility and standard legal norms. With these restrictions, I tried to develop a custom system that could be as economic, simple, effective and law-abiding as possible. So, I basically went back to the basics and studied the aforementioned optic properties of Fluorescein, in order to try to replicate a home-made filtering system. Like most centers, our department too was equipped with light filters originally designed for endoscopic procedures, aiming at the detection of skull base CSF fistulas (Karl Storz GmbH & Co, Tuttlingen, Germany). This device has a built-in thermal radiator for heat dispersion and a set of three glass interference filters: neutral, blue (centered on 490nm) and violet-blue (centered on 465nm). The filter could be connected to a light source and to a fiber optic light cable in order to deliver the “excitation light”. At first, I subsequently tried to convoy the light through our microscope built-in light cable (OPMI-4, Karl Zeiss, Oberkochen, Germany). Although this seemed a reasonable approach, the mean focal distance from the microscope stand to the surgical field was too large and led to a dispersion of the filtered light intensity.



Filtering system specs focused on the excitation and emission properties of fluorescein

Not only did this dispersion affect the fluorescence excitation, it also generated an inadequate low-light condition, especially with the violet-blue filter, which guarantees a more evident fluorescence contrast. So, I tried to deliver the violet-blue light directly near the surgical field via a standard neuroendoscopic light cable held by hand near the surgical field or fixed with VELCRO[®] disposable strips to a brain retractor, in order to obtain a stable, non-hindering placement of the light source.



(Left) Filtering system setup. Three-dimensional rendering of the filtering system employed. (A) Xenon light source with violet-blue filter for endoscopic procedures. (B) The distal end of the endoscopic light cable is fixed to a retracting blade. (C) Yellow photographic filters are superimposed to the microscope oculars. (Right) Filtering hardware. Commercially available yellow glasses. Yellow photographic filters custom-shaped to fit the microscope oculars. Endoscopic light source blue-violet filter.

Both the built-in light of the microscope and the surrounding lights of the operating room were dimmed so as not to interfere with the light modifications. Prior to human testing I verified that no significant heating took place after a prolonged filtered illumination at close range of the surfaces enlightened. In particular, no increase in temperature was recorded by a surface thermometer after a 1-hour-long exposure to the filter light at a 3cm distance. The physical explanation to this relies on the fact that the violet-blue filtering excludes most IR emissions, which are directly related to most of the heating. In order to make it employable during actual surgeries, visualization was yet to be optimized, though. I therefore set up some kind of yellow high-pass filter for the dominant violet-blue hue to be definitely excluded. Unfortunately, I immediately understood that I could not employ the original yellow filter provided in the same kit for fluorescence detection. The provided high-contrast, high-absorbance, yellow filters are clearly useful in diagnostic, all-or-nothing situations (e.g. as for the CSF fistulas detection) but limit the operability during the surgical interventions. On the contrary, I thought that photographic yellow filters, could present a good compromise between light transmittance and fluorescence contrast. Hence, for microscopic and endoscopic view I used commercial photographic filters customized to fit on top of the microscope oculars or the endoscope light patch (XSOURCE[®]), whereas for macroscopic view I employed commercial UV yellow glasses (ORAO[®]). All filters could be easily switched off or removed from the light paths during the operations. In accordance with the Clinical Engineering Service of our Hospital, I specifically aimed at providing a method that could allow neurosurgeons to perform their fluorescence-guided surgery without the need of structural modifications on the microscope. As a matter of fact, in many countries it is illegal to modify a certified OR product and industries may even withdraw their warranty and maintenance services. The fact that all the instrumentation for fluorescence excitation had already been certified for OR employment, easily allowed its immediate clinical use. All the previously reported apparatuses in the literature implied the use of non-commercial, custom

fluorescence excitation devices, potentially limiting the diffusion of this technology out of experimental trial settings. Custom blue filtering, too, has been described but I ruled it out for major heating and legal issues.

As for the economical aspect concerning my apparatus, the endoscopic fluorescence filter was already widespread in neurosurgical centers and, as a reference, in our region the cost of the commercial complete system employed was less than 1,000 euros. Both photographic filters and colored glasses were purchased for less than 50 euros. This accounted for a price ratio of at least 1:20 to 1:200 depending on the microscope technology already available. Moreover, the use of fluorescein implied a reduction in cost of the fluorophore drug by 1:20 if compared to ICG and by 1:200 if compared to 5-ALA. Even if cost issues are often cause for concern worldwide, many high-volume institutions have rapid access to new technologies. Low volume settings or, more relevantly, the developing countries may never have access to these costly technologies.

Given the above, as soon as I was able to acknowledge the usefulness of this apparatus during the first glioma surgeries, I published the technique in *World Neurosurgery* for everybody to replicate. My purpose was to demonstrate that it was possible to achieve intraoperative fluorescence at a low cost with an inexpensive drug. Unfortunately, not every feedback has been enthusiastic. On the basis of my results I polemically took part in a debate on the use of Fluorescein on *Acta Neurochirurgica*. In particular I argued with Dr. Stummer (in a conflict of interest with the pharmaceutical corporation producing 5-ALA, as he himself has declared), who had labeled Fluorescein as “poor man's fluorescence”. In this regard, I report here an extract of a “Letter to the editor” I wrote to justify a low-cost approach to the use of fluorescein in Neurosurgery:

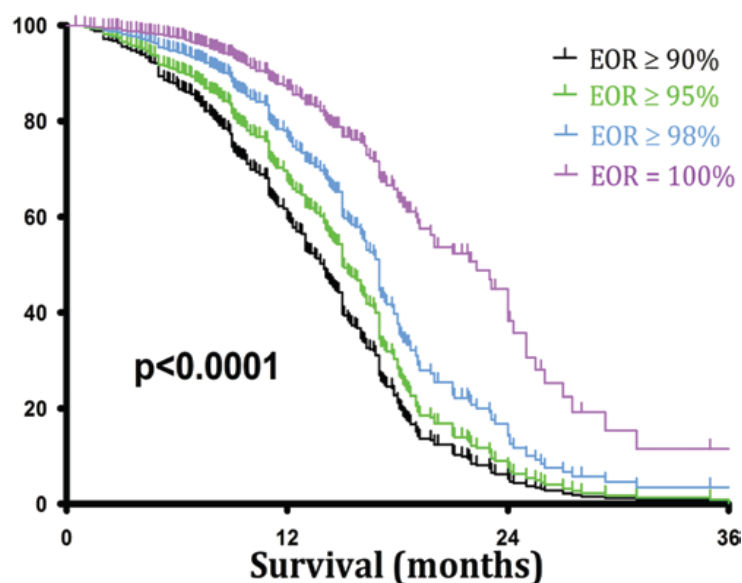
“Cost issues in fluorescence-guided surgery are, indeed, a major problem in many parts of the world where the alternative may be not between Fluorescein or 5-ALA, but between Fluorescein and nothing. In comparing different fluorescence treatments Dr. Stummer

stated: "If I were a patient with a brain tumor, I would want something that is better or equally good". Since we have reasons to believe that Fluorescein is at least better than nothing, we hope that our research will be proudly labeled as the poor man's fluorescence method."

3- High grade tumor surgery

3.1 – High Grade Gliomas

High-grade gliomas account for the vast majority of malignant brain tumors. Lesions are rapidly progressive and invasive with a poor prognosis even with optimal surgery and chemo-radiotherapy (Stupp). The gross total resection of the lesions (GTR) is possible only in a small percentage of cases (30%) because of the difficulty in identifying their margins. The radicality of the removal correlates with an increase in progression-free survival of patients, in particular the small difference between a 98% extent of resection and its totality has been reported as the most relevant in terms of gain of overall survival (Sanai).



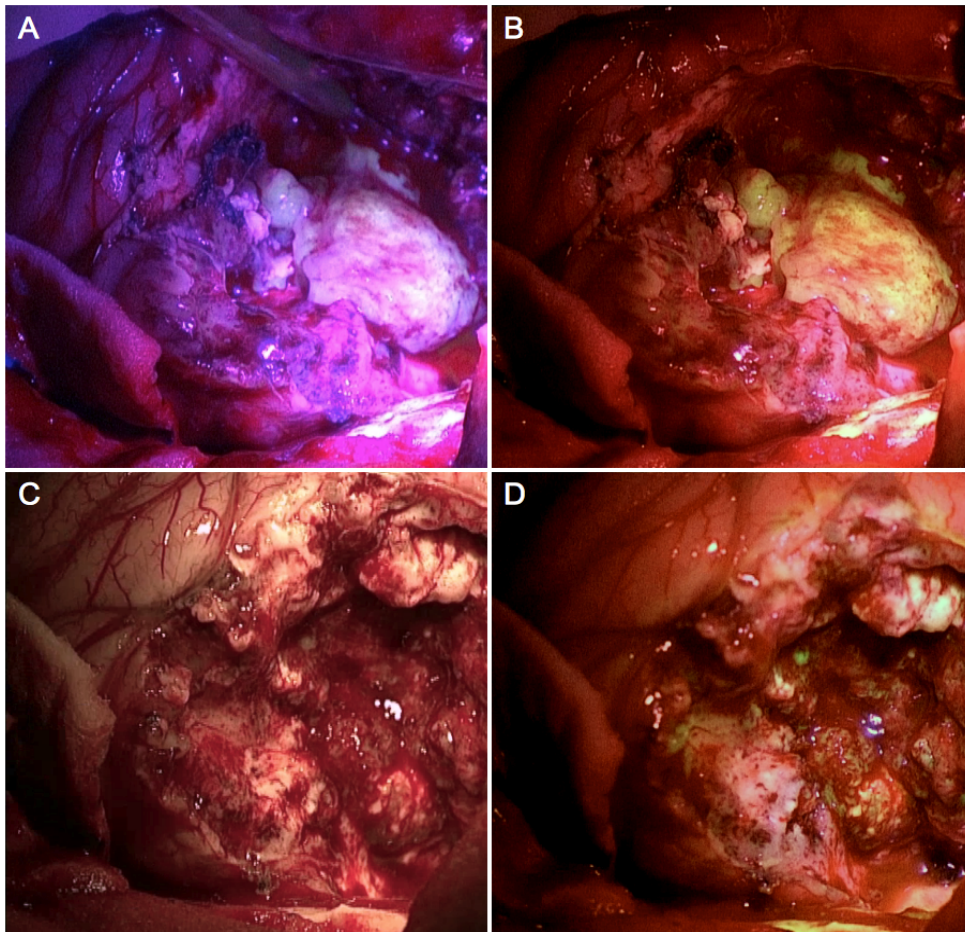
Kaplan-Mayer plotting of Overall Survival of high grade glioma patients based on Extent of Resection (EOR) (from Sanai)

In order to increase the ability to identify tumor margins, the use of some fluorophores combined with dedicated systems of optical filters has been investigated (Behbahaninia).

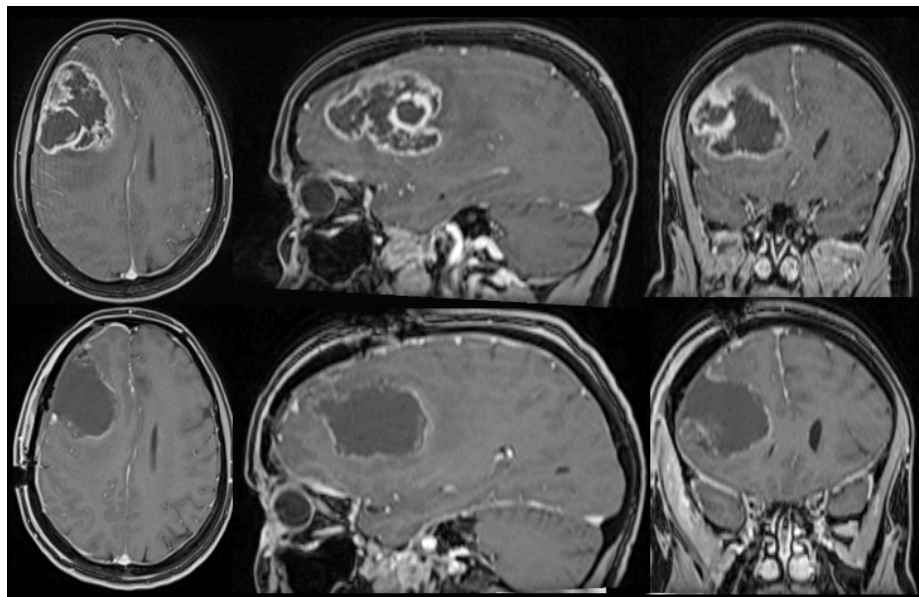
Two fluorophores in particular have been employed with the aforementioned specifications: several papers reported the advantages of the use of both 5-ALA and Fluorescein (Stummer, Acerbi). We were particularly interested in Fluorescein sodium, since it has been demonstrated to accumulate at the level of zones of breakage of the blood-brain barrier, such as in areas infiltrated by malignant gliomas, similarly to Gadolinium in contrast-enhanced MRIs (Diaz).

Given the above, I designed an ongoing Phase II, monocentric, 1-arm Clinical Trial called: "Glioma lesions outlining with optics modification assistance (GLOWOMA): a phase II trial evaluating fluorescein-aided glioblastoma surgery" (EudraCT number 2015-003402-16). The study began in January 2016 and its aim is to evaluate the effectiveness of fluorescein combined with optics modification as an actual aid for the surgeon to achieve gross-total removal (GTR) of Glioblastomas. Inclusion criteria are: adult patients with suspected high-grade gliomas amenable to GTR on pre-operative contrast-enhanced MRI. Exclusion criteria are: known contrast medium reaction, renal failure from moderate to severe, pregnancy. Specifically, a single bolus up to 10 mg/Kg of 20% sodium fluorescein solution is administered e.v. after the induction of anesthesia. Then the intervention proceeds with the aid of optic modifications in order to detect the fluorescein in the surgical field. A contrast-enhanced radiological exam (CT or MRI) is obtained in the 72 h post-op. If the diagnosis of GBM is confirmed, the patient undergoes a state-of-the-art Chemo-Radiotherapy treatment. Neuro-radio-oncological evaluations are scheduled at 1 week, 1, 3, 6, 9 and 12 months. In our series, we have been so far able to detect fluorescence in all of the cases. The dura was vividly green already from the beginning and we did not notice any dye attenuation throughout the intervention. We were under the impression, later confirmed by the use of neuronavigation, that the fluorescent staining takes place precisely in the same areas that are enhanced by gadolinium in the MRI scans, whereas necrotic areas do not retain the dye except for some degree of greenish staining of the intra-cystic fluids and necrotic melted

tissues. In our opinion, this has two major advantages. The first is represented by an easier identification of the frankly contrast-enhancing nodules, somewhat speeding the resection procedures. The second advantage lies in the last phases of the intervention when, in our experience, the fluorescein staining allows the contrast-enhancing boundaries of glioblastomas to be better identified. To date, gross total removal was achieved in all of the patients and no fluorescein-related side effects were to be noticed, even during awake surgery. Notably, the surgical time seems to be dramatically reduced given the high degree of certainty in distinguishing the pathological tissue from the normal brain.



Intraoperative video captions. Blue light, nonfiltered snapshot (A) and the same vision with added yellow detection filtering of a vividly enhancing glioblastoma multiforme nodule (B). White light, non-filtered snapshot (C) and the same vision under blue light fluorescence excitation and yellow detection filtering of the tumor margins. Spots of residual fluorescence are detectable (D).



Illustrative magnetic resonance imaging (MRI) case. Top, multiplanar volumetric contrast-enhanced MRI reconstructions of a pathologically demonstrated glioblastoma multiforme (same case of the video captions). Bottom, two-day postoperative contrast-enhanced control MRI.

4- Vascular surgery

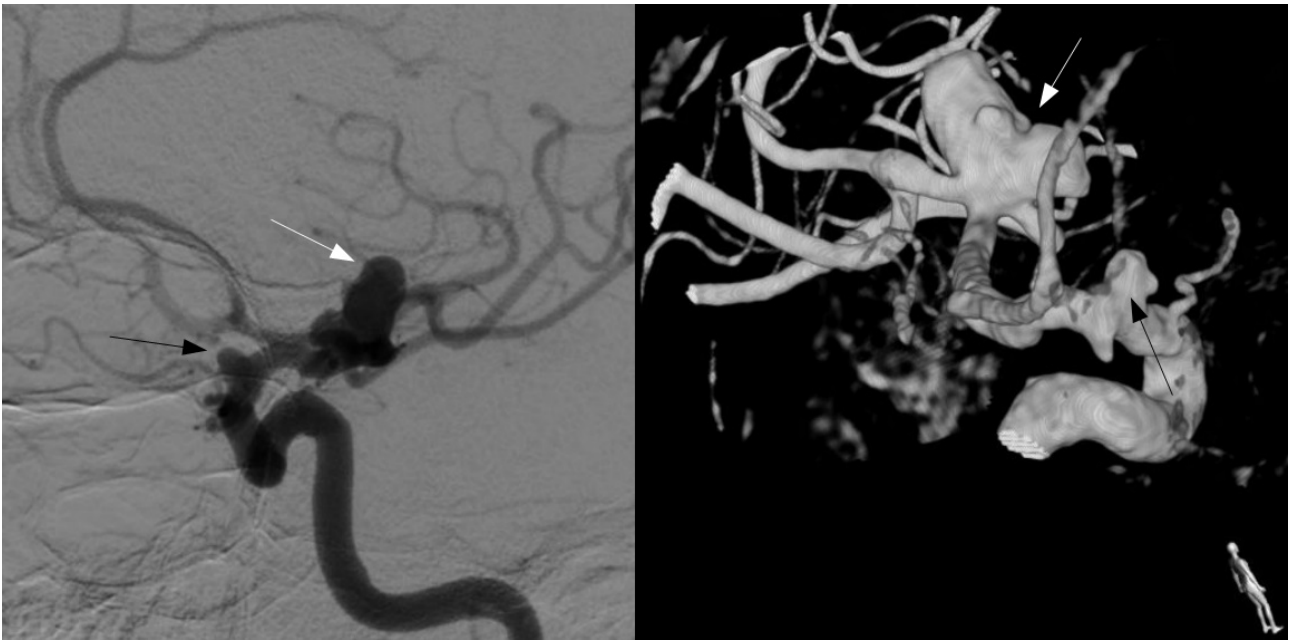
4.1 – Aneurysm surgery

The main goal of cerebral aneurysm surgery is to entirely exclude the pathological part of the vessels while preserving all of the needed blood flow in the main trunks and perforators. Unfortunately, our human visual evaluation of the actual occlusion or patency of the vessels is sometimes difficult or misleading thus implying potential aneurysm remnants or ischemic deficits. In order to overcome these issues, several techniques have been proposed through the years. In order to estimate a potential occlusion, we could monitor motor-evoked potentials (MEPs) or directly measure the flow by means of micro probes as the ones proposed by Charbel et al. In order to effectively evaluate the exclusion of the aneurysmal sac the only available solution is by visualizing its content, i.e. by staining its blood flow. The most reliable diagnostic tool, in this regard, is the use of intraoperative angiography, but it is not widely available due to its cost and the impracticality of its use in emergency settings. In order to obviate this problem, surrogates of an intraoperative angiography have been developed by employing fluorophores. As reported before, ICG has been the first fluorophore to be proposed for this specific use but in the last few years some reports have been published also on the use of fluorescein. Even though with some differences, both fluorophores vividly color blood flow. ICG has the advantage of the possibility of repeated administrations but lacks the direct real-time visualization and depth of vision. Fluorescein can be used instead under direct visualization and may be implemented to endoscopes to reach deep and even blind spots with angled optics, although its repeated administration may lead to bewildering results. As a matter of fact, fluorescein tends to stain vessel walls after repeated administrations, thus leading to potential misjudgments in aneurysm filling. Nevertheless, its better visualization potential looks promising, especially the fluorescein-

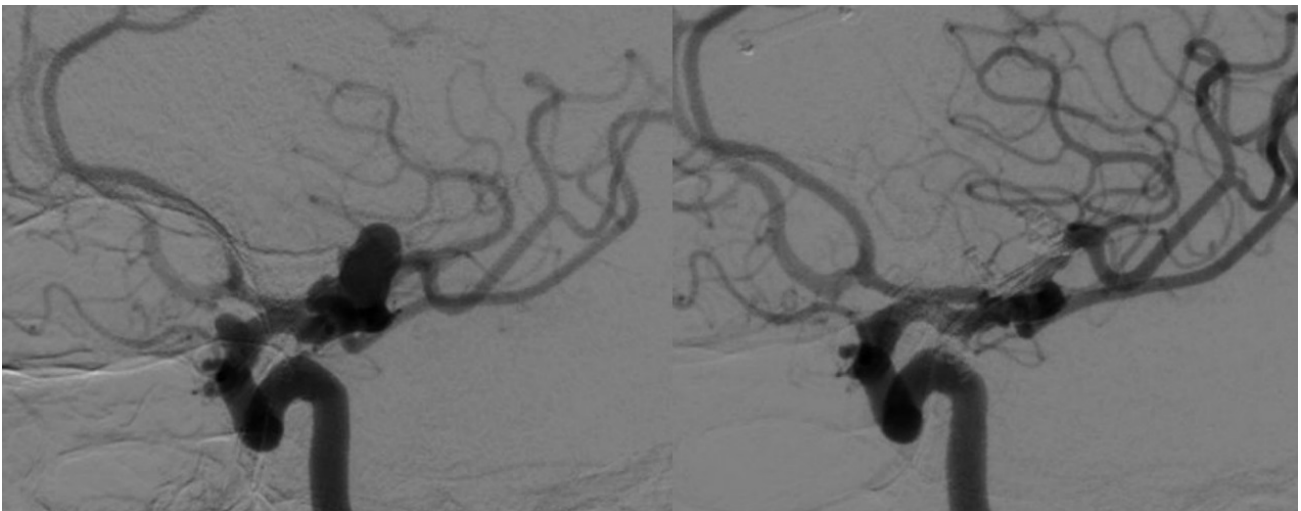
aided endoscopic evaluation of the deep perforators. Starting from these results, I will try to present the usefulness of fluorescein during intracranial aneurysm interventions by reporting here a case of a female patient with multiple intracranial aneurysms of the Internal Carotid Artery (ICA) and of the Middle Cerebral Artery (MCA) that we have recently treated. She had a large aneurysm of serious concern, with a maximum diameter of 15mm, at the MCA bifurcation with a wide neck and an irregular dome with a distal bleb. Three other small dilations were present at the level of the ICA: one, in particular, was pointing toward the homolateral optic nerve. At the neurovascular preoperative meeting with the Interventional Neuroradiologists, no endovascular indication was posed for the three little ICA aneurysms, nor for the large MCA one because of its wide neck and the complex architecture of the dome. We then treated the patient via a pterional left approach, exposing all the Sylvian fissure from ICA through the aneurysm. After the exposure of the aneurysm was carefully performed, it was evident that the bifurcation was fully involved in the wide neck of the aneurysm. We then carried on the intervention with the exclusion of the sac through the vessel reconstruction by the use of multiple "stacked" clips. In our opinion, a single, large clip would have actually occluded too much the emerging vessel to the distal branches of the MCA, thus causing potential ischemia. The drawback of such technique is that the sheer closing force of several small clips may be inferior to a large one and potential residual flow could enter the sac. After posing another mini-clip in order to exclude the little aneurysm pointing towards the optic nerve, we decided to perform a fluorescein test of the patency of the vessels. We then injected a bolus of 5 mg/Kg of Fluorescein and after about 10 seconds it became apparent that a little flow was still present at the level of the dome after the initial clipping in the large aneurysm, while a complete exclusion of the little one had already been achieved. Curiously enough, the dome of the large aneurysm became more and more vividly fluorescent as the flow continued to slow down. We interpreted this as a sign of fluorescein permeation of the vessel wall only where the flow was heavily diminished but still present,

thus giving the possibility to the fluorescein to stain the vessel interstitial space, whereas in the patent intracranial vessel an effective wash out by the circulating flow prevented this phenomenon. In the postoperative course, the patient experienced transient blurriness of speech but recovered well with anti-vasospasm therapy and was dismissed home with no neurological deficits.

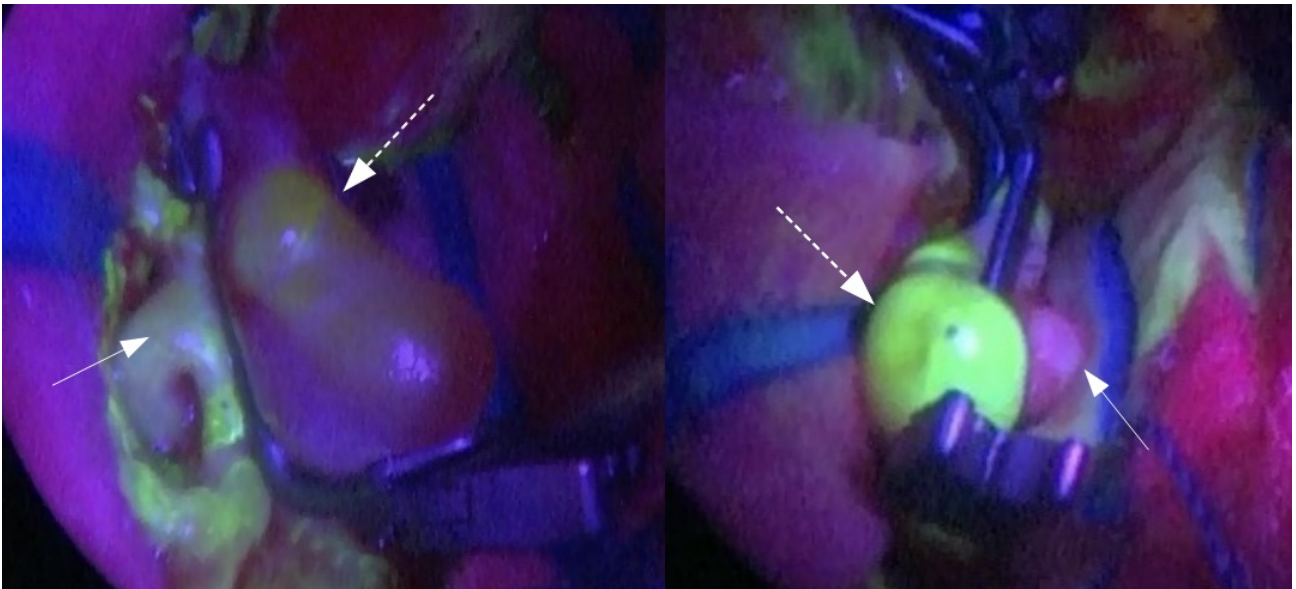
In our opinion, fluorescein is an intuitive, inexpensive way to assess the correct exclusion of aneurysmatic sacs, particularly in cases where complex clip reconstructions are needed. Moreover, its implementation to endoscopes may be a definitive advantage in visualizing deeply located perforators or visualize “blind” spots of the angioarchitecture of complex aneurysms.



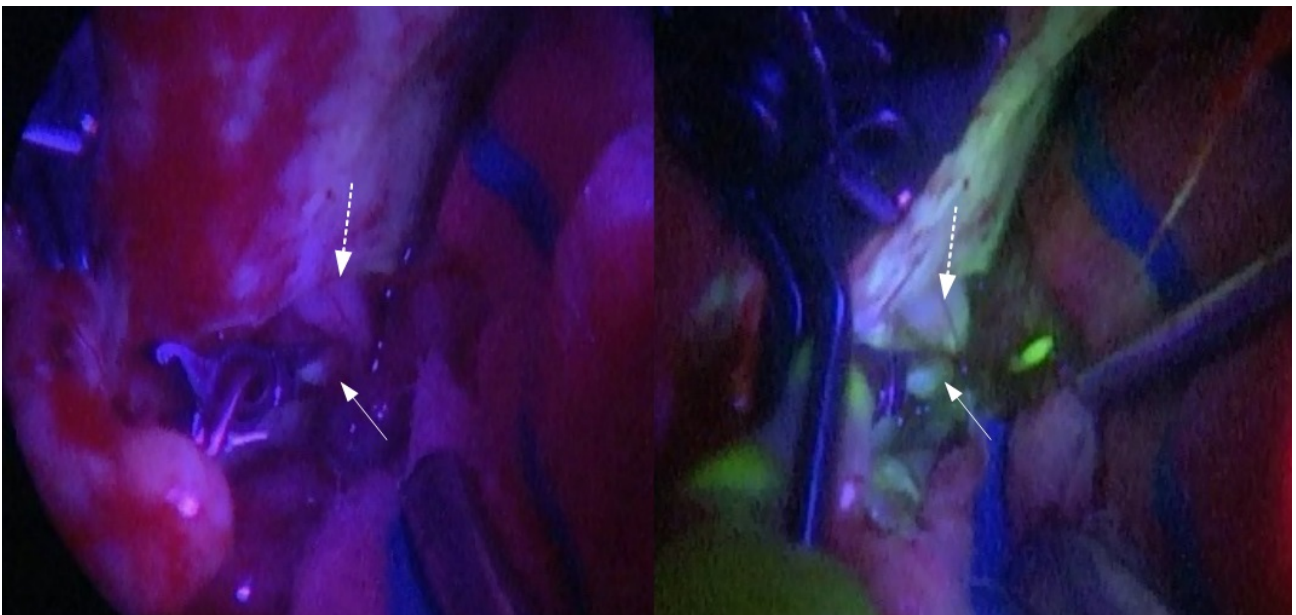
Lateral projection and 3D rendering of an angiography of a patient with multiple small intracranial aneurysms. One of the Internal Carotid Artery (ICA) was pointing the optic nerve (black arrow) while a large aneurysm, with a maximum diameter of 15mm, was present at the MCA bifurcation with a wide neck and an irregular dome with a distal bleb.



Pre- (left) and post- op (right) angiography demonstrating the complete occlusion of both the aneurysms treated.



Intraoperative visualization under fluorescein filters of the M1 segment of MCA (full line) and the aneurysmatic sac (dotted line). Note the early phase fluorescein staining (left) and the interstitial vessel staining in the latter phases after exclusion of the dome of the aneurysm (right)



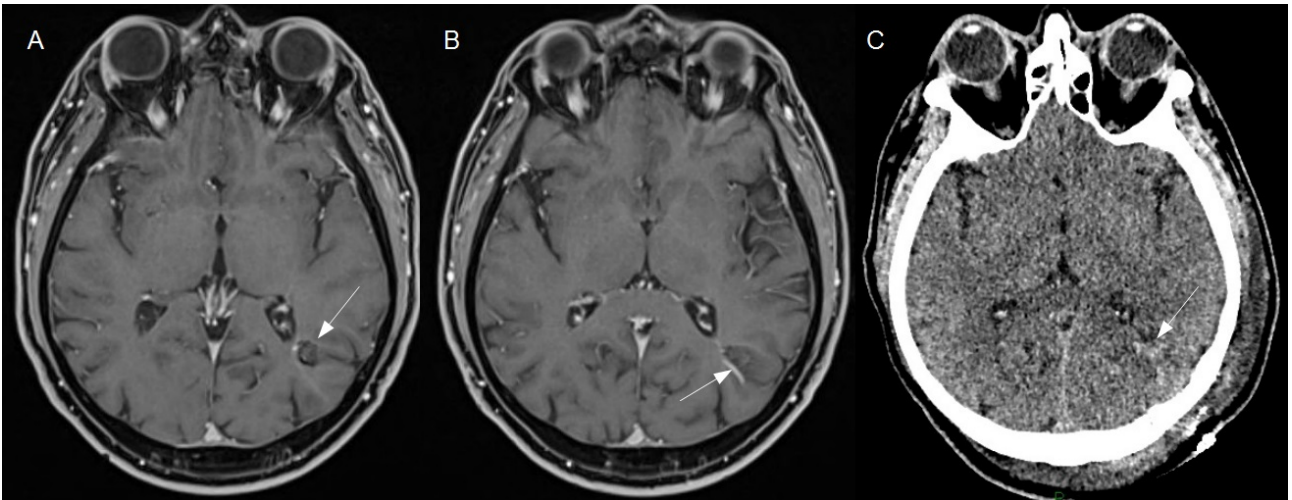
Exclusion with a miniclip of a small ICA aneurysm (full line) pointing the optic nerve (dotted line). No staining occurred both in early (left) and late (right) phases.

4.2 – Cavernous Angiomas

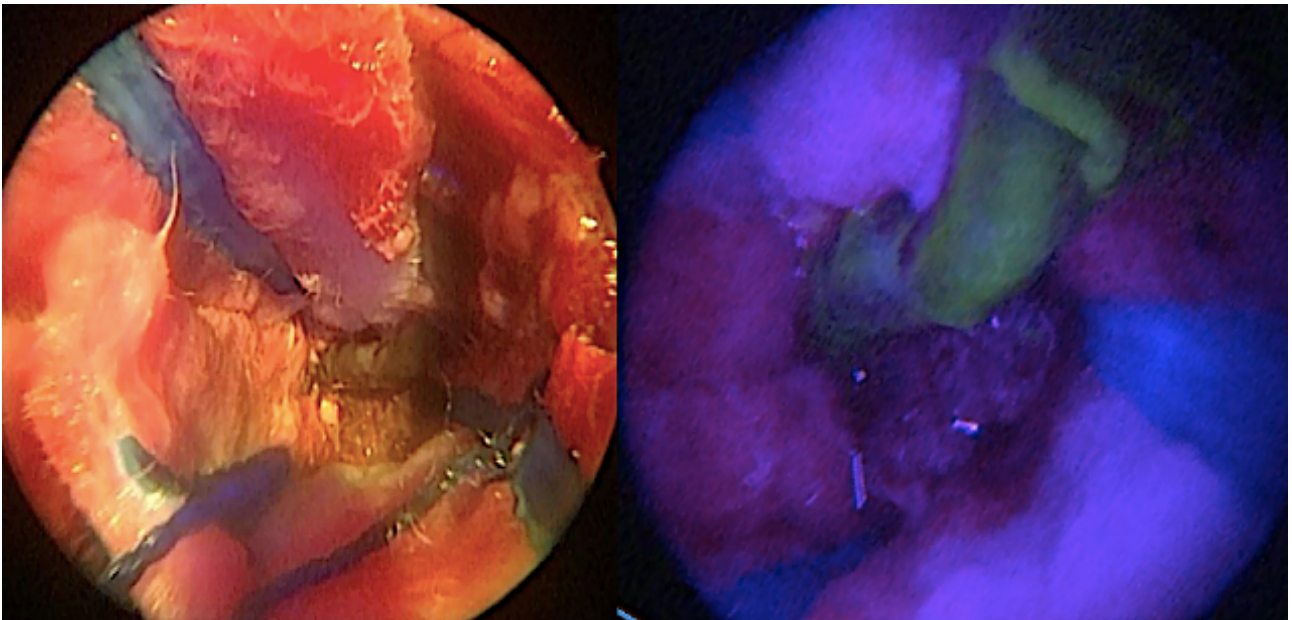
Cavernous angiomas are vascular abnormalities of the central nervous system with an incidence of 0.4-0.5% and an annual rate of hemorrhage ranging from 0.7% to 1%. Up to 18.7% of these patients suffer multiple lesions, many of which are linked to some genetic alterations. They are histologically characterized by large, adjacent capillaries with little or no intervening brain. The blood flowing through these lesions is therefore slow, thus explaining their “occult” manifestation after contrast medium injection in both MRI and angiographic studies. They are often accompanied by another kind of malformation: developmental venous anomaly (DVA). In particular almost 20% (range 8-33%) of cases of DVA are associated with cavernous malformations. A DVA is histologically a congenital malformation of veins draining normal brain and is characterized by the presence of one or multiple veins draining into a single larger collecting vein, which in turn usually drains into a dural sinus. The exact etiology of DVAs remains uncertain but it may be related to arrested development of venous structures. They are vividly enhanced in contrast sequences of both MRI and angiographic studies. These lesions may be silent as incidental findings or have a typical presentation with headache, seizures and focal neurologic deficits in case of bleeding. Surgery is an effective modality of treatment but, to date, a consensus on indications remains controversial. Almost everybody agrees on the fact that the most important, consistent risk factor for re-bleeding is a previous hemorrhage. Therefore, it is often recommended to perform surgery after a few days or weeks from the hemorrhage, provided there is no life-threatening mass effect. During the intervention, especially when in an acute setting after bleeding, it may be difficult to distinguish the actual cavernoma from the DVA. If this is the case, there may be an excessive coagulation of the vascular structures during the excision procedures, thus limiting the venous outflow of the DVA and exposing the patients to a risk of complication. Here we report our experience in the use of fluorescein

in cavernoma surgery in which we could clearly distinguish the DVA from the cavernoma in real-time vision. As already reported, Fluorescein enhancing pattern resembled the one of Gadolinium, vividly staining the DVA and sparing the cavernoma. Our interpretation of this event is that the capillary flow is so slow in the cavernoma that the wash-out effect of the surrounding vessels, including the DVA, prevents the fluorescein from reaching a sufficient amount of concentration during the bolus administration into the capillaries of the cavernoma.

We envision four potential advantages with this technique: 1- avoid coagulating unwanted structures, 2- help the arachnoidal dissection through sulci with a fluorescent guide, 3- check the complete excision of the cavernoma at the end of the procedure, 4- allow a deep visualization of the vascular structures and relative flow, which is not possible with ICG due to its limitations in the depth of the surgical field.



Para-trigonal cavernoma. Gd-MRI hypointense lesion with minor signs of previous bleeding (A), Gd-MRI hyperintense associated DVA venous outflow (B), post-operative CT scan showing excision of the lesion with no ischemic events.



Unfiltered (left) and filtered (right) intraoperative endoscopic vision of a cavernoma and its related DVA. Fluorescein enhancing pattern resembled the one of Gadolinium, vividly staining the DVA and sparing the cavernoma

4.3 – Hereditary hemorrhagic telangiectasia

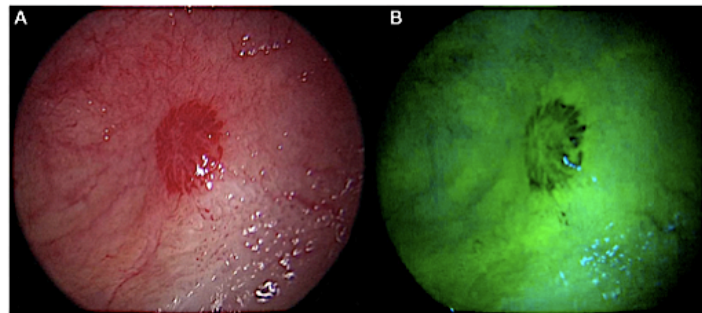
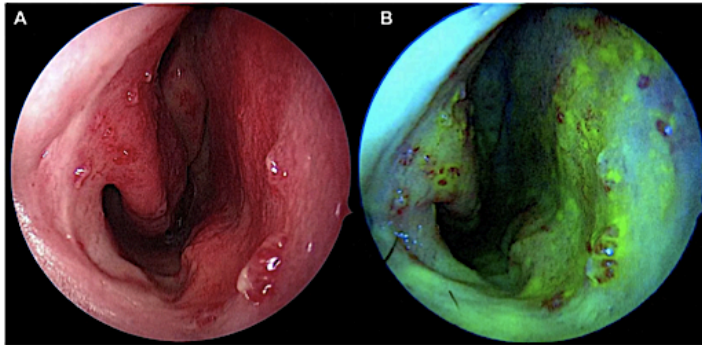
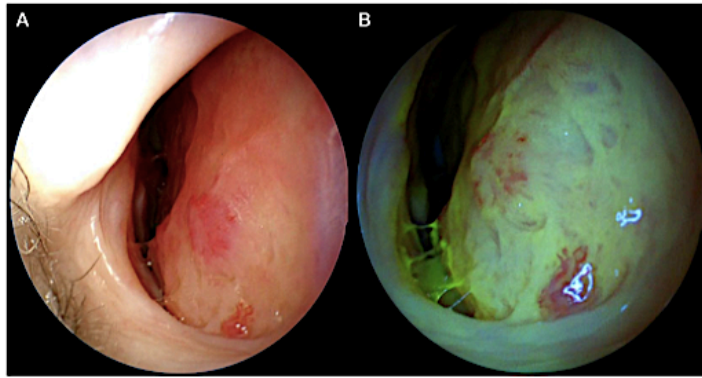
Hereditary hemorrhagic telangiectasia (HHT; Osler-Weber-Rendu disease) is a rare genetic disease with a prevalence of 1:5000 that leads to a systemic angiodyplasia. Inherited as an autosomal dominant trait, HHT results in abnormal vascular structures and commonly manifests itself with mucocutaneous telangiectasias and arteriovenous visceral malformations (AVMs). HHT is known to be related to cerebral, often multiple, AVMs, and genetically modified HHT animal models have been employed for preclinical AVM studies, since the angioarchitecture of HHT-related AVMs is indistinguishable from sporadic AVMs. Nasal telangiectasias lead to recurrent epistaxes, which affect up to 96% of patients. The complete identification of every telangiectasia in the nasal mucosa in HHT patients can be challenging, but this is a key point to avoid overlooking lesions that can be properly treated at the time of surgery. Narrow-band imaging (NBI), also known for enhancing the detail of certain aspects of the tissues in head-and-neck cancers, has recently been proposed for the morphologic study of nasal telangiectasias in HHT patients. For this reason, with the aim of enhancing the visualization of nasal vascular structures, we have developed a new method of intraoperative endoscopy based on the intravenous administration of fluorescein in collaboration with the ENT Department. Our results have been published in International Forum of Allergy and Rhinology. Briefly, Fluorescein-guided intraoperative endoscopy of the nasal mucosa in HHT patients was conducted during surgery. We selected only HHT patients who had not undergone previous treatment for their epistaxis. After informed consent, 5 to 10 mg/kg of sodium fluorescein at 20% was administered intravenously at the induction of anesthesia. We employed our endoscopic apparatus for the detection of fluorescence. We show here three series of pictures illustrating the fluorescein-guided endoscopic examinations and an argon plasma surgical treatment under fluorescein view. The length of time between fluorescein injection and dye visualization in the nasal mucosa

ranged between 10 and 20 seconds. About 60 to 90 seconds after injection, nasal telangiectasias were viewed as dark spots on a yellow background. This was the optimal time to proceed with surgery, because the maximal contrast between the telangiectasias and the surrounding mucosa has been reached at this time and kept throughout surgery. No adverse events or complications were reported.

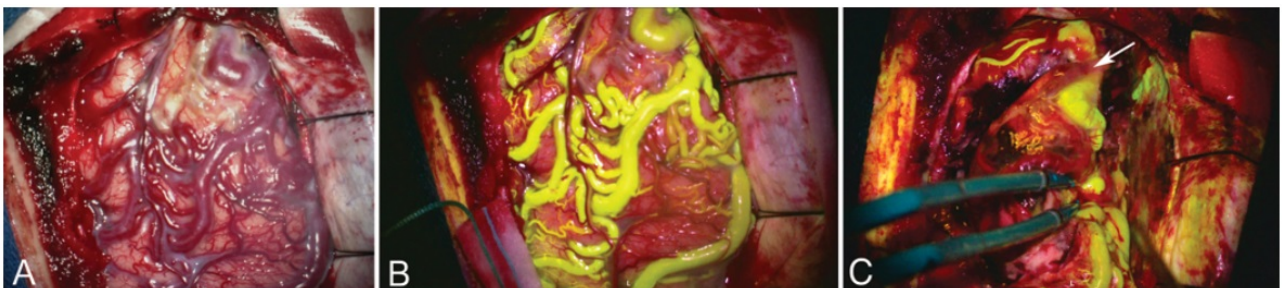
To the best of our knowledge, this technique has never been described in an ear-nose-throat (ENT) surgery and, in particular, has never been applied in HHT. In our experience, this technique has been demonstrated to be effective and safe: with the aid of filters nasal mucosa looked to be yellow and the telangiectasias were enhanced and negative, since they looked to be violet-blue (probably due to a rapid washout of the fluorescein in telangiectasias). Fluorescein-guided endoscopy provided a good definition of these lesions, with good contrast. In particular, small telangiectasias were more easily recognized and large lesions were more accurately identified, especially in the nasal valve, where they cannot always be easily recognized from the surrounding mucosa. Another potential advantage of this system may be its versatility: it can be applied in many practical operating situations, even in HHT epistaxis surgery, because blood is still red during visualization and the surrounding sino-nasal structures can be easily identified in real time. The lack of versatility during surgery was a limit of the previously described NBI endoscopy: even a small amount of blood could actually obscure the NBI visualization.

Even though we have not tested this method on intracranial AVMs, on the basis of these analogies we could argue that this visualization system is useful for the identification of the pathological vessels from the surrounding parenchyma. In this regard, Lane et al already published their preliminary results with the use of Fluorescein in cerebral AVM under microscope-dedicated filters (Lane). Two advantages may come from the use of Fluorescein-staining blood flow: 1- the possibility (contrary to ICG) of a real-time visualization of the flow into the AVM, thus better highlighting the borders of the actual lesion

from the surrounding parenchyma; 2- the realization of the different velocities of blood flow through the arteriovenous malformation, thus helping the surgeon identify the arterial feeders to be addressed before the venous components.



White-light (A) and fluorescein filtered (B) nasal endoscopy. Telangiectasias on the nasal septum enhanced as dark spots



White-light (A) and fluorescein filtered (B) AVM surgery. White arrow: flow is abolished in excluded feeders (C) (from Lane et al.)

5- Endoscopic surgery

5.1 – Pituitary adenomas

Pituitary adenomas are the third most common intracranial neoplasm although roughly only 10% are symptomatic (Ezzat). Apart from prolactin-secreting tumors, resection is the elective initial treatment, with good results in terms of clinical and endocrinological improvements and low rates of morbidity and mortality (Tabaee). Despite many surgical advances, such as the endoscope and neuronavigation, complete tumor removal is achieved only in about 80% of the patients and regrowth after an incomplete tumor removal may occur in up to 75% of cases (Tabaee, Cappabianca). Failure of surgery in macroadenomas is mainly due to the difficulty in visualizing the pathological tissue invading adjacent structures such as the cavernous sinus or the suprasellar region. On the other hand, the preoperative planning and localization for microadenoma lesions could be challenging, especially in those cases marred by a poor detection of the lesion even in magnetic resonance imaging.

Therefore, there is still need for new tools that could help the localization of pathological tissues, so as to improve their selective removal and preservation of pituitary functions. Intraoperative MRI has been proposed in order to decrease the rate of incomplete resections but it is an expensive and time-consuming technique (Paterno). Since some fluorescent agents such as 5-ALA, ICG and fluorescein are gradually entering the neurosurgical practice as intraoperative contrast agents for cerebral neoplasm (Ewelt), some reports have been published about the use of fluorophores in pituitary surgery in the recent years (Eljamel, Litwack, Sandow, Da Silva).

5-ALA

We have retrieved only one clinical experience in literature about the use of 5-ALA in pituitary adenomas. Eljamel and colleagues evaluated the use of intraoperative ALA-fluorescence to identify pituitary adenoma with two different devices: an endoscopic photodiagnosis system and a laser-based probe for intraoperative spectrometry. The sensitivity and specificity of fluorescence endoscopy with 5-ALA were of 80.8 and 75% respectively, whereas they were of 95.5 and 100%, with intraoperative spectrometry. These seemed to be promising results, though there were some limitations to this study and its applications. Firstly, no histological stratification of the results was reported, which could have shed light on the fluorescence pattern of the different pituitary adenomas. Secondly, the fluorescence visualization was possible only in poor light conditions, a limit already reported for glioma surgery (Acerbi). Another important issue is that it is not clear why 5-ALA should be more metabolized in adenomas. Interestingly, other two recent publications have investigated *in vitro* the use of 5-ALA fluorescence in pituitary adenoma cell for photodynamic therapy (Neumann, Nemes).

ICG

ICG has been used in pituitary surgery with different purposes. The first is intuitively the real-time observation of sellar vessels, whether arterious (ICAs, optic nerve vasa nervorum) or venous (cavernous sinuses) as reported by Hide and colleagues. The second application was to try and distinguish the normal pituitary parenchyma from adenomas via their different patterns of vascularization as revealed by ICG. The healthy pituitary gland is rapidly perfused, posteriorly to anteriorly, due to the lack of blood brain barrier, whereas pituitary adenomas are usually more gradually enhanced (Miki, Sakamoto, Tien, Finelli, Yuh). This may be due to the fact that pituitary adenomas show lower vascular densities compared to the non-tumorous adenohiphysis, even though with interhistological variability (Jugensburg, Turner, Viacava). 12 patients in total were evaluated with an endoscopic

approach (Litvack) and 22 with a microsurgical one (Sandow). Generally, the Authors deem their techniques to be useful for the identification of the different structures. In particular, the most useful phase for the realization of the different structures was reported to be the flash-filling phase prior the recirculation of the dye. Despite the same dosage (25mg), Litvack et al always reported a hypofluorescence of adenomas as compared to normal pituitary gland tissue, irrespective of their histology. Moreover, Sandow et al reported both 100% of hyperfluorescence of ACTH-secreting adenomas (6 on 6) and a hypofluorescence in GH-secreting adenomas for 9 cases vs 4 hyperfluorescence cases. Litvack et al. have also reported a nodular hypervascularity of the sellar dura in all cases of prolactinoma and acromegaly (n = 4). These discrepancies are difficult to interpret: some differences may be ascribed to the different visualization systems. As a matter of fact, Sandow et al acknowledge that their microscopic results showed a lower signal intensity due to the distance of the light source to the surgical field as compared with the endoscopic series (Sandow). Another factor may be the injection rate of the ICG solution. It has been demonstrated that injection rates variation from 5 ml/sec to 1 ml/sec could actually imply around a triple time to peak (45 to 135 sec) in the aortal compartment and a doubling of the time to peak (75 to 150 sec) in the hepatic compartment.

Fluorescein

There is only one report in literature on the use of fluorescein in a pituitary adenoma (da Silva): a bolus of a relatively high-dose (20mg/Kg) of fluorescein was administered with the lesion in vision under simple microscopic white light. This has been reportedly done in order to somehow increase the identification of the adenoma. Unfortunately, no histological specification or relation to the normal pituitary tissue is reported.

On the basis of the use of Fluorescein with the gadolinium-based contrast agents (GBCAs) as already reported in high-grade glioma surgery, we conjectured that fluorescein may help

the detection of pituitary lesions in real time, too. MR imaging of the gadolinium-enhanced pituitary gland is actually the elective diagnostic tool for the identification of adenomas whether in a classical or dynamical sequence acquisition (Lee). Several MRI sequences have revealed significant differences between pituitary tumors and normal gland tissue with respect to time sequences and enhancement patterns. The source of this enhancement, though, is not clear: there are relative contributions of the vascular and interstitial compartments. Several peculiarities of the pituitary tissue are to be taken into account in this regard. Firstly, the pituitary gland resides outside the blood-brain barrier and the contrast media can rapidly equilibrate between the vascular and interstitial spaces. Secondly, there are significant differences in microvascular density between normal anterior pituitary region and different adenoma histological cases. Thirdly, the relative amount of interstitial space varies between different histological cases. Fourthly, the vascular supply of adenomas is mainly arterial via neovascularization instead of a slow, portal vasculature of the normal anterior pituitary region.

Lastly, the dose and rate of administration as well as the state of hydration, age and renal function of the patient may hinder a clear-cut assessment. Generally, the gadolinium enhancement pattern develops as follows: the first enhancement occurs in the posterior lobe, followed by the pituitary stalk and finally there is a centrifugal opacification of the anterior lobe (Yuh). Microadenomas usually appear as relatively non-enhancing lesions with regard to an intensely enhancing pituitary gland. Since the entire gland shows a homogenous enhancement within 30-60 seconds, the maximum image contrast between the normal pituitary parenchyma and microadenomas is during this timeframe, hence the usefulness of early dynamic sequences after bolus administration. The peak enhancement of the pituitary adenomas occurs at 60-200 seconds, usually after the most marked enhancement of the normal pituitary gland, and persists for a longer duration (Sakamoto). Delayed scans, obtained after up to 30-60 minutes after contrast injection, may demonstrate

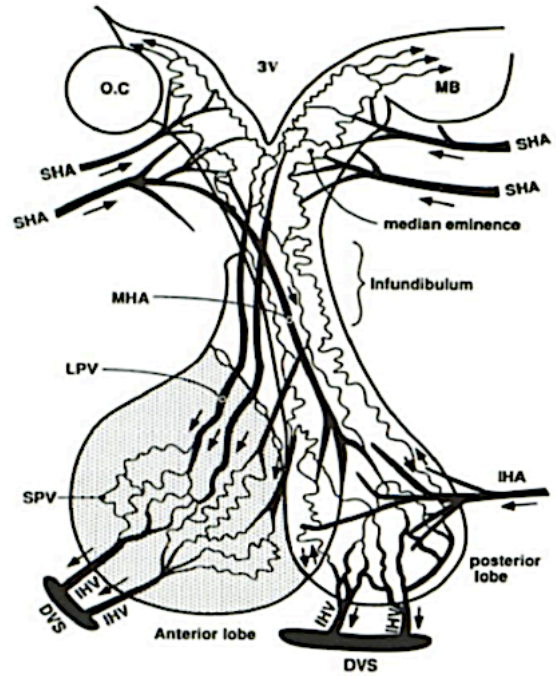
a reversal of the image contrast obtained at 30-60 seconds on dynamic scanning. A possible explanation is that the contrast from the normal pituitary gland may fade but diffuses into the microadenoma, which stands out as a hyperintense spot (Dwyer). This has some analogies with gd-MRI cardiac imaging in which imaging taken too early (e.g. less than 5 minutes after the initial contrast material injection) results in a reduced contrast difference between the infarcted and normal myocardium because insufficient contrast material has been washed out of the normal myocardium (Oshinski). Lastly, Yuh *et al.* have also documented an early enhancement in the microadenomas, way before the anterior lobe, potentially due to the fact that pituitary adenomas have a more direct arterial blood supply (Yuh, Bonneville).

Given all the above we have developed a Research Protocol in order to: 1- evaluate the early and late gd-MRI contrast enhancement patterns of different histological cases of pituitary adenomas; 2- Correlate this pattern with the intraoperative visualization of Fluorescein during endoscopic transnasal surgery with a dedicated detection filtering system. Since the proportion of gland-to-lesion contrast enhancement remains stable with different dosages of Gadolinium at about 26% (Bartynski), we chose to employ approximately half of the dose used for glioma surgery (3mg/Kg) in order to obtain a fluorescence detection more similar to the all-or-nothing setting. Briefly, every patient will undergo the standard-of-care pre-operative routine for patients with pituitary lesions including an endocrinological blood-testing routine and a Gadolinium-enhanced MRI with early (dynamic) and late (30 min) phase acquisitions. A single bolus of Fluorescein solution will be administered e.v. and the association with other drugs will be avoided.

The timing of administration (e.g. 30 minutes prior to dural opening vs. on time when the pituitary lesion is in vision) will be determined on the basis of Gadolinium-enhanced MRI study results. Different adenoma histological cases will be tested in order to try to establish a potential “contrast-enhancement pattern reference scheme” for the most common pituitary

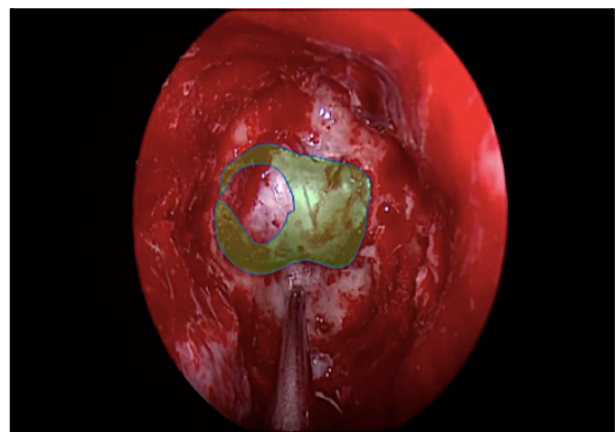
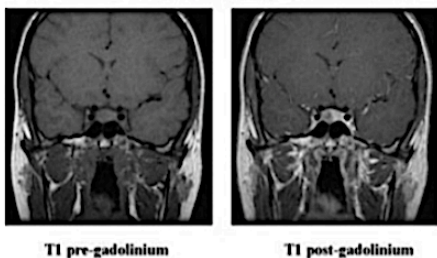
lesions. The actual trial will take place in the next few months, we present here some preliminary data about a case of Cushing Syndrome.

Fig. 6. Illustration of the blood supply and drainage of the pituitary gland. The posterior lobe is supplied predominantly by the inferior-hypophyseal artery (*IHA*) derived from the meningo-hypophyseal trunk of the internal-carotid artery. The hypothalamus is supplied by the superior-hypophyseal arteries (*SHA*), which are derived from the supraclinoid portion of the internal-carotid artery and from branches of the anterior and posterior cerebral arteries. These arteries form a ring around the infundibulum and serve as the arterial blood supply to the median eminence and infundibulum. The main blood supply to the anterior lobe is through the portal venous system. This complex system originates from specialized vascular structures located in the region of the median eminence. This system is composed of short-terminal arterioles surrounded by a dense capillary network. This network drains into the long-portal veins (*LPV*) that run along the surface of the pituitary stalk and terminate in the sinusoidal capillaries of the anterior pituitary. The sinusoidal capillaries are continuous with the deeper short-portal veins (*SPV*). The sequential enhancement after the intravenous injection of a contrast agent should be the posterior lobe first, followed by the hypothalamus-infundibulum, and last the anterior lobe. Both anterior and posterior lobes have direct venous drainage (inferior hypophyseal vein [*IHV*] to the dural venous sinus [*DVS*]). *O.C* indicates optic chiasm; *MB*, mamillary body; *MHA*, medial-hypophyseal artery; and *3V*, third ventricle.

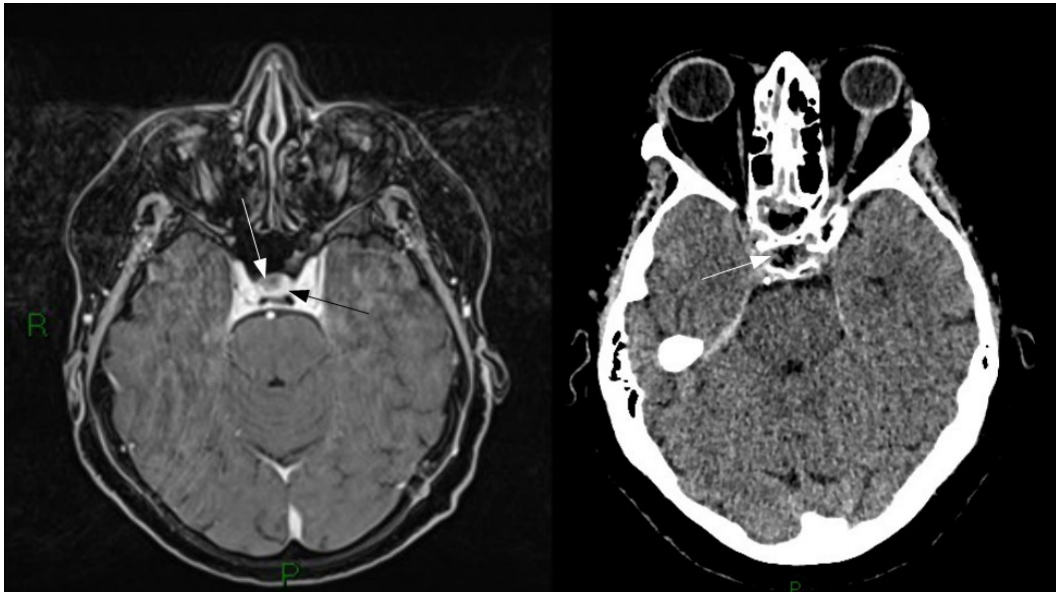


The complex vascular angioarchitecture and interstitial components of the pituitary gland influencing the contrast enhancement patterns (From Yuh)

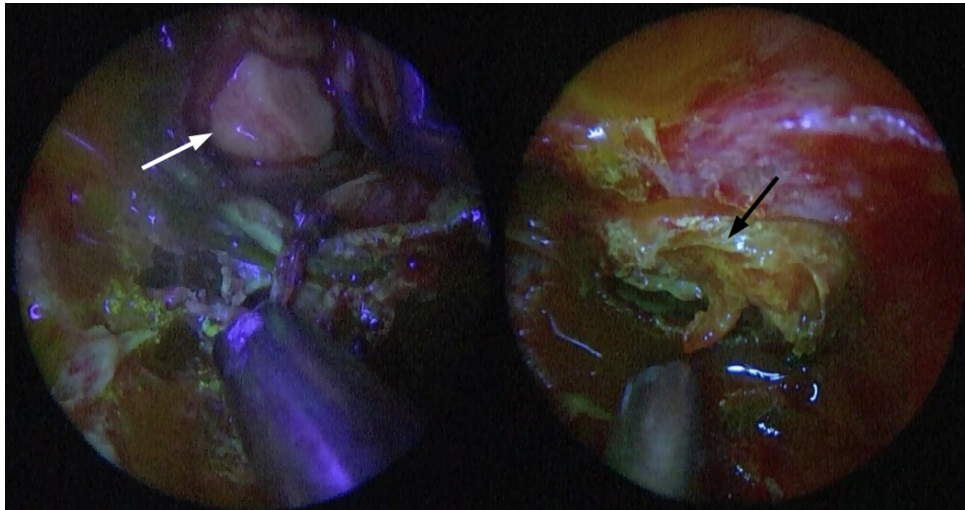
Pituitary Microadenoma MRI findings



Rationale of the use of Fluorescein as a gadolinium analogue in real time visualization



(Left) Pre-operative MRI. A small, non-enhancing lesion is present on the right part of the sellar region (white arrow) surrounded by homogeneously enhancing normal pituitary parenchyma (black arrow). (Right) post-operative CT scan showing the ablation of the lesion (white arrow)



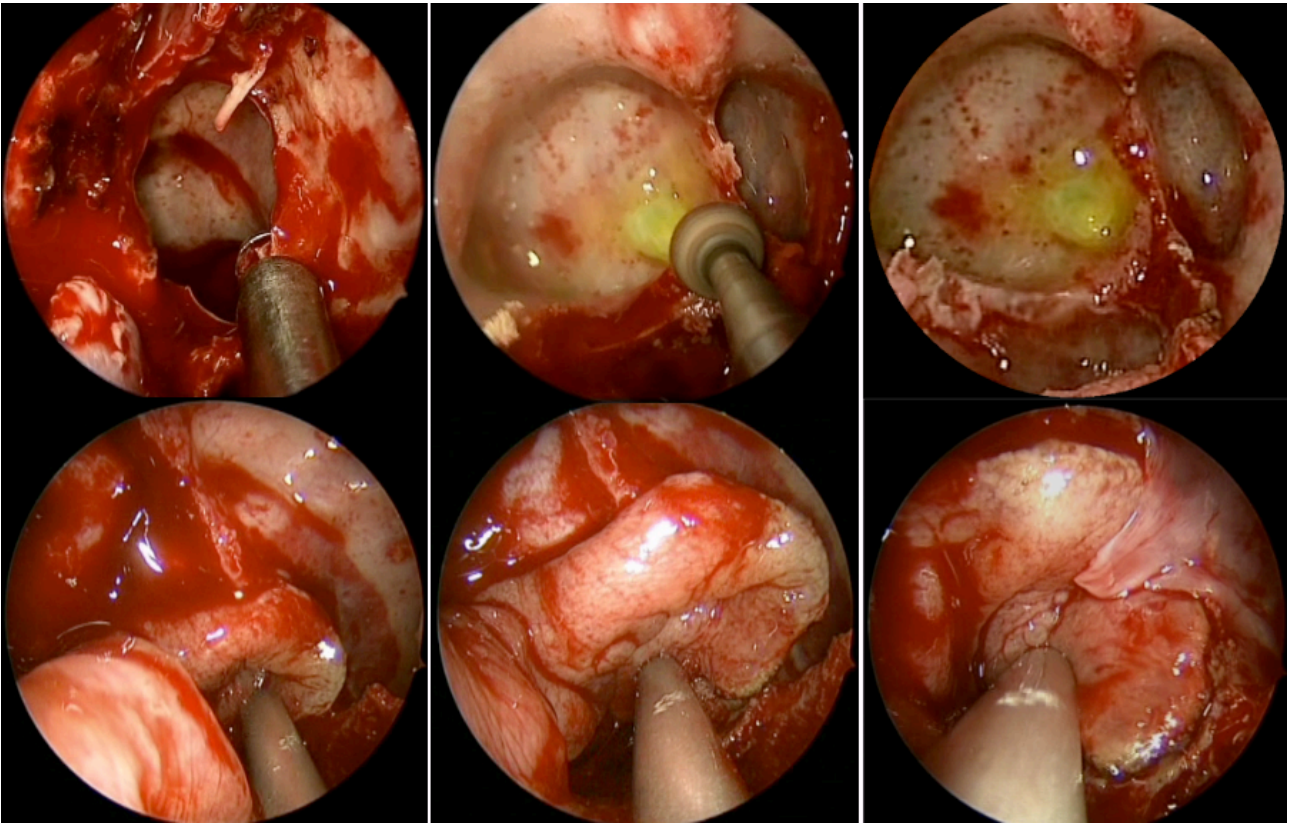
Fuorescein-filtered vision of the sellar region: non-enhancing adenoma (white arrow) and vividly enhancing pituitary parenchyma (black arrow)

5.2 – CSF leak

Cerebrospinal fluid (CSF) leak occurs in case of a dural lesion of the skull base. It may occur spontaneously or after trauma or surgery and, even if it is a relatively rare event, it should be considered a potentially life-threatening situation mainly because of infection issues. In the last decades, an endoscopic transnasal approach has been proven effective given its direct access to the fistula and relatively low invasiveness. Unfortunately, it is often difficult to identify the exact point of leakage since the active leak might be missing during the normal vision, especially in low-flow fistulas. In order to overcome this problem, the use of a fluorescent CSF tracer has been proposed. The first report of the use of Fluorescein to detect a suspected CSF leak was published in 1960, but this technique became widespread in the late '90s after Wolf et al published their extensive case series of endoscopic evaluation of fluorescein tracing CSF fistulas (Kirchner, Wolf). Briefly, fluorescein is administered via lumbar injection and a fluorescent leak may be identified endoscopically with the aid of dedicated filters. After the early dissemination of the technique, there have been several reports of complications mainly related to the intrathecal administration. The main reason for concern was seizures, although at a second analysis, most of the complications turned out to be related to an incorrect dosage. In particular, if the dose of fluorescein is equal or lower than 1 ml in a 5% mixture (a total of 50 mg) no major side effects have been reported in large series. Given the above, its off-label intrathecal use is neither indicated nor prohibited by the United States' Food and Drug Administration and the procedure is widely practiced worldwide.

In dealing with this technique, we employed our custom filtering system in several patients. In our experience, a potential advantage of our custom photographic filters over the all-or-nothing yellow filter from the industries may be in enabling surgeons to have a sufficient identification of the tracer while allowing real-time visualization. This helps to correctly define

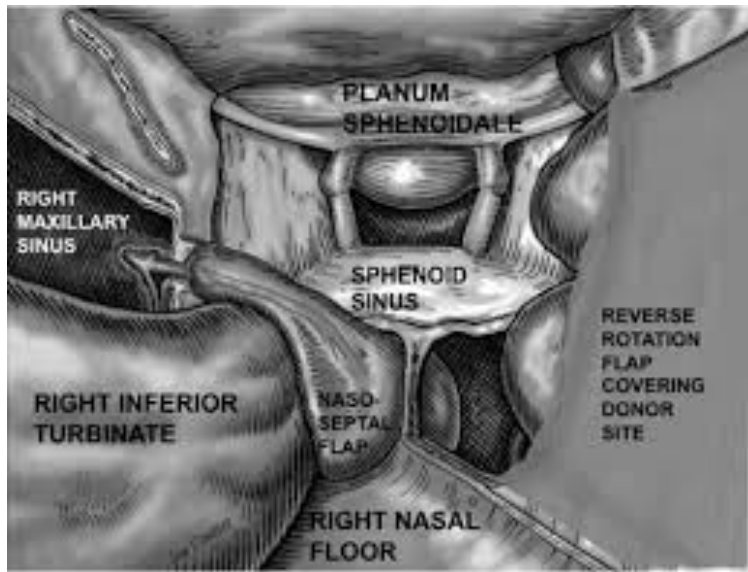
the fistula spot within the surrounding anatomy, while checking in real-time the effective repair of the leak, thus making the procedure eventually safer and faster.



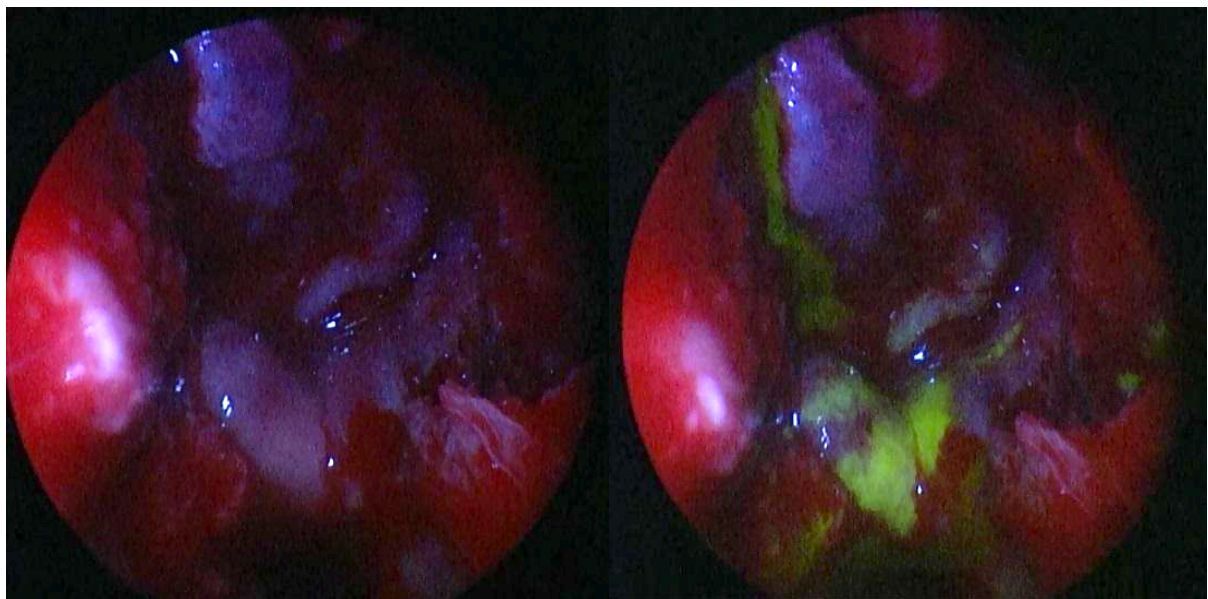
Endoscopic repair of a clival fistula. A low flow leak is identified by fluorescein staining and the defect is repaired with a pedicled nasoseptal flap and fibrin glue.

5.3 – Pedicled flaps for extended approaches

During the last decade, the evolution of transnasal endoscopic approaches extended our possibilities from the frontal sinus to the clivus and from orbit to orbit. Still, a greater invasiveness led to unacceptable CSF leak rates in the post-operative period. This was successfully addressed by the introduction in 2006 of nasoseptal pedicled flaps which foster a rapid, stable healing of the skull base scar (Hadad). In this regard, Chabot et al has recently reported that the nasoseptal flap (NSF) necrosis, rare as it may be, could be a dreadful complication in endoscopic endonasal surgery. Apart from an incorrect NSF design and / or concomitant infections, the main cause of NSF necrosis is an insufficient vascular patency. Two ischemic problems may arise: one at the time of surgery due to the kinking of the flap vessels and one in a subacute / delayed fashion due to vasospasm. This latter issue may be addressed by avoiding post-op hypotension. In dealing with the immediate vessel kinking, we have recently published a method to check the perfusion of the flap in real time by using vascular fluorescein distribution. Briefly, with the aid of our slightly modified endoscopic filtering system for the Fluorescein detection, we injected a low dose of Fluorescein (5mg/Kg). After about 15 seconds a vivid green staining became progressively visible in the perfused tissues. By comparing the synchronicity and the amount of fluorescein staining of the NSF with the residual mucosa it was possible to infer the effective perfusion of the pedicled flap. The advantage of this technique lies in the real-time visualization of the perfusion under viable operating light conditions, thus allowing to precisely identify where the perfusion might be blocked. A potential disadvantage of this technique is represented by the interstitial distribution of the Fluorescein, which stains for many hours the mucosal tissues, thus limiting repeated evaluations. This could be avoided through the use of ICG fluorescence, even if, unfortunately, it does not allow real-time visualization and implies higher operating costs.



Anatomical sketch of the design of a nasoseptal flap



Intraoperative images of a nasoseptal flap pre- (left) and post- (right) injection of 5 mg/Kg of Fluorescein.

6- Conclusions

It is predictable and desirable that the Neurosurgery of the future will be less and less invasive. Endovascular procedures will gain more indications over open surgery and perhaps tailored oncological therapies will limit our surgical role to pure bioptic diagnosticians. Although somewhat inevitable, this transition is not going to be immediate. Until then, modern Neurosurgery must be well-grounded on anatomical knowledge and maintain a strict relationship with the development and availability of new operative technologies. These should be easy to employ, as safe as possible, repeatable and possibly at a low cost. In this regard Fluorescein-aided Neurosurgery proved itself to be as helpful as easy, safe and affordable. I hope that the set up and the protocols I have developed will be disseminated to other Departments, so that many potential applications could be tested for the treatment of other pathologies, even outside the Neurosurgical field.

I had the luck and privilege to be able to almost immediately translate my “do-it-yourself experimental researches” to the actual clinical practice. In conclusion, I'd like to recall three major advantages in the process.

Firstly, I was forced to go back to the basics. Optical physics and physiology and pharmacodynamics are all unfortunately far from the daily clinical routine, especially for surgeons. The possibility to actually familiarize myself with new tools, develop theoretical speculations and to test them, fail and overcome problems, brought me back to what it's all about: to try and find new answers by experimenting different solutions. The assumption “It's always been done like that” is the worst enemy for the advancement of Medicine.

Secondly, I had to face the huge wall of bureaucracy, legal issues and trial funding. Not only did this training put my patience and perseverance to an exhausting test, it has also taught

me the best way to design a trial properly, as well as to turn my raw ideas into a valuable research. A low-budget sure is a limitation but should not impede ideas to surface.

Lastly, I honestly believe that the outcome of the patients I have treated had a major improvement with the aid of intraoperative Fluorescein. This is, by any means, what matters the most to me.

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