CASE REPORT

Esthesioneuroblastoma: the complete picture – case report and review of the literature

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Abstract
Esthesioneuroblastoma (ENB), also called olfactory neuroblastoma, is a cancerous tumor originating from the olfactory neuroepithelial cells frequently invading the brain through the cribriform plate. The optimal therapy is the multimodality treatment involving a group of physicians trained in different medical specialties. Establishing a careful histopathological diagnostic and treatment planning based on a multidisciplinary approach is of paramount importance. The treatment of ENB correlates with the extent of the lesion, with surgery being the mainstay of therapy followed by postoperative irradiation. Surgery, when complete, image-verified and associated with radiation therapy results in long-term survival and presents a very low probability of illness recurrence. We present the case of a 46-year-old female with ENB, who was operated on in the Clinic of Neurosurgery of the National Institute of Neurology and Neurovascular Diseases in Bucharest, Romania, through a bifrontal craniotomy approach. Gross total resection of the intracranial extent was performed. The pathological diagnosis revealed an aggressive olfactory neuroblastoma. Three weeks after discharge from hospital, the tumor was completely resected through a lateral rhinotomy performed by an otorhinolaryngologist. Six weeks later, the patient received adjuvant therapy (radiotherapy and chemotherapy). The outcome was favorable, with no tumor recurrence at 20 months postoperatively. Our case demonstrates that even when dealing with a visibly aggressive tumor, a correct diagnosis, accurate classification and grading along with appropriate therapy ensure a favorable outcome.

Keywords: esthesioneuroblastoma, pathological features, multimodality treatment, grading system, prognosis.

Introduction
Esthesioneuroblastoma (ENB) is an uncommon cancerous tumor emerging from the olfactory specialized neuroepithelium located in the superior aspect of the nasal vault, commonly invading not only the cranial vault and the floor of the cranial cavity, but also the eye socket [1]. Following its first depiction by Berger et al., in 1924, more than 350 cases have been illustrated in literature [2].

Portmann & Bonnard named this malignant tumor ENB, in 1929. Obert et al. (1960) proved that the malignant growth of abnormal cells emerged from the upper nasal cavity and established the neuroectodermal origin of the tumor, primarily from the olfactory epithelium [3]. It accounts for up to 3% of all intranasal tumors [1]. The occurrence of ENB usually correlates with the presence of the olfactory epithelium in that site. Isolated ENB has been identified in the upper cavity of the pharynx, ethmoidal air cells, maxillary sinus, hypophysis [4] and sphenoid sinuses [5–7]. Synchronous association of inverted papilloma with low-grade ENB has recently been reported [4].

The disease presents two types of age distribution: in the first instance between ages 10 and 20 years old and secondly between age 50 and 60 years old with a slight predominance in females; tumors showing the more classical neuroblastic Homer Wright rosettes tend to arise at an earlier age [5, 6]. The common presenting symptoms are: unilateral nasal obstruction, epistaxis, hyposmia and the presence of a fleshy, friable nasal mass. In order to identify the complexity and extent of the malignant tumor, in the preoperative phase are used complementary techniques such as magnetic resonance imaging (MRI) and computed tomography (CT) [3].

From a histological point of view, the tumor presents similarities with the cancerous injuries of the retina, adrenal medulla and sympathetic ganglia. Usual features are little, circular neuroepithelial cells disposed in lobules possibly showing scattered Homer Wright pseudorosette, and separated by fibrous elements, with scant fibrillary cytoplasm and round, dark nuclei [7]. When Flexner–Wintersteiner true rosettes are present, this morphological pattern is often referred to as ENB or olfactory neuroblastoma. Much less commonly, the tumor may show Flexner rosettes and other characteristics of classic neuroblastoma [8]. Any of the two rosette types may be absent, as well as the fibrillary stroma [9]. The histological features are included in a four grades system, according to Hyams. The high-grade lesions show anaplastic cells, with nuclear pleomorphism and hyperchromasia, increased mitotic activity and confluent foci of necrosis [10].

From an immunohistochemical (IHC) and histological point of view, the olfactory neuroblastoma is originating from the neuroendocrine cell. A diagnosis of ENB is determined using light microscopic appearance and IHC stainings [11]. The tumor cells express neuronal markers...
as synaptophysin, chromogranin or neuron-specific enolase (NSE) [12], as well as calretinin, supposed to differentiate the tumor from other small round blue cell tumor types [13]. Sustentacular cells, spindle or stellate shaped and disposed at the periphery of the lobules, are strongly reactive for S100 protein. Cytokeratins (CKs) are usually negative, even though some patchy expression could be seen in isolated cases [14].

Differential diagnosis is possible, especially in the high-grade cases, only by immunohistochemistry [9]. This includes all the small blue cell neoplasms arising in the sinonasal tract, including sinonasal undifferentiated carcinomas, squamous carcinomas, mucosal malignant melanomas, lymphomas, rhabdomyosarcoma, melanoma, pituitary adenoma, sinonasal lymphoma, etc. [15].

The optimal therapy is the multimodality treatment based on the experience of a team of physicians trained in different medical specialties. The prognosis is established according to two staging systems: the Kadish imaging scale (Table 1) and Hyams histological grading system.

| Table 1 – Kadish esthesioneuroblastoma staging system |
|---------------------------------------------|------------------------------------------|
| Stage | Description                     |
| A     | Tumor limited to the nasal cavity. |
| B     | Tumor involves the nasal and paranasal cavities. |
| C     | Tumor extends beyond the nasal and paranasal cavities. |

This classification was modified by Morita et al., in 1993, who established stage D in the classification for metastatic dissemination to the lymph nodes of the cervical region or to various remote sites (Table 2) [16–18]. Predicting the overall survival rate is only possible when using the modified Kadish grading system [19].

In an effort to define the Kadish stage 3 more precisely, in 1992, Dulguerov & Calcaterra suggested a different tumor, node, metastasis (TNM) system, which incorporates MRI and/or CT scans (Table 3).

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<th>Table 3 – Modified TNM staging system</th>
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<td>Classification</td>
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<td><strong>Tumor</strong></td>
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| **Node** | |
| N0 | No cervical lymph node metastasis. |
| N1 | Any form of cervical lymph node metastasis. |

| **Metastasis** | |
| M0 | No metastasis. |
| M1 | Distant metastasis. |

The treatment of ENB correlates with the extent of the lesion based on the Biller staging system (Table 5) with surgery being the mainstay of therapy. Intracranial surgery can be approached through a bifrontal craniotomy. Adjuvant therapy consists of radiotherapy and chemotherapy.

| Table 5 – Biller esthesioneuroblastoma staging system |
|---------------------------------------------|------------------------------------------|
| Stage | Description                     |
| T1  | Tumor in the nasal cavity and/or paranasal sinuses (except sphenoid). |
| T2  | Periorbital or anterior cranial fossa extension. |
| T3  | Brain involvement with resectable margins. |
| T4  | Unresectable tumor. |

The aim of the study was to report a case with an ENB tumor and discuss the diagnostic approaches.

**Case presentation**

We present the case of a 46-year-old female with ENB, who was operated on one year ago (2016) in the Clinic of Neurosurgery of the National Institute of Neurology and Neurovascular Diseases in Bucharest, Romania. The study was conducted complying with current proper guidelines and procedures. The informed consent was collected from the patient and all procedures were approved by the Medical Ethics Committee of the National Institute of Neurology and Neurovascular Diseases.

The patient was admitted in the hospital complaining of unilateral nasal obstruction, face and tooth pain, proptosis, anosmia and visual changes. Preoperative contrast-enhanced axial CT scan delineated osteal implication surrounding the nasal septum, orbital cavity,
and anterior basicranium (Figures 1 and 2) and indicated a Kadish stage C of the olfactory neuroblastoma.

Contrast-enhanced coronal (Figure 3) and sagittal (Figure 4) MRI studies revealed an anterior skull base tumoral mass that was hypointense to gray matter, homogeneously enhancing and hyperintense to isointense to gray matter on T2-weighted sequences, while giving details regarding intraorbital, intranasal and intracerebral extension. According to the Biller’s ENB staging system, the tumor was in T3 stage.

The tumor was resected through a bifrontal craniotomy approach. The patient was laid down in supine position with the head elevated and slightly extended. A bicoronal (Souter) incision was performed behind the hairline through the galea (Figure 5).

The scalp and galea were reflected anteriorly. An osteoplastic bifrontal craniotomy (from one superior temporal line to the other) was performed in eight burr holes and the pericranium and the temporalis muscle were reflected inferiorly (Figure 6).

Opening the frontal sinuses required frontal sinus exenteration and cranialization. For the management of epidural bleeding, the sutures were made along the margins of the craniotomy. The dural incision was made over every medial lower frontal lobe just above the anterior edge of the craniotomy and then carried medially near the edge of the sagittal sinus. The sagittal sinus was then divided between sutures, the falx was cut and the frontal lobes were retracted laterally and slightly posteriorly with a self-retaining retractor system. The blood supply that entered the tumor through numerous openings in the bone in this area was interrupted by bipolar coagulation. The gross appearance of the tumor was that of a fleshy, soft, polypoid, friable and richly vascularized mass. The anterior capsule was then opened and the tumor completely resected with the adjacent dura mater (Figures 7 and 8).

The patient was out of bed on the second postoperative day. After surgery, the patient had a good recovery and was discharged six days later. Follow-up CT scan at six months showed the complete resection of the tumor and relaxation of the brain (Figure 9).
Pathology

The tumor sample was fixed in buffered formalin, paraffin-embedded, sectioned at 4 μm and routinely processed for histological examination.

Two μm-thick sections were subsequently obtained from the most representative paraffin block. Each section was deparaffinized and hydrated in graded ethanol concentrations. Heat-induced antigen retrieval was obtained with the buffer indicated by each antibody supplier for 30 minutes. The slides were then treated with 3% hydrogen peroxide for 20 minutes, at room temperature, to block endogenous peroxidase activity. Tissue slides were incubated with primary antibodies solution overnight at room temperature, followed by two phosphate-buffered saline (PBS) changes. The list of primary antibodies used is the following: S100 protein (Dako, Glostrup, Denmark, 1:500 dilution), synapthophysin (Cell Marque, Rocklin, CA, USA, 1:500 dilution), MNF116 pan-CK (Dako, Glostrup, Denmark, 1:50 dilution), Ki67 (ImmunoLogic, Duiven, The Netherlands, 1:500 dilution). The reaction was visualized using poly-Horseradish peroxidase–goat anti-mouse/rabbit/rat immunoglobulin gamma (poly-HRP-GAM/R/R IgG) detection kit for 30 minutes (Immunologic, Duiven, The Netherlands) using 3,3’-diaminobenzidine (DAB), followed by counterstaining with Hematoxylin. Negative control was obtained by omitting the primary antibodies.

The Hematoxylin–Eosin (HE) slides showed features suggesting the presence of an olfactory neuroblastoma. These included small blue cells arranged in lobules and nests, with relatively large necrotic areas, separated by a fibrovascular stroma (Figure 10). The cells had indistinct margins.

Rosettes or pseudorosettes were not present. The nuclei were pleomorphic, with coarse chromatin and nucleoli hardly visible on usual staining. Very rare atypical mitoses were present (Figure 11).

The IHC examination revealed positivity for synaptophysin, S100 protein and negativity for CK. Synaptophysin was positive in a diffuse manner, in all tumor cells, with a cytoplasmic and membrane distribution (Figure 12). On the other hand, S100 protein was strongly expressed on the sustentacular cells located in the peripheral areas of the lobules (Figures 13 and 14).

MNF116 pan-CK was negative throughout the tumor (Figure 15).

The proliferative potential of the tumor, as determined by Ki67 immunostaining, was very high, around 45%, despite the paucity of mitotic activity (Figure 16). Ki67 also permitted a much more accurate visualization of nucleoli, which were conspicuous in almost tumor cells.

The IHC examination confirmed the diagnosis of ENB and revealed a high histological aggressiveness of the proliferation.

Since the tumor presented nuclear pleomorphism, mitoses, prominent necrotic areas and absence of calcification, we could include our case in the grade 3 in Hyams’ classification.

Three weeks after discharge, the patient was operated on by an otorhinolaryngologist who completely resected the intranasal tumor through a lateral rhinotomy. Six weeks
later the patient received adjuvant therapy (radiotherapy and chemotherapy). The outcome was favorable and 20 months postoperatively the patient had no symptoms at all and no tumor recurrence was visible on CT scans.

Figure 10 – General aspect of the tumor. The cells are arranged in a lobular pattern, separated by loose fibro-vascular stroma. Necroses are visible at some lobule centers (arrows). HE staining, ×100.

Figure 11 – High power image of the tumor. The nuclear pleomorphism is conspicuous. An atypical mitosis is visible (arrow). HE staining, ×100.

Figure 12 – Synaptophysin is expressed in a diffuse pattern by all tumor cells, with both cytoplasmic and membrane positivity. The surrounding fibrovascular stroma is completely negative. Anti-synaptophysin antibody immunostaining, ×400.

Figure 13 – At low magnification, it is obvious that sustentacular cells are strongly positive for S100 protein, while the tumor cells are slightly reactive, in a non-specific manner. Anti-S100 antibody immunostaining, ×100.

Figure 14 – Details of the reactivity for S100 protein. Peripheral sustentacular, elongated cells, in the lobules periphery, as well as rare stellate cells within the lobules strongly express S100 protein (arrows). Anti-S100 antibody immunostaining, ×400.

Figure 15 – The tumor is negative for cytokeratin (CK). As an internal control, strong positivity of the pharyngeal epithelium is seen (arrow). Anti-MNF116 pan-CK antibody immunostaining, ×100.
surgery, a preoperative ophthalmology investigation is required. The nerves are subjected during radioactivity or radical
nation, neuro-ophthalmologic evaluation and brain imaging.

Our case had a locally aggressive ENB, but no metastatic disease. [3] or spinal metastases [26]. Our case had a locally aggressive ENB, but no metastatic disease.

Patient evaluation consists of otolaryngologist examination, neuro-ophthalmologic evaluation and brain imaging. Taking into account the risk of damage to which the optic nerves are subjected during radiotherapy or radical surgery, a preoperative ophthalmology investigation is required.

In order to obtain an overview of each medical case, every patient is subjected to contrast-enhanced axial and direct coronal CT scanning, allowing for preoperative classification of the tumor, according to the Kadish classification (Table 1) [1]. CT studies usually reveal a lytic pattern and very rarely (only five cases described in the literature) dominant hyperostosis mimicking fibrous dysplasia [27]. The main advantage of CT studies is that they delineate osteal implication surrounding the nasal septum, orbital cavity, and anterior basicranium [21].

On brain imaging, ENB are strong and increasing nasal cavity masses that may express destruction into close osseous organization of the ethmoid bone, cribriform plate, and fovea ethmoidalis. In case of erosion of the floor of the anterior fossa or the orbital wall, MRI is indicated because it provides a more intracranial detail of the soft tissue disease [14].

On T1-weighted images, ENBs appears as hypointense to gray matter and homogenously enhancing tumors and hyperintense or isointense to gray matter on T2-weighted sequences. We performed both contrast enhanced CT scanning and MRI imaging in order to diagnose, define the extent of tumor, staging and surgical approach, monitoring and evaluate response to treatment.

The capacity to contour intraorbital and intracerebral expansion is a great advantage of using MR imaging. A definitive diagnosis is based on the presence of intraslesional calcifications and the existence of cysts along the edges of Kadish stage C injuries [28, 29]. Our case could be included in Kadish C stage. Angiography should reveal a tumor blush with persistent opacification and arteriovenous shunting. However, we did not perform an angiography on our patient.

If metastatic disease is suspected, the patient should undergo a bone scan and CT scan of the neck, chest, abdomen and pelvis [1]. We did not find metastatic disease on CT scan of the neck and chest.

The histological appearance of olfactory neuroblastoma is diverse. The characteristic histological features of olfactory neuroblastoma are round-to-oval-shaped nuclei with scanty cytoplasm, sharply defined chromatin, layers of malignant cells organized into lobules through slender vascular fibrous septa, true neural rosettes (Flexner–Wintersteiner type) and pseudorosettes (Homer Wright type) [1].

At low magnification, ENB presents an appearance that is often described as a small, round cell tumor and it must be distinguished from lymphosarcoma, transitional cell carcinoma, plasmacytoma, reticulum cell carcinoma, small cell undifferentiated carcinoma and Ewing’s sarcoma [30]. Judicious use of IHC stainings is sufficient to resolve most diagnostic dilemmas. Ancillary techniques such as electron microscopy or analysis of molecular markers are valuable in difficult diagnostic cases [31].

Our case showed the typical appearance of olfactory neuroblastoma, with nests and lobules of tumor cells, locally coalescent, surrounded by a fibrovascular stroma with a prominent vascular network [9]. The presence of rare mitoses but relatively extensive necrotic areas, associated to nuclear pleomorphism, scant fibrillary matrix and lack of calcifications included our case in grade 3 in Hyams’ classification.

The tumor’s neural origin was established by Trojanowski et al., in 1982, who demonstrated the presence of neurofilament proteins (NFPs) in the tumor cells [32].

IHC and histological examinations show the tumor’s reactivity with NSE, S100, synaptophysin, NFP, class III beta-tubulin and microtubule-associated protein [30] and confirm that ENB is of neuroendocrine cell origin [33]. Epithelial markers, such as CK and epithelial membrane antigen, are absent from the neuroblastoma forms of

Figure 16 – The Ki67 proliferation index of the tumor is high. Nucleoli are also visible in each tumor cell. Anti-Ki67 antibody immunostaining, ×400.
olfactory neuroblastoma [31]. In our case, the presence of the neuroendocrine marker synaptophysin, together with the peripheral expression of S100 protein, within the sustentacular cells surrounding the tumor lobules, was considered sufficient to make the correct diagnosis. Furthermore, the absence of CK reactivity excluded carcinomas from the differentials. Lymphoid neoplasms (lymphosarcoma, plasmacytoma), as well as Ewing sarcoma and rhabdomyosarcoma do not express neuroendocrine markers as did our tumor, another argument for including this tumor in the olfactory neuroblastoma category. Malignant melanoma is diffusely and strongly positive for S100 protein, which was not present in our case, where only the sustentacular cells expressed this marker.

A long-term bad prognosis in ENB is associated with an elevated mitotic index, the privation of rosettes and tumor necrosis [34]. All these aspects were present in our case.

Electron microscopy evaluation reveals presence of dense core neurofilaments and neurotubules. Neurosecretory granules having features consistent with those of catecholamines are diagnostic of ENB [30]. Catecholamine-secreting ENB can produce intraoperative hypertensive crisis during surgical resection [35]. Our case did not need ultrastructural examination, since the histological and IHC aspects were suggestive enough to make the correct diagnosis.

The genetic and molecular studies showed that translocation, a process identified in Ewing sarcoma and primitive neuroectodermal tumors (PNETs) – Ewing sarcoma/Friend leukemia integration 1 transcription factor (EWS/FLI1) genes – is also present in ENB cell lines and confirmed that olfactory neuroblastoma is a part of the malignant tumor family [36].

Combined IHC/microdissection genotyping showed that subsets of olfactory neuroblastoma demonstrate varying degrees of overexpression of wild-type tumor protein p53 (TP53) [31]. We did not perform molecular tests on our case.

It has been reported that ENB has a high proliferative index of 10–50% as demonstrated by Ki67 immunostaining and which correlates with tumor recurrence, metastatic spread and decreased survival [37]. Our case showed a high Ki67 labeling index. However, the overall prognosis does not seem to be correlated to this factor.

The neuroblastic appearance of the tumor cells suggests that they may be derived from the neuroepithelium. Bipolar neurons of the olfactory plate are considered to be the cells of origin for the tumor [10]. During childhood and adolescence, the neuroepithelium is replaced by respiratory mucosa. The expanding nasal placode of untimely embryos and which correlates with tumor recurrence, metastatic spread and decreased survival [37]. Our case showed a high Ki67 labeling index. However, the overall prognosis does not seem to be correlated to this factor.

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Great news is referring to the survival rate, which is more than 70%. Prognostic factors include Hyams histological grade, Kadish–Morita staging system, TP53 overexpression [36], lymph node involvement, treatment modality and age [38–41]. The pathological grading system proposed by Hyams is the most important prognostic factor. Based on the Kadish system, the 5-year survival rate is 86% for those patients with A and B tumor stages, and 72% in persons affected by stage C tumors. Age over 65 years old seems to have a negative impact on the outcome [42]. In a retrospective examination of the medical results achieved from the 35 patients treated with radical surgery in association with adjuvant treatment (radiation therapy with or without chemotherapy), authors from the University of Virginia found that the 8-year disease-free survival rate was 80.4%. Twenty-two patients (62.9%) of 35 were in Kadish stage C disease [3].

Conclusions

ENB is a curable slow-growing sinonasal malignancy. The optimal therapy is the multimodality treatment based on the experience of a team of physicians trained in different medical specialties. We recommend surgery as the first type of intervention and secondly, the postoperative radiation therapy. Establishing a careful histopathological diagnostic and treatment planning based on a multidisciplinary approach is of paramount importance.

We favor Hyams grading system, since it shows the most accurate prognostic capabilities and also use the Kadish staging system for the treatment protocol. Our case demonstrates that even when dealing with an obviously aggressive tumor, a correct diagnosis, accurate classification and grading and appropriate therapy ensure a favorable outcome.

Conflict of interests

The authors declare that they have no conflict of interests.

References


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