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Editorial
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Neuro-Oncological Aspects in Modern Neurosurgery of 21st Century

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Key Words: Neuro-Oncology ▪ Brain ▪ Spine ▪ Peripheral Nerves ▪ Tumors.

It is a great honor and privilege for me to serve as a guest editor for this special supplemental issue of Acta Medica Academica (AMA) of the Academy of Arts and Sciences of Bosnia and Herzegovina, and to compose this introductory editorial at the invitation of Academician Professor Dr. Husref Tahirovic, the Editor-in-Chief of the Journal.

The "Neuro-Oncological Aspects in Modern Neurosurgery of 21st Century" Symposium was held in conjunction with the Association of Neurosurgeons in Bosnia and Herzegovina and the South East Europe Neurosurgical Society and under the auspices of the Academy of Arts and Sciences of Bosnia and Herzegovina. The Symposium was also endorsed by the Semmes-Murphey Clinic, Memphis, TN, USA, the Department of Neurosurgery, University of Tennessee Health Science Center, Memphis, TN, USA, the Slovenian Neurosurgical Society, and the Croatian Society for Cerebrovascular and Endovascular Neurosurgery. We were very pleased to have had robust participation, discussions, and deliberations among neurosurgeons, neurologists, neuro-radiologists, physiatrists, orthopedic spine specialists, residents in training, and medical students from the United States, Germany, Slovenia, Croatia, Bosnia-Herzegovina, Serbia, Romania, Turkey, and Greece. Among the attendees were the neurosurgical authorities of the Balkans and World—department chairmen and presidents of national and international neurosurgical associations from the region and beyond—who participated in the Symposium. Roughly half of the lectures were selected for publication by the editors of the journal and prepared by the authors. Those papers underwent strict peer-review process as appropriate for any other scientific publication in this Journal.

Neuro-oncology is an important, ever evolving part of neurosurgery (1-3). As such, it involves central (CNS) and peripheral (PNS) nervous systems (brain, spine/spinal cord, and peripheral nerve) tumors. Albeit one of the youngest medical and surgical specialties, neurosurgery enjoys an exciting and privileged role in leading the charge in basic science research on the cellular and molecular level of tumors, epidemiological and outcomes of treatment studies, all complex aspects of diagnostics, and operative and non-operative treatment, including a vast number of technical and technological features and rehabilitation. Not surprising, in this special Supplement Issue of AMA, we are honored to present some of the newest, exciting, and original contributions to the field of neuro-oncology.

Velnar et al. (4) reported an exciting and optimized protocol for glioblastoma (GBM) cell isolation from brain resection samples, with a high yield and low risk for contamination. This isola-

tion technique provides sufficient quantities of isolated cells that may be used as an important new tool for *in vitro* research. The availability of this system will permit the study of cell properties and biochemical aspects and provides the potential of therapeutic candidates for pathological disorders in a well-controlled environment. A single-center experience and outcomes in the treatment of pediatric posterior cranial fossa tumors was presented by Hodzić et al. (5), who reviewed this ever-interesting neurosurgical topic. The authors analyzed clinico-pathological characteristics, treatments, complications, and outcomes in their study population, and they presented their results in treating both common and extremely rare lesions, which will be of interest to the readership who treat children. Bosnjak et al. (6) provided an original and important technical contribution in the treatment of deep-seated lesions of the CNS. To minimize incidences of brain retraction injury, the authors reported on a technical case in which a navigated endoport system was employed with endoscope-assisted microsurgery to resect a lateral ventricle subependymoma. This relatively simple and affordable application for intraventricular and other deep-seated brain lesions will undoubtedly find its place in the treatment of complicated, deep-seated CNS pathologies. Ravnik et al. (7) reported an exceptional retrospective series of their experience using the endoscopic endonasal approach with telemonitoring to reach lesions of the clival region. The authors provided an overview of the technique and analyzed and determined the safety, risk of post-operative complications, and surgical outcomes of this approach in patients in whom pituitary macroadenoma, craniopharyngioma, metastasis, and a prepontine neurenteric cyst were removed. On the subject of treatment of similar lesions in sellar/parasellar/suprasellar tumors, Pojskić et al. (8) (Arnautović group) provided an interesting original and "outside of the box" clinical experience in combining 2 frequently conflicting approaches—microscopy and endoscopy—for the treatment of pituitary tumors. The technical nuances of this multimodal transseptal-transsphenoid surgery are described in detail and an overview is provided of the excellent outcomes the authors experienced with the approach in a rather large cohort of patients.

Porčnik et al. (9) provided an excellent clinical study investigating the use of continuous dynamic mapping of the corticospinal tract in removing tumors from motor eloquent regions of the brain. The technique employed both continuous dynamic mapping with motor-evoked potentials to monitor patient motor function, which allowed for higher rates of gross total resection. This important study will further help in improving the safety of surgery in the proximity of the corticospinal tract. An extremely interesting cross-sectional study on the effect of antiepileptic treatments (carbamazepine and lamotrigine) on the bone health of patients with tumor-initiated seizures was provided by Mehičević et al. (10). Patients taking antiepileptics were found to have lower bone mineral density versus controls, which means that patients from this cohort should have their bone health carefully evaluated and monitored considering the risk of osteoporosis or osteopenia. This article will be of great interest to neurosurgeons, neurologists, and orthopedic surgeons who treat patients suffering from central nervous system (CNS) tumors and who are frequently at risk of developing osteoporosis and various bone fractures. Another retrospective clinical series by Ravnik et al. (11) analyzed the safety, efficacy, and surgical outcomes of an extensive posterolateral 1-step approach for very frequent neurosurgical problem—giant spinal epidural tumors. The authors' procedure included costotransversectomy, laminectomy, corpectomy, tumor resection, spinal column stabilization, and reconstruction to provide a safe and effective approach that balances the needs for invasive surgical access with sufficient exposure of the surgical site for both tumor resection and spinal column reconstruction. Matić et al. (12) (Rasulić group) provided a thorough personal experience and review article of peripheral nerve tumors, including recent advances in diagnostics, differential diagnostics, pathology, treatment option, and prognosis. While the goal of removing nerve tumors and preserving nerve function at the same time is

complicated and difficult, excellent results can be achieved with carefully planned surgery and superb technique. The article benefits from numerous color illustrations and provides an interesting read for beginners to neurosurgery to advanced peripheral nerve surgeons.

We are hopeful readers will enjoy this Supplement Issue of AMA and find it interesting and informative.

Acknowledgement: We appreciate the very hard work and efforts of both Academician Prof. Dr. Husref Tahirovic, Editor, and Ms. Nerma Tanovic, the Secretary of the Journal Acta Medica Academica, for their work on the supplement. We are also indebted to Academician Professor Dr. Lidija Lincender-Cvijetic, the Vice-President of the Academy of Arts and Sciences of Bosnia and Herzegovina, for her enthusiastic contributions and help conducting the "Neuro-Oncological Aspects in Modern Neurosurgery of 21st Century" Symposium, which was held on January 31, 2020 in Sarajevo. It is from the Symposium that the idea and content of this supplement originated. Finally, Mr. Andrew J. Gienapp of the Department of Neurosurgery, University of Tennessee and the Neuroscience Institute of Le Bonheur Children's Hospital, Memphis, TN served as a Guest Language Editor and provided an invaluable contribution to the supplement.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Original Scientific Article
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The Isolation of Human Glioblastoma Cells: An Optimised Protocol

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Abstract

Objective. The aim of this study was to establish an optimised protocol for glioblastoma (GBM) cell isolation from brain resection samples, with a high yield and low risk for contamination. **Methods.** Human GBM cells can be obtained following cranial tumour operations. In sterile conditions, the fragments of viable tissue removed during surgery were collected. The tissue was cut and mechanically coarsely decomposed. The sediment was harvested after centrifugation, the cells were seeded in suspension, and supplemented with a special medium (Advanced DMEM) containing high level nutrients and antibiotics. **Results.** In an appropriate environment, the isolated cells retained viability and proliferated quickly. Attachments were observed after ten hours, and proliferation after two days. The time to full confluence was about one week. The cells were stable. Under standard culture conditions, cell proliferation and cluster formation were observed. Cell viability was 95%. **Conclusion.** The protocol described for isolation is easy, quick and affordable, leading to stable GBM cells. The isolation technique provides sufficient quantities of isolated cells that may be used as an important new tool for *in vitro* research. The availability of this system will permit the study of cell properties, biochemical aspects, and provides the potential of therapeutic candidates for pathological disorders in a well-controlled environment.

Key Words: Human Cell Culture ▪ Glioblastoma Cell Isolation ▪ Isolation Protocol ▪ Glioblastoma.

Introduction

In recent decades, cell models have become increasingly important for the *in vitro* study of physiological and pathophysiological processes. To explore the mechanisms of neurotrauma, tumorigenesis and neurodegeneration, *in vitro* organ culture systems with live neural cells are becoming highly appealing (1, 2). Under specific conditions, the isolated cells may be maintained outside the body, in an *in vitro* environment, and included in various cell models. Since the first attempts at tissue and cell culture development in the early 20th century, which included the method of explantation, isolation techniques have greatly improved (3, 4). *In vitro* cell cultures and functional cell models are thus becoming an essential research tool. Moreover, the employment of cell cultures is becoming widely recognized due to the decreasing tendency towards animal tests and subsequently lower expense (5-7).

As glioblastomas are the most prevalent primary malignant brain tumours in the adult population, glioblastoma cells are in the focus of research (6, 8). Glioblastoma is a universally lethal disease with no effective therapy. From the biological point of view, it is a very heterogeneous tumour, displaying all the characteristics of cancer, with substantial variability among patients. One of the clinical hallmarks of glioblastoma is a high degree of invasiveness, which is evident from the extensive infiltration of the tumour into the surrounding brain parenchyma. The malignant glioblastoma cells can cross tissue barriers and invade the neighbouring tissue as individual cells or collectively, by remodelling the extracellular matrix and their cytoskeleton (6, 9). The treatment of glioblastoma is aggra-

vated, and currently includes maximally safe surgical resection, supplemented with radiation therapy and chemotherapy. In spite of combined treatment modalities, the mean overall survival is still only 21 months, depending on many patient- and tumour-related factors. The mechanisms leading to glioblastoma formation, invasion and treatment are gradually being understood in *in vitro* conditions and animal models. These results, however, have not yet been translated into clinically significant therapeutic approaches, despite the ongoing progress (6, 8). For *in vitro* research, numerous new and improved methods of isolation have been used and new ones are being developed, in order to expand and optimise the research possibilities (6, 8-10).

The preclinical glioblastoma models currently used in experimental practice are classified into three categories: genetically engineered mouse models, xenografts and syngenic murine models. Every model has its own advantages and disadvantages (11, 12). The preclinical mouse models, which are genetically engineered, are essential for the development of new therapies for glioblastoma treatment. These are used for analyses of glioblastoma biology, evaluation of new therapeutic strategies, and identification of therapeutic targets. Genetically engineered mouse models harbour mutations in the components of the main signalling pathways that are altered in human glioblastomas. These genetically engineered mouse models display a long latency to tumorigenesis and advanced tumour heterogeneity, which represents a challenge in preclinical drug testing. An orthotopic mouse model of glioblastoma is constructed by transplanting brain tumour cells derived from glioblastoma into the brain of syngeneic mice. This model develops a glioblastoma with similar features to the human disease. These include aggressive invasion of tumour cells and a high degree of vascularisation. It is frequently used for testing the therapeutic agents used in clinical trials for glioblastoma treatment, representing a valuable preclinical system for advancing present therapies, and for testing novel drugs and drug combinations against glioblastomas (12, 13).

The xenografts are classified into two categories: I) glioblastoma cell-line xenografts and II) patient-derived xenografts. The former exhibit the advantages of high engraftment and growth rates, but it is doubtful whether glioblastoma cell-line xenografts reflect the true biological nature of glioblastomas. The patient-derived xenografts preserve both the histological and genetic features of the primary tumour. As a result, they represent good preclinical models in glioblastoma research. However, they cannot fully reflect the host's anti-tumor immunity in human glioblastomas (12-14).

Glioblastoma Cell-Line Xenografts

Commercially available glioblastoma cell lines, including T98G, A172, U87 and U251, are the most common models used both *in vitro* and *in vivo*. These cell lines were derived from glioblastoma patients and are cultured in a serum-containing medium and then xenografted into immunodeficient mice. Glioblastoma cell-line xenografts exhibit the advantages of high engraftment and growth rates, reliable tumour growth and progression, and good reproducibility (12-15). The immortalized cell lines can be readily expanded for an unlimited number of passages *in vitro*, yielding a large number of tumour cells for experimental use (12). On the other hand, glioblastoma cell-line xenografts do not reflect the clinical characteristics of the original patient-derived tumours, and do not exhibit the tumour necrosis, microvascular proliferation, cell invasion and expression of integrin molecules, differing phenotypically and genotipically from the original patient tumours. Since it is possibly that glioblastoma cell-line xenografts do not reflect the true biological nature of the glioblastomas, this may present a disadvantage in preclinical trials (12, 14-16).

Patient-Derived Xenografts

Patient-derived xenografts, on the other hand, have been a recent focus of glioblastoma research and are being used extensively. They are established by injecting glioblastoma tumour spheres produced under serum-free neurosphere-culture conditions, into immunodeficient mice. The tumour spheres

have some advantages over glioblastoma cell lines in *in vitro* conditions. These models retain both the genetic and histological features of the primary tumour from which they were derived. Patient-derived xenografts cells are not subject to the stresses that can arise in cell cultures (17-20). Additionally, their molecular profile is stable over time, they are tumorigenic, and the tumour spheres retain the molecular profile, which is similar to that of the patient's original tumour (21-24). The disadvantage is that not all human glioblastomas can be successfully cultured as tumour spheres (12, 25).

The aim of the study was to establish a relatively quick and easy protocol for the isolation of glioblastoma cells from brain resection samples, with a high yield and a low risk for contamination.

Materials and Methods

The Source of Tissue

The tissue for glioblastoma isolation was obtained from adult patients following elective cranial tumour surgery. The permission for human brain tissue use was obtained from the ethical committee, and written informed consent was acquired from the patients before the experiment. The cells were isolated from samples taken from various tumour parts: I) from the resection margin on the border to macroscopically normal brain tissue, II) from the superficial tumour parts, III) from deep inside the tumour (approximately 1 cm from the tumour surface) and IV) from the tumour core. Surgically, subtotal resection was performed. Our samples exhibited the typical microscopic and macroscopic glioblastoma features: necrosis, hypercellularity, nuclear atypia, haemorrhages, thrombosed tumour vessels and vascular proliferation. Altogether, there were five donor patients. In every experiment and for every patient-donated tissue, the isolation procedure was the same as described. A fresh-frozen section during the operation confirmed the tissue as glioblastoma, WHO IV. The resected tissue specimen was examined in the operating theatre, and some of the viable tumour tissue was used for the isolation. The necrotic and haemorrhagic parts were not used for cell isolation and these were macroscopically removed during the tissue preparation in the operating theatre. The sample size of the tumour tissue varied between 2 and 3 cm^3. These tissue specimens corresponded to the cortical and subcortical regions. In sterile conditions, fragments of viable tissue removed during the operation were collected, stored in 20 ml of Advanced DMEM medium, supplemented with 100 IU/ml penicillin, 0.1 mg/ml streptomycin, 2 mM L-glutamine, and immediately taken to the laboratory.

Reagents

All materials and chemicals used were of laboratory grade. The advanced DMEM cell culture medium was purchased from Thermo Fisher Scientific (Waltham, Massachussets, USA).The heat inactivated foetal bovine serum was acquired from Gibco (by Thermo Fisher Scientific, Waltham, Massachussets, USA). The penicillin, streptomycin, L-glutamine, phosphate-buffered saline (PBS) and trypsin/EDTA were procured from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). All other chemicals were obtained from common commercial suppliers.-

Preparation of Tissue for the Cell Culture

The experiment was performed in a 2nd degree biosafety level cell laboratory. After delivery, the tissue fragments were stored in PBS containing 100 U/ml penicillin and 0.1 mg/ml streptomycin. In sterile conditions, the tissue specimen was examined again. The macroscopically necrotic parts were discarded. The blood was removed by washing the sample with the saline and trypsin/EDTA, and centrifugation, where the coarse parts and contaminates were removed. The viable part of the tumour was washed three times with PBS. The PBS was decanted and 0.25% trypsin/EDTA was added. The tissue was submerged in trypsin/EDTA throughout, in order to prevent desiccation. The tissue was then cut into small pieces (<1 mm^3) to achieve coarse mechanical decomposition. After one-hour incubation in a controlled atmosphere

at 37° C, 5% CO_2, the Advanced DMEM medium was added and the suspension was transferred into centrifuge tubes. Centrifugation at 200G-force for 5 minutes followed. The sediment was resuspended in 14 ml of cell culture medium, containing penicillin (100 IU/ml), streptomycin (0.1 mg/ml), L-glutamine (2 mM) and 5% FBS, and then plated in two T25 tissue culture flasks. The resulting cell suspension was incubated for two weeks at 37° C in 5% $CO_{2,}$ leading to the preferential proliferation and survival of glioblastoma cells. The medium was replaced twice weekly.

The Culture of Primary Glioblastoma Cells

Primary glioblastoma cells were routinely cultured in T25 flasks and incubated at 37° C in a controlled atmosphere with 5% CO_2. The density for cell culturing was 800000 cells for T25 flasks. After one week in the culture, they became 100% confluent and were split in a 1:3 ratio with 0.25% trypsin-EDTA. This was followed by centrifugation at 200G-force for 5 minutes. The cell sediment was resuspended in 21 ml of fresh medium with 5% FBS. The cell suspension was transferred to three T25 flasks. The cultures were then incubated, and growth was monitored. In this way, the cell culture of the first passage was obtained. The first passage cells were grown for five to seven days, until 100% confluent. Additionally, some cultures were frozen and then thawed again. The growth was monitored under an inverted microscope.

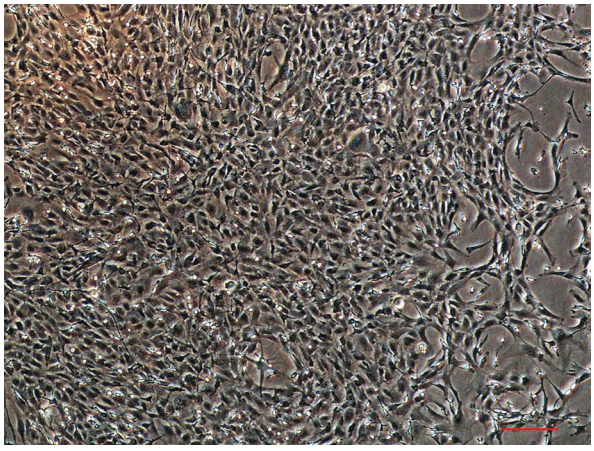

Figure 1. Primary glioblastoma cells. One week after isolation, glioblastoma cells completely cover the surface of the flasks, with the formation of strong intercellular connections (100% confluence). Images were taken at x40 magnification on a Nicon Diaphot 300 inverted microscope. Scale bar = 100 µm.

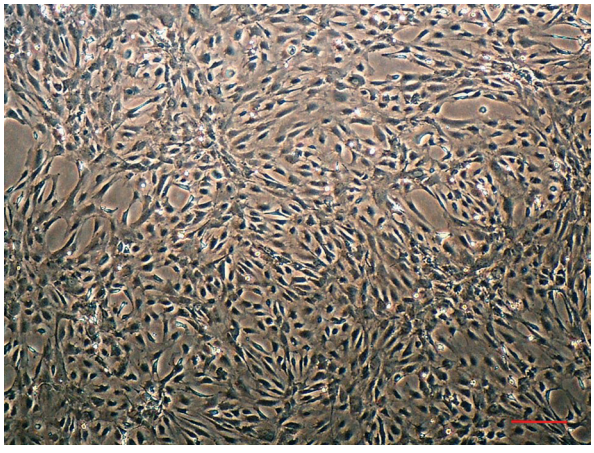

Figure 2. Glioblastoma cell culture in the first passage. One week after the first trypsinization, the glioblastoma culture was 90% confluent. Images were taken at x40 magnification on a Nicon Diaphot 300 inverted microscope. Scale bar = 100 µm.

Results

The human glioblastoma primary cultures described in the experiment consisted of rapidly growing cells that were isolated from the tumour of adult donors. The primary glioblastoma cultures were 100% confluent after one week. The cells were then split in a 1:3 ratio and transferred to culture flasks. Attachments were observed after ten hours and marked proliferation followed after two days. After five to seven days, a 90% confluent culture of the first passage was obtained. We grew the cultures up to the tenth passage. Some of these cells were stored in liquid nitrogen. Viability of 95% was observed when the cells were thawed and reseeded. These cultures also grew normally after plating (Figure 1 and Figure 2).

We performed this protocol five times, since we had five donor patients. The tissue sources came from glioblastoma patients and were taken during elective surgery. In every experiment and for every patient-donated tissue, the isolation procedure

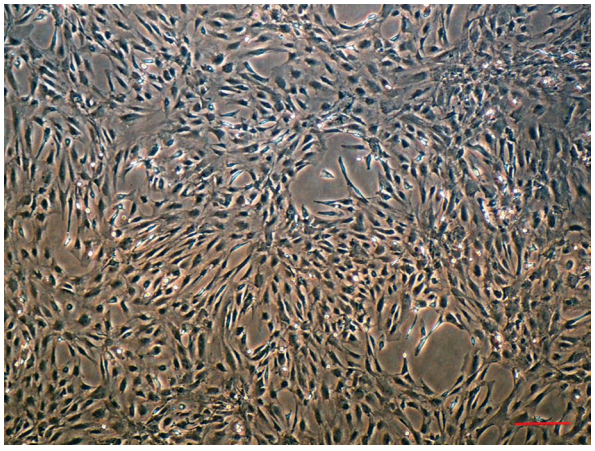

Figure 3. Glioblastoma cell culture in the first passage, isolated from the second patient. One week after the first trypsinisation, the glioblastoma culture was 90% confluent, as was the case for the first patient. Images were taken at x40 magnification on a Nicon Diaphot 300 inverted microscope. Scale bar = 100 μm.

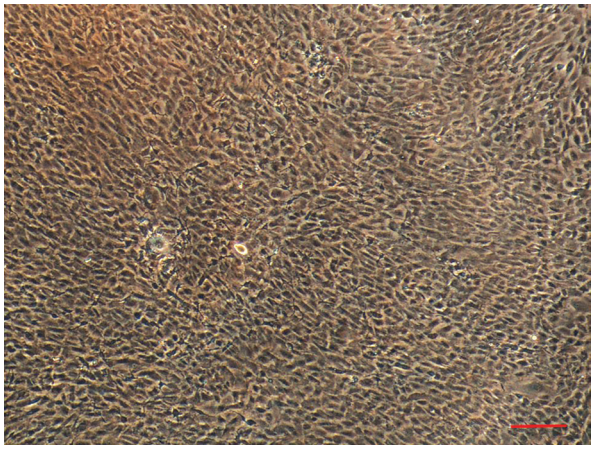

Figure 4. A fully confluent GBM culture. A characteristic polymorphic appearance can be noted. The nuclei display various shapes with scarce cytoplasm. Nicon Diaphot 300 inverted microscope. Scale bar = 100 μm.

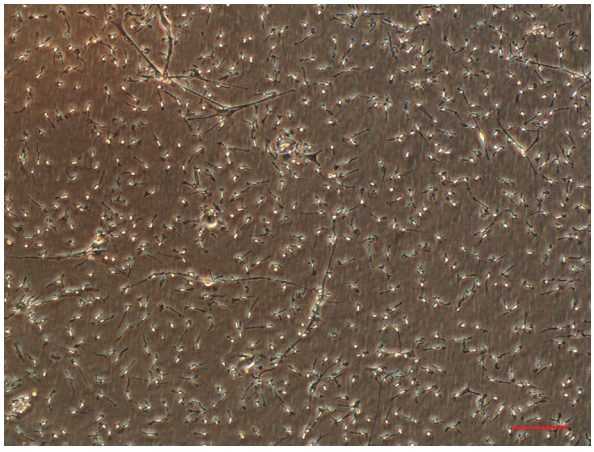

Figure 5. The cells started to attach to the substratum approximately ten hours after seeding. Some cells are still rounded and some exhibit elongated processes. Various cell shapes can be observed. Nicon Diaphot 300 inverted microscope. Scale bar = 100 μm.

was the same as described, leading to the isolation of the glioblastoma cells. The growth characteristics were very similar in all five isolations (Figure 3). According to these results, we think that the method, described in the protocol, was reproducible. Additionally, the cells were frozen and stored in liquid nitrogen, and then thawed and seeded again. These cells also grew normally.

Examination of the morphological properties of these cells showed a distinctive appearance that changed depending on the cell seeding density. The characteristic nucleus shape was polygonal to round, and triangular with scarce cytoplasm. The shape of the cells varied during the attachment process and growth, from round or oval to polygonal, which was characterised during the growth in confluent culture. After 24 hours, most of the cells were attached to the substratum. The shape alterations from round to polygonal were visible at that time. The cells grew well and were easy to maintain in culture, which had a cobblestone appearance, with defined cell borders. The average time to the formation of the confluent culture was one week (Figure 4 and Figure 5). Then, the growth did not stop due to the lack of contact inhibition and the cells started to accumulate in multiple layers. This is a typical growth property of cancer cells. Some of the patients' tumour and cell culture characteristics are presented in Table 1.

Table 1. The Patients' Tumour and Cell Culture Characteristics

Patient	Diameter*	Location	Sample†	Time‡	Number§	Per cent‖	Cell¶	Cell**
1	4/1	Right frontotemporal	3	10	800000	90	11	48
2	5/1	Right frontal	2.5	20	800000	90	9	52
3	4/1.5	Right temporal	2	15	700000	88	10	54
4	3/0.5	Right frontal	2	20	750000	90	10	48
5	6/2	Right frontotemporal	3	10	850000	94	9	46

*Approximate tumour diameter/necrotic core size (cm); †Sample size (cm^3); ‡Approximate time to cell laboratory (min); §Number of cells obtained after dissociation (for a T25 flask); ‖Percentage of confluence after one week; ¶Cell attachment (hours after seeding); **Cell proliferation (hours after seeding).

Discussion

Glioblastomas are the most common and malignant primary brain tumours in the adult population (9). They are a heterogeneous malignancy, composed of all glial cell types from the central nervous system. In addition to the inter-patient differences in glioblastoma tumours, there is also a well-known intratumoural cellular heterogeneity. This biological heterogeneity is also evident in its resistance to pharmacology, radiation and surgery, and may result in tumour recurrence (26). Since they contain multiple subclonal mutations, this makes them highly adaptable entities, resistant to all therapeutic approaches used today (5, 26-28). It is almost impossible to remove a glioblastoma surgically or treat it completely with radio- and chemotherapy. Despite their resistance to therapy, numerous drugs have shown promising results in preclinical and clinical studies. As a result, primary cultures of glioblastoma cells represent an important target for basic and translational neuroscience research, especially for *in vitro* cell models (4, 10, 29).

There have been various reports about glioblastoma cell culture isolation, each with a different technique, and advantages and drawbacks (30-33). We have developed a simplified protocol for enriched primary glioblastoma culture, derived from neurosurgical patients. In the experiment, we used resection specimens from glioblastomas, removed from the right frontotemporal lobes. All samples obtained were excess tumour tissue that corresponded to cortical and subcortical areas. Care was taken to remove the tumour necrosis, and to utilise the vital part of the tumour, which was then used for the culture preparation. This is one of the important steps in establishing a cell culture, since the necrotic parts contain few viable cells and this may lower the cell yield.

The majority of glioblastomas that are removed during surgery are taken for histological examination. We used small resection specimens for the experiment. The tissue was taken from various tumour parts, in order to ensure the most representative samples, since glioblastomas are composed of heterogonous cell populations (22-24). The macroscopically necrotic parts were avoided, since these were not suitable for cell isolation. During the isolation process, it was necessary to develop an effective technique for maintenance of the cell culture. This is often complicated and challenging, and it may take a long time to establish an efficient and reliable culture (3, 32). Our protocol was simplified, not involving neurospheres or flow cytometry for cell isolation and the culturing or density separation processes. The tissue was only involved in coarse mechanical degradation and exposed for a short period to trypsin/EDTA in order not to damage the cells to a large extent. Then, immediate plating followed. With a limited degree of tissue degradation and fast culturing, it is possible to obtain a large number of viable cells, allowing the majority of them to attach and proliferate further. We used trypsin for enzymatic digestion, which was necessary in the beginning, when the tissue was brought to the laboratory. We had superior results with trypsin use, since the tissue decomposed better, and the cell yield in the subsequent steps of the isolation process was higher. The unattached contaminant cells were removed in the course of

culture growth, during medium exchange and gentle washing of adherent cells. Other isolation protocols described so far used more complicated methods for establishing the culture, especially in the tissue decomposition procedure (22, 23, 31-35). With every additional step in isolation, the cells are exposed to potential damage, and thus the cell yield drops. Our cell yield was high, with a 100% confluency after one week and viability of 95%.

The data were compared to other research protocols, and we saw the following advantages and disadvantages of the described method: I) The tissue source for isolation came from adult donors. This means that the tissue is easily accessible, since there are many more surgical procedures that may provide tissue for experimentation; II) The transport to the laboratory may vary and is usually long in brain samples taken during surgery. They are first brought to the pathology department and the tissue is separated there. The parts are usually taken to the cell laboratory as a secondary priority. This transportation time is typically less than two hours (36, 37). In our case, the tissue transportation time was up to 20 minutes. The specimen for the cell laboratory and for pathology examination were already separated in the operating theatre, shortening the time to isolation, and improving the quality of the tissue taken. This resulted in a better cell yield during isolation in the cell laboratory; III) In our protocol we observed that it was not necessary to dissociate the brain tissue to a high extent in order not to lose cells during fine mechanical decomposition. Only coarse mechanical dissociation and a brief period of trypsin addition sufficed, causing less mechanical damage to the cells, thus improving their survival and the subsequent cell yield. It is this process that leads to the high cell survival rate, proliferation and good growth during the isolation procedure; IV) The storage and the transport procedure are extremely important, not only the time needed to the laboratory. In our case, the fragments of viable tissue were collected after surgical resection. They were stored in the Advanced DMEM medium with supplements, and brought to the laboratory immediately; V) The tumour parts chosen for the isolation were carefully selected during surgery. The tissue in our experiment was not taken unselectively from the tumour. We avoided taking the necrotic parts, as these do not add to the cell isolation yield. Only macroscopically viable tissue was taken. Necrosis may contaminate the cell suspension during the preparation, and interfere with cell growth during the isolation. Additionally, the contamination risk is higher; VI) Only 5% FBS was used, as the cells were also successfully isolated with the Advanced DMEM. Other studies used different media and therefore, the addition of 10% FVS was necessary; VII) Since the tissue was taken from the tumour, the tumorigenic nature of the isolated cells can be confirmed. The samples were taken from the tumour areas verified by neuronavigation and 5-aminolevulinic acid (5-ALA), so any doubt that healthy tissue was obtained was excluded. Additionally, it was surgically evident that the rested tissue was pathologically altered. Again, necrotic pars were excluded.

In the culture, the isolated cells grew rapidly. They were composed of firmly adherent cells of various morphologies. The culture became 90% confluent after one week, and it was possible to grow cells until the tenth passage. Although we did not cultivate them further, we assume that the cells would grow longer due to their cancer-like characteristics. After plating, attachments were observed after 10 hours, and marked proliferation followed after two days. The time to full confluence was about one week. The cells grew normally even when frozen and thawed again. When observed under a light microscope, the typical characteristics of glioblastoma cells were observed during growth. The cells were pale-staining and polymorphic, displaying round to oval or lobate nuclei, with little visible cytoplasm. The cells were grown in flasks in a single layer and after the formation of a confluent culture, the growth continued, which is characteristic for cancer cells, due to the loss of contact inhibition (7, 35). When the confluent layer was formed, the cells began to accumulate in domes, or grew in several layers. Our isolated cells exhibited a high proliferation index, which enabled the rapid formation of a confluent culture.

Due to their fast growth, the cultures required continuous monitoring of the growth conditions, like other rapidly growing cells.

During the cell isolation procedure, doubt always exists that the cells in culture may not be the target cells. It is true that when preparing the cell isolation from the brain, other contaminant cells may be present, including microglia (38, 39). The separation of microglia is done by mechanical separation using the physical properties of these cells (38, 40-44). In our case, microglia were removed when changing the medium by washing the attached cells, and removing and discarding the loosely adherent cells. It is possible to reduce the amount of microglia to to 5% or less, and according to some authors to less than 1% (39, 45, 46). The isolation process of glioblastoma cells differs from the isolation of oligodendroglia and astrocytes. It also differs from the isolation of microglia (36, 39, 47). All these protocols are more complex, and the cells are not so easily established in the culture. In our experiment, we did not observe any additional cell layer over the glioblastoma cells during the culture growth. It is probable that the contaminant cells were removed during the medium change, culture washing and in the subsequent passages. Additionally, glioblastoma cells proliferate faster than healthy oligodendrocytes and astrocytes, and the cells that are a minority in the cell culture are lost from the culture during the growth of other cells. Moreover, glioblastomas are heterogeneous tumours with mixed cell populations. It is believed that there are three cells for the origin of glioblastomas: oligodendrocyte precursor cells, neural stem cells (NSCs) and NSC-derived astrocytes and oligodendrocyte precursor cells (OPCs). In such cases of heterogeneous tumours, the contamination of glioblastoma tumour cells by microenvironmental components, such as macrophages and astrocytes, is also not important, since all the constituents are malignant and neoplastic (48).

In this study, we have described only the protocol from the tissue specimen to the cell culture. This was a new and improved protocol for a highly enriched glioblastoma culture from adult human brains. The isolation technique is quick, easy and cost-effective, and provides sufficient quantities of isolated cells. The cell proliferation rate was rapid, enabling us to reach a 100% confluent culture after one week. Later on, during the first passage, a 90% confluent culture was obtained after one week. Viability of 95% was observed. The protocol by itself is quick and relatively easy, and also the reagents used are of a standard type, which are easily obtainable. An important observation was that the cells did not need supplementation with 10% FBS, since we used Advanced DMEM. This type of medium was sufficient for the growth conditions. Since this is classified as a less-serum medium, a lower quantity of FBS is needed to supplement the mixture for cell growth. Advanced DMEM is a rich medium by itself, and therefore no additional increments of FBS are needed. When using other types of media for cell experiments, FBS is supplemented according to the protocols employed, the cell needs and the composition of the media used. Additionally, the price of the reagents was advantageous, making the possibilities for isolation processes more accessible. The intention of this article is to present an improved isolation protocol or isolation technique for glioblastoma cells *per se*. At the time of writing, we have performed this protocol five times, since we had five donor patients. The isolation procedure was the same as described, and the growth characteristics were very similar in all five isolations. According to these results, we believe that the method described in the protocol is reproducible. A very important point that needs to be taken into account in future GBM cell isolation is the need for further genetic and phenotypic characterization of the isolated cells, which was not done in our case.

Examination of the morphological properties of the growing culture illustrated that glioblastoma cells demonstrated a high degree of cellular division and high plasticity. The cells would make a useful model for research into glioblastoma treatment, and may provide a useful basis for *in vitro* studies.

Conclusion

The isolation protocol described is simple, quick and economical, leading to a viable long-term glioblastoma cell culture. The cell culture generated in this manner employs a very simple media-based culture technique that takes advantage of the adherent properties and proliferative potential of these cells, which makes them appropriate for almost all types of in vitro studies. The availability of an experimental system with glioblastoma cells will permit the study of cell properties, biochemical aspects, and the potential of therapeutic candidates in a well-controlled environment, using a human glioblastoma cell culture.

What Is Already Known on this Topic:

Glioblastomas are the most prevalent and malignant primary brain tumours in adults and glioblastoma cells are an important focus of research. For this purpose, in vitro functional cell models, employing glioblastoma cells, are highly appealing. Several methods of isolation have been used, and new ones are being developed, in order to expand and optimise the research possibilities.

What this Study Adds:

We have presented an improved technique for creating a highly enriched glioblastoma culture from adult human glioblastoma patients. The protocol for isolation described is easy, quick and affordable, leading to a stable glioblastoma cell line. Isolated cells may be used as an important new tool for in vitro research.

Authors' Contributions: Conception and design: TV and LG; Acquisition, analysis and interpretation of data: TV and LG; Drafting the article: TV, UM and LG; Revising it critically for important intellectual content: UM, RB and LG; Approved final version of the manuscript: TV, UM, RB and LG.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Combined Microsurgical, Endoscopic and Neuronavigation Assisted Transseptal-Transsphenoidal Resection of Pituitary Tumors

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Abstract

Objective. To describe the technical nuances of multimodal transseptal-transsphenoid surgery for pituitary tumors using a combination of microneurosurgery, neuroendoscopy, and electromagnetic neuronavigation. **Materials and Methods.** A transnasal approach to the sella is performed endoscopically and widely exposed by an otolaryngologic surgeon. Surgery is next performed by the neurosurgeon with microscope and neuronavigation for microsurgical resection of pituitary tumors. Neuroendoscope is also used at the end of surgery to confirm tumor resection and inspect operative site. During surgery, the patient's head, angle and height of the microscope, and position of the table are repositionable to allow for multiple angle views. Abdominal fat harvested prior to the procedure is used to ensure cerebrospinal fluid seal. **Results.** The senior author (KIA) has used the combined approach with 84 consecutive patients. Radical resection was achieved in 66 patients, subtotal in 11, and partial in 7. There were no perioperative complications. Six patients experienced postoperative transient diabetes insipidus. The pituitary gland and stalk were preserved in all cases. Visual symptoms were improved in 78% and endocrinological symptoms in 56% of cases. **Conclusion.** This combined approach is safe and effective. It increases the efficacy and radicality of surgical resection, helps to preserve the pituitary gland, and improves and resolves preoperatively altered patient hormonal function and impaired vision. It also reduces complications, provides less postoperative pain and discomfort, reduces the surgery time, and enables a shorter hospital-stay.

Key Words: Pituitary Tumor ▪ Combined Microsurgical and Endoscopic ▪ Transseptal ▪ Transsphenoid Approach ▪ Electromagnetic Neuronavigation.

Introduction

Transsphenoidal microsurgical resection has been long established as the gold standard treatment for pituitary tumors. Use of the endoscope has gained increasing popularity in the past 20 years and has been described by its advocates as being less invasive and providing better visualization of the surgical field than the microscope (especially "around the corner"), except for sacrifice of the middle turbinate (1, 2).

Although endoscopic and microscopic techniques for pituitary lesion resection are often portrayed as competitive approaches, we believe they are complementary and that both should be used to optimize the minimally invasive surgical technique. The addition of electromagnetic neuronavigation, which allows free head movement, enhances the benefit of these techniques. Furthermore, a combined interdisciplinary transseptal-transsphenoid surgery (TTA) to the sella, which involves a neurosurgeon and an otolaryngologic (ENT) surgeon, minimizes the complication rate.

All techniques described above have been used previously and are well described in the literature

with good results. Any 2 of the 3 techniques used in combination have also been described previously in the literature. However, the use of all 3 techniques combined have shown good results in our experience.

Herein, we describe our experiences and original technical steps of combining these techniques in the surgical workflow.

Materials and Methods

Appropriate Institutional Review Board (IRB) Approval was obtained prior to study. Preoperative evaluation included detailed neurological, neuroradiological, ophthalmological, and endocrine assessment. We included all patients presenting with sellar or supra/parasellar tumors who were operated on using this technique. Magnetic resonance imaging (MRI) of the head was obtained with 2 mm thin cuts, using navigation system protocol to accurately delineate the sellar region and surrounding structures (pituitary imaging protocol [PIP]). The MRI-Pituitary protocol +/- contrast was done prior to surgery, 6 weeks after the surgery, and every year subsequently. In the event that the patient cannot have an MRI, a computerized tomography (CT) scan with contrast (PIP) can be used. A neuro-radiologist evaluated MRI scans independently and their reports were used for assessment. Full endocrinological pituitary hormonal workup was done by a neuro-endocrinologist (MNQ) prior to surgery, immediately postoperatively in the hospital, and then every 6 and 12 months, respectively, or more frequently as needed. Full ophthalmologic workup was done by a neuro-ophthalmologist (JL) preoperatively, and then every 6–12 months postoperatively, or as needed.

Operative Technique

The patient was placed supine on the operating table with the head elevated approximately 15° on a donut sponge headrest. The head was unattached and free to move in all directions to improve visualization as rigid fixation limits intraoperative manipulation of the patient's head.

An AxiEM neuronavigation system (Medtronic, Minneapolis, MN, USA) was used for electromagnetic navigation. A patient tracker (cranial dynamic reference frame) was put on the ipsilateral forehead. Once the patient was registered according to the MRI or CT imaging loaded into the system, a tracker was mounted on the endoscope rod using the tip of the rod as a navigation tool. Likewise, surgical instruments can be registered and calibrated.

Neuronavigation enabled precise localization, especially in complicated cases that transgress compartments, for recurrent cases, or in patients who underwent previous nasal surgeries in which

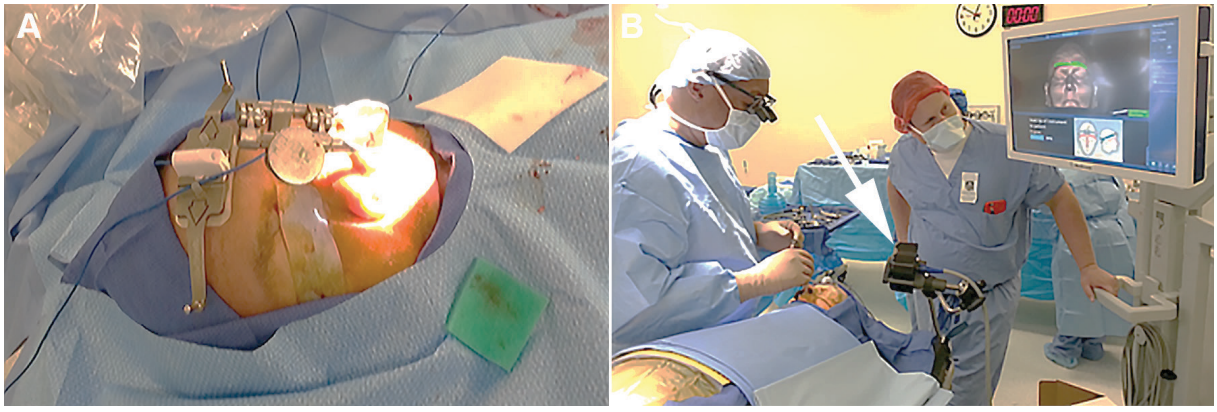

Figure 1. Operative setting for transsphenoidal combined microsurgical and endoscopic approach. (A) The patient tracker is placed on the forehead. (B) The AxiEM magnetic field emitter (white arrow) was fixed on the side of the surgical table directly and adjacent to the head of patient to obtain a stable magnetic field covering the head. The patient is registered according to MRI images, which are loaded into the system.

the anatomy was distorted due to scar tissue, there was a narrow distance between the carotid arteries, and the sphenoid sinus was poorly pneumatized (Figure 1).

A transnasal approach to the sella was performed by the ENT surgeon (NSB). Before the surgery, the nasal mucosa of the cartilaginous septum was infiltrated unilaterally with a 1% lidocaine solution containing 0.05% of adrenaline to reduce bleeding and facilitate dissection of mucosa from the septum. The patient's lower face was prepped and draped in sterile fashion. At the same time, the right abdominal region was prepped and draped for an abdominal fat graft harvest used to prevent cerebrospinal fluid (CSF) leak when closing the surgery site.

A hemitransfixion incision was made just posterior to the columella (usually on the left), and the septal mucosa was dissected from the adjacent cartilage and bone. The contiguous floor of nose mucosa can be elevated as well for better exposure. The bony/cartilaginous junction was divided and the mucoperiosteum was elevated on the contralateral side. The perpendicular plate of the ethmoid and the vomer bone were removed, exposing the sphenoid rostrum. Visualization for this portion of the procedure was facilitated with the use of a nasal endoscope (Karl Storz, Tuttlingen, Germany or Zomed/Medtronic, Minneapolis, MN, USA). A pituitary speculum was then inserted in the nose, separating the septal mucosa, facilitating optimal exposure of the rostrum, and facilitating bimanual instrumentation. The sphenoid sinus ostia were identified and used as a point of entry into the sphenoid sinus. The entire rostrum was then removed using Kerrison rongeurs and endoscopic sinus instruments, thus maximizing exposure of the operative field. Although the neuronavigation system helps identify the trajectory to the sellar floor, neuroanatomical knowledge of the region - such as the carotid prominences - is essential for establishment of safe working zone (Figure 2).

When the sellar floor was reached and bone widely opened, surgery was next performed by a neurosurgeon with microsurgical technique. At this point, the microscope was brought in (Kinevo 900 or Pentero 900, Zeiss, Oberkochen, Germany). The surface of the cavernous sinus was exposed bilaterally as needed. The dura was opened in an "X" shape. Microsurgical resection of the pituitary tumor was usually performed using curettes of various angles and sizes, bipolar electrocautery, and micro-forceps.

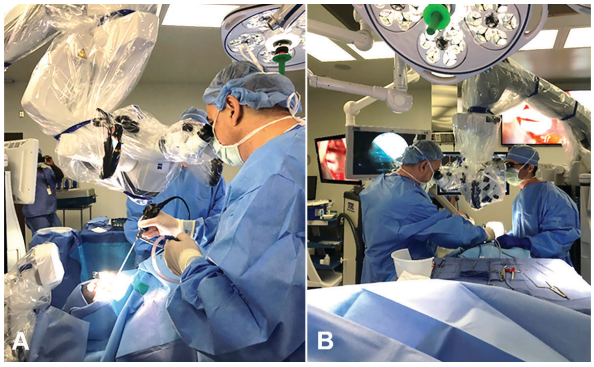

Figure 2. Operative setting with simultaneous use of (A) endoscope and (B) microscope with electromagnetic-navigation guided resection of pituitary tumor.

Resection begins with a ringed curette that loosened the tissue. We prefer usage of 30° angled curettes. It is advised to remove the laterally situated regions of the tumor first as well as lower ones, followed by the more central segments in order to avoid entrapment of the lateral portions by prematurely descending the diaphragma sellae. Superior dissection should be performed only when the tumor is freed inferiorly. Controlled suction along with bipolar forceps, micro pituitary rongeurs, micro-dissectors, and curettes were used interchangeably using 2 hands technique. If the suprasellar part of the tumor does not prolapse into view following resection of the intrasellar part, Valsalva maneuver can be used to facilitate descent of the residual tumor. The endoscope with a multiple angle lens can be used to locate any lateral fragments.

Throughout the surgery, repositioning the patient's head, angle and height of the microscope, the position of the table with microscope magnification, as well as the use of the endoscope and electromagnetic navigation with a registered pointer and instrument, allowed for a full and optimal view of the surgical field and exact localiza-

tion. This range of motion and visualization outreached the view provided by endoscope alone. If and when residual tumor was identified by endoscope, microsurgical or endoscopic technique was used to remove it as deemed appropriate intraoperatively.

If CSF leak was noted, the abdominal fat graft harvested at the beginning of surgery was placed on the sellar floor intradurally. The reconstruction can be reinforced with fibrin glue. The speculum was removed and the septum pushed into the midline from the other nostril and mucosal flap reflected over the septum and secured with absorbable suture. Endonasal trumpets, that allow breathing through the nostrils but remain unseen when looking at patient, were placed bilaterally and secured with permanent sutures. They were removed on the fourth day during an outpatient visit following surgery. The patient usually leaves the hospital on the first day following surgery after overnight monitoring for diabetes insipidus. Operative Procedure YouTube Video Clip Link: https://youtu.be/AvL03Jn1DS4

Results

In this personal series of the senior author (KIA), the combined approach was used on 84 consecutive patients (January 2011-December 2017) in cooperation with an ENT surgeon and a multidisciplinary team (endocrinology, ophthalmology). The series was continued up to the present. The mean age of the patients was 55 years (range, 16–70) with 51 male and 33 female patients. Mean follow-up was 37 months. There were 76 pituitary adenomas, 4 Rathke's cleft cyst, 1 clival chordoma, 1 renal cell carcinoma metastasis, and 2 cases of lymphocytic hypophysitis. Radical resection was achieved in 66 patients, subtotal resection in 11 patients, and partial resection/biopsy in 7 patients (based on neuroradiology reports). The mean length-of-stay was 3 days. There were no perioperative complications. Six patients experienced postoperative transient diabetes insipidus. CSF leak possibility was eliminated with the placement of fat graft. The pituitary gland and stalk were preserved in all cases. Visual symptoms were improved in 78% and endocrinological symptoms in 56% of the cases based on neuro-ophthalmology and neuro-endocrinology assessment. Six cases that had substantial supra/parasellar tumor extension were subsequently operated on via craniotomy as a second stage. We did not have any postoperative CSF leaks. There were no postoperative transient or permanent olfactory deficits (Figures 3, 4, 5, and 6).

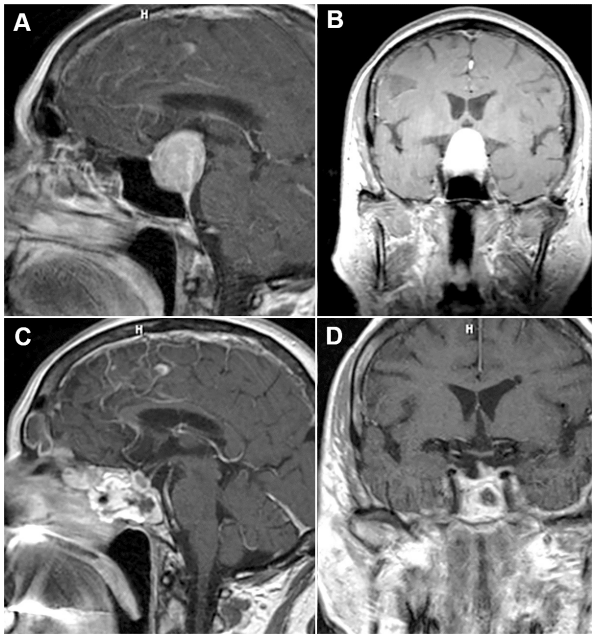

Figure 3. A woman in her late 70s presented with severe headache, VI nerve palsy, and visual decline. Combined transsphenoidal resection for hormone-inactive macroadenoma was performed. Preoperative (A) T1 sagittal and (B) T1 coronal post-contrast MRI of the head shows large intra, para, and suprasellar tumor with compression of optic chiasm and infiltration of cavernous sinus. Postoperative (C) sagittal and (D) coronal post-contrast T1 MRI of the head shows complete resection of the tumor with intact pituitary gland/stalk (note the fat graft in the sphenoid sinus-SS). The patient recovered fully her vision and double vision.

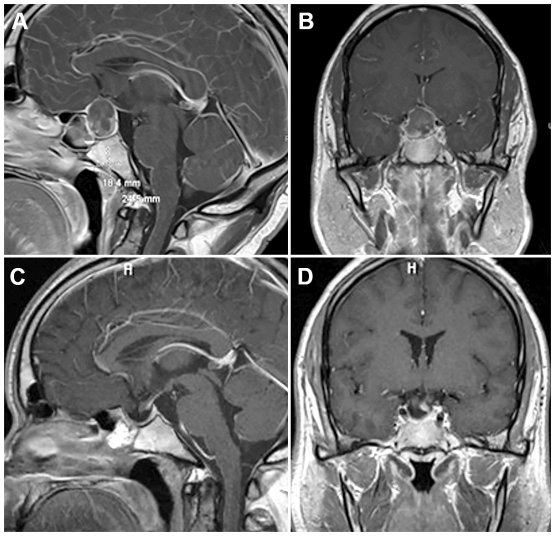

Figure 4. A man in the fifth decade of life experienced a sudden onset of severe headache and acute blindness. Combined trans-sphenoidal approach for pituitary apoplexy was performed. Preoperative (A) T1 sagittal and (B) coronal post-contrast MRI of the head shows large intra, para, and suprasellar hematoma with compression of optic chiasm and infiltration of cavernous sinus. Postoperative T1 (C) sagittal and (D) coronal post-contrast MRI of the head shows complete resection of the lesion with intact pituitary gland/stalk (note the small fat graft in the SS. Visual symptoms recovered following surgery (see also YouTube link).

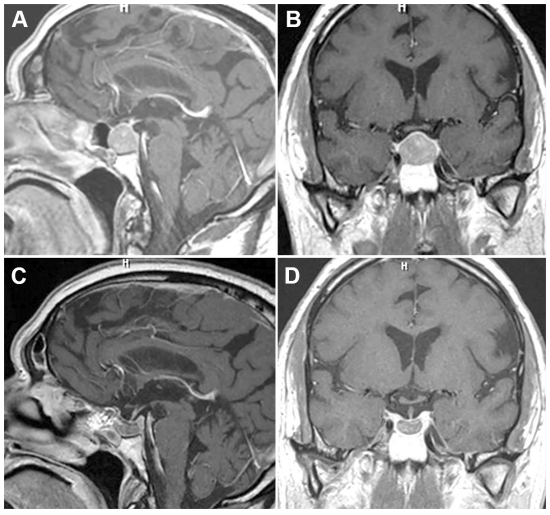

Figure 6. A man in his early 60s presented with acromegaly. Combined transsphenoidal approach was performed. Preoperative (A) sagittal (B) coronal post-contrast T1 MRI of the head shows intra, and supra-sellar tumor with intratumoral hemorrhage and compression of optic chiasm. Postoperative (C) sagittal and (D) coronal post-contrast MRI of the head shows complete resection of the tumor with intact pituitary gland/stalk. Visual symptoms recovered with remission of acromegaly.

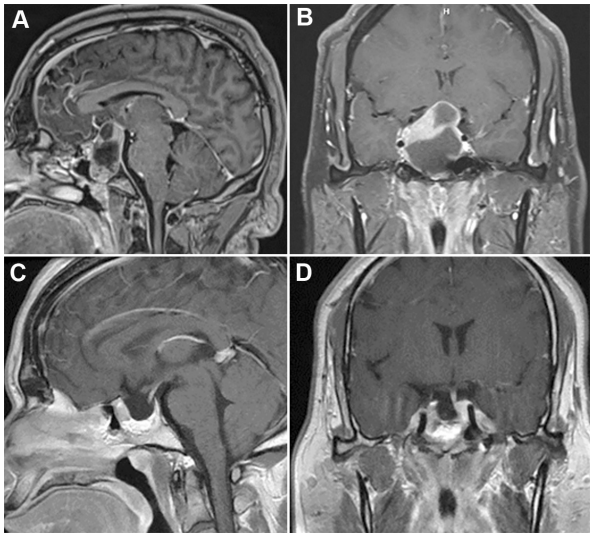

Figure 5. A man in his late 20s experienced a sudden onset of severe headache and blurred vision. Combined transsphenoidal approach for giant prolactinoma with intratumoral hemorrhage was performed. Preoperative (A) T1 sagittal and (B) T1 coronal post-contrast MRI of the head shows large intra, para, and suprasellar tumor with intratumoral hemorrhage with compression of optic chiasm and infiltration of cavernous sinus. Postoperative (C) sagittal and (D) coronal post-contrast T1 MRI of the head shows complete resection of the tumor and intact pituitary gland/stalk. Visual symptoms recovered and prolactin serum level improved significantly (preoperative, 1000 nmol/L vs. postoperative, 200 nmol/L) following surgery.

Discussion

Microsurgery vs endoscopic surgery and our experiences with combination

We utilized an original combination of a microsurgical and endoscopic approach combined with Axiem neuro-navigation to remove pituitary tumors in order to best employ the surgical and technical advantages of all 3 techniques.

The transsphenoidal microsurgical approach has been considered to be the gold standard for operative treatment of pituitary tumors (3). The technique introduced by Schoffler was modified by Hardy and Guiot in the 1960s with the introduction of microscopy and fluoroscopy and was used for many years. Guiot was the first surgeon to describe using endoscopes in pituitary surgery (4). Over time, the endoscopic approach gained popu-

larity and may have been more frequently used. It was believed to be less invasive and allowed for seeing around different angles or "around the corner." It has better visualization of the close-up surgical field, an enlarged working angle, and an increased panoramic view. Four disadvantages of the endoscopic technique are noted: the possible need to remove the normal middle turbinate, relatively high frequency of temporary or permanent loss of smell, 2D visual experience, and the definite need of additional simultaneous participating surgeons. Although there are newly developed 3-dimensional (3D) models of the endoscope, they clearly lack the 3D advantage of microsurgical technique. Gross total resection of macroadenomas with parasellar extension was reported to be significantly enhanced by the endoscopic approach (5-7). The endoscopic approach has also been reported to show better results in invasive adenomas (8), lowers the incidence of new hypopituitarism after surgery (2, 6, 9), has better intraoperative identification and preservation of pituitary gland (2), and results in shorter hospital stay and less post-op pain (10).

A significant difference in the complication rates between endoscopic and microscopic technique, however, was not found in recent studies (10-12). Also, these studies on patients who underwent transsphenoidal resection for Cushing's disease (13) and acromegaly (14) found that remission and recurrence rates were the same among patients who underwent endoscopic surgery versus microscopic surgery. Both techniques have been found to be comparable for infradiaphragmatic craniopharyngiomas, while the endoscopic approach yields better results for supradiaphragmatic lesions (15). However, a large retrospective review of 1054 patients with microsurgically resected craniopharyngioma revealed good patient outcomes without more limitations on each individual tumor with distinct features, despite the impact of recent endoscopic techniques (16). On the contrary, a recent literature review has shown that vascular complications and CSF fistulas were reduced with microsurgery compared with endoscopy (17). As a matter of fact, these 2 approaches may have driven neurosurgeons into the 2 competing camps that exclusively utilizes one procedure or the other.

Although the combined use of a microscope and endoscope for pituitary surgery was described by Bush and Halves in 1978 (18), we have hypothesized that using a combination of microscope and endoscope along with neuronavigation can capture the best advantages of all 3 techniques. An inspiring report by Al-Mefty et al. described the use of combined approach to yield benefits of both techniques (4) as the 2 approaches became more competitive among neurosurgeons over the years.

Combined approach to pituitary lesions has been previously reported. The term "combined approach" described either a combined endoscopic and microscopic transsphenoidal approach to pituitary lesions, or a combined transcranial and transsphenoidal approach. The additional support by neuronavigation for microsurgical or endoscopic approach has also been published (3, 4, 19). Combined endoscopic and microscopic management of pituitary region tumors has also been described in a technical note for pediatric surgery (20), in case reports (21, 22) and in few retrospective studies (4, 23, 24). In the pioneering work of Helal et al. in which 37 patients were treated with a combined approach, the additional use of an endoscope was highly beneficial as hidden areas could be visualized in 84% of the patients, and tumor residues were detected in 40.5% (23).

The transition from microsurgical to endoscopic approach has also been previously published (25). Laws et al. recommended that the operating microscope should always be balanced and readily available since up to 14% of endoscopic surgeries were converted from an endoscopic to microscopic approach due to tissue hypertrophy, atypical nasal airway, scar tissue, complex sphenoid sinus anatomy, mucosal bleeding, need for binocular vision in extended approach, and technical problems with the endoscope. Most of the studies, however, described the results of either microscopic or endoscopic surgery and provided a comparison of the 2 methods. According to 2 literature reviews, the endoscopic approach may be associated with a

higher rate of gross tumor movement and a lower risk of postoperative complications for treating a nonfunctioning pituitary adenoma when compared with a microscopic approach, although the rate of permanent diabetic insipidus and meningitis remains the same (26, 27). An additional single institution study of 137 patients found no differences between the 2 approaches (28). A national database study of more than 30.000 patients noted a significant increase in the endoscopic surgery group over time, yet rates of gross total resection, need for adjuvant therapy, and short-term mortality were similar (29). No differences in complication rates between endoscopic and microscopic surgery were observed in a large single institution retrospective study of 1153 patients (30).

As noted above, both endoscopic and microscopic surgeries have yielded similarly good outcomes in numerous reports. Our intention was to combine all 3 techniques simultaneously to both add and multiply the benefits of all in a single procedure. We believe that one of the clear advantages of the microscopic technique compared to the endoscopic approach is preservation of all nasal and paranasal anatomical structures. Furthermore, the surgeon can freely use both hands while applying microsurgical techniques when dissecting the tumor. Also, a single surgeon controls the situation the entire time as opposed to having 2 surgeons involved in the endoscopic approach. A microscope provides magnification, illumination, 3-D visualization, communication with operating room personnel (via microscope monitor), and intraoperative recording, which can be used for educational purposes to teach trainees.

We utilized an endoscope at the end of surgery (0°, 30°, 60°, 70°) to carefully inspect the resection cavity, find any unnoticed residual tumor, and verify anatomy and lack of complications. Furthermore, our combined approach provided preservation of all anatomic structures, no evidence of postoperative CSF leak and no evidence of postoperative transient or permanent olfactory loss thus minimizing postoperative discomfort. Overall, our experiences indicate that both microsurgical and endoscopic techniques have merits and should be used as complementary, rather than competing, techniques (32).

Neuronavigation in Transsphenoidal Surgery

Axiem neuronavigation obviates the need for rigid head fixation. We consider the ability to freely move the head to the desired position intraoperatively to be very important. This freedom of movement enables the microscope view to be adjusted in multiple directions, enhancing the visualization capabilities of microscope itself. We found that one of the benefits versus optoelectronic navigation is that there are no line-of-sight problems when tracking instruments, and accuracy is comparable to standard navigation. This has been reported by other authors, too (31). A drawback of conventional neuronavigation, however, is the necessity of focusing on 2-dimensional images in 3 planes at the same time to determine one's position in the operating field. Several reports described the use of neuronavigation in combination with microsurgical or endoscopic approach alone, but only a few reports have described the use of a combined microscopic and endoscopic approach with use of neuronavigation (3, 4, 19). Only Al-Mefty et al. (4) described the use of a free-head navigation technique in combination with microscope and endoscope. Our additional observation is that the use of neuro-navigation obviates the use of bulky C-arm fluoroscopy and the subsequent health hazards of intraoperative radiation to operative room personnel and surgeons.

Conclusion

Our experiences utilizing the original combination of microsurgical and endoscopic endonasal transsphenoidal approach and using neuronavigation indicate that it is a safe and effective practice for the resection of pituitary tumors. Our results indicate that it may increase the efficacy of resection in all relevant outcome parameters, such as increasing the radicality of surgical resection, helping to preserve the pituitary gland, and helping to improve and resolve preoperatively altered

patient hormonal function and impaired vision. Furthermore, the combined approach reduces complications (CSF leak and postoperative olfactory loss), provides less postoperative pain and discomfort, reduces the surgery time, and enables a shorter hospital-stay.

What Is Already Known on this Topic:

The utilization of microsurgical or endoscopic technique for the resection of pituitary tumors have both been proven to be effective. However, these 2 techniques are presently used competitively and not simultaneously. This antagonism deprives surgeons to use the benefits of both techniques simultaneously.

What this Study Adds:

Utilizing the original combination of microsurgical and endoscopic endonasal transsphenoidal approach and using neuronavigation proves to be a safe and effective practice for the resection of pituitary tumors. Our results on a relatively large series of patients indicate that it may increase the efficacy of resection in all relevant outcome parameters: increasing the radicality of surgical resection, helping to preserve the pituitary gland, and helping to improve and resolve preoperatively altered patient hormonal function and impaired vision. Furthermore, the combined approach reduces complications, provides less postoperative pain and discomfort, reduces the surgery time, and enables a shorter hospital-stay. Finally, combination of these 3 techniques into one accumulates and enhance their singular benefits.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Longterm Antiepileptic Therapy and Bone Health: Implications for Patients with Brain Tumors

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Abstract

Objective. The objective of our study was to investigate the effects of carbamazepine (CBZ) and lamotrigine (LTG) treatment on bone metabolism in epileptic patients. **Patients and Methods.** A cross-sectional study was performed on normal controls (N=30) and 100 patients with symptomatic epilepsy caused by a primary brain tumor, divided into two groups according to the treatment: LTG monotherapy group (N=50) and CBZ monotherapy group (N=50). For each participant serum levels of 25-OHD and osteocalcin (OCLN) were measured, and bone mineral density (BMD) was evaluated by the dual-energy X-ray absorptiometry method. **Results**. There was no statistically significant difference in the average values of vitamin D in serum between the CBZ and LTG groups (Vitamin D CBZ 17.03±12.86 vs. Vitamin D LTG 17.97±9.15; F=0.171, P=0.680). There was no statistically significant difference in the average values of OCLN between the CBZ and LTG groups (OCLN CBZ 26.06±10.87 vs. OCLN LTG 27.87±28.45; F=0.171, P=0.674). The BMD value was lower in both groups using antiepileptic agents compared to the controls, but when comparing the CBZ group to the LTG group, a statistically significant difference was only observed for the Z score (T-score CBZ: 0.08± 1.38 vs. T-score LTG: 0.37± 1.02; F=1.495, P=0.224; Z score CBZ: -0.05±1.17 vs. Z. score CBZ: 0.38±0.96; F=4.069, P=0.046) (Table 3). **Conclusion**. The choice of antiepileptic agents for treating seizures in patients with brain tumors should be carefully evaluated in relation to their impact on bone health. These patients could benefit from supplementation and regular measurement of biochemical markers of bone turnover and BMD.

Key Words: Osteoporosis ▪ Anticonvulsants ▪ Brain Neoplasms.

Introduction

Seizures are a common complication of both primary and metastatic brain tumors as they may appear as the initial manifestation or during the course of the disease (1). Such seizures are focal in origin and may either remain focal or secondarily generalize. As seizures represent an important source of morbidity and mortality in patients with primary and secondary brain tumors, they require aggressive treatment (2). Although carbamazepine (CBZ) has been considered as a first line agent for treating focal seizures with or without secondary generalization, antiseizure drugs with no or minimal hepatic enzyme-inducing properties, such as lamotrigine (LTG), are generally preferred, as these agents are considered to have a more favorable safety profile compared with older agents (3).

Long-term antiepileptic therapy has been strongly associated with negative effects on bone health. Multiple studies have reported the osteopenic effect of CYP450 inducing antiepileptic drugs (AEDs), reflected in hypocalcemia, hypophosphatemia, increased vitamin D catabolism, hyperparathyroidism, elevated markers of bone resorption and accelerated bone mass loss (4-6). Novel studies have reported that AEDs may cause bone loss in the absence of vitamin D deficiency (7). However, data on newer AEDs are insufficient, and although their use has become increasingly prevalent, their effects on bone metabolism remain poorly understood.

It has been proposed that patients with brain tumors, particularly those with a long-term life expectancy, may benefit from regular checks of bone turnover markers and BMD testing (8). Therefore, the choice of antiepileptic agents in this specific group of patients should include a critical assessment of their impact on bone health. The objective of our study was to investigate the effects of carbamazepine (CBZ) and lamotrigine (LTG) treatment on bone metabolism in epileptic patients.

Patients and Methods

A cross-sectional study was performed between 2016 and 2018, at the Neurology Clinic in Sarajevo. We prospectively recruited 100 patients with symptomatic epilepsy caused by a primary brain tumor. The subjects in the case group were further stratified according to treatment into two different patient groups: epileptic patients undergoing treatment with LTG monotherapy (N=50), and CBZ monotherapy (N=50) for a period of at least twelve months. Patients who had any condition known to affect bone metabolism (e.g., renal disease, recent fracture, hyperparathyroidism, Paget disease, osteoporosis) or who were taking any drug known to cause or treat osteoporosis, were excluded. We also excluded patients with diagnosed metastatic disease and patients who had received AEDs before the presumptive diagnosis of brain tumor. The results were compared with age-matched healthy controls with no evidence of metabolic bone disease (N=30).

All participants were asked to complete a questionnaire including medical history, information on fractures, falls and injuries, and vitamin D or calcium supplements. Bone mineral density (BMD) was evaluated by a dual-energy X-ray absorptiometry method called DXA technology. DXA was performed using a Hologic QDR-4000A densitometer (Hologic, Bedford, MA, U.S.A.). DXA measured bone mineral content (BMC in grams) and bone area (BA, in square centimeters), then calculated BMD "area" in g/cm2 by dividing BMC by BA. The T-score, the value used to diagnosis osteoporosis, is defined as the difference between the patient's BMD, and the mean reference value of the normal young population, divided by the standard deviation of the normal young population. The Z-score, used to compare the patients' BMD with a population of peers, is calculated by subtracting the mean BMD of an age, ethnicity and sex-matched reference population from the patients' BMD, and dividing it by the SD of the reference population.

For each subject, the level of vitamin D and osteocalcin (OCLN) in the serum was determined in laboratory findings. Serum 1, 25-dihydroxyvitamin D (3) (normal range, 20–74 pg/ml) was measured by radioimmunoassay. Serum osteocalcin level was determined by Elisa. Due to laboratory errors, not every test was obtained for every patient. The precise n for each test in each patient group is noted in the tables.

Ethics Statement

The study was conducted according to the standards of the Declaration of Helsinki (1975, revised 2000), and the protocol was approved by the local Bioethical Committee (decision reference numbers 0207-28784).

Statistical Analysis

Statistical analyses were performed using SPSS for Windows, version 16 (SPSS Inc., Chicago, IL). Continuous data were presented as mean ± standard deviation (SD). Student's t-test and ANOVA test were used for continuous variables as baseline comparisons between the cases and the controls. Odds ratio (OR) and 95% confidence interval (CI) were calculated. A P value of ≤0.05 was considered significant.

Results

The study involved 50 subjects on CBZ monotherapy with mean age 36.74±10.26 years, and 50 subjects on LTG monotherapy with mean age 31.82±8.84 years, compared to the age matched controls. The characteristics of the study partici-

pants are shown in Table 1. We observed a statistically significant difference in the gender structure between the LTG group and the controls ($\chi 2=4.301$; P=0.033). The average duration of epilepsy in the CBZ group was 11.18±6.87 years and in the LTG group 7.28±4.47 years. The average duration of CBZ therapy was 6.18+3.36 years. The average duration of LTG therapy was 4.72±2.52 years. A statistically significant difference in the duration of epilepsy was found (F=12.151; P=0.001.), as well as in the duration of therapy (F=6.012; P=0.016) (Table 1).

Although the average value of vitamin D in serum was significantly lower in both groups using antiepileptic agents compared to the controls, there was no statistically significant difference between the CBZ and LTG groups (Vitamin D CBZ 17.03 ±12.86 vs. Vitamin D LTG 17.97±9.15; F=0.171, P=0.680) (Table 2). Although the average value of OCLN in serum was higher in both groups using antiepileptic agents compared to the controls, there was no statistically significant difference between the CBZ and LTG groups (OCLN CBZ 26.06±10.87 vs. OCLN LTG 27.87±28.45; F=0.171, P=0.674) (Table 2).

The BMD values were lower in both groups using antiepileptic agents compared to the controls, but when comparing the CBZ group to the LTG group, a statistically significant difference was only observed for the Z score (T-score CBZ: 0.08±1.38 vs. T-score LTG: 0.37±1.02; F=1.495, P=0.224; Z score CBZ: -0.05±1.17 vs. Z. score LTG: 0.38 ±0.96; F=4.069, P=0.046) (Table 3). In regression analysis, the Z score was significantly associated with the duration of antiepileptic therapy (F=8.438, P=0.005) (Table 4).

Table 1. Clinical Characteristics of Patients and Controls

Variables		N	($\bar{x}$ ± SD)	SEM	Minimum	Maximum	F	P
Age (yr)	Controls	30	40.37±8.30	1.52	22.0	50.0	2.688*	0.105*
	CBZ	50	36.74±10.26	1.45	20	57.00	18.329†	0.0001†
	LTG	50	31.82±8.84	1.25	19.00	54.00	6.599‡	0.012‡
Gender (M), N (%)	Controls	16 (53.3)	-	-	-	-	-	0.177*
	CBZ	20 (40.0)	-	-	-	-	-	0.033†
	LTG	15 (30.0)	-	-	-	-	-	0.201‡
Duration of epilepsy (yr)	CBZ	49	11.18±6.87	0.98	2.00	35.00	-	-
	LTG	50	7.28±4.47	0.63	2.00	23.00	11.217‡	0.001‡
Duration of antiepileptic treatment (yr)	CBZ	49	6.18±3.36	0.48	2.00	15.00	-	-
	LTG	50	4.72±2.52	0.36	2.00	12.00	6.012‡	0.016‡

CBZ=Carbamazepine; LTG=Lamotrigine; *For comparison of controls and CBZ group; †For comparison of controls and LTG group; ‡For comparison of CBZ and LTG groups.

Table 2. Average Values of 25-Hydroxyvitamin Vitamin D and OCLN in Serum in Patients and the Control Group

Biochemical markers		N	($\bar{x}$ ± SD)	SEM	Minimum	Maximum	F	P
Vitamin D (ng/ml)	Controls	30	32.03±6.99	1.28	21.30	50.30	14.440*	0.0001*
	CBZ	49	17.03±12.86	1.86	3.00	72.90	12.146†	0.0001†
	LTG	50	17.97±9.15	1.31	4.10	39.10	0.171‡	0.680‡
OCLN (ng/ml)	Controls	30	19.64±6.54	1.19	11.10	36.40	8.671*	0.004*
	CBZ	50	26.06±10.78	1.52	11.90	77.10	2.422†	0.124†
	LTG	50	27.87±28.45	4.02	16.00	221.00	0.178‡	0.674‡

OCLN=osteocalcin; CBZ=carbamazepine; LTG=lamotrigine; *For comparison of controls and CBZ group; †For comparison of controls and LTG group; ‡For comparison of CBZ and LTG groups.

Table 3. BMD Values in Patients and the Control Group

BMD values		N	($\bar{x}$ ± SD)	SEM	Minimum	Maximum	F	P
T score	Controls	30	0.73±1.13	0.21	-2.70	2.10	4.853*	0.031*
	CBZ	49	0.08±1.38	0.20	-3.40	3.90	2.158†	0.146†
	LTG	50	0.37±1.03	0.14	-2.70	2.40	1.495‡	0.224‡
Z score	Controls	30	0.55±0.79	0.14	-1.00	1.80	6.750*	0.015*
	CBZ	49	-0.05±1.17	0.17	-3.10	1.50	0.663†	0.418†
	LTG	50	0.38±0.96	0.14	-3.30	2.10	4.069‡	0.046‡

BMD=bone mineral density; CBZ=carbamazepine; LTG=lamotrigine; *For comparison of controls and CBZ group; †For comparison of controls and LTG group; ‡For comparison of CBZ and LTG groups.

Table 4. Logistic Regression Analysis and Risk Factors Independently Associated with Z Score

Tests of between-subjects effects					
Dependent variable: Z score					
Source	Type III Sum of Squares	df	Mean square	F	Sig.
Gender	0.282	1	0.282	0.314	0.576
Duration of epilepsy	0.788	1	0.788	0.877	0.351
Duration of antiepileptic treatment	7.578	1	7.578	8.438	0.005

Discussion

Seizures are a frequent and overwhelming complication of brain tumors, with severe clinical and social effects, and a major negative impact on the quality of life. Additionally, the presence of epileptic seizures is considered to be associated with the long-term disability of patients with brain tumors (9). It has been reported that seizures are the initial manifestation of 20-45% of brain tumors, while another 20-45% of patients will present with them during the longer course of the disease (9).

The choice of antiepileptic agent in patients with brain tumors is mostly based upon its efficacy, tolerability and pharmacokinetic interactions (10). It has been reported that patients with brain tumors experience more side-effects of antiepileptic drugs compared to the overall population of epileptic patients, particularly bone-marrow suppression. Additionally, brain tumor-related seizures are often poorly controlled, and require the concomitant use of multiple AED, which leads to an even a higher risk of bone disease (11, 12).

Several mechanisms have been proposed to explain the negative effects of antiepileptic treatment on bone mineralization. Induction of the cytochrome P-450 system, resulting in alteration of vitamin D metabolism, is a widely accepted explanation (4-6, 13, 14). The increased vitamin D catabolism leads to consequent hypocalcaemia and secondary hyperparathyroidism, increased bone resorption and accelerated bone mass loss. This mechanism is primary linked to the use of phenobarbital, phenytoin, and carbamazepine. Several studies report the osteopenic effects of carbamazepine on bone metabolism (15-18). On the other hand, several authors did not observe negative effects of carbamazepine therapy on vitamin D levels or biochemical markers of bone turnover (19, 20).

Moreover, several studies have also suggested that non–P-450 system mechanisms may play a role in bone loss (21). A large broad-spectrum of new generation AEDs, such as lamotrigine, topiramate, tiagabine, levetiracetam, oxcarbazepine, and zonisamide, have been considered to have fewer negative effects on bone health. Meyer et al. concluded that the molecule of lamotrigine shows

good penetration into tumor tissues and therefore might be efficacious in brain tumor patients, and even, due to its excellent tolerability and auspicious safety profile, it might be considered as the first choice agent for treating seizures in these patients (22). Nevertheless, the unfavorable effects of LTG on bone, including bone loss, impaired growth in children, alterations in BMD, and elevated bone turnover markers, have been reported (16, 23, 24).

In this study, we demonstrated a significant decrease in 25-OHD levels in patients treated with antiepileptic therapy compared to the control group. However there was no statistically significant difference between the CBZ and LTG groups (Vitamin D CBZ 17.03±12.86 vs. Vitamin D LTG 17.97±9.15; F=0.171, P=0.680) (Table 2). Also, the average value of OCLN in serum was higher in both groups using AEDS compared to the controls, but there was no statistically significant difference between the CBZ and LTG groups (OCLN CBZ 26.06±10.87 vs. OCLN LTG 27.87±28.45; F=0.171, P=0.674) (Table 2).

Malignancy itself, treatments and their consequences, malnourishment, and limited motion likely all affect BMD (25). Previous research has shown that brain tumor survivors are at higher risk of bone fractures and early osteoporosis (26). Several authors have explored bone diseases related to the use of AEDs, radiation therapy, anticoagulants, chemotherapy, and hemiplegia-associated osteopenia (8).

According to our results, BMD values were lower in both groups using antiepileptic agents compared to the controls, but when comparing the CBZ group to the LTG group, a statistically significant difference was only observed for the Z score (T-score CBZ: 0.08±1.38 vs. T-score LTG: 0.37±1.02; F=1.495, P=0.224; Z score CBZ: -0.05±1.17 vs. Z. score LTG: 0.38 ±0.96; F=4.069, P=0.046) (Table 3). However, we also observed a statistically significant difference in the duration of epilepsy (F=12.151; P=0.001), as well as in the duration of therapy (F=6.012; P=0.016) (Table 1). After adjustment, the Z score was significantly associated with the duration of antiepileptic therapy (F=8.438, P=0.005) (Table 4). The duration of antiepileptic treatment has been widely recognized as an independent predictor of lower BMD values (7).

A recent publication studied the use of AEDs and the risk of fracture, and concluded that long-term use of AEDs, particularly in women, was associated with a higher risk of bone fractures, but did not observe any differences between users of AEDs that do and do not induce the cytochrome P-450 system (21). In our study, we only observed a statistically significant difference in the gender structure between the LTG group and the controls ($\chi 2$=4.301; P=0.033) (Table 1). In both groups using AEDs the female examinees dominated ($\chi 2$=1.099; P=0.201) (Table 1). The female ratio in the group with LTG was slightly higher compared to the CBZ group, which can be explained by the fact that LTG as a newer generation is preferred in women in the germinal period because of the low risk for developing congenital malformations. Both female gender and the postmenopausal period are significant risk factors for bone loss, however, young females have estrogen protection for bone metabolism (27). Pack et al. suggested that the bone loss observed in premenopausal women was associated with lower levels of estrogen (28). Unfortunately, in our study, we did not explore the levels of reproductive hormons in our patients, which may represent a major limitation.

It has been proposed that all patients with brain tumors who are initiating treatment should undergo BMD testing, particularly those with tumors compatible with a long-term life expectancy (8). Female gender may present a particularly vulnerable subgroup of these patients. Patients with verified osteopenia and osteoporosis should be treated, and it seems reasonable to avoid use of cytochrome P-450 AEDs in these patients for treating seizures (8). Further investigation of the effects of new generation AEDs on bone metabolism is necessary.

Conclusion

The choice of antiepileptic agents for treating seizures in patients with brain tumors should be carefully evaluated regarding their impact on bone

health. These patients could benefit from supplementation and regular measurement of biochemical markers of bone turnover and BMD.

What Is Already Known on this Topic:

Long-term antiepileptic therapy, especially with enzyme-inducing agents, is associated with a high risk of bone disease. Therefore, regular osteoporosis investigation is recommended for these patients.

What this Study Adds:

In patients with brain tumors and symptomatic epileptic seizures, cautious use of AEDs should be kept in mind in order to avoid complications from bone loss. Preventive strategies, including regular supplementation and control of biochemical and radiological evidence of bone disease, should be implemented.

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Extensive Posterolateral Approach for Giant Spinal Epidural Tumors

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Abstract

Objective. Our objective was to determine the safety, efficacy, and surgical outcome of an extensive posterolateral approach for giant spinal epidural tumors. **Materials and Methods.** Our clinical study included 12 patients with various giant primary tumors and metastases of the spine, who underwent surgery between 2008 and 2019. The surgical procedure consisted of costotransversectomy, laminectomy, corpectomy, tumor resection, spinal column stabilization, and reconstruction. Neurological status examination and pain assessment were recorded at the time of admission, upon discharge and at outpatient check-up. **Results.** In our clinical sample, there were no major perioperative complications. All patients were discharged from the intensive care unit back to the hospital department within a week after the surgery. Postoperative follow-up showed no deterioration of neurological status. Furthermore, there was a moderate to significant improvement of paraparesis in all patients for weeks after surgery. The most notable improvement was significant pain relief in all the patients. None of the patients had issues with failure of the implanted hardware. Two patients died less than six months after the surgery due to the progression of the primary malignant process. **Conclusion.** An extensive posterolateral approach to giant spinal epidural tumors is an effective one-step approach. It presents a good compromise between invasiveness and sufficient exposure for both tumor resection and spinal column reconstruction. Good short-term clinical improvement can be achieved, but the long term results depend on the advancement of the initial disease. Careful evaluation and selection of patients are necessary to achieve clinical improvement and prolonged life expectancy, and the best results are achieved with a multidisciplinary approach.

Key Words: Spinal Epidural Tumor ▪ Neurosurgical Procedure ▪ Posterolateral Approach ▪ Complete Tumor Resection ▪ Stabilization.

Introduction

Modern oncological treatment has resulted in the prolonged life expectancy of patients with spinal tumors, both primary and metastatic. Past studies have shown the presence of spinal metastases in 36.1% of patients dying from a neoplastic disease, whereas more recent studies have shown that this percentage has increased (1). Symptoms of epidural spinal tumors arise from spinal cord compression (SCC), and manifest most commonly as worsening pain, usually present eight weeks before the diagnosis, in up to 95% of patients. Common symptoms include motor deficit, manifesting as a weakness at the time of diagnosis (60-85%). Sensory deficits are subjectively less evident in comparison with motor deficits, but can still be detected in 40-90% patients with a thorough neurological examination. Sphincter dysfunctions can present in later stages of SCC, in concordance with motor deficits (2-4). Unfavorable clinical presentations of epidural spinal tumors, and the consequent severe impact on the quality of life (QoL), create increasing demand for medical treatment. Surgical interventions of various extents can significantly improve QoL and remain irreplaceable in the modern approach to spinal tumors (2-5). Several other studies have addressed posterior or posterolateral approaches for tumors of the thoracic spine. Relevant studies are presented in Table 1.

Table 1. Studies which have Addressed Posterior or Posterolateral Approaches for Tumors of the Thoracic Spine

Authors	Sample	Methods	Results and complications
Rong et al. (6)	14 patients with dumbbell tumors involving up to two segments of the thoracic spine.	Posterolateral approach with unilateral laminectomy, decompression, costotransversectomy. No instrumentation.	Gross total removal of tumor in 13 patients, no significant operative or postoperative complications occurred in any patient.
Gezercan et al. (8)	22 patients with tumors of either thoracic or lumbar spine.	Posterolateral approach with either unilateral or bilateral decompression, posterior stabilization and anterior reconstruction.	No complications in 14 patients (63.64%), 4 patients (18.18%) died within 20 months, neurological deficit in one patient (4.54%), failure in instrumentation in 2 patients (9.09%).
Rustagi, Mashaly, Ganguly, Ahkter, Mendel (9)	96 patients with tumors of the thoracic spine. 73 patients (76%) had single level involvement.	Posterior approach, wide laminectomy, decompression, transpedicular stabilization, vertebrectomy, anterior reconstruction. 18 patients (18.25%) had anterior reconstruction with titanium cage.	18.75% of patients had neurological improvement. 2.08% of patients had neurological deterioration. 29.16% of patients had surgical complications, 11.4% of patients had major postoperative complications. 4% of patients died within one month.
Wang et al. (10)	140 patients with various metastases in the thoracic spine.	Posterolateral approach, wide laminectomy, decompression, transpedicular stabilization, and vertebrectomy - one segment in 104 pateints (74%), two segment in 27 patients (19%) and three segment in 4 patients (2.9%) and anterior reconstruction.	134 patients (94%) had significant pain improvement. Overall median survival was 7.7 months. 20 patients (14.3%) had major postoperative complications, 6 patients (3.7%) died within 30 days. 7 patients (5%) had instrumentation failure.
Joubert et al. (11)	34 patients with spinal metastases – 30 in the thoracic spine and 4 in the lumbar spine.	Posterior or posterolateral approach, wide laminectomy, decompression, transpedicular stabilization, unilateral costotransversectomy, anterior reconstruction.	Neurological improvement in 23 patients (67.6%), no neurological deterioration. Median overall survival was 13.7 months. One patient had major postoperative complication. No failure of instrumentation was observed.

Despite the relative abundance of results, there is little uniformity among studies in terms of patient selection, their condition, the size of the tumor, and surgical technique. Therefore direct comparison between the studies listed is not a viable option.

Our clinical study analyzed the surgical treatment of primary or metastatic thoracic spinal tumors involving the anterior or posterior column. It challenged the otherwise well-proven and effective transthoracic anterior, posterior, or a combined approach as the surgical approach of choice. A giant spinal epidural tumor is defined as a spinal epidural lesion that extends over two or more vertebral levels.

The aim of our study was to determine the safety, efficacy, and surgical outcome of the extensive posterolateral approach for giant spinal epidural tumors. The data obtained can be used for better preoperative planning, and improving the treatment strategy as a whole.

Methods

Patients

In our clinical study we included 12 patients with giant spinal epidural tumors, who underwent surgery during the period between 2008 and 2019. There were five male and seven female patients, with a mean age of 51 years, ranging from 16 to 70 years. Eight patients had various multilevel spinal metastases. In all eight patients the metastases were large, extensively vascularized, and in direct proximity to the dura, a lung, the aorta, vena cava, or a combination of the above. Two patients had been diagnosed with chondrosarcoma, one patient with synovial sarcoma, and one patient with an an-

Table 2. Patients with Giant Spinal Epidural Tumors, who Underwent Surgery during the Period between 2008 and 2019

Age (yrs)	Gender	Tumor type	Remarks
16	Male	Aneurismatic bone cyst Th 7-9	Complete removal, fast recovery.
34	Male	Synovial sarcoma Th 3-4	-
40	Female	Metastasis of breast carcinoma Th 3-5	Death within 6 months.
47	Male	Chondrosarcoma Th 5-7	Complete removal with dissection of the tumor.
50	Male	Metastasis of mixed testicular carcinoma Th 3-5	Complete removal, single metastasis, in remission without neurological impairment.
51	Male	Diffuse spinal plasmocytoma C6-Th1	-
56	Female	Metastasis of kidney clear cell carcinoma Th 6-8	Significant blood loss, long postoperative recovery.
61	Female	Metastasis of colorectal carcinoma Th 5-6	-
62	Female	Chondrosarcoma Th 6-7	»En bloc« resection achievable.
63	Female	Metastasis of malignant melanoma Th 9-10	Death within 6 months.
66	Female	Metastasis of lung carcinoma Th 6-7	Long postoperative recovery.
70	Female	Metastasis of breast carcinoma Th 5-6	-

eurismatic bone cyst. In all patients with primary tumors they were formed in a dumb-bell shape. The patients are presented in Table 2.

At the time of admission, neurological status examination in all patients revealed progressive paraparesis and prominent pain to various degrees. Pain was assessed using the Visual Analogue Scale (VAS). All patients had a generally good performance index, and underwent imaging diagnostics (magnetic resonance imaging – MRI and computer tomography – CT).

Examples of the preoperative MRIs of selected patients are presented in Figure 1 and Figure 2.

All patients underwent surgery using the one-step posterolateral approach. The main goal for the operative outcome in patients with metastases was radical tumor resection, and total tumor resection in patients with primary tumors.

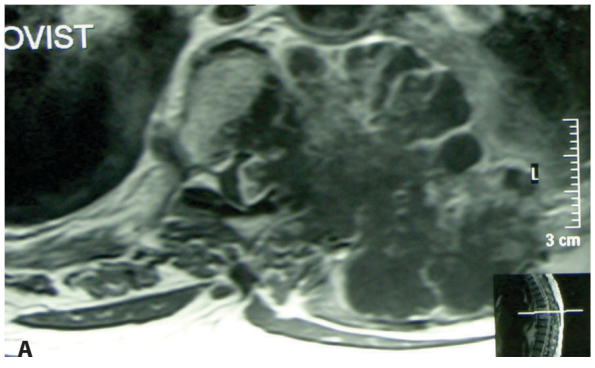
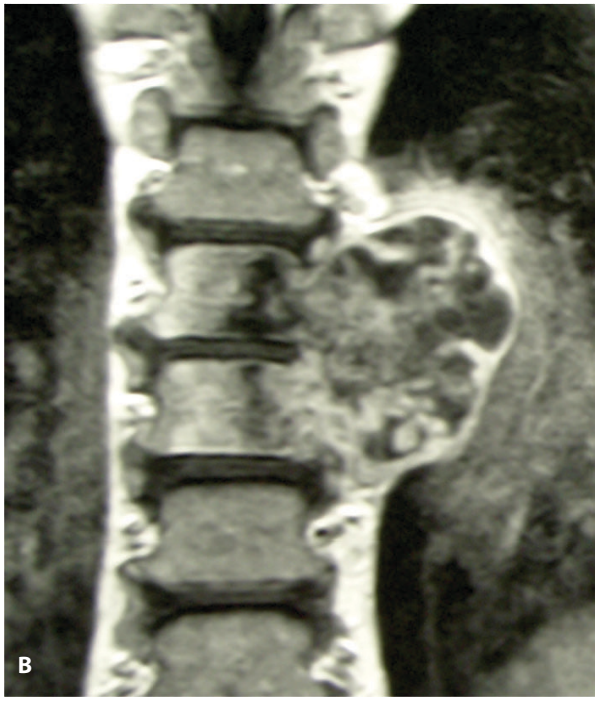
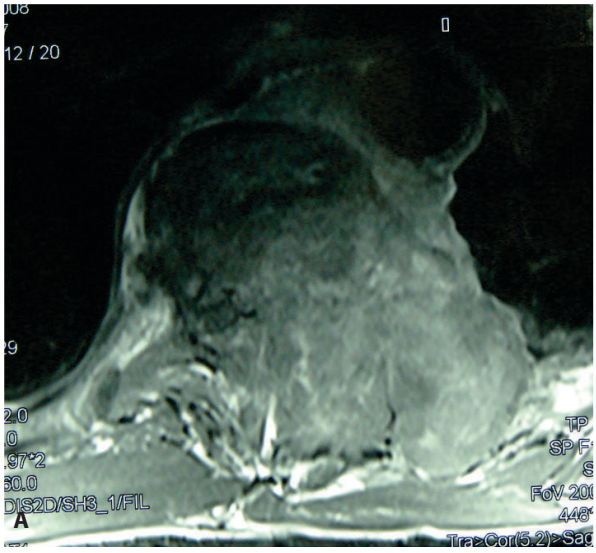


Figure 1. Preoperative MRI of a Patient with Chondrosarcoma of 6th and 7th Segments of the Thoracic Spine. A: Axial View. B: Coronal View.

Surgical Technique

A multidisciplinary approach was necessary, so surgeries were performed in tandem with a neurosurgeon and a trauma surgeon. After induc-





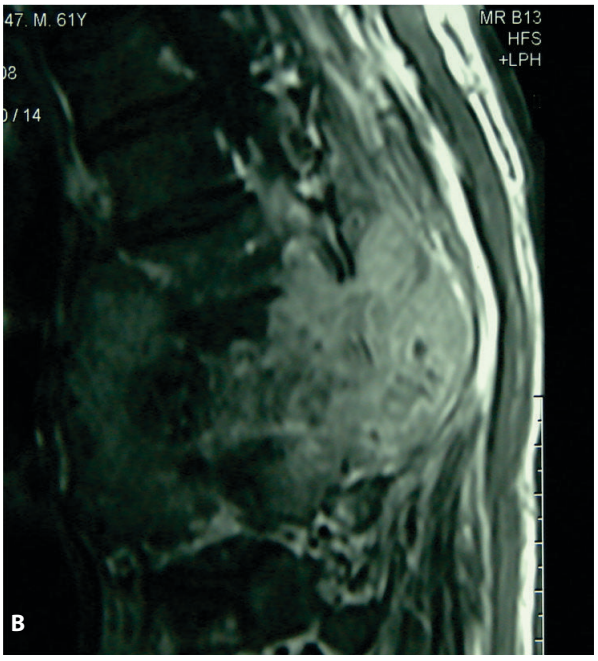


Figure 2. Preoperative MRI of a Patient with Metastasis of a Colorectal Carcinoma of the 5th and 6th Segments of the Thoracic Spine. A: Axial View. B: Sagittal View.

tion of anesthesia, all patients were turned into the prone position. An extended midline incision was made, and the paravertebral muscles were detached from the spinous processes to expose the affected vertebras. To obtain sufficient exposure, muscle detachment of at least 2-3 neighboring segments in caudal and cranial directions was necessary. Laminectomy and total gross resection of the tumorous posterior column were performed, followed by bilateral costotransversectomy on all the affected vertebras so that the pleura was sufficiently exposed and free of the tumor. Subsequent corpectomy was performed – total in six patients, and partial in six patients. Leksell, Kerrison, and Ferris-Smith rongeurs were used in this process. Hemostasis at this point was challenging, and we used a bipolar coagulator and various haemostatic materials. Next, discectomy of neighboring segments was performed. In 11 patients, a titanium cage of appropriate size was inserted to reconstruct the anterior column. In one patient, we used a temporary polymethyl methacrylate interbody spacer. In this patient, we later performed another operation using a transthoracic approach for removal of the polymethyl-methacrylate interbody spacer, followed by anterior interbody fusion with an iliac crest tricortical graft, and an anterior plate fixation system.

At the stage when discrete manipulation of the dura and spinal cord was necessary, neuromonitoring was used to control the deterioration of motoric and somatosensoric evoked potentials. Neuromonitoring allowed us to adjust the technique of resection or titanium cage insertion promptly, when harmful manipulation of the dura and spinal cord was registered, thus avoiding any long-term iatrogenic neurological deterioration. In all patients, posterior column stabilization was necessary. We used Universal Spinal System (USS) screws, and a rod fixation system on 2-3 neighboring segments in the cranial and caudal direction.

Neurological status examination and pain assessment were repeated upon discharge and at outpatient check-ups approx. 3 weeks after discharge. All the procedures used in our study were in accordance with the Declaration of Helsinki from 1975 and its amendments from 1983.

Results

Our results showed that costotransversectomy, laminectomy, corpectomy, spinal column stabili-

zation, and reconstruction via an extensive posterolateral approach were successful in the treatment of giant spinal epidural metastases and primary tumors.

Examples of spinal stabilization and fixation with USS, as performed in all the patients, are presented in Figure 3, Figure 4 and Figure 5.

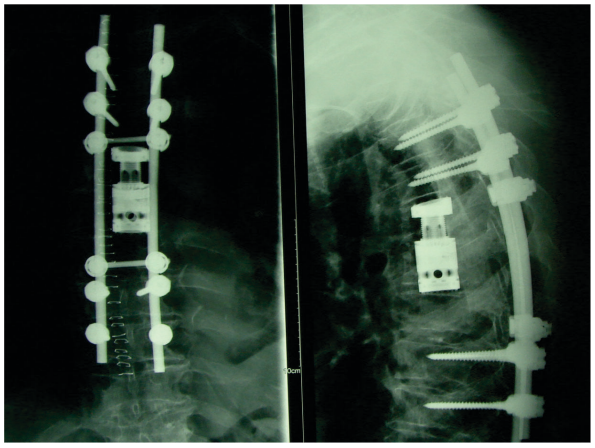


Figure 3. Postoperative CT of a Patient after Vertebrectomy of the 5th and 6th Segments of the Thoracic Spine, Cage Insert and Fixation with the Universal Spine System between the 2nd and 9th Segments of the Thoracic Spine (coronal and Sagittal View).

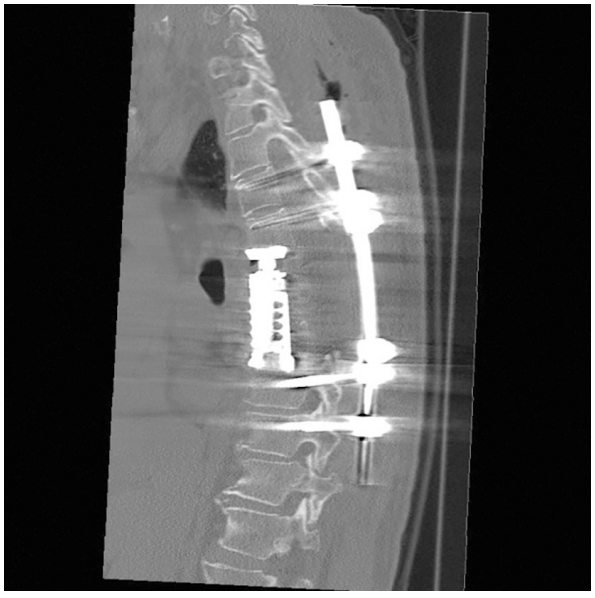


Figure 4. Postoperative CT, Sagittal View, of a Patient after Vertebrectomy of the 6th, 7th and 8th Segments of the Thoracic Spine, Cage Insertion and Fixation with the Universal Spine System between the 4th and 10th Segments of the Thoracic Spine.

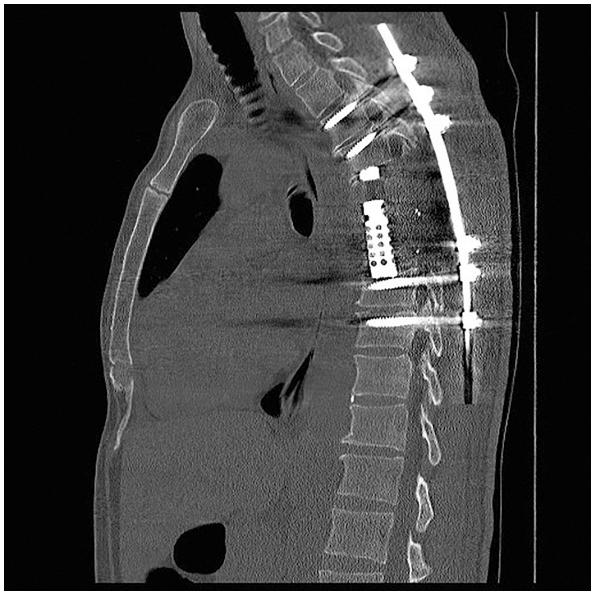


Figure 5. Postoperative CT, Sagittal View, of a Patient after Vertebrectomy of the 5th, 6th and 7th Segments of the Thoracic Spine, Cage Insertion and Fixation with the Universal Spine System between the 3rd and 9th Segments of the Thoracic Spine.

Despite the extended duration of the operation and significant blood loss during tumor resection and vertebrectomy, there were no major perioperative complications. All patients were discharged from the intensive care unit back to the hospital department within a week after the surgery.

Postoperative follow-up showed no deterioration of neurological status. Furthermore, there was a moderate to significant improvement of paraparesis in all patients for weeks after surgery. The most notable improvement was significant pain relief in all of the patients. None of the patients had issues with failure of the implanted hardware. Two patients died less than six months after the surgery due to the advancement of the primary malignant process.

Discussion

In the present study we demonstrated the suitability of an extensive posterolateral approach in cases of giant spinal epidural metastases and primary tumors such as chondrosarcomas, synovial sarcomas and aneurismatic bone cysts.

Using the extensive posterolateral approach enabled us to achieve maximal tumor resection and spine instrumentation in a one-step procedure. Similar results were achieved in other recent clinical studies that addressed the posterolateral approach (6-12). Despite its relative invasiveness, we confirmed that such an approach is safe and predictable as it is more familiar to neurosurgeons than the transthoracic approach, that usually requires the assistance of thoracic surgeons as well. Surgical treatment of patients with cervical spine tumors favors the extensive posterolateral approach even more as it facilitates septation of the vertebral artery, and enables complete exposure of lateral portions of dumb-bell tumors similar to those in other segments of the spine. In their clinical study, Zhao et al. achieved complete resection of the tumor with insignificant blood loss (13).

The selection of patients for operative treatment was challenging since no scoring system can reliably evaluate every patient with a spinal tumor. Our selection process focused on predicting patients' QoL and life expectancy. The decision-making process is usually multidisciplinary, and the NOMS, Tomita, Tokuhashi, and Karnofsky scales have to be taken into consideration.

Despite favourable results in the early postoperative phase in our study, there was susceptibility for general complications for patients with spinal metastases, which is in line with the study by Choi et al. (14). Due to the anatomical challenges in spine surgery, severe complications, such as injury to neural structures, the lungs, or vascular and visceral injuries, might occur. Mohme et al. and Bellut et al. showed better postoperative pain improvement in patients with a higher degree of stenosis, and those with an associated degenerative condition of the spine (15, 16). The histological type of the tumor does not predict early postoperative outcome but has an impact on prognosis. The anatomical location of the tumor affects the postoperative outcome. In essence, patients with SCC in the cervical spine benefited from surgical treatment the most; patients with SCC in the thoracic spine had less favourable prognostic factors in terms of postoperative pain, while patients with primary tumors in the sacral region had less favourable overall survival (15).

In our study, no patient required revision. In the study by Quraishi et al., reoperation rates of spinal metastases remained relatively low (10.7%), with surgical site infection accounting for 42%, failure of instrumentation 26%, and local recurrence 16% of revisions (17). The difference between our study and Quraishi et al. is a result of the smaller number of cases.

In general, surgical treatment should be offered to selected patients with primary spine tumors or spinal metastases, as it provides rapid improvement in pain, and to a certain extent, improved neurological performance. Indications for surgery are wide, since even less extensive palliative procedures significantly improve QoL. Postoperative follow-up in similar clinical studies showed improvement or at least stabilization of the patients' symptoms, with very few patients deteriorating in the months following the surgery. Age >80 years contributes to less neurological improvement after surgery; however, age itself should not be the determinative factor in the decision for surgical treatment. Functional outcome and QoL can be significantly improved despite a short survival rate (18).

Conclusion

In our study, we showed the suitability of an extensive posterolateral approach with costotransversectomy, laminectomy, corpectomy, spinal column stabilization, and reconstruction as a safe and effective one-step approach. It represents a good compromise between invasiveness and sufficient exposure for both tumor resection and spinal column reconstruction, for selected patients with extradural tumors with spinal canal involvement.

Good short-term clinical improvement can be achieved, but the long term results depend on the advancement of the initial disease. Careful evaluation and selection of patients are necessary to achieve clinical improvement and prolonged life expectancy. The best results are achieved with a multidisciplinary approach.

What Is Already Known on this Topic:
Various surgical approaches, such as anterior transthoracic, posterolateral, and posterior are in use for the treatment of giant spinal epidural tumors. Universal consensus on which surgical approach should be practiced has not yet been reached, nor as to which is the safest and least invasive, while still providing sufficient tumor exposure and allowing adequate instrumentation.

What this Study Adds:
An extensive posterolateral approach to giant spinal epidural tumors is a safe and effective one-step approach, allowing sufficient exposure for both tumor resection and spinal column reconstruction.

Authors' Contributions: Conception and design: JR, JŠ and JK; Acquisition, analysis and interpretation of data: JR and JŠ; Drafting the article JŠ; Revising it critically for important intellectual content: JR and JK; Approved final version of the manuscript: JR, JŠ and JK.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Diagnosis and Treatment of Pediatric Brain Tumors

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Abstract

Objective. Tumors of the brain and spine make up about 20% of all childhood cancers; they are the second most common form of childhood cancer after leukemia. Brain tumors are the most common solid tumor in children. Symptoms depend on a variety of factors, including location of the tumor, age of child, and rate of tumor growth. The aim of study was to present our experience with the diagnosis and treatment of brain tumors in children. **Patients and Methods**. The aim of this study is to analyze clinicopathological characteristics, treatments, complications, and outcomes in children with brain tumors. This study is a retrospective analysis of 27 consecutive patients younger than 16 years and hospitalized for surgical treatment of brain tumors. Intracranial hypertension, neurological status, radiological computerized tomography (CT) or magnetic resonance imaging (MRI) findings, tumor localization, type of resection, hydrocephalus treatment, histopathology, complications, and outcome were analyzed. **Results**. Twenty-seven surgeries were performed in patients for brain tumors. There were 9 females and 18 males. The average patient age was 7.8 years. There were 11 (40%) children with astrocytoma; of these, there were 9 (82%) pilocytic astrocytomas and 2 (18%) ordinary histopathological subtypes of high-grade tumors. **Conclusion**. As with any cancer, prognosis and long-term survival vary greatly from child to child. Prompt medical attention and aggressive therapy are important for the best prognosis. Continuous follow-up care is essential for a child diagnosed with a brain tumor.

Key Words: Pediatric Brain Tumors ▪ Diagnosis ▪ Treatment.

Introduction

Tumors of the nervous system represent a diverse spectrum of underlying molecular biological subtypes, prognostic categories, age distribution, and treatment recommendations. Pediatric central nervous system tumors are the most common solid tumors in children and are the leading cause of cancer-related morbidity and mortality. Among all childhood cancers, brain tumors are second only to leukemias in incidence (20%) and are the most common solid pediatric tumor (1), comprising 40–50% of all tumors (2). The annual incidence is 2–5 cases per 100,000 children. The most common pediatric brain tumors are gliomas (cerebellum, brain stem, and optic nerve), pineal tumors, craniopharyngiomas, teratomas, granulomas, and primitive neuroectodermal tumors (PNETs; primarily medulloblastomas) (3).

Signs and symptoms depend on a variety of factors, including the location of the tumor, age of child, and rate of tumor growth. It has traditionally been taught that most pediatric brain tumors (60%) are infratentorial, and that these are equally divided among brain stem gliomas, cerebellar astrocytomas, and medulloblastomas. In reality, the ratio of supratentorial to infratentorial tumors is dependent on the specific age group studied. Supratentorial tumors are more common in infants and children up to 3 years of age and again after age 10, while infratentorial tumors are more common between the ages of 4 and 10 (4). Younger children have a higher incidence of tumors of em-

bryonal origin, whereas older patients tend to have tumors of glial origin. Astrocytomas are the most common supratentorial tumor in pediatrics as in adulthood.

The goal of this study was to present our experience with the diagnosis and treatment of pediatric brain tumors. Also, we discuss the presentation, localization, histology, therapy, and outcome of 2 pediatric brain tumor cases.

Patients and Methods

We completed a database search for records over an 8-year period and identified 27 patients who were surgically treated for pediatric brain tumors. Tumor diagnosis was confirmed at surgery—all cases were included in this study. Demographic, clinical, radiological, and operative data of this patient population were reviewed from the hospital charts and a computerized data bank. In addition, we describe outcome and follow up after discharge from the hospital.

Each brain tumor patient was evaluated by pediatricians, radiologists, neurosurgeons, and oncologists. Clinical findings and ultrasound investigation, computerized tomography (CT), and/or magnetic resonance imaging (MRI) scans of the spine and head were done during diagnosis and at follow-up. Tumor size, anatomical location, and histopathology results were noted. Associated conditions (such as large head size, suggesting underlying hydrocephalus), comorbidity, and associated congenital anomalies were also noted. Detailed neurological examination was performed, noting specifically the presence or absence of any neurological deficit.

Surgical treatment for brain tumor included craniotomy for tumor removal when possible. Depending upon the anatomical location of the tumor, either complete resection or partial resection was done. The goal of surgery was to remove the tumor with preservation of brain function and without neurological deficit.

Results

The neurosurgical data of 27 patients with brain tumor who were admitted over the 8-year period and treated in the Department of Neurosurgery of our institution were retrospectively studied. There were 9 females and 18 males (ratio, 1:2). The average age at the time of presentation was 7.8 years, ranging from 6 months to 16 years. The most common tumor types by localization are shown in Table 1. The most frequent tumor was pilocytic astrocytoma in 9 cases (34%), followed by craniopharyngioma in 4 cases (15%), and ganglioglioma in 4 cases (15%). Patient's characteristics and treatment modalities are shown in Table 2. There was no surgical mortality in this study.

Table 1. Type of Pediatric Brain Tumors

Type of Tumor	Infratentorial (N)	Supratentorial (N)	Total, N (%)
Pilocytic astrocytoma	7	2	9 (34)
Craniopharyngeoma	-	4	4 (15)
Ganglioglioma	-	4	4 (15)
Medulloblastoma	3	-	3 (11)
PNET	2	-	2 (7)
Astrocytoma gr. II	2	-	2 (7)
Hemangioma	2	-	2 (7)
Choroid plexus carcinoma	-	1	1 (4)
Total	16	11	27 (100)

PNET=Primitive neuroectodermal tumor.

Table 2. Characteristics of Patients and Treatment Modalities

Type of tumor	Age (yrs)	Sex, M/F	Type of resection Total/Subtotal	Adjuvant therapy
Pilocytic astrocytoma	10.5	7/2	8/1	-
Craniopharyngeoma	6.2	2/2	3/1	Radiotherapy
Ganglioglioma	4.1	3/1	3/1	-
Medulloblastoma	7.1	2/1	2/0	Radiochemotherapy
PNET	11.4	1/1	2/0	-
Astrocytoma gr. II	12.2	1/1	1/1	-
Hemangioma	7.3	1/1	2/0	-
Choroid plexus carcinoma	3.8	1/0	1/0	Radiochemotherapy

PNET=Primitive neuroectodermal tumor.

Case 1

A previously healthy 13-year-old girl with adequate neuropsychomotor development was admitted to the hospital with a 10-day-long history of headache, vomiting, and deteriorating consciousness. Skull CT and MRI scans showed a deep-seated, irregularly shaped, mural and cystic, expansive and compressive lesion in the right cerebellar hemisphere with contrast uptake that was impinging on and obstructing cerebrospinal fluid (CSF) pathways (Figure 1). A craniotomy with total tumor removal was performed. The histopathology characteristics of the tumor showed a pilocytic astrocytoma. The patient's level of consciousness improved after surgery and her symptoms disappeared. The patient was discharged 8 days after the procedure without neurological deficit. Postoperative MRI showed satisfactory result without tumor remnant (Figure 2).

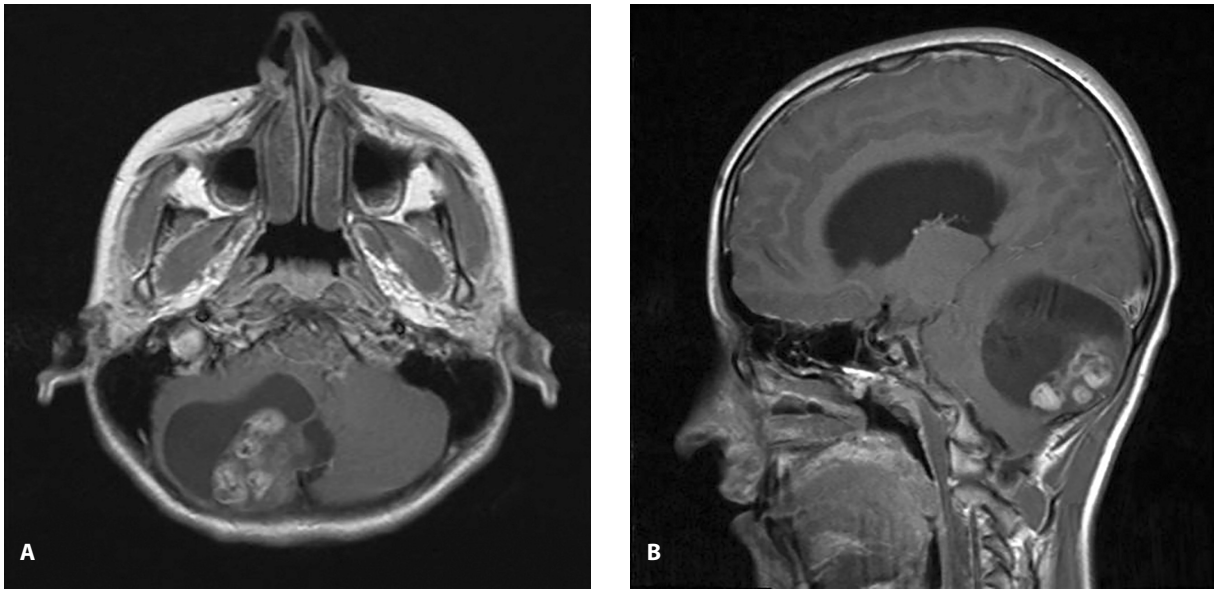

Figure 1. Axial (A) and sagittal (B) MRI showing irregularly shaped, mural and cystic, expansive and compressive lesion in the right cerebellar hemisphere with contrast uptake (pilocytic astrocytoma).

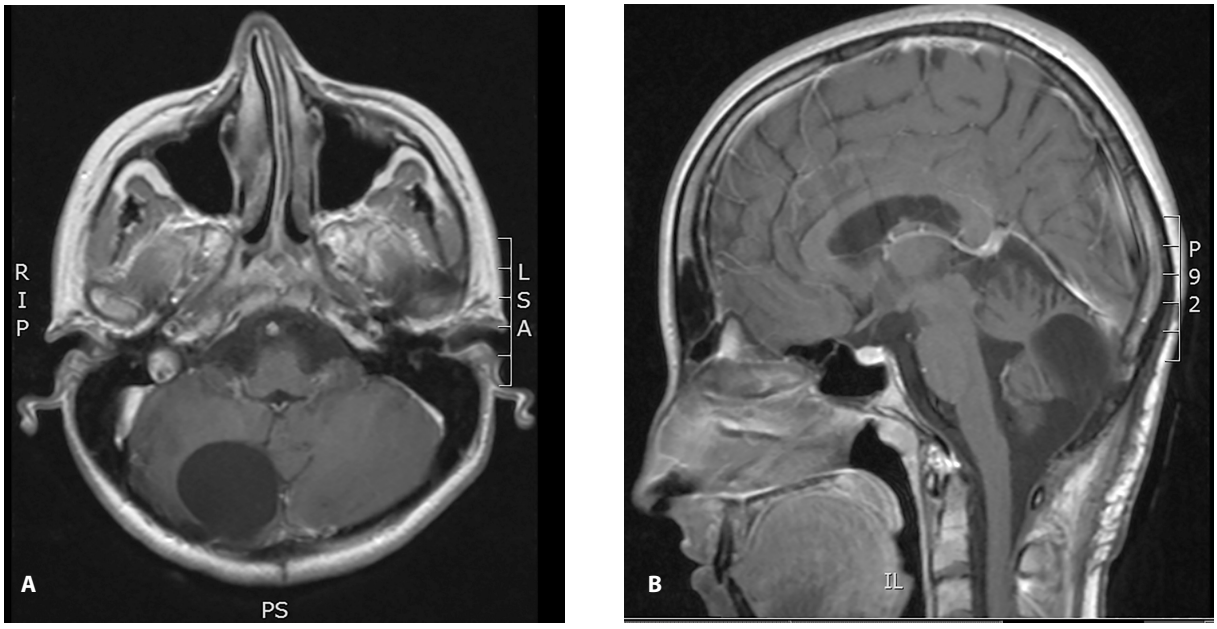

Figure 2. Postoperative axial (A) and sagittal (B) MRI without tumor remnant.

Case 2

A previously healthy 10-year-old boy was admitted to the hospital with a 20-day-long history of headache and vomiting, as well as seizure on the day of admission to the hospital. A skull CT and MRI scans showed a deep-seated, irregularly round, expansive, and compressive brain lesion in the right temporo-occipital region with contrast uptake (Figure 3). A right temporo-occipital craniotomy with total tumor removal was performed. The histopathology characteristics of the tumor showed a ganglioglioma. The patient's symptoms disappeared, and the patient was discharged 8 days after surgery without neurological deficit. Postoperative MRI showed satisfactory resolution without tumor remnant (Figure 4) and the patient had good seizure control.

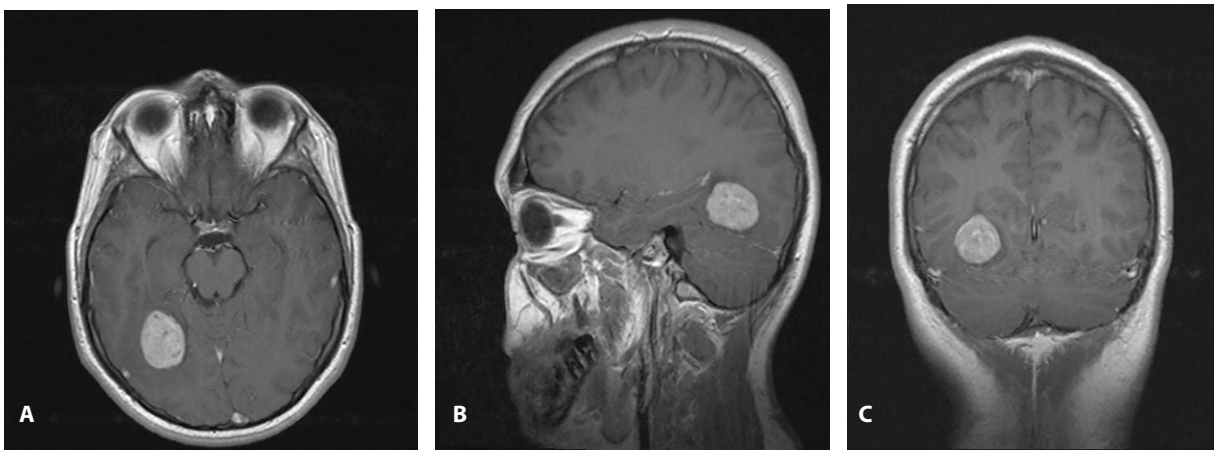

Figure 3. Axial (A), sagittal (B), and coronal (C) MRI showing irregularly round solid expansive and compressive brain lesion in the right temporo-occipital region with contrast uptake (ganglioglioma).

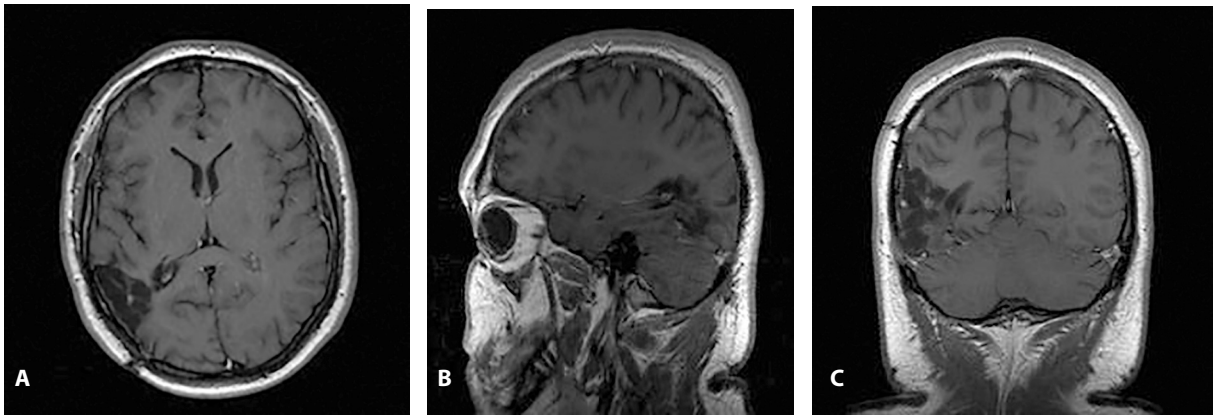

Figure 4. Postoperative axial (A), sagittal (B), and coronal (C) MRI.

Discussion

We analyzed 27 consecutive patients who were operated on for pediatric brain tumor. Patients were admitted at a children's hospital for symptoms of high intracranial pressure, seizure, or neurological deficit. After radiological diagnosis and preoperative preparation, patients were transferred to the department of neurosurgery for tumor resection. In 2 cases, an emergency surgery was done for a ventriculoperitoneal shunt as a first treatment step. The most common form of treatment was surgical removal. Follow-up and additional treatment with irradiation and chemotherapy depended on the result of histopathology. All our cases presented as single grade tumors. Some brain neoplasms only manifested as a single grade — for example, pilocytic astrocytoma, subependymoma, subependymal giant cell astrocytoma, myxopapillary ependymoma, and most glioneuronal tumors (5). In our study, the tumors manifested as a single grade. Among histopathologically benign tumors there was no spontaneous anaplastic transformation.

Regarding embryonal tumors, the categorization of medulloblastomas has undergone extensive changes since publication of the 2007 World Health Organization (WHO) tumor classification. There are now 5 subtypes based on genetic and expression profiles that correspond to histological subtypes only to an extremely limited extent (6). Histological stratification of medulloblastomas based on the 2007 WHO classification has indeed limited prognostic value, although it has long been recognized that desmoplastic and extensive nodularity variants carry a better prognosis (7), and the large cell anaplastic variants carry a worse prognosis. Also, the degree of anaplasia correlates significantly with clinical outcome (8). Following radiotherapy, chemotherapy, or both, medulloblastomas with extensive nodularity occasionally undergo maturation to tumors dominated by ganglion cells (9).

Three medulloblastoma (11%) were found in our study. Following tumor resection, patients were treated by radiotherapy and chemotherapy. Radiological follow-up implied MRI of the complete neuroaxis.

Diffuse midline glioma was first introduced as diffuse intrinsic pontine glioma (DIPG). Patients are typically young children with brainstem symptoms; signs of CSF obstruction rapidly develops within a few months. On MRI, DIPGs often present as a large pontine mass, which may encase the basilar artery (10). Contrast enhancement is usually focal. Infiltration of neighboring structures has frequently been observed. These tumors are diverse histopathologically, although they commonly show a uniform population of cells that resemble neoplastic astrocytes. Necrosis and vascular proliferation are also seen in some cases (11). Extrapontine lesions typically affect older children and occasionally adults. Since most cases contain the typical mutational profile, the term proposed by the WHO Working Group is a diffuse midline glioma H3 K27M-mutant (3).

Among the astrocytomas in our study, the most frequent tumor was pilocytic astrocytoma (34%) and then Grade II astrocytoma (7%). The astrocytomas were located in cerebellar hemispheres and they were completely removed during surgery. There was no tumor remnant found during the follow-up period. One-third of pediatric brain tumors in our study were pilocytic astrocytomas. These tumors were often cystic with a solid part, and they tended to be well-circumscribed. Tumor location could prohibit access to the neoplasm and lead to an incomplete resection. Removal of the tumor will generally allow functional survival for many years (12).

Craniopharyngiomas are histologically benign neuroepithelial tumors of the central nervous system (CNS) with malignant behavior, which are predominantly observed in children aged 5 to 10 years. These lesions tend to invade surrounding structures and to recur after a total resection (13, 14). Two of 4 craniopharyngiomas in this study showed a recurrence during the follow-up period, and reoperation was needed.

Ganglioglioma is a common seizure-associated tumor, and surgery is currently considered the treatment of choice. During the follow-up period, all 4 ganglioma patients in this study became seizure-free. These data indicated that surgical treatment might result in excellent seizure control for patients with ganglioglioma (15). Genetically based classifications are a great step forward and a valuable basis for future clinical trials. From a clinical point of view, the relevance of tumor grades may not be as significant as before.

Pediatric brain tumor symptoms vary according to the size, type, and location of the tumor. In our study the most frequent symptoms were headache and vomiting. Symptoms may occur when a tumor presses on a nerve or damages certain parts of the brain. They may also occur when the brain swells or there is fluid build-up in the skull. The most common symptoms include the following: headaches (usually worse in the morning); nausea or vomiting; changes in speech, vision, or hearing; problems balancing or walking; changes in mood, personality or ability to concentrate; problems with memory; muscle jerking or twitching (seizures or convulsions); and numbness or tingling in the arms or legs.

Brain tumors presenting during the first year of life is a different subset of tumors than those presenting later in childhood. In a busy neurosurgical unit in a children's hospital, they represented approximately 8% of the children admitted with brain tumors, an average of only ~3 admissions per year (16). Ninety percent of brain tumors in neonates are of neuroectodermal origin, teratoma being the most common. Other supratentorial tumors include astrocytoma, choroid plexus tumors, ependymomas, and craniopharyngiomas. Some of these tumors may be congenital (17).

The youngest patient in this study was 6-months-old and treated surgically for a large compressive ganglioglioma of the left-brain hemisphere. Postoperative functional and psychophysical development in this patient proceeded normally without neurological deficit. There was 1 patient with a large compressive choroid plexus carcinoma (CPC) who was treated first via tumor resection, followed by radiotherapy and chemotherapy. CPCs are rare neoplasms of neuroectodermal origin that correspond to WHO Grade III tumors. Due to their rarity, reports on CPCs most often focus on single cases or single-institution experiences with a limited number of patients

Many posterior fossa tumors escape diagnosis until they are large in size due to the elasticity of the infant skull, which is proof of the adaptability of the developing nervous system to compensate for deficits, and of the difficulty in examining a patient with a limited neurologic repertoire and an inability to cooperate. The most common presenting manifestations are vomiting, arrest or regression of psychomotor development, macrocrania, and poor feeding or failure to thrive. They may also present with seizures as one of significant long-term sequelae that influences treatment-related morbidity (18).

A small sample size in this study is a limitation in statistical power and conclusions. We presented our experience in study of 27 consecutive patients who were admitted to department of neurosurgery

for a surgical treatment. We did not include patients who are not treated surgically for extensive infiltrative brain tumors and infiltrative brain stem tumors with poor general and neurological status. These patients have been treated and followed up by pediatrics, neuro-oncologists, and radiation oncologists.

We decided to present the surgical results of 2 cases: one of the most common (pilocytic astrocytoma — WHO Grade I) and one of the rarest (choroid plexus carcinoma — WHO Grade III) pediatric brain tumors with a different supratentorial and infratentorial localization and a different further prognosis. The tumors were removed completely with a good postoperative radiological result on MRI. Further radiological follow-up showed good results in patients with astrocytoma and a recurrence in patients with choroid plexus carcinoma.

Conclusion

Pediatric brain tumors are the most common solid tumors in children and the leading cause of cancer-related morbidity and mortality. Over the past decades, considerable advances have been made in neurosurgery, radiotherapy, and chemotherapy that result in improved survival and cure rates for children with brain tumors. Prompt medical attention and aggressive therapy are important for the best prognosis.

What Is Already Known on this Topic:

Prognosis and long-term survival of pediatric brain tumors are based upon the type of tumor, tumor location and grade, the length of time the child has exhibited symptoms, the speed of growth, and treatment options. While survival and cure rates have improved, treatment-related morbidity remains high and significant long-term sequelae are common.

What this Study Adds:

Surgery is a first step in treating brain tumors in children with the goal to remove all or as much of the tumor as possible while maintaining neurological function. A close collaboration between neurosurgery, pediatrics, neuro-oncology, radiation oncology, and diagnostic radiology was essential for a good treatment and prognosis of our patients.

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Endoscopic Endonasal Approaches to the Clival Region

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Abstract
Objective. Our main objectives were to analyze and determine the safety, risk of post-operative complications, and surgical outcome of the endoscopic endonasal approach to the clival region. **Methods**. From May 2011 to May 2019, we operated on 19 patients using the endoscopic endonasal approach to the clival region. Their pathologies were diverse: pituitary macroadenoma, craniopharyngioma, metastasis, and a prepontine neurenteric cyst. The first operations were supervised by an experienced center using telementoring. We explained our surgical technique and analyzed the patients' data, which were included in our study. **Results.** We managed to achieve complete removal of the pathological process in 14 patients. There were no deaths in the perioperative and early post-operative period. The most common complication was a cerebrospinal fluid leak, which was successfully managed in all of the cases. There were no deaths or significant morbidities in the post-operative period. **Conclusion.** An endoscopic transnasal approach to the clival region is safe and effective. It provides better visualization of that region compared to other transcranial approaches. The risk of post-operative complications is significantly lower with the help of modern reconstructive techniques. CSF leak is the most frequent complication.

Key Words: Endoscopic Endonasal Approach ▪ Clival Region ▪ Operational Technique ▪ Complications ▪ Differential Diagnosis of Clival Pathology.

Introduction

The posterior cranial fossa, especially the clival region, is in the skull base, which is often difficult to access surgically (1). Managing clival lesions presents numerous challenges due to the close proximity of surrounding critical structures, including the basilar and internal carotid arteries, brain stem, and the cranial nerves (2). Important region considerations are the depth of the surgical approach and the effect of the tumor on the surrounding structures (3-6). The endoscopic endonasal approach has recently become one of the most frequently used methods in the surgical management of clival lesions at leading skull base centers (7-10). With the help of the endoscopic endonasal approach, operating procedures in the clival region have become safer, faster, and easier to perform, compared to transcranial methods.

The development of endoscopic endonasal surgery for different pathologies in the skull base region has made significant progress in the last 10 years. Skull base regions are more accessible with the help of the extended endonasal approach, especially the clival region. The endonasal approach to this region is faster and safer than transcranial techniques. It provides a direct view of the median structures of the skull base without applying traction to various structures of the brain (3-6). Surgical telementoring from experienced centers is able to provide considerable help in achieving a higher level of surgical proficiency (11).

With the help of modern reconstructive techniques, the rate of post-operative complications

is lower, especially cerebrospinal (CSF) leak (12). The results of the endoscopic endonasal approach to the clival region may thus be equal or superior to the results of transcranial approaches (1).

The most common lesions involving the clival region are: chordomas, chondrosarcomas, meningiomas, pituitary adenomas, metastatic tumors, and plasmacytomas. The first endoscopic endonasal approach to the clival region for these diverse pathological processes for our institution, the University Medical Center, Maribor, was performed in May 2011. All operations were performed with a collaborating team of ear-nose-throat (ENT) specialists and neurosurgeons. Surgical telementoring from the University of Pittsburg Medical Center was utilized in the first two years when performing complex endonasal procedures. This paper presents our results in the 8-year period.

Our main objectives were to analyze and determine the safety, risk of post-operative complications, and surgical outcome of the endoscopic endonasal approach to the clival region.

Methods

Surgical Technique

All patients underwent surgery using the extended endoscopic endonasal approach (EEA). Two surgeons (a neurosurgeon and an otorhinolaryngologist) cooperated throughout the procedure. Two nostrils were used to insert the endoscope and surgical instruments throughout the procedure. The leading surgeon was determined on the basis of the stage of the procedure. To start with, we prepared the nasoseptal flap on one side of the nose and performed complete ethmoidectomy with a median meatotomy. We saved the flap in the maxillary sinus and continued the approach with posterior septectomy (resection of the vomer) and bilateral sphenoidotomy. We saved the epithelial tissue of the nasal septum on the opposite side for reconstruction of the remainder of the nasal septum. We proceeded with the removal of epithelial tissue of the sphenoid and posterior ethmoidal sinuses, and the complete resection of the rostrum. We resected the epithelial tissue of the nasopharynx when necessary. With the resection of the clivus, we created a corridor between both paraclival carotid arteries. We drilled the bone until we reached the basilar plexus. Bleeding in this region was usually stopped with liquified hemostatic materials. After complete hemostasis, we proceeded from the extradural to the intradural stage of the operation in cases where the pathology lay subdurally.

The intradural technique of tumor removal was the same as the usual microscopic technique. The only difference was that it was performed with endoscopic visualization. The instruments were longer and modified for the endoscopic approach. Resection of critical structures demanded good cooperation between the two surgeons. We had to maneuver the location and angles of the endoscope to achieve the best possible visualization of critical structures. When bleeding occurred, coagulation was possible with the use of a modified coagulator. Applying sutures using endoscopic techniques was especially challenging. We used 5-0 nylon interrupted sutures. It was found that interrupted sutures were more suitable for a tight dural closure than running sutures, which were technically harder to place. Some authors did not use sutures at all, but use fibrin glue and mucosal flaps instead (13). Others, such as Yudo et Al., used a technique similar to ours (14). To achieve hemostasis, we sometimes used miniature surgical staples.

We then proceeded with the multi-layered reconstruction of the defect. For the intradural avascular layer, we used fascia lata. The second avascular layer was placed extradurally. It covered the defect and the surrounding bone. Resection of the clivus created a deep defect in the bone, which we filled with autologous fat from the patient's thigh. We then covered the avascular reconstruction with the vascularized nasoseptal flap, which we had prepared earlier on. The flap covered the entire avascular reconstruction and the resected bone, from which we removed all the epithelial tissue. Patients with more severe clival defects required the insertion of a lumbar drain and the opening of the prepontine cistern.

The case of one patient with an intradural lesion is presented in Figures 1-3.

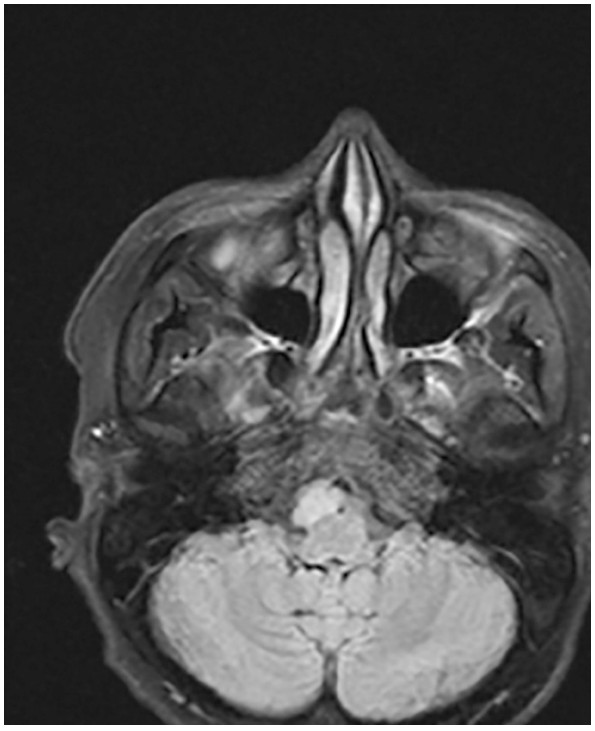

Figure 1. Preoperative head MRI of a 48-year old female patient with right abducens nerve palsy and a neurenteric prepontine cyst. The cyst was removed entirely and the abducens nerve palsy normalized.

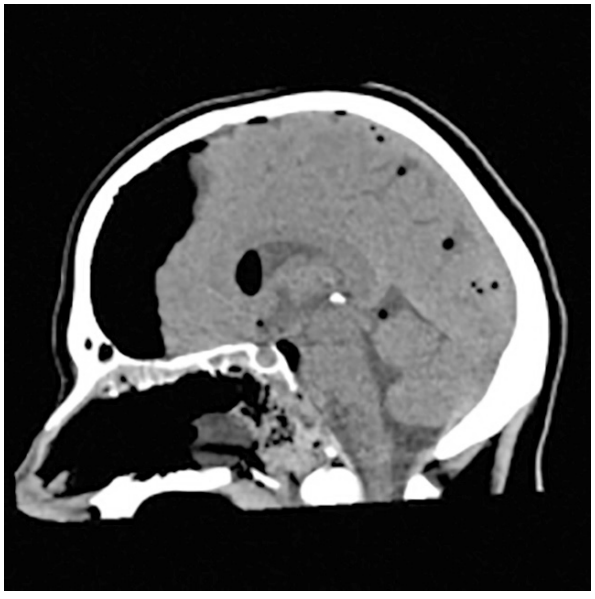

Figure 2. Post-operative head CT of the patient presented in Figure 1, which was taken on the first post-operative day. Most of the clivus has been resected due to the approach to the cyst. Pneumocephalus is seen, which did not manifest clinically and later spontaneously resolved.

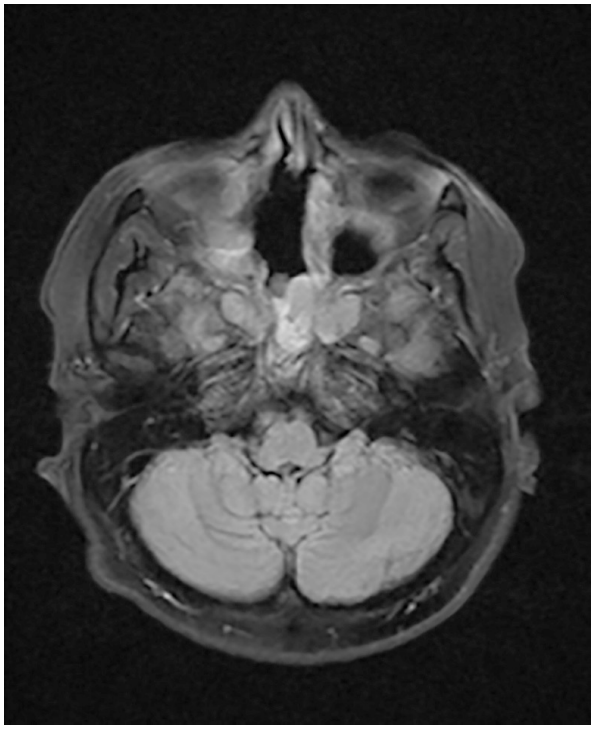

Figure 3: Post-operative head MRI of the patient presented in Figure 1, showing the completely removal of the prepontine cyst.

Results

Patients

From March 2011 to May 2019, we operated on 19 patients (10 females, 9 males). The average age of the patients was 52 years (standard deviation of 15 years). Nine patients had pituitary macroadenoma (6 of them had a secreting tumor with acromegaly, 3 of them had non-secreting tumors). In all of them the pituitary tumor invaded the clivus. Other pathologies were less frequent. Patients' characteristics, surgical variables, outcomes and complications are summarized in Table 1.

Table 1. Patients' Characteristics

Patient	Gender	Age (year)	Pathology	Type of resection	Reconstruction	Outcome	Death*	Intradural growth	Dural opening	Complications
1	M	40	Pituitary adenoma (secreting tumor)	Complete removal	Multilayer	Following up	No	No	No	-
2	F	53	Pituitary adenoma (secreting tumor)	Complete removal	Multilayer	Following up	No	No	No	-
3	M	37	Pituitary adenoma (secreting tumor)	Complete removal	Multilayer	Following up	No	No	No	-
4	F	64	Pituitary adenoma (secreting tumor)	Complete removal	Multilayer	Following up	No	Yes	Yes (1 cm)	CSF leak
5	F	66	Pituitary adenoma (secreting tumor)	Subtotal resection	Multilayer	Following up	No	Yes	No	-
6	M	68	Pituitary adenoma (secreting tumor)	Complete removal	Multilayer	Following up	No	Yes	Yes (1 cm)	-
7	M	64	Pituitary adenoma (non-secreting tumor)	Subtotal resection	Multilayer	Following up	No	Yes	Yes (1.5 cm)	CSF†
8	F	48	Pituitary adenoma (non-secreting tumor)	Complete removal	Multilayer	Following up	No	No	No	-
9	F	64	Pituitary adenoma (non-secreting tumor)	Subtotal resection	Multilayer	Following up	No	No	No	-
10	M	49	Chordoma	Partial resection	Multilayer	Died 1 year after surgery	No	Yes	Yes (2 cm)	-
11	M	44	Chordoma	Complete removal	Multilayer	Following up	No	No	No	-
12	F	68	Chordoma	Subtotal resection	Multilayer	Died 3 years after surgery	No	No	No	Bleeding‡
13	F	38	Chordoma	Complete removal	Multilayer	Following up	No	No	No	-
14	M	67	Metastasis (origo prostate cancer)	Complete removal	Multilayer	Died 1 year after surgery	No	No	No	-
15	F	58	Metastasis (origo breast cancer)	Complete removal	Multilayer	Died 1 year after surgery	No	No	No	-
16	M	61	Craniopha-ryngioma	Complete removal	Multilayer	Following up	No	Yes	Yes (3 cm)	Bleeding§
17	F	48	Neurenteric cyst	Complete removal	Multilayer	Following up	No	Yes	Yes (2.5 cm)	-
18	M	45	Solitary fibrous tumor	Complete removal	Multilayer	Following up	No	Yes	Yes (3 cm)	CSF†
19	F	6	Cholesteatoma	Complete removal	Multilayer	Following up	No	No	No	-

*Perioperative or Postoperative period; †CSF leak, revision surgery, ‡Minor post operative bleeding; §Bleeding continued with procedure one week later.

None of our patients died in the perioperative or early post-operative phase. One patient with chordoma died one year after the operation because of disease progression, while one patient died three years after the procedure due to myocardial infarction. Two patients with metastatic disease died one year after the operation because of the progression of the primary disease. The rest of the patients are still alive and are being followed up with regular MRI scans and hormone testing if necessary. Acromegaly has been well controlled after the surgery in six patients, where three of them need additional medical therapy. We achieved complete removal (no evidence of tumor tissue on postoperative CT or MRI scan) of the pathological process in 14 patients. One patient with chordoma had partial resection using the endoscopic transnasal approach, while the rest of the tumor in the pontocerebellar angle was removed using the suboccipital retrosigmoid approach. Subtotal removal (the volume of the remaining tumor tissue is less than 20% of the original tumor size) in the remaining 4 patients, one with chordoma and three with pituitary adenoma. The most common reason for subtotal removal was adherence of the tumor to the surrounding vital structures.

We dealt with intradural tumor growth in 8 cases (44% of pituitary adenomas, 25% of chordomas), and the average size of the dural opening was 1.75 cm. The most common complication was a cerebrospinal fluid (CSF) leak from the nose, which occurred in three patients. We inserted a lumbar drain in all of these patients for one week. Two of them needed revision surgery, and in one patient, the CSF leak stopped once the external lumbar drainage was inserted. We had to stop the procedure on one patient with craniopharyngioma due to severe bleeding from the venous plexuses in front of the clivus. We managed to achieve hemostasis, but we did not continue with the intradural stage of the operation. We continued with the procedure one week later when there was no excessive bleeding, and we successfully removed the tumor. One patient developed minor post-operative bleeding, which needed no revision surgery. There were no other major complications in our series.

We correlated complications with time and found that 2 out of 3 patients with CSF leakage had undergone surgery among the first 11 cases, and the 3rd patient with CSF leakage was the 13th patient to undergo surgery. We did not have any major complications in the last 6 cases. The mean surgical time in the first 10 cases was 342 minutes, while the mean surgical time in the last 9 cases was 297 minutes.

Discussion

On the basis of our results, we can say that the endoscopic transnasal approach to the clival region, performed at our institution, is safe and effective. None of the patients died in the perioperative or early post-operative phase. Complete removal of the pathological process was achieved in most of the patients. Apart from CSF leakage, there were no other major complications in our series.

In our series, the rate of post-operative CSF leakage was 16% (3 out of 19 patients), or 37.5% of patients with an intraoperative dural opening (3 out of 8 patients). We managed to stop the CSF leak in all cases. We inserted a lumbar drain in all of these patients for one week, and two of them needed revision surgery. One patient with a CSF leak developed bacterial meningitis, which was cured with appropriate antibiotic therapy. For skull base reconstruction and to reduce the risk of CSF leakage, we used a nasoseptal flap. We reconstructed the dura mater with two avascular layers and filled the clival defect with autologous fat tissue. Then, we covered the avascular reconstruction with the nasoseptal flap.

From a review of the literature, we found that the clival region can be divided into the upper, middle, and lower sections by the transverse lines located at the level of the dural entrance of the abducent and glossopharyngeal nerves. That separation is based on the concept of three neurovascular complexes in the posterior cranial fossa (3, 15). Approaches to the upper, middle, and lower sections of the clivus provide access to the anteromedial region of these three neurovascular complexes (1, 15, 16). An extended approach to the upper

section of the clivus allows access to the midbrain, the upper half of the pons, the superior cerebellar artery, and the oculomotor and trigeminal nerves in the upper neurovascular complex. Approaches to the middle section of the clivus provide access to the lower half of the pons, the anterior inferior cerebellar artery, and the abducent, facial and vestibulocochlear nerves in the middle neurovascular complex (1). The paraclival carotid artery represents the lateral limit of this approach. Extended approaches to the lower section of the clivus provide access to the medulla oblongata, the posterior inferior cerebellar artery, and the glossopharyngeal, vagus, accessory, and hypoglossal nerves in the lower neurovascular complex (1). The foramina lacera are the lateral limits of this approach at the lower section of the clivus.

The most common lesions involving the clival region are chordomas, chondrosarcomas, meningiomas, metastatic tumors, plasmacytomas, and lymphomas. Other paraclival lesions that can extend into the clivus are invasive pituitary adenomas, invasive nasopharyngeal carcinoma, and juvenile angiofibroma (17). Surgical resection is the primary treatment of most lesions. Radiation therapy may be considered, either as primary therapy for poor surgical candidates, or as adjuvant therapy for aggressive or recurrent disease (2).

Median tumors of the skull base are challenging targets for surgical treatment using standard transcranial approaches (18, 19). The endoscopic endonasal approach is a fast and effective method, characterized by high radicality, low risk of post-operative complications, and low mortality, and it is suitable for surgeons skilled in endoscopic surgical techniques (12, 18, 19).

Doglietto et al.'s quantitative anatomical study showed that endoscopic transnasal approaches to the clivus provide larger working volume and broader exposure of the clivus, compared to »classic« lateral approaches (20).

It is essential to understand the relationship between the extent of the involvement of the pathological process in the clivus or surrounding neurovascular complexes, and whether the process is located intradurally, extradurally, or both. The transclival approach can also be used for different pathologies, not just for tumors in the clival region. It can be implemented for clipping centrally located posterior cranial fossa aneurysms, originating from the superior cerebellar artery, the anterior inferior cerebellar artery or the posterior inferior cerebellar artery (15).

The repair of clival skull base dural defects is often optimized with a multilayer closure, which is challenging on account of the inability to exert forceful pressure posteriorly, where critical structures are located (basilar artery, brain stem) (2). Multilayer closure can also help prevent pontine herniation through the clivus defect (21).

The extended endonasal transclival approach has several advantages compared to transcranial approaches. Some of the benefits are the patients' quick recovery, short hospital stays, and minimal post-operative discomfort (22-26). An endoscope allows a significantly wider and better-illuminated field of view (27). As a fully endoscopic procedure, there is no need to perform traction on various structures of the brain, and it provides a wider angle of exposure and a direct view of the midline structures. There is no need to displace the vertebral artery. It provides a well-lit surgical corridor and adequate visualization of even the most inaccessible regions (3-5, 28, 29). Since the endonasal surgical corridor is not associated with resection of the oropharynx and the soft palate, the risk of bacterial contamination and infection is low. The patients have a low risk of post-operative swallowing and speech disorders, and are capable of oral food intake, without the risk of dysphagia, immediately after surgery. There are also some drawbacks to the extended endoscopic transnasal approaches to the clivus, which can be challenging to perform in cases of atypical topography of the neurovascular structures located medially and anteriorly in relation to the tumor (1). Some other disadvantages are the limited working space, reduced maneuverability, and the need for special instrumentation (27). The use of the transnasal approach can also be associated with the risk of damaging the lateral parts of the cranial nerves (oculomotor, abducens, glossopharyngeal, and the vagus nerve)

(29). A relative contraindication for this approach is the significant lateral displacement of the tumor at the level of the foramen magnum, posterior to the occipital condyle, because there is a risk of craniocervical instability and injury to the caudal group of nerves (30). In cases of significant lateral extension of the lesion, it is preferable to choose a staged approach, combining a craniotomy with an endoscopic technique. A combined open and endoscopic approach allows the surgeon to deal with different components of the tumor most appropriately and directly (25). Absolute contraindications include tumors with invasion of the orbit, requiring exenteration, or involving the skin or anterior wall of the frontal sinus, and those that require microvascular reconstruction (31).

Extended endoscopic transnasal approaches can be considered as an independent and universal approach to pathological lesions of the skull base. The choice of approach should be based on the location of the tumor, the anatomical and clinical characteristics of the individual patient, as well as the level of the surgeon's proficiency in using endoscopic approaches (29, 32).

When we first started endoscopic skull base surgery at the University Medical Centre, Maribor, a surgical telementoring program was established with an experienced skull base team at the University of Pittsburgh Medical Center in Pennsylvania. The two-way video and audio streaming provided real-time communication between the surgical teams. The most frequent mentoring interventions concerned the identification of anatomy, the extent of exposure, the extent of resection, and the surgical technique. The assessment of the efficiency of this surgical telementoring program showed many benefits. (11). We correlated complications and surgical time with time. We showed that all of the complications happened among the first 13 out of 19 cases, while mean surgical time in the last 9 out of 19 cases were on average 45 minutes shorter. We still have limited data because of the low number of cases, but the results show the efficacy of the telementoring program, with fewer complications and shorter surgical time over time.

Endoscopic surgery in the clival region has potential complications (33). Intracranial complications can result from direct injury to the brain, cranial nerves, meninges, blood vessels, or venous sinuses. In the case of the brain and cranial nerves, the resulting deficits reflect the loss of function of the damaged structures. In the case of damage to blood vessels, resulting hematomas can create a mass effect, or loss of vascular supply can lead to stroke. Severe injuries of the internal carotid artery can be catastrophic and lethal. CSF leakage can cause symptoms directly and predispose to meningitis. Pneumocephalus can also cause mass effect symptoms (33).

The most frequent immediate complications are CSF leak, intraoperative bleeding, and injury to the brain-stem and/or intracranial nerves. Wide trepanation of the clivus and significant defects of the dura mater increase the risk of post-operative CSF leakage (1). The most frequent delayed complications are meningitis, delayed bleeding, and delayed CSF leak. There is also a possibility of transitory or permanent endocrinological complications that may result from manipulation, compression, or traction of the hypophysis (33). It is important to recognize and diagnose pituitary insufficiency early, and manage this condition promptly.

There are many techniques for skull base reconstruction, and to reduce the risk of CSF leakage. Some of them are the use of a balloon catheter, microsurgical duroplasty, and nasoseptal flap, which we use. With the use of these modern techniques, the risk of post-operative CSF leakage can be diminished to 0% - 9.5% (34-36).

Conclusion

The endoscopic transnasal approach to the clival region is safe, effective and provides better visualization of that region compared to other transcranial approaches. CSF leak is the most frequent complication. Successful endoscopic surgeries in this region are based on the development of new endoscopes, surgical instruments and operative materials, but more importantly, also on the sur-

geon's knowledge of anatomy, the mastering of endoscopic surgical techniques, and a multidisciplinary approach. In our series, we showed that the transnasal approach to the clival region can be effectively performed, even in small neurosurgical departments, for different kinds of clival pathologies. At first, this was made possible through a surgical telementoring program, established with an experienced skull base team.

What Is Already Known on this Topic:

The endoscopic endonasal approach has recently become one of the most frequently used methods in the surgical management of clival lesions, and is safe, effective and provides better visualization of that region compared to other transcranial approaches. With the help of modern reconstructive techniques, the rate of post-operative complications is lower, especially CSF leak. The most common lesions involving the clival region are: chordomas, chondrosarcomas, meningiomas, pituitary adenomas, metastatic tumors, and plasmacytomas.

What this Study Adds:

In our series, we showed that the transnasal approach to the clival region can be performed effectively, even in small neurosurgical departments, on a relatively small number of patients. In the beginning, the rate of complications was higher and the mean surgical time was longer. We showed that the learning curve can be steep with the help of a surgical telementoring program, established with an experienced skull base team.

Authors' Contributions: Conception and design: JR, BH, and BL; Acquisition, analysis, and interpretation of data: JR, BH, and BL; Drafting the article: JR, BH; Revising it critically for important intellectual content: JR, BL; Approved final version of the manuscript: JR, BH, and BL.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Peripheral Nerve Tumors as an Ongoing Challenge in Neuro-oncology: An Overview of Their Biological and Technical Nuances

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Abstract
This paper aims to provide an overview of recent advances in the diagnosis and treatment of peripheral nerve tumors (PNTs) with regard to biological and technological nuances, and to highlight some recommendations for achieving better outcomes in the treatment of patients suffering from PNT. PNTs are probably the most challenging entity in the field of peripheral nervous system surgery. The goal of removing a nerve tumor while also preserving nerve function at the same time is often complicated, regardless of the surgeon's experience. Still, in most cases, high-quality results can be achieved upon carefully planned surgery. Clinical presentation, diagnosis, and indications for a specific type of treatment of PNTs still remain a topic of debate. Recent technological advances have led to an exponential improvement in the field with utilization of intraoperative ultrasound, neurostimulation devices, and intraoperative electrophysiological monitoring, along with the development of modern surgical techniques, whereby a multidisciplinary and individually shaped approach is necessary. **Conclusion.** These advances, however, still remain limited, and recent research is focused on the development of biological therapy. Biologically targeted therapies will emerge when there is a better understanding of the genetic and molecular mechanisms driving the development and growth of PNTs.

Key Words: Peripheral Nerve ▪ Tumor ▪ Surgery ▪ Biology ▪ Technology.

Introduction

Peripheral nerve tumors (PNTs) are probably the most challenging entity in the field of peripheral nervous system surgery. The goal of removing the nerve tumor while simultaneously preserving nerve function is sometimes extremely complicated, despite the surgeon's experience. Still, in most cases, high-quality results can be achieved upon carefully planned surgery (1). Different types of tumors require different surgical and conservative treatment strategies; therefore, a good working knowledge of anatomy and pathophysiology of the various tumors is mandatory, as well as familiarity with the modern surgical techniques (2).

Recent technological advances have led to an exponential improvement in the field of PNT treatment with the utilization of intraoperative ultrasound, neurostimulation devices, and intraoperative electrophysiological monitoring, as well as with the development of the modern surgical techniques. Multidisciplinary cooperation among the neurosurgeon, orthopedic surgeon, vascular surgeon, plastic surgeon, physiatrist, physiologist, neurologist, and radiologist is essential for further development of PNT treatment. Nevertheless, these technological advances still remain limited, and recent research is now focused on the development of biological therapy (3).

This paper aims to provide an overview of recent advances in PNT diagnosis and treatment with regard to biological and technological nuances, and to highlight some recommendations for achieving better outcomes in the treatment of patients suffering from PNTs.

General Characteristics and Classification

The clinical presentation of PNT is commonly asymptomatic, but typical presenting signs (or a combination of signs) include a palpable mass adjacent to the nerve and nerve palsy with or without pain. Nonspecific symptoms related to mass effect are components of a clinical presentation in symptomatic patients, but it is important to have an understanding about the nature of the tumor before surgery. The diagnostic assessment comprises of the patient's history and a clinical evaluation, followed by neuroradiological imaging and electrophysiological studies. Most commonly, the histological origin of these tumors is from the nerve sheath, but they can also be derived from neural structures (4) (Table 1).

Table 1. World Health Organization Classification of Nerve-sheath Tumors

Benign	Malignant
Schwannoma (including variants)	Malignant peripheral nerve sheath tumor
Melanotic schwannoma	Epithelioid malignant nerve sheath tumor
Neurofibroma (including variants)	Malignant Triton tumor
Plexiform neurofibroma	Malignant granular cell tumor
Perineurioma	Ectomesenchymoma
Malignant perineuriomas	-
Granular cell tumor	-
Dermal nerve sheath myxoma	-
Solitary circumscribed neuroma	-
Ectopic meningioma	-
Nasal glial heterotopia	-
Benign Triton tumor	-
Hybrid nerve sheath tumors	-

Benign PNT lesions are much more common than malignant lesions. Benign peripheral nerve sheath tumors (BPNSTs) represent approximately 1% of all soft tissue tumors. While they are more frequently found in the upper limbs, mostly affecting large nerve trunks, any nerve can ultimately be affected. BPNSTs most often include schwannomas and neurofibromas. They can appear spontaneously or within different syndromes (neurofibromatosis-1 and 2 [NF1 and NF2], Schwannomatosis, etc.) (5, 6). Benign tumors and tumor-like lesions of nonneural origin are rare lesions (usually ganglion cysts, vascular tissue or desmoid tumors), and were sporadically reported within the nerves (7-11). Hemangioblastomas of peripheral nerve were also reported (12).

Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive, soft-tissue sarcomas that originate from nerve structures. Although these tumors are rare, they are clinically challenging to treat with a 5-year survival of 50% (13). Patients with NF1 and previous radiation exposure are at highest risk for the development of MPNST.

Current evidence suggests that in most cases, benign and malignant lesions should be differentiated pre-operatively based on clinical and radiographic characteristics (14). In malignant tumors, loss of function and pain are far more common and neurological deficit is usually more severe. Some degree of pain is present in about 75% of all patients with PNTs. Positional pain or pain induced by pressure (Tinel's sign) are common in both; pain at rest is a far more specific symptom for MPNST. A possible explanation for this relationship is due to the chemical nature of pain because of the release of mediators (H+, proteolytic enzymes, cytokines, GF, etc.) by malignant cells that stimulate chemoreceptive pain fibers. Additionally, an invasion of the perineurium is necessary for the development of pain at rest (15). Rapid mass enlargement is also strong predictor for malignancy. Nevertheless, this preoperative differentiation is not always possible, and many of the tumors remain indistinct even after resection (Figure 1).

Overall, surgery is successful without function impairment in 90% of BPNST, and about 10%

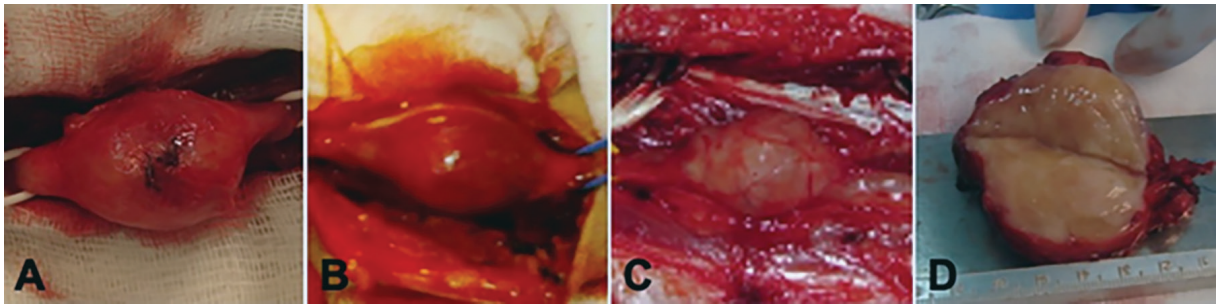

Figure 1. Intraoperatively indistinct morphologic macroscopic appearance of the solitary tumors of the peripheral nerves: (A) radial nerve Schwannoma; (B) ulnar nerve hemangioblastoma; and (C) tibial nerve MPNST within the nerve and (D) extirped and transected to find the internal structure without any signs of malignancy (homogenous solid tumor tissue).

of patients presented with post-operative motor weakness (6). Patients with larger tumor size have a higher risk for developing neurological deficit (16). The majority of postoperative neurological symptoms are transient and do not lead to permanent disability, but they can be a problem when considering patient satisfaction with the surgery. The cause of postoperative neurological deficit in BPNST remains unclear, although several mechanisms have been proposed, like tumor compression, iatrogenic direct lesion during surgery, or ischemia of the nerve associated with the surgery (17). Takase et al. have the view that damaging the fascicles around the tumor during extracapsular excision was one of the main causes of new postoperative neurological deficits (18).

Nuances in the Evaluation and Management of Peripheral Nerve Tumors

Complex biological nuances are related to the specific features of the PNT, and these are strongly interrelated with numerous controversial points considered as technical nuances at every stage of the assessment and treatment of these patients.

Imaging

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is the main imaging modality for diagnosis, evaluation, and treatment planning. Clinical and imaging characteristics can point toward the diagnosis of a benign or malignant tumor. The boundary between the tumor and the nerve is sometimes difficult to determine because the nerve may be distended and stretched over the mass. MRI evaluation consists of different sequences (e.g., T1, T2, FAT SAT, and IV Gadolinium), with images in the same plane (usually axial) supplemented by at least one other sequence in an orthogonal (i.e., sagittal or coronal) plane (19).

Positioning the patient and area for exploration at the magnet center is essential for ensuring high quality images. These can be further improved by contrast enhancement, 3D or 4D images, tractography, or by magnetic resonance angiography (MRA), depending on the type of lesion. Gadolinium-enhanced images must always be obtained to provide conclusive differentiation between a cystic and a solid lesion. A normal nerve has an intermediate signal intensity that is identical to muscle on T1 and T2 sequences. It is best viewed on T1 sequences in fatty layers or on T2-weighted fast spin-echo and T2 fat-suppressed sequences in which the nerve appears hyperintense compared to muscle. There is no nerve enhancement after contrast injection. When dealing with a mass located in the region of a nerve, MRI allows for a diagnosis in 75% of cases. Coronal and sagittal images provide individualization of the nerve's entry and exit from the tumor.

Figure 2 depicts the issue of indistinct features in MRI.

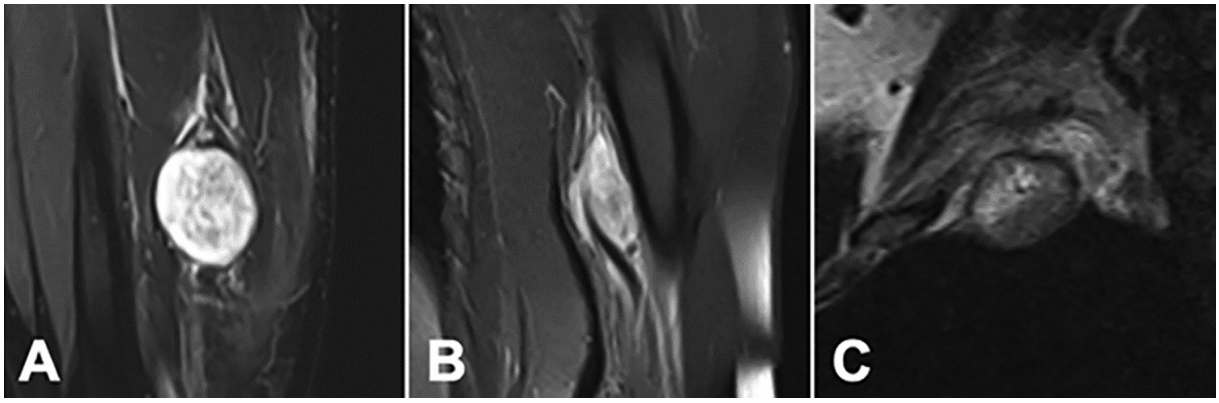

Figure 2. Indistinct features of benign and malignant peripheral nerve tumors on contrast enhanced MRI: (A) tibial nerve Schwannoma in the proximal part of the lower leg; (B) median nerve MPNST in the forearm; and (C) brachial plexus MPNST as seen on MRI without contrast. Note the homogenous structure and similar contrast enhancement of both benign and malignant PNTs.

Ultrasound

Ultrasound (US) is the imaging modality of choice for the diagnosis of BPNST. As a general rule, peripheral nerves are more visible when surrounded by the tissues with different echo features. Peripheral nerves appear as tubular structures made of hypo-echoic nerve fascicles embedded in a hyperechoic connective tissue esponding to the epineurium. Longitudinally, US images present a fascicular pattern, and transversely, fascicles appear rounded or oval in shape, giving the nerve its typical honeycomb appearance. US diagnosis of a nerve tumor is based on the existence of a mass in continuity with the nerve at its proximal and distal poles (14).

One drawback of US is the fact that differentiation between lymphadenopathy, neuroma, or liquid tumor (hematoma, abscess, or thrombosed aneurysm), is more operator-dependent than MRI, and US imaging of nerve lesions is rarely specific. US cannot replace MRI for determining the topography, the exact extent of tumor formation, or the presence of invasion. However, its features enable the physician to analyze the entire nerve course, evaluate vascularization in real time, and look for other remote tumors. Additionally, US is increasingly used intraoperatively (20).

Computerized Tomography and Radiography

Computerized tomography (CT) is rarely performed because of its low resolution for soft tissues. It shows the nerve only as a non-specific structure of intermediate density. CT is a complementary tool and may be helpful in visualizing an underlying bone abnormality (21).

Indication for Surgical Treatment

For tumors with a benign appearance, indications for surgery include pain, neurological deficit, and local mass effect. Relative indications are patient motivation and aesthetic effect. Regular follow up is acceptable for small tumors with benign appearance and a minimum of symptoms; however, this asymptomatic state is temporary and will progressively develop into functional impairment as the tumor size increases (16). Also, neurosurgeons should remember that surgery of smaller benign tumors is technically easier and will prevent further functional damage (1).

Fascicular Invasion

Because of the intimate relationship between functioning nerve fascicles and tumor tissue, these lesions should undergo careful surgical resection during surgery. The main characteristic of Schwannoma is the displacement of fascicles rather than their infiltration, with the exception of the origin fascicle from which they arise (22, 23). Neurofibromas present as more diffuse expansions

of the nerve than schwannomas. They may have single or multiple fascicles that enter and leave the nerve. In the majority of cases, the nerve of origin is nonfunctional at the time a diagnosis is established. Plexiform neurofibromas have a predominant interfascicular histologic growth pattern with redundant loops of expanded nerve fascicles. The presence of axons within the tumor helps to distinguish a neurofibroma from a schwannoma.

When possible, the main treatment goal should be complete tumor resection with preservation of nerve function. The surgeon is usually able to perform gross total marginal resection in cases of benign PNSTs. For Schwannomas, gross total resection starts by incising the capsule surrounding the tumor, enucleating the tumor mass, and sectioning the parent nerve fascicle that gave rise to the tumor under magnification. If the capsule is firmly adherent to the parent nerve, it should be partially left behind (23). Avoiding damage to the surrounding fascicles is mandatory, which will result in the resolution of symptoms and preservation of function in the majority of cases (22). For benign PNT, especially of the plexiform type, dissection of the tumor and nearby nerve must be undertaken with extreme care. Intraoperative neurophysiologic monitoring plays a considerably important role during surgery for plexiform Schwannomas or neurofibromas to determine whether the nerve associated with the tumor has motor function. If electrical stimulation of a nerve fascicle adjacent to tumor produces a motor response at 1 mA, the fascicle should not be sacrificed.

Recently, Stone and Spinner proposed a "Go for the Gold" technique for benign peripheral nerve sheath tumors (24). This technique favors preservation of all functional fascicles through the identification of all pseudo capsule layers before removing of the mass with the true capsule, leaving all *en passant* functioning nerve fascicles and epineurial vessels preserved.

Intraoperative Neuromonitoring

Intraoperative neuromonitoring (IONM) is a proven and useful tool in neurosurgery, but its usage in PNT surgery only came years after brain surgery. Main purpose for using IONM is to find a safe entry zone for intracapsular enucleation of a schwannoma since the tumor can be surrounded by a bundle of nerve fascicles (16, 25). Direct nerve stimulation (DNS) is, therefore, especially useful. Another IONM modality is utilization of nerve action potentials (NAPs), which is an electrical potential recorded from an exposed nerve in response to its electrical stimulation. NAPs allow for intraoperative monitoring of the functional integrity of peripheral nerve during peripheral nerve tumor surgery (26).

Motor evoked potentials (MEPs) alone is not able to predict postoperative loss of motor function, so usage of MEPs alone is not recommended. However, use of MEPs combined with free-run electromyography or direct nerve stimulation may improve the accuracy of nerve monitoring (17).

Fluorescein

Sodium fluorescein has been used in medicine for several decades, but its use in neurosurgery is recent. It was first used in brain tumor surgery 20 years ago, while the possible implications for its use in PNT surgery had been suggested only recently. Precise usage in terms of timing and dosage is still controversial, but some authors found sodium fluorescein useful for better identification of tumor tissue and distinguishing it from intact fascicles (20, 27). Another potential important usage is to identify residual tumor when resection is performed in a piecemeal fashion (28). Sodium fluorescein has been tested only for resection of Schwannoma so far, and further studies are needed to determine precise dose and timing of application for other PNTs.

Indications for Functional Sacrifice

Neurologic complications can occur after excision, including pain and loss of motor or sensory function (22, 29), even when a complete resection is achieved and adjacent fascicles were preserved. Complete (or *en bloc)* peripheral nerve tumor

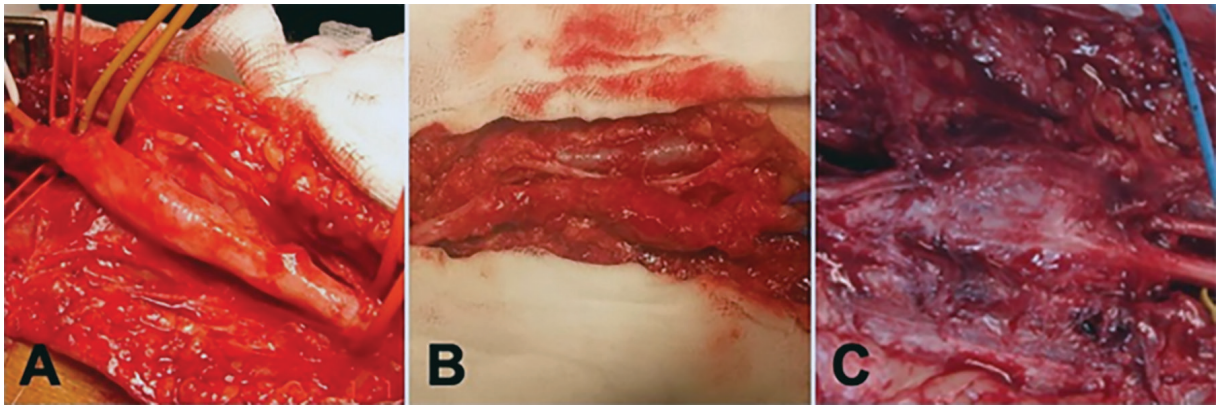

Figure 3. Intraoperatively indistinct fusiform lesions of the peripheral nerves: (A) Peroneal nerve intraneural ganglion cyst; (B) Median nerve neurofibroma; and (C) Median nerve MPNST within the soft tissues of the arm.

resection is only indicated for malignant or premalignant cases and implies resection of the entire segment of the affected peripheral nerve stem, including up to 2 cm of healthy nerve, with conscious functional sacrifice. In general, treatment decisions must consider whether or not the mass is symptomatic and has a high risk of malignancy. Surgical excision is the safest option in cases of symptomatic or asymptomatic growing PNTs. Operative techniques for the excision of probable benign PNTs should consider mass location, size, and the possibility of malignancy. Microsurgical technique and intraoperative identification of functional nerve fibers with electrical stimulation are always mandatory. Complete surgical resection without damaging functional fascicles is the goal. Most patients report significant pain relief and improvement of sensory motor deficits after surgery. Nevertheless, developing or worsening pain and neurological deficits can occur at follow-up. Recurrence rates may vary according to different types of benign PNTs and predisposing conditions. Figure 3 presents the similar appearance of the 3 completely different tumor classes.

In cases of neurological deficit and loss of function, reconstruction is mandatory (except in cases of MPNST). Reconstruction of the nervous stem in the same surgery is the best option and includes nerve grafting, nerve transfer, or other procedures for certain nerve types in a second stage surgery, such as tendon transfer. It was also reported that early radiotherapy may be applied without reinnervation process compromise; however, this remains controversial (30).

Biopsy

Soft tissue tumors in general may represent a diagnostic challenge, which is why diagnostic biopsy is performed. For PNTs, the role of biopsy is still controversial. Biopsy can be performed by needle, with or without guidance (CT or US), or in an open fashion. A needle biopsy can be done by a surgeon or radiologist. The main drawback of needle biopsy is risk of injuring intact nerve fascicles in the needle trajectory (29), which is not always avoided, even when US guidance is used (31). Another problem is risk of inconclusive sample for pathohistological analysis due to cellular pleomorphism (32).

Biopsy for benign PNTs is associated with an unacceptable risk of neurological deficit and is associated with a higher risk of postoperative deficits (23, 33). Higher postoperative deficits in previously biopsied tumors is explained by scar-tissue forming, which makes the dissection plane less clear (33). While keeping these criticisms in mind, biopsy is still recommended in cases with highly suspected malignancy (23, 34). The avoidance of biopsy is recommended in cases of expected benign pathology (22). Nevertheless, some authors still recommend biopsy for selected cases (35).

Management of MPNST

Surgical treatment for malignant tumors includes radical resection, followed by adjuvant treatment modalities, such as radiotherapy and chemotherapy. In some cases, radical resection means amputation of any extremities with disarticulation. Chemotherapy agents used in MPNST treatment include erlotinib, sorafenib, imatinib, dasatinib, alisertib, ganetespib/sirolimus, bevacizumab/RAD001, pexidartinib/sirolimus, and selumetinib/sirolimus. MPNST is nevertheless a relatively chemoresistant tumor. Evidence suggests that in partially resected or unresectable tumors, chemotherapy produces regression in less than 33% of cases. NF1 patients also have a lower response rate to chemotherapy than sporadic cases—17.6% compared to 55.3%, respectively (36).

Radiotherapy provides local control and may delay the onset of recurrence, but it has little effect on long-term survival and should be given whenever possible for intermediate to high-grade lesions, and for low-grade tumors after a marginal excision. Postoperative radiotherapy involves irradiation of the entire operative field with a 5 cm field margin, and preoperative radiotherapy involves irradiation of the overt tumor alone again with a 5 cm margin (37).

Overall, the side effects of MPNST management include secondary malignancies, dermatitis, cellulitis, fibrosis, and risk of relapse due to failure to control local or metastatic disease. Complete resection can lead to local deformities and a risk of damage to critical internal structures (36). Overall prognosis is poor: the 5-year rate of survival ranges from 15% to 50% (35). Metastases are extremely rare, and may include lungs, pleura, the breast, or skin, among other localizations. Chemotherapeutic treatment is emerging, although with only limited success—the overall survival is only 40% within 1 year of diagnosing metastatic MPNST (38).

Biological Therapy

A more thorough understanding of the genomic, epigenetic, signaling pathway, and microenvironment changes during the plexiform neurofibroma-to-MPNST malignant transformation has offered hope for the development of more effective diagnostic and therapeutic strategies for MPNST. Recent clinical trials conducted in patients with NF1 for the treatment of symptomatic plexiform neurofibromas using inhibitors of the mitogen-activated protein kinase (MEK) have shown very promising results (39). Unfortunately, MEK inhibitors do not work in all patients and have significant side effects. In addition, preliminary evidence suggests single agent use of MEK inhibitors for MPNST treatment will fail, which is why it is important to find targets other than MEK for treatment of MPNST.

Conclusion

Peripheral nerve tumors can be extremely challenging lesions to manage. Magnification, use of intraoperative recordings, and knowledge of the gross and microscopic pathology are important to the surgeon undertaking tumor resection. An understanding of the tumor's disposition, coupled with an awareness of the fascicles that may be spared, can improve the surgeon's ability to resect benign nerve sheath tumors. The clinical presentation, diagnosis, and indications for a specific type of treatment for PNTs still remain a topic of debate. However, all collected data show that both an individual and multidisciplinary approach is necessary in the treatment of these tumors. Targeted therapies will emerge when there is a better understanding of the genetic and molecular mechanisms driving the development and growth of PNTs. Early referral and evaluation by a surgical specialist with expertise in peripheral nerve tumors can mitigate negative long-term sequelae.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

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Continuous Dynamic Mapping of the Corticospinal Tract in Motor Eloquent Tumor Surgery: Our Experience and Evaluation of the Method

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Abstract

Objective. The aim of this article is to present our experience with continuous dynamic mapping (CDM) of the corticospinal tract (CST) when removing tumors in motor eloquent regions. **Methods.** We studied 44 patients with a brain tumor adjacent to the CST where CDM was used. The mapping probe was integrated at the tip of the suction device. Thresholds for eliciting MEPs were recorded. In all patients, along with CDM, MEPs to direct cortical stimulation were also monitored throughout the operation. Motor function was assessed preoperatively, after the procedure and on discharge. **Results.** In the series, there were 37 patients with gliomas, six with brain metastasis, and one with cavernoma. The threshold to elicit MEPs in CDM was >20 mA in 17 cases, 16–20 mA in six cases, 11–15 mA in six cases, 6–10 mA in nine cases and 2–5 mA in six cases. MEPs to direct cortical stimulation were preserved in all patients. In three cases a new temporary motor deficit was noted. No new permanent motor deficit occurred. Gross total resection was reached in 57% of cases. **Conclusions.** From our experience, the combined use of CDM and MEPs to direct cortical stimulation improves the safety of surgery in the proximity of the CST, and at the same time offers the possibility of higher rates of gross total resection.

Key Words: Subcortical Mapping ▪ Corticospinal Tract ▪ Tumor Surgery ▪ Motor Evoked Potential ▪ Intraoperative Monitoring.

Introduction

Operating in the proximity of the corticospinal tract (CST) represents a challenge to every surgeon, i.e., removing as much of the tumor tissue as possible while ensuring the preservation of motor function. There is increasing evidence that maximizing the extent of the resection improves survival in low and high grade gliomas (1-4)the prognostic significance of eloquent brain tumor location and the role of functional mapping during resective surgery in presumed eloquent brain regions are unknown. Methods. We performed a retrospective analysis of 281 cases involving adults who underwent resection of a supratentorial LGG at a brain tumor referral center. Preoperative MR images were evaluated blindly for involvement of eloquent brain areas, including the sensorimotor and language cortices, and specific subcortical structures. For high-risk tumors located in presumed eloquent brain areas, long-term survival estimates were evaluated for patients who underwent intraoperative functional mapping with electrocortical stimulation and for those who did not. Results. One hundred and seventy-four patients (62%. On the other hand, better postoperative performance status without new motor deficits has been shown to be an independent prognostic factor (5).

The assistance of intraoperative neurophysiological techniques is proving invaluable in such circumstances. Traditionally, to evaluate the integrity of the CST, motor evoked potentials (MEPs) are used, elicited by transcranial electrical stimulation, and detected as electromyographic (EMG) responses in the muscles of the upper and lower limbs. In the case of brain tumors, as the brain is

exposed, MEPs are best elicited by direct cortical or subcortical stimulation. Strategically, neurophysiological intervention may be viewed as either mapping or monitoring. Mapping may begin with stimulation of the cortex and recording MEPs in the muscles of the face, hands and legs, primarily to divide eloquent motor areas from non-eloquent, and to find the optimal point of entry into the tissue. After that, a stationary electrode may be positioned over the eloquent area of the cortex to enable continuous monitoring of MEPs during surgery. Mapping may then continue subcortically with stimulation of the walls of the emerging cavity throughout the removal of the tumor, thus enabling the estimation of the proximity of the CST.

In addition to intermittent and punctiform mapping, a new technique called "continuous dynamic mapping" (CDM) was introduced in 2014 (6-8). The mapping probe is integrated into the tip of a suction device which enables continuous stimulation, while the EMG responses in the corresponding facial muscles and muscles of the upper and lower limbs are detected in order to evaluate the proximity of the CST. In comparison to older techniques, CDM gives more precise temporal and spatial coverage of the surgical site, and better real time feedback of the location of the CST during tumor removal (6, 8, 9).

Knowing the distance from the CST provided by the CDM, and the integrity of the CST provided by monitoring through direct cortical stimulation, offers the possibility of more radical tumor resection, and at the same time the preservation of motor function.

In this article we present our experience with these methods in a series of patients from our own clinical practice.

Methods

Patient Population

We conducted a prospective study on 44 patients with brain tumors located less than 20 mm away from the CST (measured on MRI) in whom CDM and monitoring were used intraoperatively. Clinical and neurological parameters were evaluated preoperatively. The size and location of the tumor was assessed on the basis of preoperative magnetic resonance imaging (MRI). To estimate the size of the tumor, we measured the volume of hyperintense areas in FLAIR sequence for Grade II gliomas and the volume of contrast-enhancement in T1 sequence for Grade III and IV gliomas. All patients underwent tumor resection at the Department of Neurosurgery of the University Medical Centre, Ljubljana, between December 2016 and March 2020. All patients signed informed consent.

Intraoperative Data and the Use of Dynamic Cortical Mapping During Tumor Removal

We performed either awake brain surgery (11 patients) or surgery under general anesthesia (33 patients). Neuronavigation was used in all cases. Additionally, 5-aminolevulinic acid (5-ALA) was used in all high grade glioma cases.

The intraoperative neurophysiological approach consisted of monitoring and CDM, using the Nicolet Viasys Endeavor system (Figure 1). Both monitoring and mapping utilized an EMG signal recorded by pairs of monopolar needle electrodes, inserted into standard target muscles of interest, namely the m. orbicularis oris, m. abductor digiti minimi and m. tibialis anterior. For monitoring, anodal stimulus was applied to the cortical surface using a strip electrode placed on the precentral gyrus, and for mapping, cathodal stimulus was applied subcortically using the mapping probe integrated into the tip of a suction device. In both monitoring and mapping, monopolar stimulation was used with the other pole being a corkscrew electrode placed on the nearby scalp. A short train stimulus was used, consisting of four pulses, each pulse of 0.5 ms pulse duration, with an interpulse interval of 4 ms, frequency of 2 Hz between the trains, and stimulation intensity up to 20 mA. Anesthesia was induced and maintained with propofol and remifentanil, and a bolus of short acting relaxant was applied for the purpose of intubation (6, 9, 10)

CDM during tumor removal started at 15–20 mA. The appearance of EMG responses was observed at a sensitivity of 100 microvolts per unit. When a re-

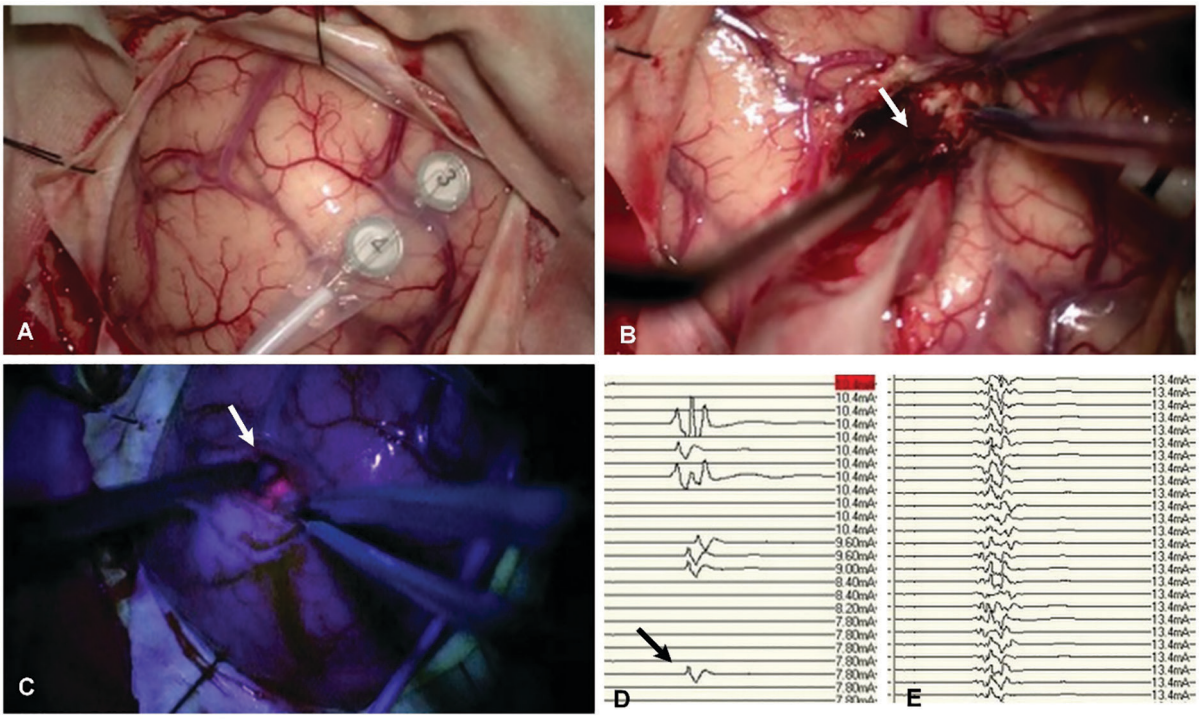

Figure 1. For direct cortical stimulation for MEP monitoring, a strip electrode is placed over the motor cortex (A). For subcortical continuous dynamic mapping, the mapping probe is integrated into the tip of a suction device (white arrow) - shown under white (B) and blue light (C). During tumor resection, the motor thresholds provided by mapping (D) and direct cortical (E) MEPs are monitored. In this case, the lowest motor threshold was 7.8 mA (black arrow).

peatable EMG response appeared, the resection was stopped, and the stimulus intensity was reduced in 2 mA steps, until the response disappeared, and the tumor removal continued. When 5 mA was reached, stimulus was reduced in 1 mA steps. Resection was stopped at 3–5 mA. If there was the possibility of total tumor removal, resection was continued, but not below 2 mA. The final lowest stimulation amplitude (in mA) needed to evoke an EMG response was noted as the lowest motor threshold.

During the whole procedure, the EMG responses from direct cortical stimulation were monitored. A 50% or more decrease in amplitude of the MEPs was considered meaningful. If this occurred, the resection was paused for 15 minutes and stopped if the decrease persisted after the 15-minute pause.

Postoperative Data

Within 48 hours after surgery MRI was performed in glioma patients, and MRI or CT in patients who underwent surgery for metastasis. The extent of resection was calculated as the difference between the preoperative and postoperative tumor volume. It was classified according to the proportion of the resected volume as: gross total resection (resection of the whole volume), subtotal resection (at least 90% of the volume) or reduction (less than 90% of the volume). Postoperative clinical evaluation was performed one day after the operation and on the day of discharge from the hospital. Pathohistological diagnosis was established and complications were noted. Data are presented as absolute value, proportion (%) or mean ± standard deviation.

Results

Patient Characteristics

We included 44 patients, of whom 30 were men and 14 were women (Table 1). The mean age was 53±17 years. The brain tumor was newly diag-

nosed in 42 patients, and two patients were suffering from recurrence. The most common presenting symptoms were epileptic seizures and hemiparesis. The mean preoperative Karnofsky Performance Scale was 88±11.

Perioperative Findings

Gliomas were present in 84% of patients, and metastatic or vascular lesions in 16%. Data on pathohistological diagnosis are presented in Table 1.

All procedures were technically successful. Gross total resection was achieved in 57%, subtotal resection in 23% and reduction in 20% (Figure 2). In 19 patients, gross total resection was not achieved. In 16 of those, partial resection was already planned preoperatively because of the infiltration of other eloquent structures, such as the basal ganglia, thalamus, optic radiation, speech areas etc. In the remaining three patients the resection was stopped at the motor threshold of 3–5 mA, after we recognized that complete resection was not possible. The lowest motor thresholds reached are presented in Table 1. The amplitude of the MEPs remained stable or was reduced by less than 50% throughout the whole operation in all cases.

Table 1. Characteristics of Presented Cases

Characteristic	Number of patients
Gender male/female	30/14
Presenting symptom	
Epileptic seizure	24
Hemiparesis	14
Cognitive decline	3
Memory deficit	1
Headache	1
Dysphasia	1
Pathohistological diagnosis	
Glioma WHO IV	22
Glioma WHO III	7
Glioma WHO II	8
Metastasis	6
Cavernous angioma	1
Extent of resection	
Gross total resection	25
Subtotal resection	10
Reduction	9
Lowest motor threshold	
>20 mA	17
16–20 mA	6
11–15 mA	6
6–10 mA	9
2–5 mA	6
New postoperative motor deficit	
Temporary	3
Permanent	-

Postoperative Complications

New postoperative motor deficit was noted in 7% of patients (N=3) on the first postoperative day. On the day of discharge, the postoperatively acquired deficits had reversed in all cases. There was one case of intracerebral hematoma and one case of postoperative abscess, which both needed reoperation. Neither patient presented with neurological deficits on discharge. One patient with metastasis died in the postoperative period due to sepsis, and one patient suffered from a pulmonary embolism.

Discussion

Every physician should aspire to offer his patients as much as possible and constantly strive to improve the results of their work. In our opinion, an optimal result in tumor surgery close to the CST cannot be achieved with surgical techniques alone. Methods that provide information on the location of the tumor and eloquent motor regions, and the integrity of the CST have already proved helpful. With their use, we can improve our results beyond those achieved by even the most accomplished surgical technique.

To localize relevant structures, neuronavigation and, in some centers, even intraoperative MRI can be used. Additionally, 5-ALA can be used to localize high grade gliomas. The disadvantage

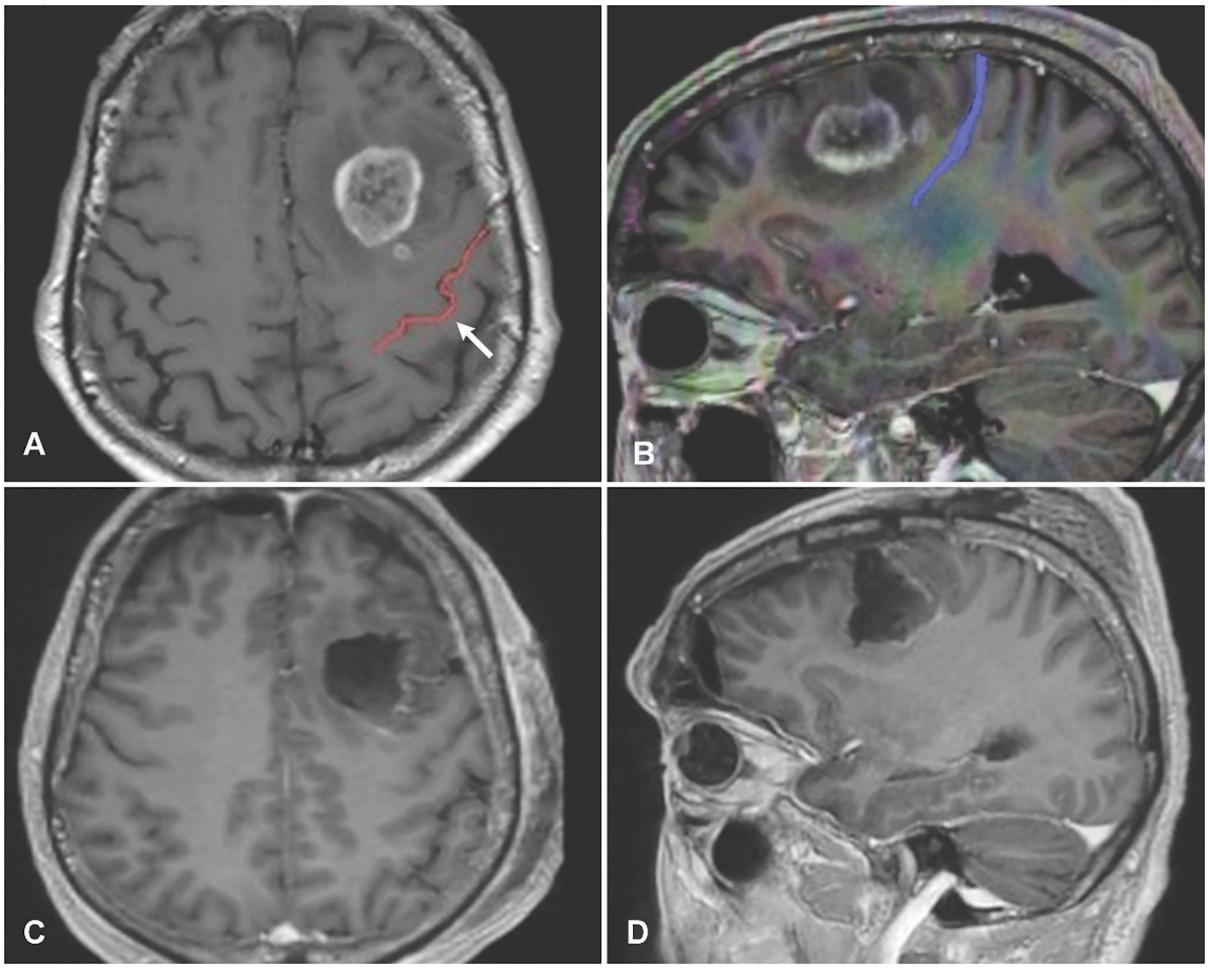

Figure 2. Preoperative contrast-enhanced MRI (A) and tractography (B) of a patient with a glioblastoma in the left precentral area. The arrow indicates the central sulcus. Tractography (B) shows the corticospinal tract less than 10 mm away from the tumor. Postoperative MRI (C, D) reveals gross total resection (C, D). No new postoperative motor deficit was noted.

of neuronavigation is that after the release of the cerebrospinal fluid, and during removal of the tumor, the brain shifts and the method is no longer so precise. Intraoperative MRI is not widely available, it is time consuming, and it does not offer continuous feedback. Furthermore, 5-ALA fluorescent tissue may surpass the area of contrast enhancement on preoperative MRI by up to 10 mm (11). Fluorescence guided surgery can therefore lead to greater resection than the preoperative MRI shows. Moreover, all these methods only provide location information and do not test the function of eloquent motor regions. On the other hand, combined neurophysiological techniques of mapping and monitoring offer information on both. Therefore, we chose this approach to prevent possible CST damage during tumor removal.

Mapping was performed using a stimulation system integrated into the suction device, and it continuously provided information on the distance between the CST and the exact point of tumor removal (6-8). In assessing the distance to the CST, the rule of thumb, mainly derived from the results of intermittent mapping, is that "1 mA correlates to 1 mm" (6-8, 12, 13). The application of this rule to the CDM during tumor surgery is also supposed to be safe, however it has not yet been studied extensively (6, 7, 9, 11).

In our series, the lowest motor threshold reached at the end of tumor removal was below

6 mA in 14% and below 11 mA in 34%. No new permanent motor deficit occurred in our series, however, we must acknowledge that the number of patients included was small. In previous studies with the use of intermittent subcortical mapping, a new permanent motor deficit was noted in 3.5–17% of patients, and it was more common when the lowest motor threshold was below 5 mA (9, 13-17)in an operation theater equipped with an integrated high-field intraoperative magnetic resonance imaging and a neuronavigation system. Diffusion-tensor imaging-based tractography of the CST was implemented preoperatively and intraoperatively. When the surgically manipulated area came close to the corticospinal pathway, MEP responses were elicited by subcortical stimulation. Responsive areas were compared with the locations of fibers traced by preoperative and intraoperative tractography. Imaging and functional outcomes were reviewed. Results Intraoperative tractography demonstrated significant inward or outward shift during surgery. MEP responses were observed around the tract at various intensities, and the distance between MEP responsive sites and intraoperative tractography was significantly correlated with the stimulation intensity ($P<0.01$). The use of CDM stimulation permitted even more radical tumor resection, with motor thresholds as low as 1–3 mA, and new permanent motor deficit in 1.7–3% of patients (6, 9). According to a previous study, permanent motor worsening is mainly due to vascular injury and not so much to mechanical injury (6).

In our series, three patients presented with a new temporary motor deficit. The lowest motor threshold was below 11 mA in all three of them. This is in accordance with other studies, where either temporary or permanent motor deficits usually occurred below 11 mA (6, 7, 9). In another report, temporary motor deficit was present in 30% of patients, which is more than was noted in our series (6). The lowest motor threshold in that report was below 5 mA in 59% of patients. Therefore, we assume that the distance from the tumor to the CST was greater in our series, which may explain the difference in the lowest motor threshold and the rate of motor deficit. However, we cannot exclude the possibility of a more radical approach in tumor removal in that report.

To evaluate the integrity of the CST, we used direct cortical monitoring throughout the operation. The alteration in the amplitude of MEPs was a warning sign of impending CST damage, and led us to consider pausing or stopping the resection, even in amplitude reductions smaller than 50%. Gross total resection was achieved in 57% of cases. Similar previous studies reported gross total resection in approximately 70% of patients (6, 8, 13, 14). These results are difficult to compare because of the differences in tumor types, and their size and distance from the CST.

In our opinion, mapping and monitoring enable us to operate safely in the proximity of the CST. They proved to be useful in different kind of tumors. In cases of metastases and cavernomas, which are generally well-defined, they still influenced our resection strategy, tissue manipulation and hemostasis technique. Some metastases can be very adherent to the white matter, but in our series all were well-defined, and gross total resection was achieved in all cases. On the other hand, in gliomas, which are infiltrative tumors, mapping and monitoring are of the utmost importance in preventing damage to the CST by tumor removal. In order to operate without interruption, Raabe et all. proposed the use of a sound alert system that alerts us when the subcortical EMG response is elicited by the suction device (6). In our practice, continuous feedback was provided by a clinical neurophysiologist.

Conclusion

From our experience, the combined use of subcortical CDM and monitoring of cortically elicited MEPs improves the safety of operations in the proximity of the CST, and offers the possibility of higher rates of gross total resection. It provides confidence to the team participating in the procedure, and improves patient outcome and satisfaction.

What Is Already Known on this Topic:

The rate of resection and preservation of motor function are known prognostic factors for the survival of patients with brain tumors. Therefore, the aim of tumor surgery in the proximity of the corticospinal tract (CST) is to remove as much tumor tissue as possible, while ensuring the preservation of motor function. The assistance of intraoperative neurophysiological techniques is proving invaluable in such circumstances. Continuous dynamic mapping (CDM) was introduced is 2014, and its use is related to a higher rate of gross total resection and a higher rate of preservation of motor function in some studies.

What this Study Adds:

We show that the combined use of subcortical CDM and monitoring of cortically elicited motor evoked potentials resulted in a satisfactory level of safety of surgery in the proximity of the CST, and offered the possibility of higher rates of gross total resection.

Authors' Contributions: Conception and design: AP, JP and ZR; Acquisition, analysis and interpretation of data: AP, JP, TŽ and ZR; Drafting the article AP and JP; Revising it critically for important intellectual content: ZR, BK and BP; Approved final version of the manuscript: AP, JP, ZR, BK and BP.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Case Report
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Navigated Endoport in the Purely Endoscopic Microsurgery of Intraventricular and Other Deep-Seated Brain Lesions: A Case Report

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Abstract

Objective. Brain parenchyma retraction is often necessary to reach various deep brain lesions during surgery. In order to minimise the incidence of the brain retraction injury, an endoport system may be employed. We present a report of a navigated endoport system in conjunction with an purely endoscopic microsurgery that was used in a patient with a deep-seated subependymoma. **Case Report.** A navigated endoport with purely endoscopic microsurgery were used in a patient with a tumour located in the frontal horn of the left lateral ventricle. The endoport channel was made of a polyvinyl sheet that was cut into a 7 cm square, rolled into a tubular structure that was wrapped around the neuronavigational probe, and inserted in the access trajectory to the tumour. The endoport tube was then expanded with a balloon to a diameter of 7 mm and a surgical corridor was thus formed. During the purely endoscopic microsurgical lesionectomy, the tumour was completely removed from the frontal horn. The foramen of Monro was released and the septum pellucidum was perforated for better cerebrospinal fluid circulation. Histopathological examination confirmed the tumour as subependymoma. The recovery of the patient was unremarkable. **Conclusion.** The expandable endoport system supplemented with neuronavigation is a safe and efficient option for deep-seated tumour removal. The tubular shape of the retractor enables standard microsurgical techniques through minimally invasive approaches and offers an excellent visualization of the underlying lesion.

Key Words: Neuronavigation ▪ Endoport ▪ Neuroendoscopy ▪ Microsurgery ▪ Deep Brain Lesion.

Introduction

Brain retraction is often necessary in order to remove deep-seated intraaxial and intraventricular lesions. Conventional microsurgical retractor systems, such as Greenberg (Symmetry Surgical Inc., Nashville, TN, United States) and Leyla (Aesculap, Hazelwood, MO, United States) retractors, have been designed with one or two retraction spatulas that may produce significant disruptive forces to the surrounding brain tissue. The incidence of retraction injury to the cerebral cortex, white matter, and vasculature, therefore, remains high. The resulting consequences can include brain laceration with critically decreased regional blood flow leading to ischemia, permanent tissue damage, and clinically caused seizures, focal neurological deficits, cerebral swelling, and cognitive impairment (1-6). In addition to mechanical trauma from prolonged traction, venous infarction may also appear in a delayed fashion. From a technical point of view, retracted brain tissue often herniates back between the blades, obstructing the operative corridor and interfering with the surgical instruments and illumination. With the advent of modern neuroendoscopy, endoscopically-assisted techniques, customized instruments, and neuronavigation, the approach to deep-seated brain lesions under direct vision through a burr hole or a minimal craniotomy has become routine. Although a fully endoscopic approach is the least traumatic, it does not enable a bimanual microsurgical technique and may not lead to a complete lesion removal (7-9).

The introduction of cylindrical retractors with microscopic and, more recently, endoscopic visualization follows the idea of a more symmetrical and less harmful distribution of pressure along the operating tube or so-called endoport (9-20). The term endoport describes a cylindrical or tube-shaped retractor system, either fixed or expandable, used as a corridor during endoscopically assisted microsurgical removal of a deep-seated brain lesion (11, 13, 14, 22, 23). Neuronavigational systems and modern diagnostic imaging have greatly improved the precise targeting of these lesions and safety of the trajectory. A deep-seated brain lesion that was previously accessible only by a stereotactic biopsy can now be removed through an endoport with the help of endoscopic visualization. These deep locations in the brain that are often problematic for the surgical access, include the following: the ventricles, the basal ganglia, the pulvinar and posterior thalamus, the insular cortex, and basal cisterns. Reports of the endoport technique have already been published that describe the removal of astrocytomas, gangliogliomas, cavernous angiomas, gliomas, ependymomas, papillomas, brain abscesses, intracerebral hemorrhage, massive ventricular bleed, haematocephalus, intraventricular meningiomas, metastasis, colloid cysts, neurocytomas, and choroidal arteriovenous malformations (10-25).

We present a novel navigated endoport system in combination with an purely endoscopic technique that was used for the removal of an intraventricular tumour.

Case Presentation

Patient Description

A 75-year old gentleman presented at our neurosurgical department with occasional vertigo and insecure gait. As a result, he experienced numerous falls. Family members also noticed emotional numbness and behavioural changes. During one of the falls, he sustained a head injury. At admission, computer tomography (CT) scan revealed an expansive lesion in the left lateral ventricle with no intracranial bleeding. Magnetic resonance imaging (MRI) of the head followed a few weeks later, which confirmed a tumour located in the frontal horn of the left lateral ventricle, measuring 3.5 cm × 3 cm × 1.5 cm (Figures 1 and 2).

Posteromedially, the tumour obstructed the left foramen of Monro; however, the ventricles were not enlarged. MRI also revealed a 3 cm thick chronic

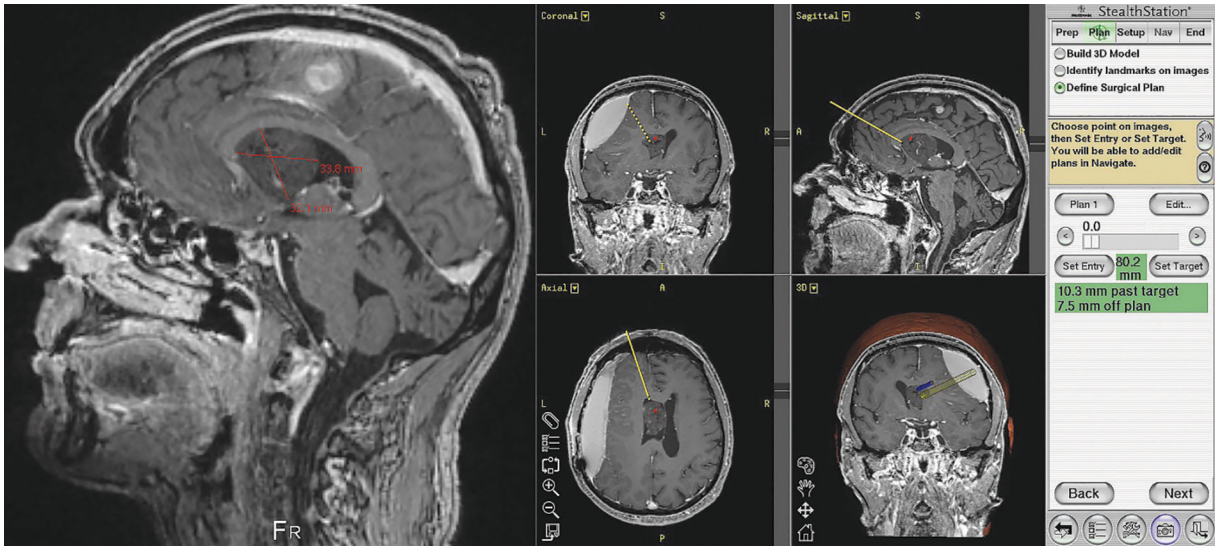

Figure 1. The surgical planning for the image-guided purely endoscopic removal of the intraventricular tumour through the endoport. A chronic subdural hematoma and a small falx meningioma are also seen.

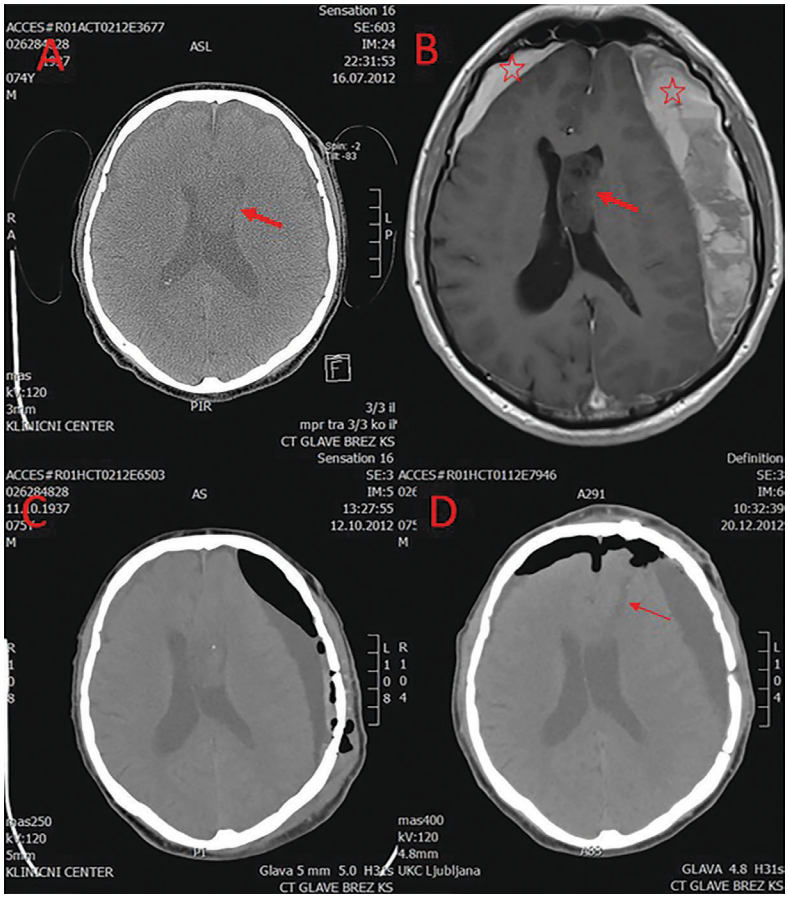

Figure 2. Initial CT revealed an accidental tumour in the left horn of the lateral ventricle (thick arrow) (A). An MRI two months later revealed chronic subdural hematomas bilaterally (star). On the left side, the haematoma is compressing the brain and causing the brain shift across the midline (B). Control CT scan after the left haematoma evacuation. On the left side, some air and fluid are still present as the brain tissue has still not expanded completely. The tumour can be seen in the left lateral ventricle (C). The last CT shows the situation 24 hours after the endoport removal of the tumour. The trace of the endoport is barely visible (thin arrow) (D).

subdural haematoma over the left hemisphere and a smaller one frontally on the right. Clinically, the patient presented with discrete right-sided hemiparesis and dysphasia. The haematoma on the left was also compressing the brain and was therefore evacuated with open surgery. One month later, the patient was operated on again in order to remove the intraventricular tumour. An endoscopically assisted minimally invasive endoport approach was planned and the tumour was removed. After the surgery, the patient became talkative and lively, and the family confirmed that his previous personality returned. The postoperative course was uneventful. The patient was discharged home a week after the operation. The histopathological examination confirmed the tumour as subependymoma.

Surgical Technique

After draping and neuronavigational setup, a 1.5 cm skin incision was made in the forehead wrinkle supraorbitally. The skull bone was exposed and a burr hole of 1.5 cm in diameter was made. The residual hematoma cavity was also explored and the thick capsule of the hygroma was fenestrated into the subarachnoidal space. Minimally invasive endoport access to the ventricles included a special expandable micro-roll retractor, which was made of a polyvinyl sheet. The sheet was cut into a 10 cm x 7 cm square and rolled into a tubular shape, which was then wrapped around the neuronavigational probe (diameter of 4 mm) and fixed to the probe through two stitch threads with releasable notches on the poles (Figure 3).

When inserted stereotactically via a minimal brain cortical incision and released from the probe, the roll measured about 7 mm in diameter. With the inflation of a 14 French Fogarty balloon attached to the catheter, the roll uncoiled to a 1 cm diameter, which was just enough for a spacious operative manipulation that encompassed the endoscope and surgical instruments. A 4 mm endoscope (Hopkins 0 degrees, Karl Storz, Tuttlingen, Germany) was introduced first, locating the lesion in the ventricle. In the meantime, the expanded endoport was held in place manually, leaning to the endoscope tube (Figure 3). The

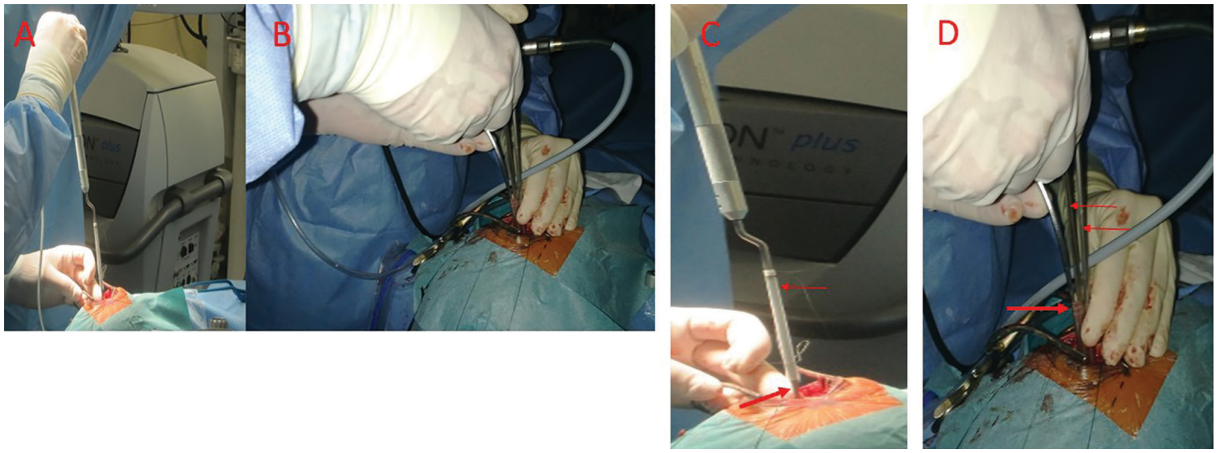

Figure 3. The intraoperative procedure of the endoport insertion under neuronavigational guidance: the neuronavigational probe with the retracted endoport just before the insertion into the brain (A) and the expanded flexible working channel with surgical instruments (B). The soft vinyl sheet was rolled three times around the neuronavigational probe (diameter, 4 mm) (thin arrow), secured with releasable stitches at both ends, and introduced into the brain to the target. The thick arrow denotes the neuronavigational shaft around which the soft endoport sheet was rolled. The retracted endoport can clearly be seen (C). After releasing the stitches and deployment, the tube was expanded with a Fogarty catheter to a 10 mm diameter and held manually to the endoscope tube. During bimanual work, the tube adopted an oval and funnel shaped configuration (thick arrow), facilitating the introduction of aspirator and grasping forceps (thin arrows) (D).

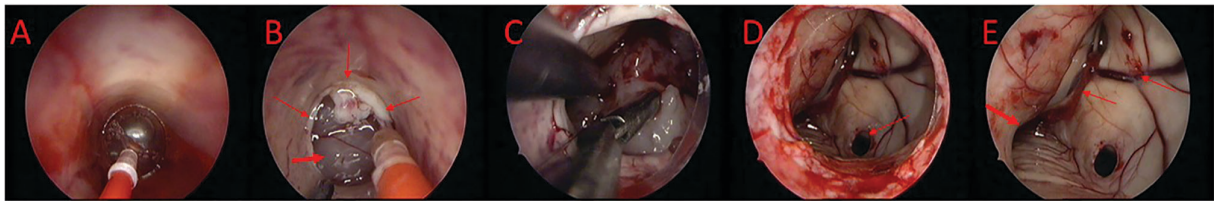

Figure 4. After insertion, the tube was expanded with a Fogarty catheter. The polyvinyl sheet, which surrounds the Fogarty catheter, is unrolling and broadens the working cannel of the expandable endoport (A). The entry into the left lateral ventricle and exposure of the tumour. The tumour tissue is grey and avascular (thick arrow) and is located just below the rim of the endoport (thin arrows). The Fogarty balloon is deflated and is removed (B). The tumour was removed by aspiration in a piecemeal fashion (C). After the tumour was removed completely, the interventricular septum was perforated in order to achieve a communication between the ventricles (arrow) (D). The foramen of Monro (thick arrow) and the thalamostriate and septal veins (thin arrows) are visible after the complete tumour removal (E).

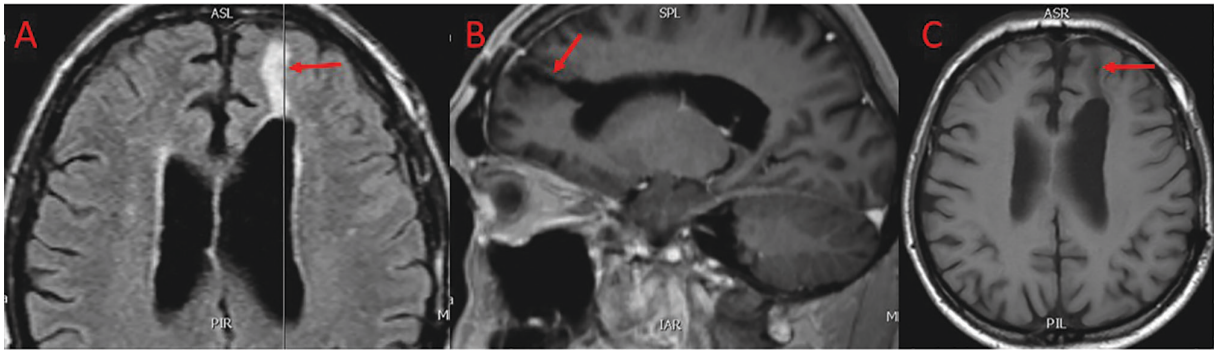

Figure 5. Control MRI three months after the operation. The tumour has been removed completely and the trace of the endoport can still be seen (arrow), morphologically confirming that very little brain trauma has been inflicted with such approach. The subdural haematoma has been resorbed completely (A, B). Follow-up MRI three years after the surgery shows a barely visible trace of the endoport access (arrow) (C).

expanded endoport acquired an oval shape; half of the lumen was occupied with endoscope and the other half was free for introducing the surgical instruments. Two pituitary bayonet instruments were used for tumour removal through the endoport. When reaching the grey tumour mass, the endoport was first detached from the surrounding tissue and then gently aspirated until the frontal horn was completely free. The foramen of Monro was released, the third ventricle inspected, and the septum pellucidum was perforated for better cerebrospinal fluid circulation (Figure 4). Postoperative oncological treatment was not necessary; the patient is being followed-up regularly (Figure 5). The full operative video is available online at: *https://www.youtube.com/watch?v=zMno8C07txk*.

Discussion

With the development of the neuroendoscopic and neuronavigational technology, especially in the last decade, minimally invasive neurosurgery of deep-seated lesions has progressed significantly. Surgery is becoming more patient-friendly, due to its minimal invasiveness and technological support, and also more convenient for the surgeon. Intraventricular lesions are especially difficult to reach surgically due to their deep location, demanding accessibility, and the close proximity to vital brain structures (Table 1). In the 1980s, these lesions were approached transcortically with 20 mm or 30 mm tubular cylinders that enabled binocular vision and bimanual microsurgical technique under microscopic magnification. Twenty years ago with the introduction of endoscopically-assisted microsurgery by Perneczky, the endoscope was used as an adjuvant tool for angled visualization of the whole operative field (19, 20). Thus, the fibre-optic based ventriculoscopy has significantly reduced the amount of cortical and white matter disruption during the surgical approach. Here, the tumours may be biopsied or removed through a working channel of the endoscope.

However, the feasibility of definitive lesion removal with fully endoscopic procedures is limited by a mono-manual technique and the restricted movements of the instruments, including operating the instrument back and forth through the tube as well as manoeuvring the endoscope shaft sidewise and up or down. Highly vascularized and firm tumours present an unacceptably high risk for complications. It is also known that prolonged or excessive brain retraction causes damage to the brain tissue and its vasculature (2, 4-6). Self-retaining retractor blades, a revolutionary assistant instrument for achieving a corridor

Table 1. The Advantages and Disadvantages of the Endoport System for Deep Brain Lesion Surgery

Advantages	Limitations
- Neuronavigation compatible - Precise - Offers direct access to the deep lesion - Less brain retraction - Forces to the brain tissue are equally transmitted intermittent brain retraction - Flexible working channel - Possibility of moving and angulating the working channel, targeting it into the lesion centre and to its edge - Lower possibility to damage brain vessels - Lower possibility for consequent ischemia and permanent tissue damage - Compatible with endoscopic and endoscopically assisted techniques - Cheap and affordable - Single use - Minimal craniotomy - Better aesthetic results - Shorter wound healing course	- Possibility of endoport shift - Designed for endoscopically trained surgeons - Requires dedicated assistance - Narrow working channel - Learning curve for mastering the endoport-assisted operation

through the brain parenchyma or for lobar retraction, was soon recognized as being hazardous, especially with long-term surgical procedures. As a result, self-retaining retractors were replaced by an intermittent retraction technique in which the retraction is performed with working instruments only. The transfissural and transsulcal approaches are further innovations for minimizing surgical trauma to the brain parenchyma (4, 5). On the other hand, the endoport access offers the possibility of endoscopic visualization and bimanual microscopic technique and minimizes trauma to the surrounding brain parenchyma (21, 23).

As the intermittent retraction with a bipolar forces and aspirator is not suitable for entering soft brain parenchyma several centimetres deep, the idea of a tubular retractor was developed and includes evenly distributed forces to the brain tissue around the retractor. In comparison to animal studies in which a significant reduction in regional cerebral blood flow with a retraction pressure of 30 mmHg has been studied, the retracting pressure around a cylindrical endoport up to 2 cm in diameter never exceeded 10 mmHg (4, 18). Postoperative T2 MRI, fluid-attenuated inversion recovery (FLAIR), and diffusion restriction/apparent diffusion coefficient (ADC) signal are the best imaging modalities to determine the extent of resection and the extent of white matter injury along the surgical trajectory (11, 13).

Tubular retractors can be fixed-size or expandable. Some fixed-size models are funnel-shaped for additional operative freedom. In others, the bayonet-shaped pituitary instruments are preferable. The funnel-shaped or various sizes of dilators that are inserted into the brain tissue to the target successively are further improvements in reducing brain trauma during the operation. Image-guided tubular endoport retractors have enabled the removal of many deep-seated lesions, which was also a feasible alternative to stereotactic biopsies. Additionally, surgical morbidity was decreased, and no mortality was observed (11, 13, 14, 19, 21-23, 25). In the recent series of Engh et al. in which resection of 32 colloid cysts and 15 intraventricular tumours was performed through an 11.5 mm transparent conduit, gross or near total resection was achieved in 31 (96.9%) of the 32 colloid cysts and in 80% of intraventricular tumours, respectively. No permanent neurological morbidity occurred (11). The endoport technique has also been recently applied in posterior fossa surgery via the lateral transcerebellar route (26-28).

Neurosurgical planning is of vital importance for a successful surgical outcome. The planned trajectories during neuronavigational employment may be affected by brain shifts and dislocations, especially after cerebrospinal fluid aspiration or leak, or after larger resections. If trajectories are not planned carefully, the targeted lesions may be missed, especially when they are small and in a deep location. In our patient, planning for the tumour surgery was done after the operation of the chronic subdural haematoma on the left, which can clearly be seen from the preoperative MRI plans. The subdural haematoma was evacuated first as this was the acute cause for patient's neurological symptoms. In the second phase, MRI-based planning was performed to determine the trajectory for the tumour removal. The surgical trajectory was not planned through the haematoma and the associated brain tissue, but according to the tumour location itself. In this case, the brain shift was not important for the navigation and did not affect the trajectory, since the trajectory started in the entry point on the skull bone (which is immobile) and terminated in the tumour, the location of which did not change. We did not experience any problems during the operation and the navigation was precise, targeting the endoport directly into the tumour.

Outside of rare commercially available models, most tubular retractors can be assembled with equipment and material already available at many institutions (13, 15, 19, 22). Our polyvinyl roll used for endoport was a low-cost modification of an expendable transparent polyvinyl tube introduced by Jho in 2002 (17). The tube was rolled from a very soft 10 cm x 7 cm polyvinyl sheet and was then uncoiled with a Fogarty catheter from the initial 4 mm to the final 10 mm diameter. During the bimanual work, the tube adopted a more

oval and funnel shaped configuration that facilitated the introduction of curved teardrop aspirators and grasping forceps. Because the polyvinyl roll had several turns, it never unrolled completely during the surgery. Its elastic structure enabled rolling with each retrieval of the instruments and unrolling with each introduction of instruments—its diameter never exceeded 1 cm. This dynamic effect further minimized pressure to the surrounding brain parenchyma.

According to reports in the literature, the first endoscopic removal of a third ventricular tumour through a polyvinyl sheath rolled into a cigarette shape and inserted with image-guided stereotactic assistance into the lateral ventricle and foramen Monro was reported in 2002. This polyvinyl tube was expanded with a balloon dilatation technique (17). A rigid 7 mm polypropylene tube was used to remove a neurocytoma with aspiration, a unipolar coagulating aspirator, and visualization with a 2.7 mm 0-degree optics (10). A longitudinally cut silicone tube has been reported as a low-cost endoport (29). Selected tumours, however, can be removed with a fully endoscopic approach to the third ventricle using a diode laser for vaporization of the tumour (20). To avoid thermal injury to the brain stem, several authors prefer mechanical removal of the tumour, which is possible with a microsurgical bimanual technique. The endoscopic single-port or dual-port approaches offer this possibility. A side-cutting instrument, combining variable aspiration and cutting function from a lateral aperture, has been used successfully in keyhole craniotomies, endoscopic endonasal approaches, and more recently in transcranial port surgery (30). The tubular retractor system used in spinal surgery (METRx; Medtronic, Minneapolis, MN) has been used microscopically to remove deep-seated parenchymal lesion (12, 13, 31). This system includes a progressive dilatation of the corridor using a set of dilating tubes.

Conclusion

The expandable endoport surgery minimizes skin incision, craniotomy size, and retractor-induced trauma to brain tissue. It has the potential for improving treatment outcome and lowering complications in endoscopic neurosurgery of intraventricular and deep-seated brain lesions with the use of standard microsurgical techniques under endoscopic visualization.

What Is Already Known on this Topic:

In an operation of deep-seated intraaxial and intraventricular lesions, brain retraction is necessary and may produce disruptive forces to the brain tissue. The consequence can be brain laceration that leads to ischemia and the tissue damage. With the introduction of modern neuroendoscopy, endoscopically assisted techniques, customized instruments, and neuronavigation, these lesions can be removed less invasively and in a more patient-friendly manner.

What this Case Adds:

Deep brain lesions may be safely and effectively managed by the endoport technique. Expandable endoport surgery minimizes the skin incision, craniotomy size, and retractor-induced trauma to the brain. It has the potential to improve surgical outcome and lowering complications associated with endoscopic neurosurgery of intraventricular and deep-seated brain lesions.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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