Neuroendocrine neoplasms (NENs) of the head and neck are a rare group of heterogeneous epithelial neoplastic proliferations arising in virtually all of the different organs of this region, particularly in the nasal cavity, the paranasal sinuses, the nasopharynx, the larynx, the salivary glands, and the middle ear. They encompass a wide spectrum of entities ranging from very indolent neuroendocrine tumors to highly aggressive neuroendocrine carcinomas. They may represent a challenge for radiologists, oncologists, and pathologists and a correct diagnosis is crucial for the management of patients. The nomenclature and classification of cervicocephalic NENs is currently under debate and for this reason a different diagnostic terminology has been used over the years, creating confusions among clinicians and pathologists. Olfactory neuroblastoma is a rare neuroectodermal neoplasm arising in the nasal cavity showing some challenging diagnostic aspects. In this review we give an update of the more relevant criteria for diagnosing head and neck NENs and olfactory neuroblastomas focusing on the critical use of morphological parameters and immunohistochemical staining.

**Key words**

Neuroendocrine neoplasm • Olfactory neuroblastoma • Carcinoid • Neuroendocrine carcinoma • Head and neck

**Summary**

Neuroendocrine neoplasms (NENs) of the head and neck are a rare group of heterogeneous epithelial neoplastic proliferations arising in virtually all of the different organs of this region, particularly in the nasal cavity, the paranasal sinuses, the nasopharynx, the larynx, the salivary glands, and the middle ear. They encompass a wide spectrum of entities ranging from very indolent neuroendocrine tumors to highly aggressive neuroendocrine carcinomas. They may represent a challenge for radiologists, oncologists, and pathologists and a correct diagnosis is crucial for the management of patients. The nomenclature and classification of cervicocephalic NENs is currently under debate and for this reason a different diagnostic terminology has been used over the years, creating confusions among clinicians and pathologists. Olfactory neuroblastoma is a rare neuroectodermal neoplasm arising in the nasal cavity showing some challenging diagnostic aspects. In this review we give an update of the more relevant criteria for diagnosing head and neck NENs and olfactory neuroblastomas focusing on the critical use of morphological parameters and immunohistochemical staining.

**General approach to the pathology of head and neck neuroendocrine neoplasms and olfactory neuroblastoma**

**NEUROENDOCRINE NEOPLASMS OF THE HEAD AND NECK**

Neuroendocrine neoplasms (NENs) of the head and neck are a rare group of heterogeneous epithelial neoplastic proliferations arising in virtually all of the different organs of this region, including the nasal cavity, paranasal sinuses, nasopharynx, larynx, salivary glands, and middle ear. Their morphological and clinical features mainly depend on the degree of differentiation and on the site of origin. According to the 2017 WHO classification, they are currently subdivided into: typical carcinoid (well differentiated neuroendocrine carcinoma), atypical carcinoid (moderately differentiated neuroendocrine carcinoma), and poorly differentiated neuroendocrine carcinoma (small and large cell subtype) 1. In addition to these pure neuroendocrine neoplasms, rare cases composed of neuroendocrine and non-neuroendocrine components have been described, although they are not currently classified as separate entities, with the exception of the larynx where they are defined as combined neoplasms 1. The term mixed neuroendocrine/non-neuroendocrine neoplasms (MiNENs) has been recently proposed to designate such rare neoplasms 2. The heterogeneity of the clinic-pathological features of head and neck NENs represents a challenge for both pathologists and oncologists and a correct diagnostic approach is mandatory for the management of patients. Indeed, NENs encompass a wide spectrum of neoplasms, from indolent tumors to highly aggressive carcinomas. Accordingly, the morphological features of these neoplastic proliferations are variable and must be carefully identified in order to produce a correct histopathological report, which represents the starting point for the choice of the more appropriate treatment. NENs are morphologically heterogeneous and, from a general point of view, the diagnosis is firstly based on

**Acknowledgements**

The authors thank dr. Igor Letovanec (Institute of Pathology, Lausanne University Hospital, Lausanne, Switzerland) for providing the material used for Figure 4 and dr. Nikolaos Papanikolaou (Department of Pathology, Multimedica Hospital, Milan, Italy) for providing the material used for Figure 6.

**Correspondence**

Stefano La Rosa, Institut Universitaire de Pathologie, CHUV, 25 Rue de Bugnon, 1011 Lausanne, Switzerland - Tel: +41 21 3147162 - Fax +41 21 3147115 - E-mail: stefano.larosa@chuv.ch
the differentiation degree, which separates these neo-
plasms in two broad categories: well-differentiated neu-
roendocrine tumors (carcinoids) and poorly differen-
tiated neuroendocrine carcinomas. As above mentioned,
a third category is represented by neoplasms with both
a neuroendocrine and a non-neuroendocrine component
(usually squamous cell carcinoma or adenocarcinoma)
and it is defined as MiNENs 2.
Well differentiated neuroendocrine tumors (carcinoids)
are characterized by an organoid proliferation of uni-
form cells, with moderately abundant granular and eo-
sinophilic cytoplasm containing numerous secretory
granules. Nuclei are generally round, with clumped or
finely granular (“salt and pepper”) chromatin and small
nucleoli. Tumor cells may grow forming small nests,
trabeculae or pseudoglandular structures. The distinction
between typical and atypical carcinoids follows the cri-
teria proposed for the classification of lung neuroen-
docrine neoplasms and includes mitotic count and the pres-
ence of necrosis 3. Typical carcinoids show < 2 mitoses
X 10HPF and lack any evidence of necrosis. Atypical
carcinoids are characterized by 2-10 mitoses x 10HPF
and/or presence of necrosis, which is generally focal.
Since a subset of carcinoids may behave in an aggressive
fashion, it is important to look for other morphological
cues that can be associated with higher propensity to
disseminate, such as lymphovascular and perineural in-
filtration. The neuroendocrine nature of the neoplastic prolifera-
tion has to be confirmed by immunohistochemical analyses using antibodies di-
rected against general neuroendocrine markers, since
other non-neuroendocrine poorly differentiated neo-
plasms of the head and neck may mimic NECs (i.e. pe-
ripheral neuroectodermal tumors, Ewing sarcoma, des-
moplastic small round cell tumors, lymphoid neoplasms,
NUT midline carcinoma, adenoid cystic carcinoma, and
basaloid carcinoma).
MiNENs are neoplasms with both a neuroendocrine and
an epithelial non-neuroendocrine component, each rep-
resenting at least 30% of the tumor mass 2. The spectrum
of MiNEN encompasses different possible combinations
between neuroendocrine neoplasms (carcinoids and
NECs) and other epithelial tumors (adenocarcinomas and
squamous cell carcinomas).

**Olfactory neuroblastoma**

Olfactory neuroblastomas (ONBs) are rare nasal neo-
plasms thought to originate from the olfactory membrane
because of the specific site of origin and the expression
of proteins typically expressed by the olfactory epithe-
lium 5 6. They can show different morphological features
which permit to classify tumors in grade categories us-
ing the Hyams’ histological score (see below) 1. ONBs
need to be differentiated from nasal NENs because of
the different therapeutic approach and prognosis. While
for low grade ONBs this differential diagnosis is not dif-
ficult and can be performed on H&E stained sections,
the differential diagnosis between high grade (grade IV)
ONBs and NECs is generally problematic and always
requires immunohistochemical investigations. A de-
tailed description of the morphological and immunohis-
tochemical features of ONBs will be presented below in
the pertinent paragraph.

**Immunohistochemical approach
to the diagnosis of head and neck
neuroendocrine neoplasms and olfactory
neuroblastoma**

Immunohistochemistry is a crucial step in the diag-
nostic work up of NENs and ONBs. Both neoplasms
express general neuroendocrine markers including
chromogranins and synaptophysin. The chromogranin
family includes different proteins such as chromo-
granin A, chromogranin B and secretogranins which
are stored in secretory granules together with hor-
mones 7. Chromogranin A is widely used in routine
practice because it is a very specific neuroendocrine
marker 8 and may be released in the blood stream de-
termining an increased level that may be useful for
monitoring the follow-up of patients. However, chro-
mogranin A is not as sensitive as other markers, es-
specially in the case of poorly differentiated neuroen-
docrine carcinomas: indeed, the sensitivity is mainly
related to the number of secretory granules contained
in the cytoplasm of neoplastic cells, which can be re-
duced when cells are poorly differentiated. Synap-
tophysin is associated with intracytoplasmic synaptic-
like vesicles and is a highly sensitive neuroendocrine
marker 9, albeit not as specific as chromogranin A,
since some non-neuroendocrine tumors, like adrenal
cortical neoplasms, can also express it. CD56 is an-
other broad spectrum neuroendocrine marker directed against the cell adhesion molecule NCAM. It is less specific than chromogranin A and synaptophysin, but it is used in diagnostic practice as an additional marker, when chromogranin A or synaptophysin expression is absent or questionable. It is worth noting that CD56 is not only expressed in neuroectodermal neoplasms but also in other malignancies including T/NK lymphomas which can arise in the head and neck region. Protein Gene Product 9.5 (PGP 9.5), which belongs to the carboxy-terminal hydrolase family, has been proposed as a neuroendocrine marker but it is not highly specific. The last marker deserving mention for historical reason is neuron specific enolase (NSE), which was the first broad spectrum neuroendocrine marker to be employed in the pathology work up of NENs. However, because of its relative low specificity, its use is not currently recommended.

For all the reasons mentioned above, the most useful immunohistochemical markers for the assessment of the neuroendocrine nature of a neoplasm are chromogranin A and synaptophysin, which must be used in routine practice as recommended by several guidelines. It is worth noting that immunostaining for these two markers is especially essential in the diagnosis of poorly differentiated NECs, in which the neuroendocrine phenotype may be not evident on routine hematoxylin and eosin stained slides. It is also recommended in well differentiated NETs, when the diagnosis on morphology alone is not straightforward. Synaptophysin immunoreactivity is generally diffuse in both carcinoids and NECs whereas chromogranin A expression in NECs, especially in the small cell subtype, may sometimes be absent or focal, showing a paranuclear dot-like pattern of immunoreactivity and not the granular diffuse pattern observed in carcinoids. For this reason, care must be taken in the use of chromogranin A alone in the diagnostic pathway of NECs. The use in the routine practice of other general neuroendocrine markers including NSE, PGP 9.5, and CD56 has been discouraged, as their specificity is questionable, albeit they can be useful in the diagnostic management of those NECs in which chromogranin A is absent of only focally expressed and synaptophysin immunoreactivity is the only clue to a neuroendocrine differentiation.

The transcription factor achaete-scute homolog 1 (ASH1) has recently proved to be a marker of lung and extra-pulmonary NECs, its expression being almost exclusively restricted to NECs and absent in well differentiated NETs. For this reason, ASH1 has been proposed as a marker of poor differentiation in the workup of NENs, also in the evaluation of small biopsy specimens where morphology is compromised and the differential diagnosis between well differentiated and poorly differentiated NENs may be challenging.

Neoplasms of the nasal cavity and paranasal sinus

**Neuroendocrine neoplasms of the nasal cavity**

NENs of the nasal cavity are rare and their classification is still under debate. However, the widely accepted classification scheme now used by the majority of pathologists reflects the classification of lung NENs which includes the following entities: carcinoids (typical and atypical) and NECs (small and large cell subtype). In addition, cases of mixed neoplasms composed of a neuroendocrine and non-neuroendocrine component (MiNENs) have been described and they need to take into account, although they have not been included in the WHO classification.

**Carcinoids**

Typical and atypical carcinoids of the nasal cavity and paranasal sinuses are extremely rare with typical carcinoids being the least common NEN type in this site. Patients’ age ranges from 13 to 83 years. Common symptoms are nasal obstruction and epistaxis. Due to the rarity of such neoplasms, definitive data regarding the outcome and the best therapeutic approach are lacking. In typical carcinoids (TC), tumor cells do not show cytologic atypia and/or necrosis and growth forming cords or nests similarly to carcinoids or well differentiated neuroendocrine tumors located elsewhere. By definition, TC show < 2 mitoses per 2 mm²/10HPF and Ki67 index is generally low. The most important differential diagnosis includes ectopic pituitary adenomas that can arise in the nasal cavity, nasopharynx, sphenoid and ethmoid sinuses. Immunohistochemistry, including pituitary hormones (ACTH, prolactin, TSH, FSH, LH, and GH), is mandatory for the diagnosis. Atypical carcinoids (AC) generally show trabecular, glandular, and acinar pattern of growth and neoplastic cells are generally larger than those of the TC. AC shows 2-10 mitoses per 2mm²/10HPF and/or foci of necrosis. The Ki67 labeling index in generally comprised between 3% and 20%.

**Neuroendocrine carcinoma**

High grade poorly differentiated NECs resemble the lung counterpart. Most of the reported cases are of small cell type (Fig. 1), since the large cell variant has been only recently described. Large cell NEC (LCNEC) is a challenging entity that needs to be separated from AC due to the different behavior and different therapeutic approach. Indeed, similarly to lung LCNECs, sinonasal LCNECs show the same behavior of small cell NECs, which is significantly worse than that of AC. While small cell NECs are easy to identify in H&E stained sections, LCNECs diagnosis requires a careful evaluation of the mitotic count for the differential diagnosis with AC. It is worth noting that some sinonasal small cell NECs may show CK20 and Merkel cell polyomavirus immunoreactivity and they need to be differentiated from metastases of Merkel cell carcinoma elsewhere located. An accurate clinical and radiological investigation is mandatory in these
cases. Sinonasal NECs do not show sex, racial or geographic predilection and seem not to be associated with smoking history or radiation exposure. The average age at diagnosis has been reported to be 49 years with a 26-77 years range. Most of the cases arise in the superior portion of the nasal cavity and extend to paranasal sinuses and intracranial fossa.

**Mixed neuroendocrine/non-neuroendocrine neoplasm (MiNEN)**

Nasal MiNENs are extremely rare and as few as 12 cases have been published in the English literature, to date; the non-neuroendocrine component was represented by squamous cell carcinoma, adenocarcinoma, adenosquamous carcinoma, and inverted papilloma. The neuroendocrine component consisted of a high grade poorly differentiated NEC in all cases with the exception of one AC. Symptoms are generally unspecific at presentations and include nasal stuffiness, epistaxis, rhinorrhea and headache without any relationship with professional exposure to carcinogens. The neuroendocrine component is positive for general neuroendocrine markers (synaptophysin, chromogranin A, and CD56), whilst the immunophenotype

---

**Fig. 1.** Nasal small cell neuroendocrine carcinoma characterized by a proliferation of small to medium-sized round to oval cells with scant cytoplasm and hyperchromatic nuclei with inconspicuous nucleoli forming large nests (A). Neoplastic cells are positive for synaptophysin (B), chromogranin A (C), and Cytokeratin 8/18 (D).
of the non-neuroendocrine component depends on the tumor type. Adenocarcinomatous components are immunoreactive for carcinoembryonic antigen (CEA) and may be variably positive for cytokeratin (CK) 7, CK 8/18, CK20 and CDX2, according to the intestinal or non-intestinal differentiation; the squamous cell component express CK5 and p63. The molecular profile of nasal and paranasal MINENs has not been extensively analyzed. In a case of mixed intestinal type adenocarcinoma/NEC, concurrent copy number changes in both components at the TP53, MLH3 and KLK3 regions have been found. Since additional gains and losses of other genes, as well as aberrant methylation, were detected only in the neuroendocrine component, it has been suggested that MINENs derive from the proliferation of a single precursor cell with divergent differentiation and that the molecular and morphological progression implies a pathway going from a non-neuroendocrine towards neuroendocrine cell pathway and not vice-versa 23. Nasal and paranasal MINENs are generally locally advanced (T4a or T4b) and aggressive cancers with poor survival despite the employment of multimodal therapies, including surgery, radiotherapy and platinum-based chemotherapy 7.

**Olfactory Neuroblastoma**

ONB is a rare malignant neoplasm arising in the upper portion of the nasal cavity accounting for about 2-3% of nasal neoplasms with an annual incidence estimated to be 0.4 cases per million population 1 24. A bimodal age distribution has been noted in the 2nd and 6th decade of life, although ONBs can be observed in almost of all ages ranging from 2 to 90 years 1. There are not well identified etiological agents in humans and, to date, there are not data suggesting an association with wood dust or other occupational exposure.

ONBs arise in the upper portion of the nasal cavity although rare cases in other nasal region including lower nasal cavity and maxillary sinus have been described. Moreover, cases of intracranial and intrasellar ONBs without an apparent intranasal component have also been reported 25 26.

From a clinical point of view, patients frequently present epistaxis and symptoms of unilateral obstruction which generally precede the diagnosis by 6-12 months. Other less frequent symptoms are mainly related to the extent of the disease and may include anosmia, headache, proptosis, visual field defects, and epiphora 27. ONBs can extend within the nasal cavity and paranasal sinuses and in about 10% to 30% of cases they are metastatic at locoregional lymph nodes or distant sites.

Macroscopically, tumors present as a mucosa-covered highly vascularized polypoid mass with variable size, ranging from 1 cm to large masses occupying the nasal cavity with possible extension to the paranasal sinuses and in more advanced cases to the orbit and/or nasal fossa.

Histologically, ONBs are submucosal proliferations growing in lobules with a more or less well represented neurofibrillary matrix, separated by a richly vascularized fibrous stroma. Vessels frequently show a peculiar plexiform or glomeruloid appearance. Although there is a great case-to-case variability in morphological features, tumor cells are generally uniform in size with scant cytoplasm and small round nuclei showing coarse to fine chromatin with the typical “salt and pepper” feature. Nucleoli are generally absent and inconspicuous. Necrosis is absent and mitotic activity is low or absent. Tumor cells show tangles of neuronal cell processes which appear as a neurofibrillary matrix, around which pseudorosettes of the Homer-Wright type can be observed. Much more rare are true rosettes forming gland like structures (the so called Flexner-Wintersteiner-type rosettes). It is worth noting that these rosettes are not diagnostic by themselves. However, these are the typical features of low grade ONBs. Indeed, in high grade cases nuclear pleomorphism with prominent nucleoli, high mitotic activity and necrosis are observed. The combination of architectural structure, nuclear pleomorphism, presence of neurofibrillary matrix, mitotic activity, and necrosis are currently used to grade ONBs into four grade groups 1 (Tab. I). As a rule, grade 1 ONBs are the most differentiated while grade 4 are the poorly differentiated counterpart (Fig. 2). The Hyams’ grading system has been demonstrated to have a good correlation with prognosis 28.

Immunohistochemistry is mandatory in the work-up of ONB to confirm the diagnosis. Tumor cells are typically diffusely and intensely positive for synaptophysin and chromogranin while they are typically negative for cytokeratins, although some cases may show focal reactivity (Fig. 3). S100 immunostaining is typically limited to

<table>
<thead>
<tr>
<th>Microscopic features</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lobular architecture</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Pleomorphism</td>
<td>Absent</td>
<td>Present</td>
<td>Prominent</td>
<td>Marked</td>
</tr>
<tr>
<td>Neurofibrillary matrix</td>
<td>Prominent</td>
<td>Present</td>
<td>Low</td>
<td>Absent</td>
</tr>
<tr>
<td>Rosettes</td>
<td>HW rosettes</td>
<td>HW rosettes</td>
<td>FW rosettes</td>
<td>No</td>
</tr>
<tr>
<td>Mitoses</td>
<td>Absent</td>
<td>Present</td>
<td>Prominent</td>
<td>Marked</td>
</tr>
<tr>
<td>Necrosis</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
<td>Prominent</td>
</tr>
<tr>
<td>Calcification</td>
<td>Variable</td>
<td>Variable</td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>

HW: Homer Wright; FW: Flexner-Wintersteiner
sustentacular cells which are located at the periphery of tumor lobules. CD99, desmin, HMB45 and hematolymphoid markers are negative and can help in the differential diagnosis with tumor mimickers which can include lymphoma, rhabdomyosarcoma, Ewing-sarcoma, and melanoma.

Molecular alterations of ONB are not well known. Somatic TP53 mutations have not been documented, although p53 immunohistochemical expression has been demonstrated in some cases. It has been suggested that p53 alterations probably occur at late stage of tumor growth and progression. Sequencing analyses have been performed in only a few cases and for this reason the available data are not conclusive. Although cytogenetic alterations have been studied in the last years using different techniques, the number of cases analyzed is limited. However, ONBs seem to show high level of chromosomal instability which, paradoxically, seems to be associated to a relatively indolent behavior.
Neuroendocrine neoplasms of the larynx

NENs of the upper respiratory tract are extremely rare, accounting for less than 1% of malignant tumors in this site. Nevertheless, since the first report of a carcinoid tumor of the larynx in 1969, a number of single cases and small series of NENs have been described in the larynx and, to date, these neoplasms are the most frequently diagnosed, after squamous cell carcinoma and its variants. Atypical carcinoid is the most frequent laryngeal NEN, followed by small cell NEC, paraganglioma and typical carcinoid. The disparity of nomenclature and classification criteria used in the literature along the years complicate the assessment of the exact number of each subtype. The terminology needs standardizing because the terms used to indicate laryngeal NEN are too various and confusing. Regardless of their different names, laryngeal NENs appear to be clinically characterized by a common predilection for elderly male patients (in their 6th and 7th decades) with a male to female predominance and a preferential supraglottic location. Paraganglioma deserves a separate mention, because it generally occurs in females and local excision guarantees healing with an excellent prognosis.
A smoking history is considered as a risk factor while a history of significant alcohol intake or exposure to environmental carcinogenic substances are not correlated with the incidence of laryngeal NENs. Clinical symptoms, in order of decreasing frequency, include hoarseness, dysphagia with voice change, sore throat or throat irritation, foreign-body sensation in the throat, hemoptysis and neck mass. When otalgia is present, it is related to throat irritation. Laryngoscopic examination and radiologic studies are diagnostic, revealing polypoid, nodular, pedunculated, exophytic, fungating, papillomatosus, granular, warty or erythematous masses; they are most frequently identified in the supraglottic region, in particular arytenoids and epiglottis. Some patients are asymptomatic and laryngeal lesion is found during incidental intubation for unrelated surgery. Paraneoplastic syndromes are rare but can cause severe symptoms: 10 cases were reported in the English literature, with an incidence of 2.3%. These include five cases of carcinoid syndrome, three cases of Schwartz-Bartter syndrome, one case of ectopic ACTH syndrome and one case of Eaton-Lambert syndrome.

As already mentioned, the classification and terminology of laryngeal NENs have been very heterogeneous since they have been firstly described. As an example, the term small cell carcinoma, widely used in the past to designate poorly differentiated carcinoma, in analogy with lung tumors, is equivocal because not all of laryngeal carcinomas composed by small cells show a neuroendocrine differentiation. The importance of using a correct nomenclature is not merely of academic interest but has determinant clinical implication in terms of diagnosis, prognosis and therapy. According to the World Health Organization (WHO), laryngeal NENs are divided into two broad categories, based on their tissue of origin: epithelial and neural. Among the epithelial-derived tumors, four subtypes are identified: typical carcinoid, atypical carcinoid, small and large cell neuroendocrine carcinoma and combined small cell neuroendocrine carcinoma. The neural-derived tumor category only includes the paraganglioma, in its benign and malignant forms. The WHO classification also uses synonyms for each epithelial-derived NEN, allowing the use of the term “neuroendocrine carcinoma”, grade I (well differentiated, for typical carcinoids), grade II (moderately differentiated, for atypical carcinoids) and grade III (poorly differentiated, for small cell carcinoma, neuroendocrine type) (Tab. II). However, this terminology may be confounding since, in the spectrum of NENs of other sites, the term “neuroendocrine carcinoma” is never used to define well differentiated neoplasms, which are called “carcinoids” in the lung and “neuroendocrine tumors” in the gastroenteropancreatic system. In fact, the word carcinoma, in the neuroendocrine context is reserved for poorly differentiated, high-grade, highly aggressive and early-metastasizing neoplasms, such as small cell and large cell NECs. This is to strengthen the concept that well differentiated neuroendocrine tumors, or carcinoids, are indolent or low-grade neoplasms, with a good prognosis and with a totally different clinical history and management compared to high-grade NECs.

Based on all the above mentioned consideration and on recently published proposal of classification, in the present review we will describe laryngeal NENs using a modified classification proposed in Table III, where the nomenclature of pulmonary NENs is adopted.

### Typical Carcinoid

Typical carcinoid tumor of the larynx is a very uncommon neoplasm and it account for only 3% of laryngeal NENs. It is a well-differentiated neuroendocrine tumor, presenting macroscopically as a submucosal mass ranging in diameter from 0.3 to 4 cm. Histologically, it is composed of nests or chords of uniform polygonal cells with regular round or oval centrally placed nuclei, finely dispersed chromatin, small nucleolus and granular eosinophilic cytoplasm. This organoid growth is accompanied by fibrovascular or hyalinized stroma. Mitotic figures are fewer than 2 per 10 mm² and necrosis and cellular anaplasia are absent. Oncocytic and mucinous changes may be observed, as well as the focal presence of “Zell-ballen”, rosettes and foci of squamous differentiation. Immunohistochemically, general neuroendocrine markers are strongly positive, and neuropeptide markers, in particular serotonin, bombesin, calcitonin and somatostatin, may be present. Low-molecular-weight cytokeratins (CK7-8-18-19-20), epithelial membrane antigen (EMA) and carcinoembryonic antigen (CEA) are consistently expressed.

### Atypical Carcinoid

Atypical carcinoid is much more common than typical carcinoid and it is the most frequent type of laryngeal NEN. The macroscopic aspect is similar to typical carcinoid, with a submucosal nodule or polyp, which can, however, show ulceration and hemorrhage. Histologically, although, like typical carcinoids, they are composed of nests or chords of polygonal cells, these cells...
are generally larger and the nuclei are often vesicular and contain prominent nucleoli; mitotic index ranges are from 2 to 10 per mm², punctate necrosis and lymphatic vessels invasion are often present (Fig. 4). When necrosis is found, it is usually related to surface ulceration. The careful distinction between typical and atypical carcinoids is of great prognostic importance, as well as a correct differential diagnosis with other entities. Paraganglioma, malignant melanoma and medullary thyroid carcinoma are the most common differentials. In particular, the distinction from metastatic medullary carcinoma may be challenging, because of the partially overlapping immunophenotype of the two entities, which are both positive for synaptophysin and CEA. In addition, laryngeal carcinoids may be also positive for calcitonin. However, TTF1 expression, which is constantly observed in medullary carcinoma, is absent in laryngeal carcinoids. Imaging studies of the thyroid, as well as serum calcitonin and CEA levels (both higher in laryngeal carcinoids than in laryngeal carcinoids) may help in differentiating the two entities.

**LARGE CELL NEUROENDOCRINE CARCINOMA**

Laryngeal large cell NEC is a newly recognized entity, which shows peculiar morphologic and clinical features. Macroscopically, this neoplasm is usually characterized by a prevalently submucosal mass, often ulcerated. The histopathological criteria for recognizing large cell NEC in the larynx are the same used in the lung: tumor cells with neuroendocrine morphology and immunophenotype, showing moderate to abundant cytoplasm, vesicular nuclei with prominent nucleoli; mitotic activity >10 per mm² and zonal to extensive necrosis. The most important parameter that differentiates large cell NEC from atypical carcinoid is mitotic index. At present, there is no definitive evidence that Ki67-related proliferative index may be used in the distinction between these two neoplasms, nor it can be incorporated in the classification of laryngeal NENs. The distinction from small cell NEC is based on cell morphology, but it can be difficult on small biopsies, where scratch artifacts may be present. Even in this situation, the most reliable criteria to differentiate small cell from large cell carcinoma is the nuclear to cytoplasmic ratio, which is higher in the small cell type. However, this is not a great clinical issue since it has been reported that these two laryngeal high grade NECs do not differ in terms of survival.

**SMALL CELL NEUROENDOCRINE CARCINOMA**

Small cell NEC represents more than one third of laryngeal NENs. These neoplasms may reach a large size (up to 5 cm) and are macroscopically undistinguishable from squamous cell carcinomas. Histologically, this neoplasm is similar to poorly differentiated small cell neuroendocrine carcinoma of the lung and other sites. It is composed of sheets or, occasionally, interconnecting ribbons of small to intermediate sized cells with high nuclear/cytoplasmic ratio, hyperchromatic oval, round or spindle-shaped nuclei with delicate chromatin, small inconspicuous nucleoli and a minimal rim of cytoplasm. Mitotic and apoptotic body are numerous. “Geographic chart” necrosis is the rule and vascular and/or perineural invasion are commonly seen. Rosette formation may be observed. The differential diagnosis of small cell neuroendocrine carcinoma includes other laryngeal NENs (see above), basaloid squamous cell carcinoma and the solid variant of adenoid cystic carcinoma. In these latter cases, the use of general neuroendocrine immunohistochemical markers is mandatory to reach a correct diagnosis.

**MIXED NEUROENDOCRINE NON-NEUROENDOCRINE NEOPLASMS (MiNENs)**

To date, 19 cases of laryngeal MiNENs have been reported in the English literature and all but one were composed of squamous cell carcinoma and small cell NEC. The remaining MiNEN was a combined atypical carcinoid with squamous cell carcinoma. Histologically, the two components may be well recognizable from each other when the squamous component is well differentiated. However, when the squamous component is poorly differentiated, the only way to differentiate the two components is using immunohistochemistry. It should be noted that the recognition of a poorly differentiated neuroendocrine component in a laryngeal squamous cell carcinoma is of capital importance in terms of prognosis, as the patients’ outcome is heavily influenced by the former and it is significantly worse than that of pure squamous carcinoma. In these setting, an appropriated panel including general neuroendocrine markers, p63, p40 and cytokeratins is recommended.

**PARAGANGLIOMA**

Macroscopically, this tumor has the appearance of red or
blue submucosal mass of 1 to 6 cm in size with an average diameter from 2 to 3 cm; the consistency is firm with a red or brown cut surface and areas of hemorrhage or streaks of fibrous tissue are often present. Microscopic findings include two cell types: chief and sustentacular cells. The former are polygonal cells with eosinophilic cytoplasm and inconspicuous nuclei, arranged into alveolar or “Zellballen” pattern. Pleomorphism may be seen and mitoses are not frequent. The latter have a round body with elongated dendritic projection which are arranged around the edges of the “Zellballen”. Around the tumor a highly vascular, fibrotic capsule is frequently identified. Uncommon findings are vascular invasion, perineural involvement and necrosis and when are found in this tumor, they are not necessarily related with a malignant behavior.

Management of laryngeal NENs is guided by the histologic subtype. Typical carcinoid is treated with surgery alone, in the form of conservation surgery: supraglottic subtotal laryngectomy or CO2 laser resection. Total laryngectomy may only be performed for large tumors. There is no need to perform elective neck dissection because lymph node metastases are infrequent. Irradiation and chemotherapy are ineffective. Typical carcinoid rarely metastasizes; however, its clinical course may be not indolent because cases with distant metastasis have been described, frequently involving the liver. The 5-year survival rate is 48.7% in a large series reported by Soga. The gold-standard for the atypical carcinoid is surgical excision, in the form of partial or total laryngectomy depending on site, size and extent of the tumor. Elective neck dissection is recommended in view of the high incidence of both early cervical metastasis and subsequent involvement of cervical nodes. Radiotherapy does not appear to be effective. According to a recent meta-analysis of a 436 reported cases published in 2014, 30% of patients have distant metastasis at presentation, the recurrence rate is at 62.5%, with a 5-year disease-free survival and overall survival of 52.8% and 46%, respectively. Small and large cell NECs are high-grade tumors and a multimodality therapy is preferred. Total laryngectomy may control the primary tumor but the loss of voice is not justifiable in the face of other therapies that control, in the same way, primary tumor: the role of surgery, in fact, is limited. The combination of primary radiation therapy and adjuvant chemotherapy resulted in
median survival of 55 months, significantly longer than with any other treatment regimen. Small cell NEC of the larynx has the worse prognosis of all laryngeal tumors; more of 90% of patients with this tumor develop metastatic disease. The prognosis of combined small cell is comparable to that of a pure small cell neuroendocrine carcinoma.

Paraganglioma is a benign tumor; surgery is preferable to radiation because may be curative without loss of laryngeal function, so partial laryngectomy remains the mainstay of treatment. Laser surgery is not recommended because of highly vascular nature of paragangliomas. When possible, extramucosal excision of tumors arising in the paraglottic and/or supraglottic space is preferred. The prognosis is excellent with a radical excision.

**Merkel cell carcinoma**

Merkel cell carcinoma (MCC) is a rare primary neuroendocrine carcinoma of the skin, which shows an aggressive biological and clinical behavior. It was firstly described by Toker in 1972, but its neuroendocrine features have led to the assumption that it derives from Merkel cells of the skin. About 50% of all cutaneous lesions arise in the head and neck. The cervicofacial district includes also most of the unusual extracutaneous primary sites of MCC, among which the parotid gland is the most common, followed by the submandibular gland, nasal cavity, mucosa of the lip and mandibular gland, nasal cavity, mucosa of the lip and nasal cavity.

Macroscopically, cutaneous MCC typically presents as a painless, rapidly enlarging, red or purplish nodule, more often in sun-exposed areas. Histologically, MCC is composed of sheets or nests of small to medium sized cells with a small amount of eosinophilic cytoplasm (Fig. 5). The nuclei are round/oval with dispersed chromatia; the nuclear membrane is well evident and nucleoli are inconspicuous. The mitotic index is often high with some atypical mitoses. The immunohistochemical stainings (Fig. 5) show intense positivity for neuroendocrine markers (synaptophysin and chromogranin A) and a peculiar dot-like pattern of immunoreactivity for CK20.

Clinically, MCC shows an aggressive behavior with spread to local lymph nodes. The treatment is complex and, depending on stage, involves surgical resection, radiotherapy, and chemotherapy. As stage seems to be the most important prognostic factor in this disease, a staging system for cutaneous Merkel cell carcinoma and regional lymph nodes is reported in the 8th edition of the AJCC cancer staging manual. The AJCC staging thresholds between pT1, pT2 and pT3 are at 20 mm and 50 mm, whereas invasion of bone, muscle, fascia or cartilage are determinant for stage pT4. A positive node with microscopic disease is stage n1a and with macroscopic disease n1b. Negative immunohistochemistry is required to designate a node as free from carcinoma. According to the guidelines of the Royal College of Pa-
thologists, negative immunohistochemistry is required to establish a NO staging. In-transit metastasis defines stage N2. The criteria for in-transit metastasis are different from those for melanoma, and it is defined as any discontinuous nest of cells greater than 0.05 mm in diameter that is clearly separated from the main invasive carcinoma by at least 1 mm of normal dermis. The most important factors in establishing the prognosis of MCC are stage (such as maximum diameter, lymph node metastases, in-transit metastases), the presence of a second malignancy, margins status and lymph node metastasis.

Neuroendocrine tumor of the middle ear

Literature published in the last years has indicated that the so-called “middle ear adenoma” and “middle ear carcinoid” represent the same entity. Both terms are not correct because several papers have demonstrated, using different techniques, that this tumor type is composed of both a glandular (exocrine) and solid (neuroendocrine) component, making the neuroendocrine tumor of the middle ear a mixed neoplasm, for which the term MiNENs may be more appropriate. This is a very rare neoplasm showing equal sex distribution and occurring more frequently in the third to fifth decades of life (range
20-80 years). The most common symptoms is unilateral conductive hearing loss. Pain, optic discharge and facial nerve paralysis are rare, demonstrate a locoregional extension and are associated to an aggressive behavior. No etiologic factors have been identified to date. At otoscopic examination, the tympanic membrane is generally intact. The tumor generally appears as a gray-white to red brown firm mass and appears easy to remove. Histologically, the tumor is unencapsulated showing a commingling of glandular/tubular and solid/trabecular structures (Fig. 6). Rarely, a predominant papillary architectural pattern can be observed. Tumor cells are cuboidal with eosinophilic cytoplasm and round to oval nuclei with “salt and pepper” aspect, sometimes containing eccentrically located small nucleoli. Mitoses are absent or rare.

Tumor cells are CK-positive and express neuroendocrine markers including synaptophysin and chromogranin A. Interestingly, middle ear neuroendocrine tumor can show an immunophenotype similar to that of hindgut-derived well differentiated neuroendocrine tumors including the expression of pancreatic polypeptide-related peptides, glucagon-related peptides, serotonin, CAR5, and prostatic acid phosphatase, but the reason of this is not clear.

Data on patients’ prognosis are limited by the rarity of such neoplasms. Recurrence has been reported for cases with incomplete local surgical excision.

**Neuroendocrine neoplasms of the salivary glands**

The spectrum of the salivary gland NENs is almost totally covered by poorly differentiated NECs of the small cell and large cell types. Only a few cases of well differentiated neuroendocrine tumors (carcinoids) have been reported.

*Small cell neuroendocrine carcinoma (SCNEC)* of the salivary glands accounts for about 2% of all salivary glands malignancies and is the most common type of salivary gland NEN. Minor salivary glands are rarely the primary site, and most of the cases arise in the parotid gland as poorly circumscribed nodules. Histologically, these neoplasms are very similar to small cell carcinoma of the lung and are composed of sheets, ribbons,
or nests of round, oval to spindle cells measuring as large as or larger than (up to 2 times) lymphocyte diameter, with scant cytoplasm. In some cases, rosette formation may be observed. Crash artifacts and molding are common features, as well as high mitotic index, zonal necrosis and invasion of vascular and perineural spaces. Ductal and squamous differentiation may be seen and also skeletal muscle differentiation has been observed in these neoplasms 1. The immunohistochemical stainings are positive for at least one general neuroendocrine marker. CKs are, at least focally, expressed, usually with a dot-like paranuclear pattern 84. The expression of CK 20 in more than 70% of SCNECs of the salivary glands has lead to the concept that these CK20-immunoreactive neoplasms were related to Merkel cell carcinoma, and to the subclassification of primary SCNECs of the salivary glands in Merkel cell type and pulmonary type 85. In fact, it has been reported that Merkel cell type SCNECs behave less aggressively than their pulmonary type counterpart, suggesting that CK20 immunostaining should be included in the immunohistochemical panel when evaluating a small cell carcinoma of the parotid 1 84. There is obviously an overlap between the so called “primary Merkel cell carcinomas of the salivary glands” and the Merkel cell type of SCNEC of these sites. SCNECs of the salivary gland may also express CK7, epithelial membrane antigen (EMA) and neurofilaments. Vimentin may be positive, whereas immunoreactions for S100 and HMB45 are always negative.

The differential diagnosis of SCNECs includes a number of epithelial and non epithelial malignancies, both primary and metastatic. Immunohistochemistry is a useful tool, in addition to morphology, to differentiate these neoplasms from other blue cell tumors, such as non Hodgkin lymphomas, basaloid carcinomas, and the solid variant of adenoid cystic adenocarcinoma. Also metastatic melanoma may be easily recognized using the appropriate panel of immunostainings. In contrast, the distinction from small cell neuroendocrine carcinomas metastatic from other sites, in particular from the skin or, more frequently, from the lung, may be challenging and should also rely on careful clinical and imaging studies. A salivary gland metastasis may be the initial presentation of an occult neuroendocrine carcinoma of another site and immunohistochemistry may be positive only with careful clinical examination.

The prognosis and the clinical history of LCNEC are similar to that of SCNEC and tumor size greater than 4 cm is an adverse prognostic indicator 87.

Well differentiated neuroendocrine tumors (carcinoids) of the salivary glands are exceedingly rare, with as few as seven cases reported until now, including 2 TC 80 81 and five AC 22 82 83. Interestingly, the totality of cases occurred in the parotid gland. The histological features of these neoplasms are those described for pulmonary carcinoids and well differentiated NETs of the gastroenteropancreatic tract. They show an organoid growth, with cords, nests or pseudoglands, composed by uniform neoplastic cells with moderately abundant eosinophilic cytoplasm round nuclei with salt-and-pepper chromatin pattern and small nucleoli. In typical carcinoids, mitotic figures, pleomorphism, and necrosis are characteristic, whereas atypical carcinoids usually show these features. Strong and diffuse immunoreactivity for neuroendocrine markers is a consistent feature of these tumors, but a systematic analysis of amine and peptide hormones production has not been carried out on reported cases. Pan-cytokeratin is always expressed, whereas CK 20 was negative in all cases 22.

The differential diagnosis of salivary glands carcinoids includes metastatic carcinoids from other sites, which needs to be excluded on clinical bases, and LCNECs, in which proliferative index and cytologic atypia are greater. In addition, metastatic melanoma and can be ruled out using appropriate immunostainings, as well as other primary tumor of the salivary glands, first of all adenoid cystic carcinoma. Due to the small number of the reported cases, no definitive conclusion about patients’ outcome may be drown,
however, the observed follow up data suggest that salivary gland carcinoids are less aggressive than poorly differentiated neuroendocrine carcinomas of this site 22 80-83.

References

6 Gonzalez-Kristeller DC, Gutiyama LM, Campos AH, et al. Odor-


Lloyd RV. Practical markers used in the diagnosis of neuroendocrine tumors. Endocr Pathol 2003;14:293-301.


