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TITLE:

Role of Diffusion MRI Tractography in Endoscopic Endonasal Skull Base Surgery

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KEYWORDS:
endoscopic endonasal surgery, skull base tumors, morbidity, tractography, optic pathways, cranial nerves, presurgical planning.

SUMMARY
We present a protocol to integrate diffusion MRI tractography in patient work-up to endoscopic endonasal surgery for a skull base tumor. The methods for adopting these neuroimaging studies in the pre- and intra-operative phases are described.

ABSTRACT
Endoscopic endonasal surgery has gained a prominent role in the management of complex skull base tumors. It allows the resection of a large group of benign and malignant lesions through a natural anatomical extra-cranial pathway, represented by the nasal cavities, avoiding brain retraction and neurovascular manipulation. This is reflected by the patients' prompt clinical recovery and the low risk of permanent neurological sequelae, representing the main caveat of conventional skull base surgery. This surgery must be tailored to each specific case, considering its features and relationship with surrounding neural structures, mostly based on preoperative neuroimaging. Advanced MRI techniques, such as tractography, have been rarely adopted in skull base surgery due to technical issues: lengthy and complicated

processes to generate reliable reconstructions for inclusion in the neuronavigation system.

This paper aims to present the protocol implemented in the institution and highlights the synergistic collaboration and teamwork between neurosurgeons and the neuroimaging team (neurologists, neuroradiologists, neuropsychologists, physicists, and bioengineers) with the final goal of selecting the optimal treatment for each patient, improving the surgical results and pursuing the advancement of personalized medicine in this field.

INTRODUCTION

The possibility to approach the skull base midline and paramedian regions through an anterior route, adopting the nasal fossae as natural cavities, has a long history, dating back more than one century[1]. However, in the last 20 years, the visualization and operative technologies have improved enough to expand their possibility of including the treatment of the most complex tumors such as meningiomas, chordomas, chondrosarcomas, and craniopharyngiomas[1] due to the (1) introduction of the endoscope, which gives a panoramic and detailed 2D/3D view of these regions to the surgeon, (2) the development of intraoperative neuronavigation systems, and (3) the implementation of dedicated surgical instruments. As painstakingly demonstrated by Kassam et al. and confirmed by multiple reviews and meta-analyses, the advantages of this surgical approach are mainly represented by its chances to resect challenging skull base tumors, avoiding any direct brain retraction or nerve manipulation, thus reducing the risk of surgical complications and long-term neurological and visual sequelae[2-12].

For multiple skull base and pituitary-diencephalic tumors, the ideal surgical goal has changed in the last years from the most extensive tumor removal possible to the safest removal with preservation of the neurological functions to preserve the patient's quality of life[3]. This limitation could be compensated by innovative and effective adjuvant treatments, such as radiation therapy (adopting massive particles such as proton or carbon ions when appropriate) and, for selected neoplasms, by chemotherapy as inhibitors of the BRAF/MEK pathway for the craniopharyngiomas[13-15].

However, to pursue these goals, a careful preoperative assessment is crucial, to tailor the surgical strategy to each case's specific feature[2]. In most centers, the MRI preoperative protocol is usually performed only with standard structural sequences, which provide the morphological characterization of the lesion. However, with these techniques it is not always possible to assess the anatomical relationship of the tumor with adjacent structures reliably[3]. Moreover, each patient may present different pathology-induced functional reorganization profiles detectable only with diffusion MRI tractography and functional MRI (fMRI), which can be used to provide guidance both in the surgery planning and in the intraoperative steps[16,17].

Currently, fMRI is the most commonly used neuroimaging modality for mapping brain functional activity and connectivity, as guidance for surgical planning[18,19] and to improve the patients' outcome[20]. Task-based fMRI is the modality of choice to identify "eloquent" brain regions that are functionally involved in specific task performance (e.g., finger tapping, phonemic fluency), but is not applicable for the study of skull base tumors.

Diffusion MRI tractography permits in vivo and noninvasive reconstruction of white matter brain connections as well as cranial nerves, investigating the brain hodological structure[21]. Different tractography algorithms have been developed to reconstruct axonal pathways by linking water molecule diffusivity profiles, evaluated within each brain voxel. Deterministic tractography follows the dominant diffusivity direction, whereas probabilistic tractography evaluates possible pathways' connectivity distribution. Additionally, different models can be applied to evaluate diffusivity within each voxel, and it is possible to define two main categories: single fiber models, such as the diffusion tensor model, where a single fiber orientation is evaluated, and multiple-fiber models, such as spherical deconvolution, where several crossing-fiber orientations are reconstructed[22,23]. Despite the methodological debate about diffusion MRI tractography, its utility in the neurosurgical workflow is currently established. It is possible to evaluate white matter tract dislocation and distance to the tumor, preserving specific white matter connections. Moreover, diffusion tensor imaging (DTI) maps, especially fractional anisotropy (FA) and mean diffusivity (MD), can be applied to assess microstructural white matter alterations related to possible tumor infiltration and for longitudinal tract monitoring. All these features make diffusion MRI tractography a powerful tool both for pre-surgical planning and intra-operative decision making through neuronavigation systems[24].

However, the application of tractography techniques to skull base surgery has been limited by the need for specialized technical knowledge and the time-consuming work-up to optimize diffusion MRI sequence acquisition, analysis protocol, and incorporating tractography results in neuronavigation systems[25]. Finally, further limitations are due to the technical difficulties extending these analyses from intraparenchymal to extra-parenchymal white matter structures, as cranial nerves. Indeed, only recent studies presented preliminary results attempting to integrate advanced MRI and skull base surgery[26-28].

The present paper presents a protocol for the multidisciplinary management of pituitary-diencephalic and skull base tumors using diffusion MRI tractography. The implementation of this protocol in the institution resulted from the collaboration between neurosurgeons, neuro-endocrinologists and the neuroimaging team (including clinical and bioinformatics expertise) to offer an effective integrated multi-axial approach to these patients.

In the center, we have integrated multidisciplinary protocols for managing patients with skull base tumors, to provide the most informative description possible, and to tailor and personalize the surgical plan. We show that this protocol can be adopted both in the clinical and the research setting for any patient with a skull base tumor to guide the treatment strategy and to improve the knowledge on the brain modifications induced by these lesions.

PROTOCOL

The protocol is following the Local Research Committee's ethical standards and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

1. Selection of the patients

1.1. Adopt the following inclusion criteria: patients older than 18 years old, fully collaborating, presenting a tumor of the skull base, or pituitary-diencephalic region.

1.2. Exclude patients with contraindication to MRI (i.e., a pacemaker or ferromagnetic material) or presenting with emergent clinical conditions (i.e., intracranial hypertension, the acute visual loss that requires immediate surgery), or pregnant women, or patients with mental illness, or those who explicitly refuse to participate in this protocol.

2. Preparation for the MRI exam

2.1. Before the MRI exam, administer the safety form to exclude significant contraindication to the exam and contrast agent injection: no ferromagnetic materials in the body, evaluation of MRI devices, safe or conditional, no pacemaker, no eye contact lenses on.

2.2. If the scanner used for the MRI acquisition is a high field (e.g., 3 T, see **Table of Materials**), consider any potential additional contraindications related, for example, to neurostimulation devices.

2.3. Check whether the patient has claustrophobia.

2.4. Ensure that the patient has read and signed the MRI consent form to acknowledge the imaging exam's risks and benefits.

2.5. Have a neuropsychologist perform a general evaluation and a targeted neurocognitive assessment based on the tumor location.

2.6. Administer the Edinburgh inventory to evaluate handedness dominance[29].

3. Positioning of the patient in the scanner

3.1. Give earplugs to the patient to reduce MRI noise.

3.2. Head movements can affect imaging quality; thus, use foam pads to reduce head movements, immobilizing the head inside the MRI coil.

3.3. Provide an emergency alarm button to the patient in case of need to interrupt the exam.

3.4. Switch on the camera and microphone inside the scanner to monitor, speak, and listen to the patient from the MRI acquisition room outside the scanner.

4. Brain MRI protocol setting and acquisition parameters

4.1. Acquire a standardized multimodal MRI protocol high-field scanner (1.5 T or 3T). The following sequence parameters refer to a 3 T MRI, using a head-neck high-density array coil (64 channels).

4.2. Acquire high-resolution and volumetric anatomical sequences: T1-weighted pre- and post-gadolinium contrast agent administration and FLAIR T2-weighted.

4.3. Acquire continuous sagittal slices providing isotropic resolution of 1x1x1 mm^3 scanning time of about 5 min per sequence.

4.4. Acquire a high-resolution T2-weighted sequence and localize the tumor area for cranial nerve visualization: a volumetric CISS (Constructive Interference in Steady State) with voxel dimension of 0.5x0.5x0.5 mm^3 (scanning time of about 9 minutes).

4.5. Acquire diffusion-weighted sequences using single-shot echo-planar images (EPI), voxel dimension of 2x2x2 mm^3, 64 magnetic gradient directions with b-value of 2000 s/mm^2, echo time of 98 ms, and relaxation time of 4300 ms.

4.6. Acquire five volumes with null b-value at the beginning of the diffusion-weighted acquisition with phase encoding direction set to anterior-posterior (scanning time of 5 minutes).

4.7. Additionally, acquire three volumes with null b-value but reversed phase encoding direction, posterior-anterior, to correct imaging distortions due to the EPI acquisition (scanning time of 42 seconds). Continuous near-axial slices are acquired.

4.8. Acquire additional sequences to investigate specific tumor features, such as multi- or single-voxel MRI-spectroscopy localized in the tumor area.

NOTE: The total scanning time duration is about 30 minutes, excluding patient preparation for the MRI exam.

5. Brain MR images pre-processing

5.1. Convert the MRI data from the imaging format adopted by MRI acquisition consoles, DICOM (.dcm), to the NIFTI format (.nii) used in advanced imaging analyses.

5.2. Run the dcm2niix function (https://github.com/rordenlab/dcm2niix). Set as input files dicom images and as output the corresponding .nii files: T1.nii, Flair.nii, T1_contrast.nii, DTI_b2000.nii and DTI_b0_flip.nii.

5.3. Install the FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki) and MRtrix3 (https://www.mrtrix.org) software needed for the advanced imaging analyses.

5.4. Register the Flair.nii and T1_contrast.nii to the T1.nii image by running the FSL-flirt function, which performs a linear image registration.

5.5. Register the DTI_b2000.nii image to the T1.nii by running the FSL-epi_reg function, which takes into account EPI imaging distortion artifacts.

5.6. Run the FSL-topup function to correct phase encoding direction artifacts presenting the DTI_b2000.nii image. Set the DTI_b0_flip.nii inverse phase encoding acquisition as the "in_main" input file.

5.7. Run the MRtrix3-dwidenoise function for imaging denoising with a principal component noise modeling.

5.8. To correct eddy current and signal drop-out artifact, run the FSL-eddy function, and for MRI coil-induced signal inhomogeneities, the MRtrix3-dwibias correct function.

5.9. Run the FSL-bet function to remove the scalp signal presenting the T1.nii image and rename the output file by using the "_brain" suffix: T1_brain.nii.

6. Tumor segmentation

6.1. Install the itk-snap software (http://www.itksnap.org) [30].

6.2. Once the itk-snap software is installed, press **File - Open Main Image** and select the T1.nii image, then press **File - Add Another Imager** and upload the Flair.nii and T1_contrast.nii images, setting the semi-transparent overlay option.

6.3. Inspect the tumor in the T1.nii, Flair.nii, and T1_contrast.nii images. Choose the anatomical plane to follow when drawing the lesion, e.g., axial.

6.4. Place the pointer in one axial slice to start. In the **Main Toolbar**, select the **Polygon Inspector** icon and start drawing tumor boundaries by using the **Freehand Drawing Style - Smooth curve or Polygon**.

6.5. Once finished drawing the tumor perimeter, close the curve linking the first and last dots, press **Accept**, and continue drawing in the next slice. For large tumor lesions, to accelerate the drawing process, skip some axial slices (e.g., three), and draw the lesion perimeter in interleaved slices.

6.6. At the end of the lesion perimeter drawing, select **Tools – Interpolate Labels**, set the **Label to/with interpolate** as the tumor lesion and the **Interpolate along a single axis** as the axis orientation followed in drawing the tumor boundaries.

6.7. Select **Segmentation – Save Segmentation Image** and name the tumor segmentation as Tumor_mask.nii by selecting the Nifti format option to save.

7. Tractography analysis

7.1. Run the FSL-dtifit function to model diffusivity and the different spatial directions and obtain the following diffusion tensor maps: FA.nii, MD.nii, and V1.nii. Evaluate these DTI maps to access abnormal diffusivity values that may occur in the presence of tumor edema or infiltration.

7.2. Run the MRtrix3-tckgen function with the default setting "ifod2" to perform probabilistic tractography and reconstruct the white matter pathways by modeling crossing-fibers issues[31].

7.3. Adopt a seed-target approach by setting the "-seed_image" and "-include" options based on a priori anatomical knowledge.

7.4. Manually draw regions of interest (ROIs) set as seed or target for tractography. Alternatively, use atlas-based ROIs. See Mormina et at.[32] for the optic radiation tractography, Hales et al.[33] for the optic chiasm and optic cranial nerves, and Testa et al.[34] for the pyramidal tracts.

7.5. Launch the FSL-fsleyes image viewer, select **Open**, and choose images to inspect visually.

7.6. In the FSL- fsleyes viewer, go to **Setting – Ortho View 1** and activate the **Edit Mode** tool.

7.7. Click the FSL-fsleyes pencil icon and draw the tractography ROIs.

7.8. Install the Freesurfer (https://surfer.nmr.mgh.harvard.edu) software.

7.9. Run the Freesurfer-Recon-all function on the T1.nii image to obtain the automatic cortical region segmentation to use as tractography ROIs.

7.10. Run the FSL-epi_regregistration function, setting as input image the T1.nii, and reference image the DTI_b2000.nii, save the registration output matrix (T1_onto_DTI.mat).

7.11. Use the obtained T1_onto_DTI.mat matrix to register the segmented ROIs to the DTI_b2000.nii image.

7.12. Run the tractography using the MRtrix3-tckgen function.

7.13. Run the MRtrix3-tckmap function to convert the ".tck" streamlines tractography output in the "-template FA.nii" image.

7.14. Run the FSL-flirt function to linearly register the T1.nii image to the MNI152_T1_2mm_brain.nii template.

7.15. Save the output matrix as T1_onto_MNI.mat. Run the FSL-convert_xfm function setting the "-concat" option as T1_onto_MNI.mat and T1_onto_DTI.mat, save the output matrix as DTI_onto_MNI.mat.

8. Tractography: along-tract analysis

8.1. For an accurate description of DTI parameters, use along-tract algorithms, such as the Matlab-based algorithm that models the surface tract geometry with the Laplacian operator properties[35].

8.2. Install the Matlab software (https://matlab.mathworks.com) and request the along-tract code to the developing authors[35].

8.3. Alternatively, use the MRtrix3-tcksample function for along-tract analysis as Matlab requires a license.

9. 3D-rendering visualization

9.1. Install the Surf Ice software (https://www.nitrc.org/plugins/mwiki/index.php/surfice:MainPage).

9.2. In the Surf Ice command panel, click on **Advanced - Convert voxelwise to mesh**, select the nifti image to convert, save the resulting .obj file.

9.3. In the Surf Ice command panel, click **File – Open**, and select the .obj file to visualize the 3D volume rendering.

10. Preoperative clinical examinations

10.1. Perform bio-humoral endocrinological assessment, consisting of prolactin, TSH, freeT4, ACTH, cortisol, GH, LH, FSH, and serum tests total testosterone/estradiol, respectively in men and women.

10.2. Analyze the 24-hour urine volume and serum and urine osmolality and sodium levels to determine the presence of diabetes insipidus.

10.3. Perform an ophthalmological evaluation, including visual acuity measurement, computerized visual field assessment, and retinal optical coherence tomography (OCT).

10.4. Perform a neurological physical examination, with a collection of anamnestic information about weight gain, the sensation of hunger, continuously monitoring rectal temperature every 2 min for 24 h using a portable device to evaluate the circadian temperature rhythm, and 24 h sleep-wake cycle recording (including an electroencephalogram, right and left electro-oculogram, electrocardiogram, and electromyogram of mylohyoid and left and right tibialis muscles)[36-38].

11. Surgical planning

11.1. Discuss in a collegial team meeting each patient candidate to surgery, based on the results of tumor segmentation and relationship with the functional eloquent neural structures (optic nerves and chiasm, pituitary stalk, third ventricle, internal carotid artery, anterior cerebral artery–anterior communicating artery (ACA-ACoA) complex, basilar artery, cranial nerves III, IV, VI, mammillary bodies, white matter tracts, and functional cortical areas) to determine the most appropriate surgical approach.

11.2. Select the surgical corridor with the minimal risk of injuries of neural structures[39].

11.3. Define the safe resection area for each case, localizing the critical neural structure (such as chiasm, mammillary body) under whose proximity the resection must be arrested to avoid permanent damage[39].

11.4. Merge the most relevant MRI sequences and import them into the operative phase's neuronavigation system.

12. Surgery preparation

12.1. Induce general anesthesia adopting total intravenous anesthesia with propofol and remifentanil (it has been demonstrated that the other anesthetic agents are among the most critical factors affecting intraoperative monitoring reliability, increasing the false-negative rate), avoiding myorelaxant[40].

12.2. Perform oro-tracheal intubation with gauzes in the oropharynx to prevent blood or fluid leakage in the stomach or airways[41].

12.3. Set up the neurophysiological monitoring, with continuous recording of motor evoked potentials (MEPs) and somatosensory evoked potentials (SEPs) and free-running electromyography (EMG) for cranial nerves.42

12.4. Import the MRI data, including the tractography reconstructions, in the neuronavigation system (**Table of Materials**).

12.5. Select the brain surgery electromagnetic registration modality on the neuronavigation system.

12.6. Register the neuronavigation system on the patient, adopting a free-tracking technique or external markers.

12.7. Control the accuracy of the achieved registration, checking the position of external markers (i.e., ear or nose) on the imported MRI; if the result is not acceptable, repeat the registration.

12.8. Place the patient in a semi-sitting position; Mayfield's use to fix the head is not needed[43].

12.9. Administer corticosteroid (endovenous flebocortid, dosage depending on the patient's weight) and antibiotics (2 g of amoxicillin-clavulanic acid)[44].

13. Endoscopic endonasal surgery

13.1. Start with a 0° endoscope (**Table of Materials**).

13.2. Harvest the naso-septal flap[45].

13.3. Perform an anterior sphenoidotomy, followed by posterior septostomy and ethmoidectomy with preservation of the middle turbinate, when possible[43].

13.4. Open the sellar and tuberculum bone[41].

13.5. Incise the dura layer with an H-shape, after coagulation of the superior intercavernous sinus[41].

13.6. Cleave the tumor by the arachnoidal plane[43].

13.7. Centrally debulk the tumor[43].

13.8. Remove its capsule from the surrounding diencephalic neural structures, arresting the resection in case of tumor adhesion to eloquent structures visualized under neuronavigation guidance[43].

13.9. Explore the surgical cavity with angled optics (**Table of Materials**)[46].

13.10. Ensure hemostasis with bipolar coagulation or hemostatic agents.

13.11. Close the osteo-meningeal opening with an intradural intracranial layer of dural substitute[43].

13.12. Place an extradural intracranial layer of dural substitute, scaffolded with abdominal fat and eventually bone (**Table of Materials**)[43].

13.13. Cover the closure with the naso-septal flap[43].

14. Histological examination

14.1. Fix tumor samples with 10% formalin and embed them in paraffin immediately after surgery.

14.2. Cut tissue into sections of 4 µm thickness and stain with hematoxylin and eosin. The histological diagnosis must be based on the most recent version of the WHO classification of brain tumors (2016)[47].

14.3. Perform specimen immunohistochemical staining by an automated immunohistochemical staining instrument, using avidin-biotin labeling and diaminobenzidine as a detection reagent. For craniopharyngiomas, adopt anti-beta-catenin, anti-BRAF v600E mutant epitope, and anti-Ki67 antibodies for immunohistochemical staining (**Table of Materials**).

14.4. Evaluate the Ki-67 index through the manual count of positive tumor cells[48].

15. Post-surgical patient management

15.1. Wake the patient immediately after surgery.

15.2. Restore spontaneous breathing from the mouth by filling nasal cavities with absorbable and non-absorbable material.

15.3. Monitor vital parameters (blood pressure, heart rate, oxygen saturation and consciousness state) for the following 6-12 hours in ICU.

15.4. Restore oral feeding after 12 hours.

15.5. Perform a CT scan after 6-9 hours.

15.6. Maintain bed rest for three days with heparin treatment.

15.7. Control fluid balance every 12 hours and assess serum electrolytes every 24 hours.

15.8. Administer corticosteroid therapy (endovenous flebocortid in the first 24 hours, and then oral cortone acetate 30 +15 mg/day).

15.9. Perform an MRI with/without gadolinium within 72 hours after surgery.

15.10. Discharge the patient on the 4th day.

16. Early follow-up

16.1. Repeat the complete endocrinological assessment 30 days after surgery[43].

16.2. Repeat the ophthalmological assessment three months after surgery[43].

16.3. Repeat the neurological physical examination and temperature and sleep-wake rhythms function investigations three months after surgery[46].

16.4. Perform the MRI with/without gadolinium three months after surgery[46].

17. Adjuvant therapy

17.1. Evaluate the presence of early tumor progression, and if it is indicated, refer the patient to radiation therapy[43].

18. Long-term follow-up

18.1. Repeat the clinical, endocrinological, and ophthalmological assessments annually[43].

18.2. Perform yearly MRI with/without gadolinium: in case of recurrence, the patient can be re-operated on and then referred to radiation therapy or directly referred to radiotherapy[43].

REPRESENTATIVE RESULTS

A 55-year-old woman presented with progressive visual deficits. Her medical history was unremarkable. On ophthalmological evaluation, bilateral reduction of visual acuity (6/10 in the right eye and 8/10 in the left eye) was revealed, and the computerized visual field showed complete bitemporal hemianopia. No further deficits were evident on neurological examination, but the patient reported persistent asthenia and an increase in hunger and thirst sensation in the previous 2-3 months, with a weight gain of 4-5 kg and frequent awakenings in the night for the need to urinate. On endocrinological evaluation, central hypercorticism and diabetes insipidus were revealed. The patient was treated with corticosteroids (hydrocortisone 30+15 mg/day and desmopressin 30+30 µg/day). On 24 h sleep-wake cycle and temperature

monitoring, no significant alterations were noticed after the hormonal substitute therapy's optimization.

Brain MRI demonstrated a suprasellar tumor occupying the opto-chiasmatic cistern and invading the 3rd ventricle, with an irregular polycystic morphology, enhancing after gadolinium, suspected as the first hypothesis for a craniopharyngioma (**Figure 1A-C**). Advanced imaging analyses were performed, as illustrated in the current protocol. The tumor core segmentation highlighted the gadolinium uptake and corresponded to a volume of 7.92 cm^3 (**Figure 1D-E)**.

The visual pathways were the most critical to evaluate in the pre-surgical planning of this patient. The pyramidal tracts were also reconstructed to assess the microstructural correlate of the signal increase detected on the FLAIR T2-weighted image at the level of the right tract.

The optic pathway tractography reconstruction was investigated, particularly the optic chiasm dislocation in the presence of the tumor mass. The bilateral optic cranial nerves were also reconstructed. In the interface between the brain, bones, and blood vessels, susceptibility artifacts did not allow for full reconstruction of the fibers connecting the optic chiasm to the optic nerves (**Figure 2**).

The pyramidal tracts diffusivity profile was investigated with along-tract DTI map statistics. At the level of the right posterior limb of the internal capsule, a focal FLAIR T2-weighted hyperintensity was present, corresponding to a 5% increase of the right MD measure (5^{th}-7^{th} segments) compared to the left side (**Figure 3**).

By considering such relationships between tumor and neural structures, the endoscopic endonasal extended transplant/transtuberculum approach was chosen[36]. The tumor removal was performed with a microsurgical two-hands technique. Initially, the tumor was centrally debulked, also draining its cystic component (**Figure 4**). Afterward, it was possible to progressively detach the craniopharyngioma from the neural structures, adopting the arachnoid as a cleavage plane (**Figure 5**). At the end of the surgery, complete tumor removal with the hypothalamus's anatomical preservation was achieved (**Figure 6**). The repair of the osteo-dural defect was performed with abdominal fat and naso-septal flap (**Figure 7**).

The postoperative course was uneventful, and the patient was discharged after four days in the right clinical conditions. The tumor turned out to be an adamantinomatous craniopharyngioma (WHO grade 1) on histological examination.

The patient developed complete panhypopituitarism at follow-up and was under complete substitution therapy with hydrocortisone, desmopressin, and levothyroxine. Visual deficits wholly regressed, and no alterations on neurological examination, 24 h sleep-wake cycle, and temperature monitoring were detected. Three months of brain MRI demonstrated a complete tumor removal, with no remnant or recurrence. Therefore, no adjuvant treatment was advised, and the patient is followed up with yearly clinical and neuroradiological examinations (**Figure 8**).

Figure Legends

Figure 1. **Preoperative anatomical MRI sequences (F/55 years).** Axial view of T1-weighted (**A**) and FLAIR T2-weighted (**B**); axial (**C, D**) and sagittal (**E**) T1- after gadolinium administration (0.1 mm/kg). The tumor segmentation (red) overlaid to the gadolinium-enhanced T1-weighted image is shown in **D** and **E**.

Figure 2. **Preoperative 3D rendering of optic pathways tractography and tumor segmentation.** (**A**) Axial slice of the FLAIR T2-weighted image overlays the optic chiasm tractography, localized anteriorly to the tumor. (**B**) 3D volume rendering of the FLAIR T2-weighted image, selecting an axial plane and overlaid the optic pathways tractography. (**C**) 3D volume rendering of the brain surface, optic pathways tractography, and tumor segmentation in red. All the panels' tractography streamlines are colored by the RGB directionality color map (red: lateral-lateral, green: anterior-posterior, and blue: inferior-superior).

Figure 3. **Pyramidal along-tract DTI measure analysis.** (**A**) 3D rendering of the bilateral pyramidal tracts or corticospinal tract (CST), colored based on the Laplacian inferior-superior segmentation gradient. (**B**) Right (red) and left (blue) CST mean diffusivity (MD) profiles resulting from the partitioning of the tract into twenty segments displayed in the color maps in A; segments start at the level of the pons towards the precentral gyrus (PrCr). The black box highlights the segments at the posterior limb of the internal capsule (PLIC) (5^{th}-7^{th}). (**C**) Axial view of FLAIR T2-weighted image at the PLIC level, with and without the right CST connectivity map, where a brighter red intensity corresponds to a higher streamline density.

Figure 4. **Intraoperative endoscopic images.** (**A**) 0° scope, after dural opening, the tumor was initially detached by the chiasm, adopting the arachnoid as a cleavage plane. (**B**) and (**C**), afterward, it was centrally debulked, and the cyst was progressively drained.

Figure 5. **Intraoperative endoscopic images.** (**A**) 0° scope, the craniopharyngioma is cleaved by the arachnoidal plane with the help of neuronavigation, showing the tumor and the neural structures (identified according to our current protocol). Therefore, the mammillary bodies can be spared to avoid permanent hypothalamic damages. (**B**) and (**C**) afterward, it was possible to resect the tumor by the medial hypothalamic surfaces, avoiding any tractions not to injure such neural structure. (**D**) During the removal of the tumor's intra-ventricular portion, particular care was paid in re-opening the cerebral aqueduct and Monro foramina to avoid postoperative acute hydrocephalus.

Figure 6. **Intraoperative endoscopic images.** (**A**) and (**B**) 30° scope, at the end of the surgery, the neural structure of the 3^{rd} ventricle has been explored with angled optics to confirm the complete tumor removal and demonstrate its anatomical integrity. (**C**) At the bottom of the surgical field, it was possible to identify the CN III, under the Liliequist membrane: its function, as the MEPs, SEPs, and other CNs, had been continuously controlled with intraoperative neurophysiological monitoring.

Figure 7. **Intraoperative endoscopic images.** (**A**) 0° scope, closure of osteo-dural defect requires a multilayer technique, adopting dural substitute, abdominal fat, eventually bone, and naso-septal flap. The first layer is constituted by intracranial intradural positioning of the first layer of a dural substitute. (**B**) The following step is

represented by abdominal fat placement to fill the surgical cavity; particular care should be paid to avoid overpacking. (**C**) The second layer of dural substitute is adopted to cover the fat, and it can be maintained in position thanks to a rigid scaffold, as a piece of bone or cartilage (gasket seal technique). (**D**) Finally, the naso-septal flap or a free graft of septum or middle turbinate is used to cover the multilayer closure.

Figure 8. **MRI, sagittal view T1-weighted after gadolinium administration (0.1 mm/kg).** (**A**) Preoperative MRI demonstrates the tumor. (**B**) Post-operatively, the complete tumor removal with the mammillary bodies' anatomical preservation and the hypothalamic structures are visible.

DISCUSSION

The application of the presented protocol resulted in a safe and effective treatment of one of the most challenging intracranial tumors such as a craniopharyngioma invading the 3rd ventricle, possibly opening up a new horizon for a lesion that was defined by H. Cushing about a century ago as the most baffling intracranial neoplasm[1]. The combination of accurate preoperative planning, integrating advanced MRI techniques, and multidisciplinary clinical assessments have permitted us to tailor the surgical strategy, identifying the most appropriate surgical corridor and minimizing the risk of neural structure damage[2,49-51]. Unlike other MRI protocols reported in the literature, the inclusion of fast sequences, such as phase reverse encoding scans for diffusion-weighted images, allows advanced post-processing corrections[52]. This procedure should always be adopted, especially at high intensity field (e.g., 3 T or higher) where imaging distortions are present.

Moreover, the use of a probabilistic tractography approach based on constrained spherical deconvolution allowed an increase in fiber reconstruction quality compared to other deterministic tractography models[53]. Besides, the proposed 3D rendering, and quantitative analyses increased the accuracy of the preoperative patient assessment. This neuroimaging study, together with neurophysiological monitoring, represented a guide for the surgeon, helping him/her to decide whether and where to stop the surgical resection with the final goal of avoiding patients' permanent neurological deficits.

Indeed, the most aggressive tumor resection for craniopharyngiomas has been recently progressively abandoned in favor of a hypothalamic-sparing technique, consisting of arresting the tumor removed before any permanent neural damage[54]. However, in standard clinical practice, it is often complicated for the neurosurgeon to decide when to stop the tumor removal from achieving the maximal safe resection, exposing the patient to the risk, on the one hand, of leaving a tumor remnant larger than planned or, on the other hand, of inducing a permanent hypothalamic injury, with consequent quality of life detriment.

The presented protocol has provided a model of integrating clinical and neuroradiological data intending to provide a practical and easy-to-adopt method for the management of pituitary-diencephalic and skull base tumors. However, we underline that it presents some critical points: the need for adequate equipment, such as high field (3 T) magnet, high-resolution channel coil, and advanced pre/processing imaging software.

The MRI sequences in the presented protocol are also acquirable at 1.5 T, but acquisition parameters reported in Step 4 have to be modified to achieve a good signal to noise ratio: for the diffusion-weighted sequences, a lower b-value is suggested (e.g., 1000 s/mm^2). Moreover, the implementation of the proposed neuroimaging analyses and their introduction in the clinical practice required both clinical and MRI technical and computer science expertise, in particular for the imaging processing. The majority of the reported software is freely available (e.g., FSL, MRtrix3), but the development of homebrew pipelines is required to manage specific datasets or imaging analyses.

Moreover, the further critical point is that, although this technology represents crucial support for the surgeon, it could not replace their learning curve. For these reasons, this advanced surgery should be reserved for few or tertiary referral centers, highly specialized and dedicated specialists.

Finally, the future goal is to improve the reconstruction of extra-parenchymal white matter structures, as cranial nerves. Tractography of these structures is currently impaired by the small dimension of the cranial nerves and by the presence of susceptibility artifacts that dramatically reduced the MRI signal for the presence of air and bone[55].

In conclusion, the synergistic collaboration between neurosurgeons and the neuroimaging team is crucial for clinical and research purposes, allowing planning with the highest accuracy the most effective surgical strategy for each patient and contributing to the advancement of personalized medicine in this field.

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None

DISCLOSURES:
The authors have nothing to disclose

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Figure

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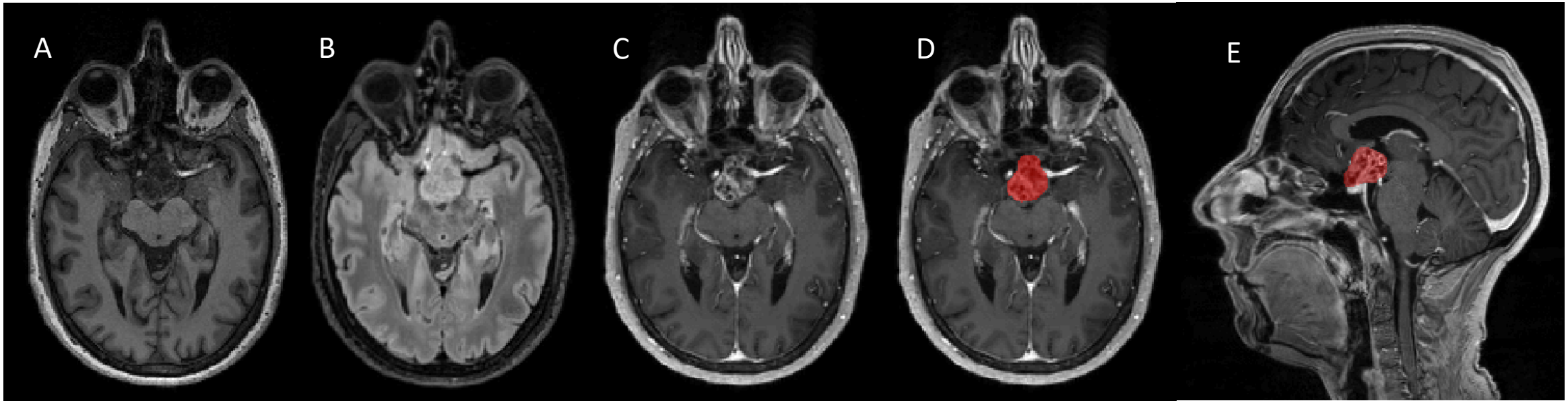

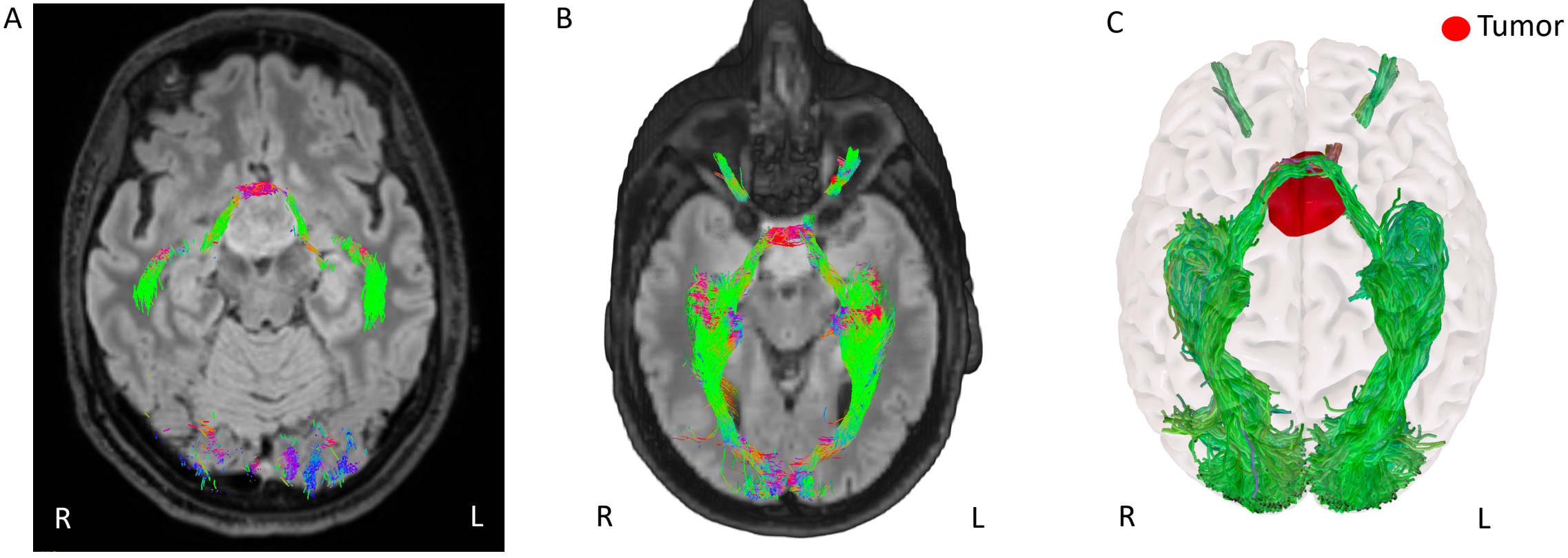
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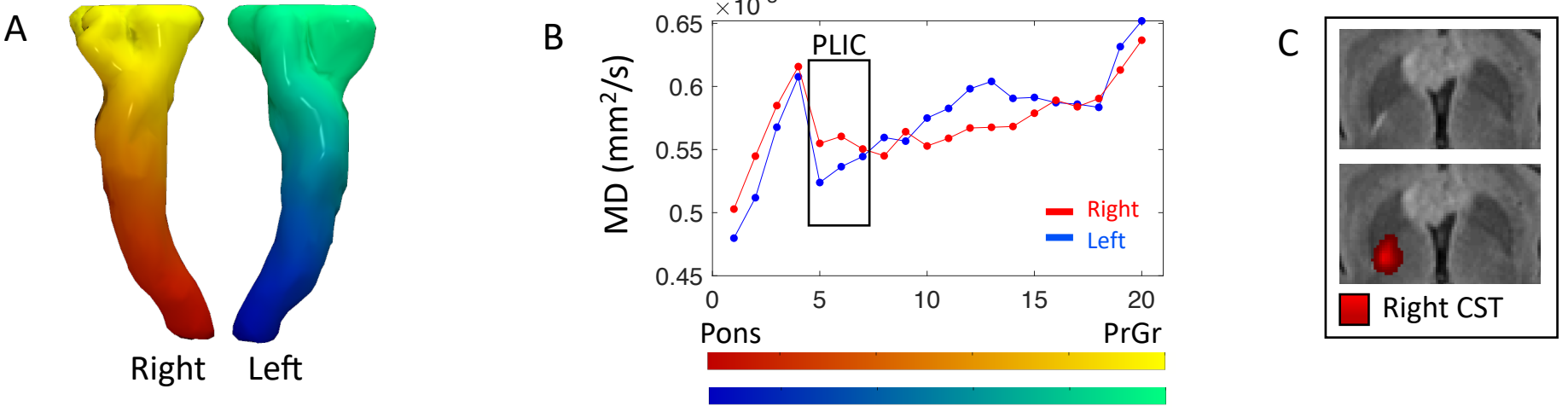
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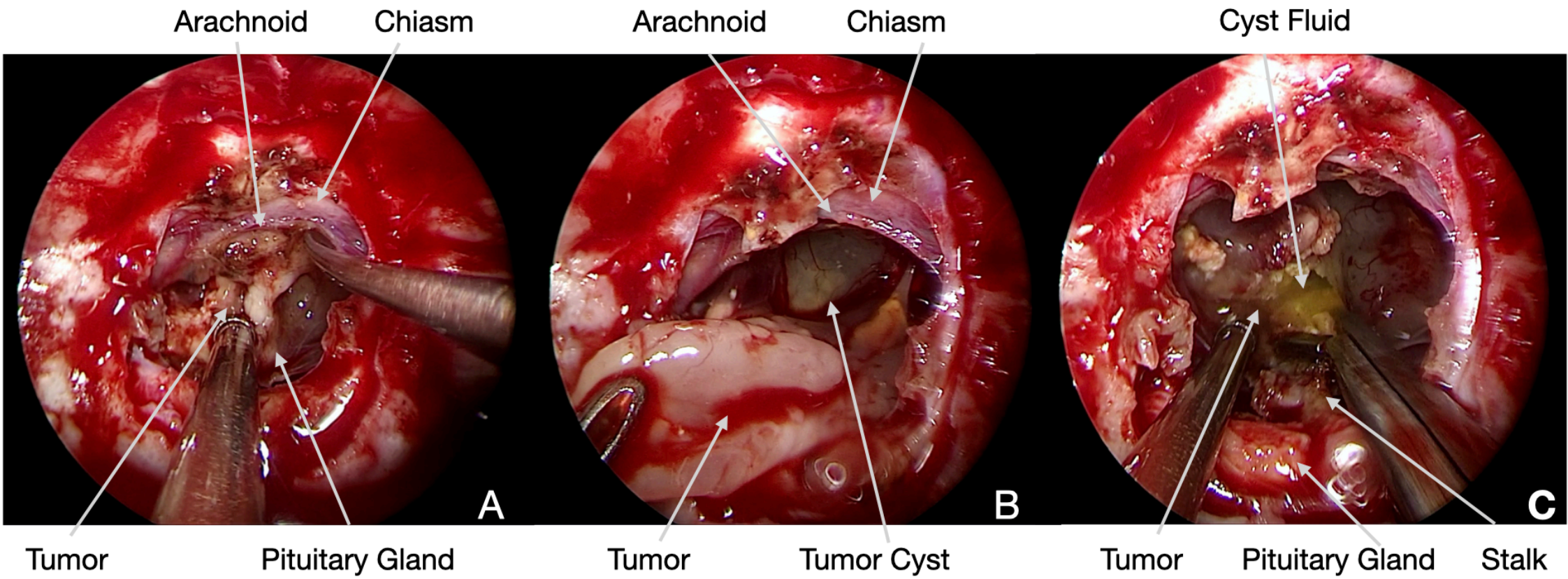

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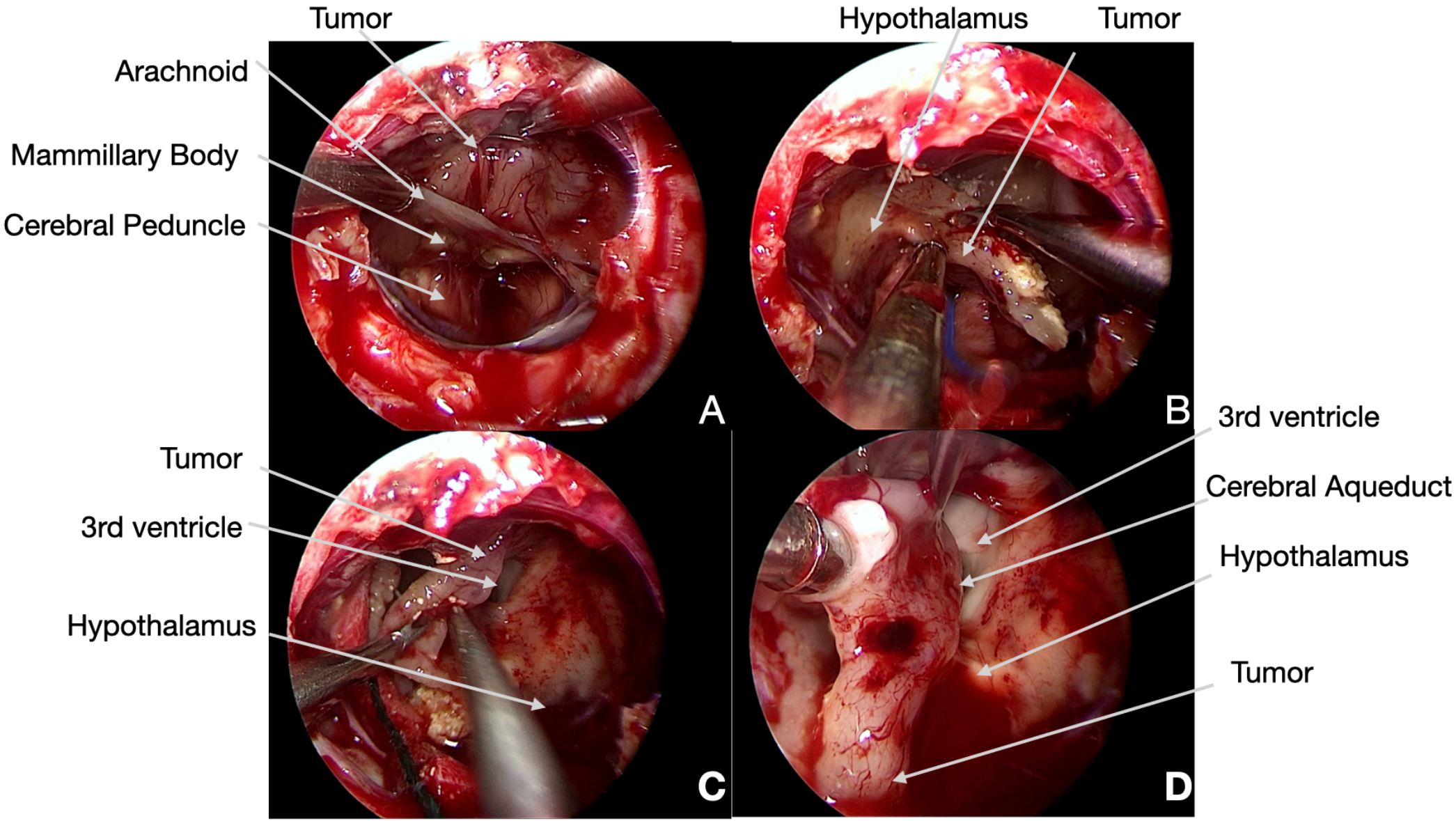

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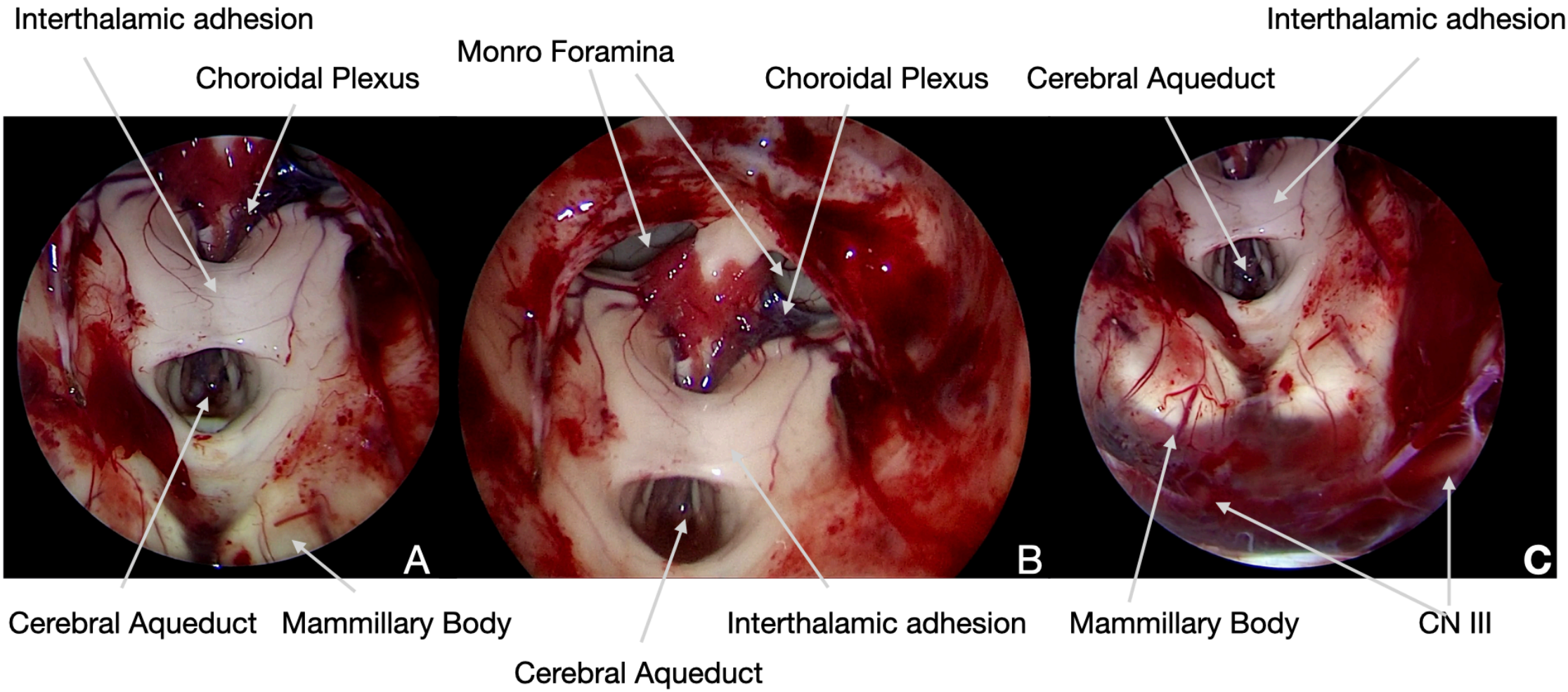

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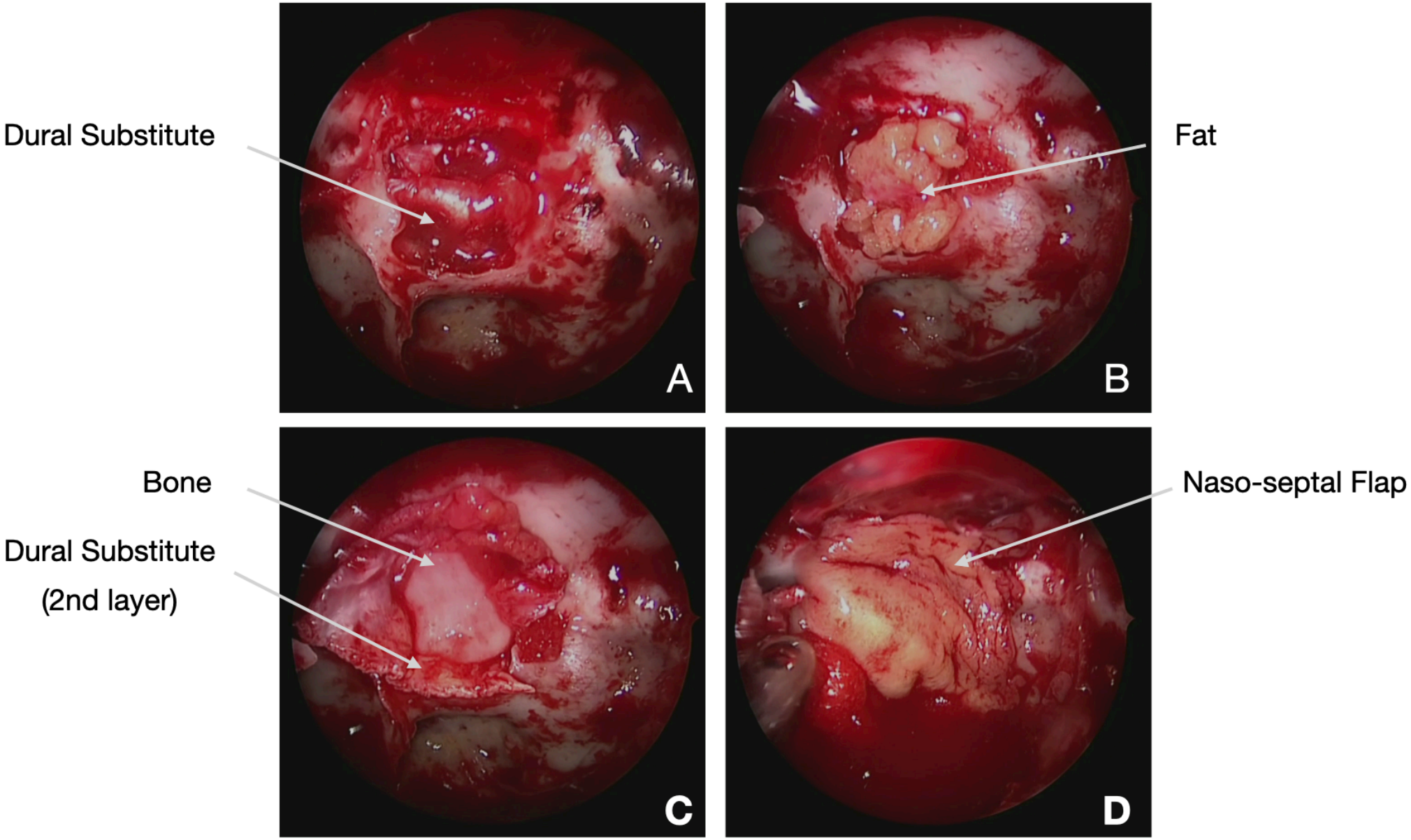

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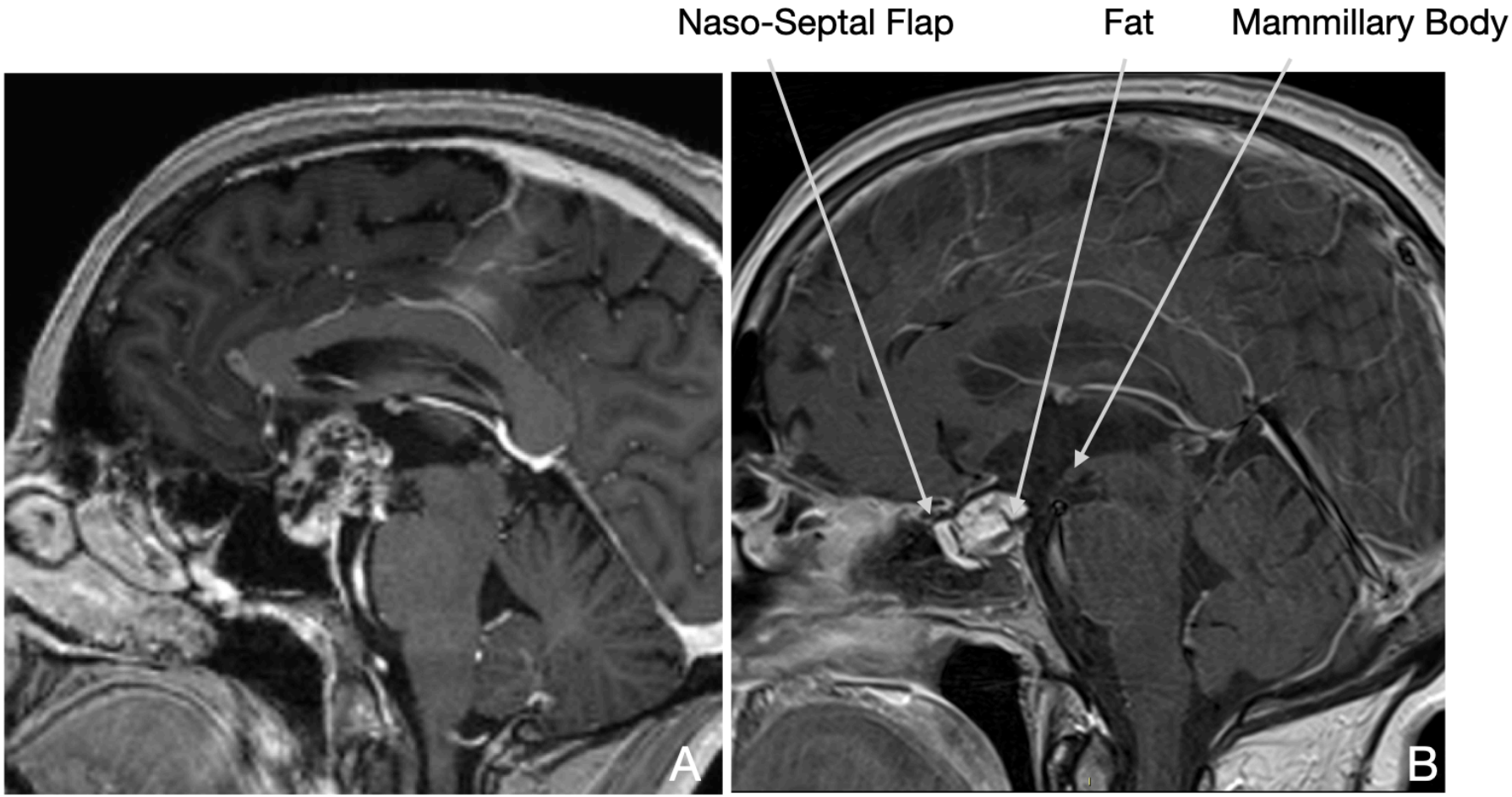

Material	Company
BRAF V600E-specific clone VE1	Ventana
Dural Substitute	Biodesign, Cook Medical
Endoscope	Karl Storz, 4mm in diameter, 18 cm in length, Hopkins II – Karl Storz Endoscopy
Immunohistochemical staining instrument	Ventana Benchmark, Ventana Medical Systems
MRI	3T Magnetom Skyra, Siemens Health Care
Neuronavigator	Stealth Station S8 Surgical Navigation System, MEDTRONIC

Editorial Team

JoVE

Bologna, 10 November 2020

Revision of the manuscript JoVE61724R1, entitled "Role of advanced MR imaging in minimally invasive skull base surgery,"

Dear Editorial Team,

thank you for the opportunity of submitting a revised version of the manuscript. We are grateful to the Editor and Reviewers for their stimulating comments and observations that allowed us to ameliorate our paper. We have deeply revised our manuscript and we provided our point-to-point replies.

Editorial comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues, and that all abbreviations have been defined:

Please complete the sentence in line 100.

DTI has been used for Diffusion Tensor Imaging in line 98 and for diffusion tensor model in line 114.

Authors' reply: We have corrected all spelling and grammar mistakes in the manuscript. The statement in line 100 has been rephrased as following: *"Moreover, each patient may present different pathology-induced functional reorganization profiles detectable only with diffusion MRI tractography and functional MRI (fMRI), which can be used to provide guidance both in the surgery planning and in the intraoperative steps."* Abbreviation DTI is for Diffusion Tensor Imaging, therefore the erroneous abbreviation in line 114 for Diffusion Tensor Model has been removed.

2. Please provide email addresses for all authors.

Authors' reply: The e-mail addresses of all Authors have been added in the first page of the manuscript.

3. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.

Authors' reply: All the company's name has been removed from the manuscript. A Table of Materials and Reagents has been added to the manuscript.

4. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step (e.g., 7.5).

Authors' reply: The steps of the Protocol have been simplified, according to Editor's suggestion.

5. Please provide more details for 10.3: what is the Mini-logger portable device? How is it used? Please provide a citation if this is described elsewhere. Please note that commercial products are to be referenced in the Table of Materials.

Authors' reply: The monitoring of rectal temperature can be recorded by a portable device, connected with the temperature probe. A specific citation has been provided and the statement has been modified as follows: "*…continuously monitoring rectal temperature every 2 min for 24h using a portable device to evaluate the circadian temperature rhythm…*".

6. In line 627, What do you mean by "specialized scientist equipe"?

Authors' reply: We thank the Editor(s) for this question, which permits us to better explain how the implementation of accurate and reliable DTI sequences requires a team work by multiple experts, including neuroradiologists, for their knowledge of the brain normal and pathological anatomy and informatics, for their knowledge of the computer science needed to process these images. The statement has been rephrased as following: *"Moreover, the implementation of the proposed neuroimaging analyses and their introduction in the clinical practice required both clinical and MRI technical and computer science expertise, in particular for the imaging processing."*

7. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then et al. The journal (source) should be written out fully.

Authors' reply: References have been corrected accordingly.

8. Please use uppercase letters in all figure panels consistently.

Authors' reply: All the futures have been fixed, according to this suggestion.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

The authors used DTI to inform the relationship of a lesion and used this information to guide an endonasal approach for an extra axial lesion.

Major Concerns:

There is nothing new described in the manuscript.

Abstract: there is not a clear objective stated. DTI is not an advanced technique and is broadly used. The reason skull base surgeons rarely rely on it is that cranial base tumor are quite uncommonly intra parenchymal. Hence the effort in expanding DTI to cranial nerve imaging, which indeed would be of major help in the field.

Authors' reply: We thank the Reviewer for his/her comment, which prompted us to refocus this study's aim. Our goal was to report and comment on our protocol of synergic work-up between multiple specialists to diagnose and cure patients with pituitary-diencephalic and skull base tumors. We admit that the single actions included in the proposed protocol are not new "per se," indeed the adopted DTI technique and the extended endoscopic endonasal approach for craniopharyngiomas and their outcome have been already reported. However, few reports have focused on the integrated management of these tumors, describing how different skills, from basic science and bio-informatic to neuroradiological, neurosurgical, and neurological expertise, can improve patient outcomes. Indeed, in recent years it is more and more relevant to evaluate the results of skull base tumors treatments not limiting to the oncologic, neurologic, and endocrinological point of view but including the patient's quality of life as well as their return to an active and productive social, lavorative and familiar life. We firmly believe that a multidisciplinary integrated approach to such complex patients is the key to success in pursuing this goal. Therefore, this study is intended to present how all these different skills may be integrated into our center, giving our contribution to this field.

The point has been clarified with the following statements, respectively, in the Abstract and Introduction paragraphs:

"This paper aims to present the protocol implemented in our Institution and highlights the synergistic collaboration and teamwork between neurosurgeons and the neuroimaging team, including neurologists, neuroradiologists, neuropsychologists, physicists, and bioengineers, with the final goal of selecting the optimal treatment for each patient, to improve the surgical results and pursue the advancement of personalized medicine in this field."

and *"In our center, we have integrated multidisciplinary protocols for managing patients with skull base tumors, to provide the most informative description possible and tailor and personalize the surgical plan. Indeed, we would like to show how this protocol can be adopted both in the clinical and*

the research setting for any patient with a skull base tumor to guide the treatment strategy and to improve our knowledge on the brain modifications induced by these lesions."

Moreover, in line with the reviewer suggestion we update the paper's title: *"Role of Diffusion MRI tractography in endoscopic endonasal skull base surgery"*.

We also modified the sentences regarding the limits of application of DTI in skull base surgery, including the Author suggestion that a further limit for the adoption of this neuroradiological technique in this field is the difficulty to visualize white matter extra- parenchymal structure as cranial nerves: "*All these features make diffusion MRI tractography a powerful tool both for pre-surgical planning and intra-operative decision making through neuronavigation systems.*[24]

However, the application of tractography techniques to skull base surgery has been limited for the need for specialized technical knowledge and the time-consuming work-up to optimize diffusion MRI sequence acquisition, analysis protocol, and incorporating tractography results in neuronavigation systems.[25] *Finally, further limitations are due to the technical difficulties extending these analyses from intraparenchymal to extra-parenchymal white matter structures, as cranial nerves. Indeed, only recent studies presented preliminary results attempting to integrate advanced MRI and skull base surgery.*[26-28] ,,

Reviewer #1: Introduction: endonasal approaches are NOT necessarily minimal invasive, in fact the term minimally invasive can ver quite misleading. Also, although for some lesions endonasal procedure do yield a better neurological outcome the evidence about this only applies to discrete number of lesions and the authors should not rely on a appear published more than 20 years ago. For instance, in pituitary adenoma surgery, the use of the endoscope was better only in preserving pituitary function. Endonasal approaches also carry a minuscule risk of seizures, another clear advantage. But certainty about other neurological outcomes is less clear.

Authors' reply: We thank the reviewer for his/her comment. Indeed, we agree that the concept of minimal invasiveness is vague and sometimes misleading. Therefore we followed the Reviewer suggestion, avoiding to use this term both in the title and in the text. The endoscopic endonasal approach is gaining a relevant role in skull base approaches. It permits to resect skull base tumors avoiding any skin incision or possible unaesthetic bone demolition, needing cosmetic reconstruction.
Moreover, it allows reaching some deep-seated skull region, avoiding brain manipulation and retraction and vascular-nervous structures handling. Indeed, the rapid and substantial evolution of their technique in the last decades had led to be considered suitable for resection not only sellar lesions (e.g., pituitary adenomas), but also: suprasellar tumors (e.g., craniopharyngiomas), clival and paraclival tumors (e.g., chordomas and chondrosarcomas), craniovertebral junctions diseases (e.g., basilar invagination), and

intradural neoplasms (e.g., the large number of meningiomas) (Schwartz TH, et al. 2019). Notably, for clival and paraclival tumors, (both with intra- and extradural extension) the endoscopic endonasal approach is considered as the first surgical choice for its improved outcome (Labidi M et al. 2016). Moreover, larger series and comparative studies have pointed out that this approach is characterized by a reduced overall morbidity and faster recovery in comparison to transoral approach for tumoral or non-tumoral craniovertebral junction lesions (Cannizzaro D et al. 2020; Fujii T, et al. 2015; Tubbs et al. 2016). Moreover, an improved visual outcome for craniopharyngiomas and anterior skull base meningiomas has been assessed by recent meta-analyses, and promising results are reported for middle and posterior fossa meningiomas (Cossu et al. 2020; Komotar RJ et al. 2012; Clark AJ et al. 2013; Ditzel Filho LF et al. 2015). Finally, in a recent review of the literature performed by our group, the favorable visual outcome and improved morbidity rate of this approach also for orbital tumors has been demonstrated (Zoli M et al. 2020). All the appropriate literature references supporting the endoscopic endonasal approach for skull base tumors have been added to the manuscript.

Reviewer #1: The notion that a anatomy is important to operate does not need to be reported.
Authors' reply: the notion has been removed.

Reviewer #1: Why is it necessary to tract the CST in an extra axial lesion?
Authors' reply: We apologize for the possible confusion. With the CST, we would like to give an example of the possible application of our protocol. We agree that CST has a limited role in craniopharyngioma and skull base surgery in general. Therefore, we modified the Case Description, reporting our protocol's results on another tract as the visual pathway.
"The visual pathways were the most critical to evaluate in the pre-surgical planning for this patient. The pyramidal tracts were also reconstructed to assess the microstructural correlate of the signal increase detected on the FLAIR T2-weighted image at the level of the right tract."
Moreover, we are improving our protocol for cranial nerve tractography, which is of paramount importance in skull base surgery. This point has been added to the Discussion section with the following statement:
"Finally, this protocol's future perspective is to improve the reconstruction of extra-parenchymal white matter structures, as cranial nerves. Tractography of these structures is currently impaired by the small dimension of the cranial nerves and by the presence of susceptibility artifacts that dramatically reduced the MRI signal for the presence of air and bone. "

Reviewer #1: Minor Concerns:
An extensive language review is needed
Authors' reply: Language has been re-edited by a professional English interpreter and translator.

Reviewer #2:

Manuscript Summary:

This article details the protocol of pure endoscopic surgery for resection of sellar tumors under the guidance of multimodal images. Writing is relatively standard.

Major Concerns:

The protocol involves DTT technology and uses corticospinal tract reconstruction as an example, which I think is unreasonable. For sellar surgery, the reconstruction of the visual pathway may be more clinically meaningful.

Authors' reply: we agree with the Reviewer and we apologize for the confusion. We were aimed to provide an example of DTI reconstruction with our protocol, but we admit that CTS has a marginal role in craniopharyngioma's surgery. The DTI reconstruction of the visual pathway has been added to the Results Section to give a more realistic example of the proposed protocol.

"The visual pathways were the most critical to evaluate in the pre-surgical planning for this patient. The pyramidal tracts were also reconstructed to assess the microstructural correlate of the signal increase detected on the FLAIR T2-weighted image at the level of the right tract."

Reviewer #2 Minor Concerns:

Figure needs to increase the surgical photos of the saddle reconstruction, because this is a very important part of the protocol.

Authors' reply: a figure showing the reconstruction phase has been added (Figure 7).

Reviewer #3:

Manuscript Summary:

This is a protocol for including tractography/ DTI MRI data into surgery planning/ neuronavigation for complex skull base tumors.

Major Concerns:

None

Minor Concerns:

Is the patient´s head fixed in Mayfield clamp? If yes please specify.

Authors' reply: head is not fixed in Mayfield clamp in our protocol, because electromagnetic neuronavigator does not require such fixation. This point has been clarified in the protocol point 12.8 "*Place the patient in a semi-sitting position, the use of Mayfield to fix the head is not needed*".

Reviewer #3: You mention that the preoperatively prepared MRI data are used for neuronavigation but this is not mentioned in chapter 12. or 13?

Authors' reply: the upload and the registration of the pre-operative MRI data into the neuronavigation has been added into the point 12.4-12.7: "*Import the MRI data, including the tractography reconstructions, in the neuronavigation system*", "*Select the brain surgery electromagnetic registration modality on the neuronavigation system*", "*Register the neuronavigation system on the patient, adopting a free-tracking technique or external markers*" and "*Control the accuracy of the achieved registration, checking the position of external markers (i.e. ear or nose) on the imported MRI, if result is not acceptable, repeat the registration.*"

Reviewer #3: There are a few stylistic/ grammatical issues:
Reviewer #3: Page 3, line 100: The sentence is incomplete.
Authors' reply: We apologize for spelling and grammar mistakes in manuscript, which have been corrected. The statement in line 100 has been rephrased as following: *"Moreover, each patient may present different pathology-induced functional reorganization profiles detectable only with diffusion MRI tractography and functional MRI (fMRI), which can be used to provide guidance both in the surgery planning and in the intraoperative steps."*

Reviewer #3: Two references (p4, line 172 and p13, l 597 are formatted in a different style than the others, please adjust.
Authors' reply: References have been corrected.

Reviewer #3: In general, superscripted reference numbers are usually put after punctuation.
Authors' reply: references have been moved after punctuation.

Reviewer #3: Please put a space between numbers and Units (e.g. on page 5).
Authors' reply: a space has been put between numbers and units in all the protocol.

We hope that, after the extensive revision performed, the manuscript is now suitable for publication in *JoVE*. If it is permitted, we would like to update the author's order after reconsidering the submitted paper's contributions.

Looking forward to receiving your decision.

On behalf of all the authors.
Yours sincerely,
Prof. Caterina Tonon