

An Update on Sinonasal Round Cell Undifferentiated Tumors

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Abstract The sinonasal cavities host a wide variety of undifferentiated malignancies with round cell morphology, including neoplasms of epithelial, mesenchymal, neuroectodermal, and hematolymphoid lineage. The differential diagnosis may be difficult, especially in small biopsy material, due to overlapping morphology, but their correct classification is clinically relevant. The aim of this review is to provide practical guidelines for the differential diagnosis of these malignancies, with emphasis on recently described entities and special reference to the role of ancillary techniques.

Keywords Nasal cavity · Paranasal sinuses · Undifferentiated tumors · Diagnosis · Immunohistochemistry · Molecular biology

Introduction

In the sinonasal tract, a wide range of malignancies may histologically appear as formed by neoplastic cell with no definitive differentiation, round cell morphology with high nuclear to cytoplasmic ratio, high mitotic and apoptotic activity, and necrosis. The differential diagnosis is challenging, particularly on small biopsy specimens, and includes a whole range of neoplastic entities, both epithelial and non-epithelial. However, it is important to separate them because their prognoses and management are different. Moreover, poorly differentiated neoplasms may spread to the sinonasal

region from adjacent sites (oral cavity, nasopharynx, or skull base) or from distant sites, further complicating the approach to the differential diagnosis. Thus, the clinical history and the results of imaging studies should be always available for an accurate differential diagnosis.

Although no definitive data on the relative frequency of undifferentiated sinonasal malignancies is available, it can be expected that it should reflect the distribution of all histotypes in the general population, where epithelial tumors are by far most frequent than non-epithelial tumors [1]. On the other hand, in the pediatric population rhabdomyosarcoma and olfactory neuroblastoma are the most frequent histotypes, followed by haematolymphoid malignancies, whereas epithelial malignancies are extremely rare [2]. These differences should be taken into account when approaching the diagnosis of an undifferentiated sinonasal neoplasm.

With the refinement of diagnostic criteria and the use of immunohistochemical and molecular markers, new sinonasal malignancies with poorly differentiated morphology have recently been described. Moreover, several new immunohistochemical and molecular markers have been tested on these neoplasms, and they facilitate, in combination with light and ultrastructural morphology, their correct classification. This review is focused on the differential diagnosis of recently described entities in the group of sinonasal undifferentiated/poorly differentiated tumors with round cell morphology, with special reference to the role of ancillary techniques.

Epithelial Neoplasms

Table 1 summarizes the immunohistochemical features of sinonasal carcinomas that may present with undifferentiated round cell morphology.

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Table 1 Summary of the diagnostic features of sinonasal poorly differentiated/undifferentiated carcinomas

	Histologic features	CK ^a	P63	P40	S100	Syn	HPV	EBV	Diagnostic gene alterations
SNUC	Sheets, nests or ribbons of small to medium-sized cells, no evidence of squamous or glandular differentiation	7+; 5/6–	–	–	–	–	– (+ in rare cases)	–	ND
Lymphoepithelioma	Syncytial growth pattern, prominent lymphoid infiltrate	5/6+, 13+	+	+	–	–	–	+	ND
NUT midline carcinoma	Sheets of monotonous undifferentiated round cells, foci of mature keratinized squamous cells occasionally seen	5/6+, 7+, 20+ in rare cases	+	+	–	–	–	–	t(15;19) NUT + by immunohistochemistry
Neuroendocrine carcinoma	Closely packed small cells with scant cytoplasm, extensive necrosis, and brisk mitotic activity; in large cell NEC, cytoplasm is abundant, nuclei are vesicular with prominent nucleoli, moderate pleomorphism is present	5/6 rarely+	–/+	–	–	+	–	–	ND
Basaloid squamous cell carcinoma	Nests of basaloid cells, peripheral palisading, comedo necrosis, foci of squamous differentiation	5/6+, 34βE12+	+	+	–	–	+	–	ND
Solid adenoïd cystic carcinoma	Solid nests of basaloid cells	5/6+, 7+, 34βE12+, 20–	+	–/+	+	–	–	–	t(6;9)(q22–23;p23–24)
HPV-related carcinoma with adenoïd cystic-like features	Similar to adenoïd cystic carcinoma; myoepithelial differentiation, squamous differentiation in surface epithelia	AE1/AE3+	+	ND	+	–	+	–	ND
SMARCB1 (INI-1)-deficient sinonasal carcinoma	Nests of basaloid cells with peripheral palisading and variable amounts of rhabdoid cells	5+	+	+		Focal+	–	–	SMARCB1 gene alterations; SMARCB1 negative by immunohistochemistry

CK cytokeratin, Syn synaptophysin, ND not determined

^a All carcinomas are positive for pan-cytokeratins, only specific classes are reported

Sinonasal undifferentiated carcinoma (SNUC) is a rare clinically aggressive neoplasm, which more frequently originates from the ethmoid region and invades the adjacent sinonasal structures, the orbit, the skull base, and the brain [3]. Histologically, it is composed of sheets, nests or ribbons of small to medium-sized cells, which according to the original description should lack evidence of squamous or glandular differentiation [4] (Fig. 1), whereas neuroendocrine features have been repeatedly reported [5, 6].

Immunohistochemically, SNUC is positive for epithelial markers, such as simple epithelia cytokeratins (CKs) and

epithelial membrane antigen (EMA) [7]. Variable reactivity can be seen with neuron specific enolase (NSE), chromogranin, and synaptophysin. SNUC usually shows only limited positivity for p63 and p40 [8–11], and this is a useful feature to separate it from poorly differentiated SCC and lymphoepithelioma. SNUC is typically negative for Epstein–Barr virus (EBV) [5, 6], while association with HPV has been reported with variable, generally low, frequency [12, 13]. However, a recent study reported a higher frequency of p16 positivity in SNUC (78.6%) with detection of HPV DNA in 64.3%, and p16 positive tumors had a significantly improved survival [14].

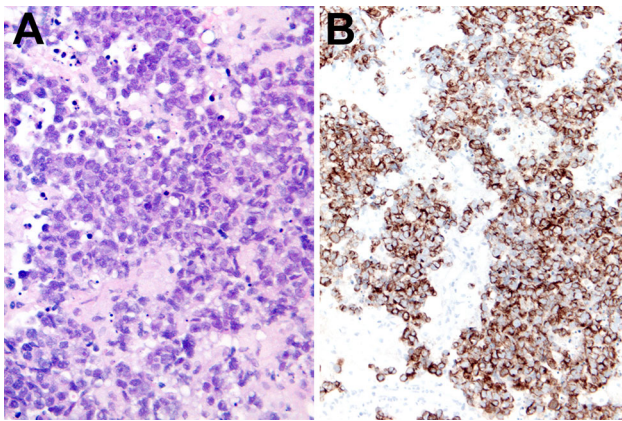


Fig. 1 Sinonasal undifferentiated carcinoma. **a** The tumor consists of a proliferation of undifferentiated round cells, with a high nuclear/cytoplasmic ratio. Mitotic figures and apoptotic bodies are present. Tumor cells are positive for cytokeratin 8 (**b**)

Being mainly a diagnosis of exclusion and due to the lack of specific criteria for the diagnosis, it is likely that the diagnosis of SNUC may have been applied to a heterogeneous group of carcinomas, and further refinement in diagnostic criteria and the availability of new molecular markers may lead in the future to the separation of specific tumor types from this group of tumors.

Lymphoepithelial-like carcinoma primarily involving the sinonasal tract is extremely rare, while an extension of a nasopharyngeal lesion is more frequent [15]. The distinction from SNUC can usually be done on morphological grounds, because neoplastic cells grow in a syncytial growth pattern, with indistinct borders, and have markedly vesicular nuclei with prominent nucleoli. A prominent lymphoplasmacytic cell infiltrate is seen in most cases. The expression of CK5/6, CK13, p40, and p63 supports the diagnosis of lymphoepithelioma, whereas these markers are negative or focally positive in SNUC [9, 11]. EBV markers are positive in LEC but not in SNUC [5, 6].

NUT midline carcinoma (NMC) is a recently described highly aggressive carcinoma defined by a reciprocal chromosomal translocation, which in most cases involves the NUT (nuclear protein in testis) gene on chromosome 15q14.6 and the BRD4 (bromodomain-containing protein 4) gene on 19p13.1 [16]. NMC represents 2 % of all carcinomas involving the sinonasal tract [17], and occurs at all ages with a predilection for young adults. Histologically, it consists of a proliferation of sheets of monotonous undifferentiated round cells, but foci of mature keratinized squamous cells may be occasionally seen abruptly juxtaposed to the undifferentiated component. Brisk mitotic activity, apoptotic bodies, and areas of tumor necrosis are present. The diagnosis requires the demonstration of NUT rearrangement, by FISH or RT-PCR, but immunohistochemical staining for NUT appears to be sensitive and

specific in the distinction of NMC from other carcinomas [18]. In addition, NMC stains for cytokeratins, p63, CD34 (in approximately half of the cases), while it is negative for S100, HMB45, desmin, myoglobin, smooth muscle actin, muscle actin, chromogranin, synaptophysin, CD45, placental alkaline phosphatase, alphafetoprotein, neuron specific enolase, CD57, and CD99 [16]. HPV and EBV were also negative in all cases tested.

Sinonasal neuroendocrine carcinoma is a rare and aggressive tumor type, which is similar to the pulmonary counterpart. It occurs over a wide age range, and it has an aggressive clinical course with frequent local recurrences and metastatic spread to lymph nodes, lung, liver, and to the skeleton. Histologically, it may present with “small cell” or “large cell” morphology (Fig. 2). It is positive for pan-cytokeratins and neuroendocrine markers, including CD56, synaptophysin, and chromogranin, although with variable frequency. CD56 or neuron-specific enolase (NSE) immunoreactivity alone is not considered sufficient to demonstrate neuroendocrine differentiation. Cytokeratin 5/6, p63 and p40 are either negative or focally positive. The differential diagnosis with atypical carcinoid (i.e., moderately differentiated neuroendocrine tumor), which is extremely rare in this anatomic site, is mainly based on mitotic count [19]. Positivity for cytokeratins allows the distinction from other undifferentiated tumors, which may show neuroendocrine differentiation, including olfactory neuroblastoma and melanoma. Finally, sinonasal neuroendocrine carcinoma may occur in combination with squamous cell carcinoma or adenocarcinoma [20, 21].

A group of poorly differentiated sinonasal carcinomas may present with a “basaloid” morphology, being formed by solid nests of small to medium sized cells, with scant cytoplasm and dense hyperchromatic nuclei, with frequent peripheral palisading and central necrosis. Neoplastic cells may be associated with extracellular matrix production (hyalinized pericellular basement membrane like material) or with microcystic spaces containing a basophilic myxoid material. The differential diagnosis of this group of tumors include basaloid squamous cell carcinoma (BSCC), solid adenoïd cystic carcinoma (ACC) and two recently described entities, the HPV-related carcinoma with adenoïd cystic-like features and the SMARCB1 (INI-1)-deficient sinonasal carcinomas [22–24].

BSCC is an aggressive variant of SCC, often presenting in advanced stage, which involves the upper aerodigestive tract, including the sinonasal cavities. Histologically, foci of squamous differentiation can be identified in conjunction with the basaloid component, although these may represent a minor component and include both intraepithelial or invasive SCC. Areas of comedo necrosis are often seen (Fig. 3). Cribriform, gland-like or cystic pattern can also be recognized. Immunohistochemically, p63, p40,

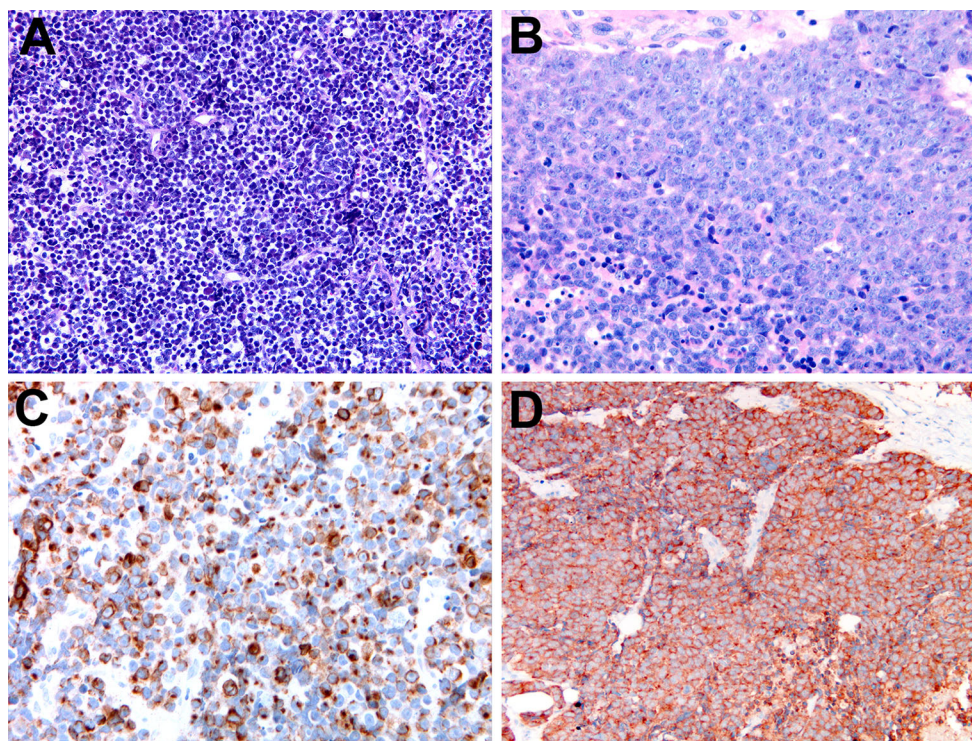


Fig. 2 Small cell neuroendocrine carcinoma of the maxillary sinus (**a**). The tumor is composed of closely packed cells with inconspicuous cytoplasm and nuclei with dense chromatin. In sinonasal large cell neuroendocrine carcinoma, neoplastic cells have larger nuclei

with prominent nucleoli (**b**). These malignancies are positive for cytokeratins, often with a dot-like paranuclear pattern (**c**) and synaptophysin (**d**)

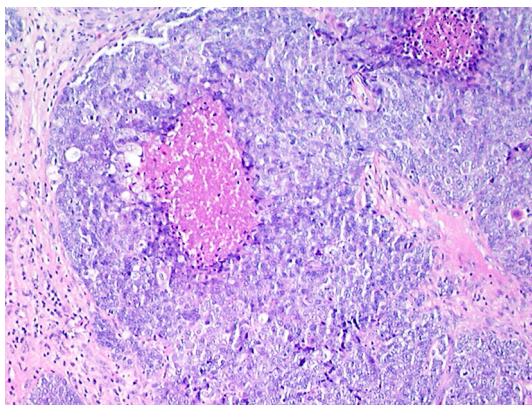


Fig. 3 Basaloid squamous cell carcinoma with comedo necrosis

cytokeratin 5/6 and 34betaE12 [10, 25, 26] are diffusely positive in nearly all cases. Solid ACC shows a similar immunohistochemical profile, although p63 is usually positive at the periphery of the tumor nests, and p40 expression is more limited. However, the neoplastic population is more uniform, not pleomorphic and no squamous differentiation is seen. Identification of the t(6;9)(q22–23;p23–24) chromosomal translocation, which is specific for adenoid cystic carcinoma, can help to separate sinonasal ACC from other mimics [27].

The HPV-related carcinoma with adenoid cystic-like features presents significant histologic and immunohistochemical overlap with solid adenoid cystic carcinoma (Fig. 4), but the presence of surface intraepithelial dysplasia, absence of MYB gene rearrangement, and association with HPV allows a separation of the two entities [24].

A subset of poorly differentiated sinonasal carcinomas is characterized by inactivating alterations of the SMARCB1 tumor suppressor gene [22, 23]. These tumors are composed of an admixture of basaloid cell nests with peripheral palisading and variable amounts of rhabdoid cells. They are positive for pan-cytokeratins and show variable expression of p63 and p40. SMARCB1 (INI1) is negative and fluorescence in situ hybridization shows SMARCB1 deletion. No HPV, EBV, or NUT association has been documented so far. The small number of cases reported precludes any definitive conclusion on the clinical importance of their recognition.

Finally, other carcinomas may exceptionally present with a high grade undifferentiated morphology, but can still be diagnosed based on their immunoprofile. Petersson et al. [28] reported a case of anaplastic myoepithelial carcinoma with anaplastic morphology, but showing a distinct myoepithelial immunohistochemical phenotype including positivity for smooth muscle actin, p63, S100 protein, calponin, cytokeratin 14, vimentin and cytokeratins AE1/AE3 and 5/6.

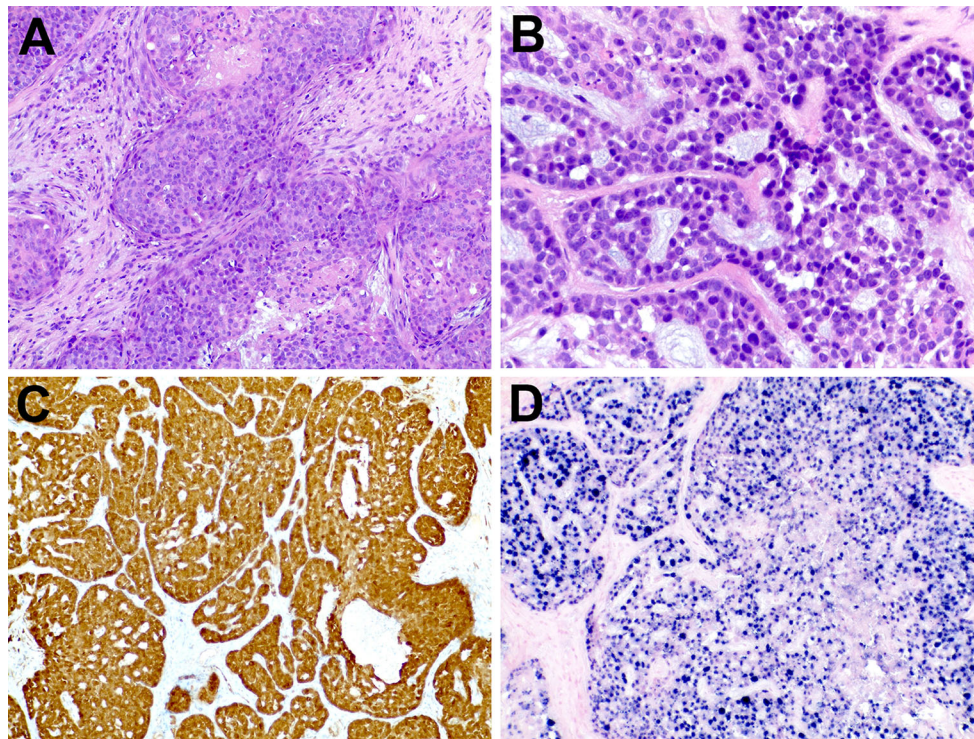


Fig. 4 HPV-related carcinoma with adenoid cystic-like features presents areas of solid growth of basaloid cells (a) and cribriform areas (b). P16 is diffusely positive (c), and high risk HPV can be detected by in situ hybridization (d)

Non-epithelial Neoplasms

Melanoma

Sinonasal melanoma accounts for 5–10 % of all sinonasal malignancies, and affects mainly adult patients. Histologically, it may present with variable morphology, including epithelioid, pleomorphic, spindle cell, and only rarely a round cell morphology (Fig. 5). Melanin pigment may be present either in neoplastic cells or in melanophages. Sinonasal melanoma is positive for S100 protein and for melanocytic markers, including MART-1/melan-A, tyrosinase, HMB-45, Mitf, and SOX10. Since none of these markers has a 100 % sensitivity, they should be employed in a panel [29]. It should also be remembered that melanomas might show anomalous expression of markers of neuroectodermal differentiation, as well as of intermediate filament, thus potentially causing diagnostic confusion (see below for discussion).

Sarcomas

Sinonasal sarcomas are a group of rare tumors, representing 10–15 % of all sinonasal malignancies. According to a recent analysis of data from the SEER database, the maxillary sinus is the most frequently involved site, followed

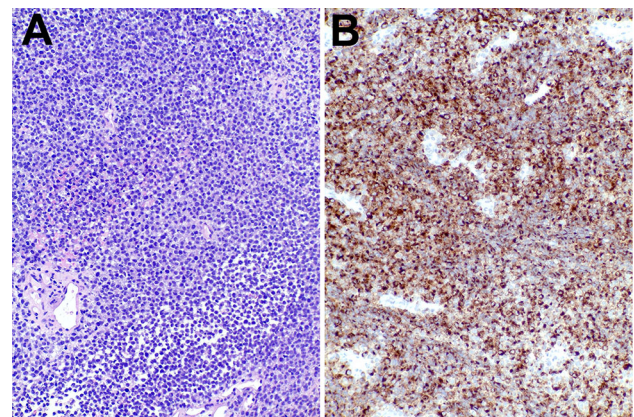


Fig. 5 Sinonasal melanoma presenting with round cell undifferentiated morphology (a). The tumor is positive for HMB45 (b)

by the ethmoid and the sphenoid sinus, and rhabdomyosarcoma is the most common tumor histology [30]. Limiting the discussion to round cell sarcomas, these tumors affect mainly, but not exclusively, children and young adults. Since specific therapeutic protocols may be very effective in some of these entities, their proper classification is crucial for the appropriate patient management.

The differential diagnosis includes rhabdomyosarcoma, Ewing sarcoma, desmoplastic small round cell tumor [31,

Table 2 Summary of the diagnostic features of sinonasal “round cell” sarcomas

	Histologic features	Immunohistochemical markers	Diagnostic gene fusion(s)
Rhabdomyosarcoma	Loose myxoid areas, subepithelial cellular areas, solid, alveolar	Myogenin, desmin, actin	PAX3 or PAX7-FOXO1 (Alveolar Rhabdomyosarcoma)
Ewing sarcoma	Solid, pseudorosettes	CD99, variable expression of neuroectodermal markers and cytokeratins	EWSR1-FLI1 or other ETS family members
Desmoplastic small round cell tumor	Solid areas separated by desmoplastic stroma	Cytokeratins, desmin, WT1, neuron-specific enolase, CD99	EWSR1-WT1
Synovial sarcoma (poorly differentiated)	Solid, prominent vascular channels	Cytokeratins, EMA, TLE1	SS18-SSX1 or SSX2
Mesenchymal chondrosarcoma	Biphasic, small cells and islands of cartilage	SOX9	HEY1-NCOA2
Small cell osteosarcoma	Solid, osteoid deposition	SATB2	Not determined

[32], poorly differentiated synovial sarcoma, mesenchymal chondrosarcoma [33], and small cell osteosarcoma. Table 2 summarizes the key diagnostic features of this group of tumors.

Rhabdomyosarcoma is the most common sinonasal malignancy of the pediatric age, and the embryonal variant represents the most frequent subtype in the head and neck area. In a recent analysis of 48 sinonasal sarcomas arising in adult patients, alveolar rhabdomyosarcoma was the most frequent histologic type (33.3 %), and all round cell sarcomas were rhabdomyosarcomas [34]. Histologically, these tumors consist of a population of primitive cells with variable degrees of skeletal muscle differentiation, and scattered rhabdomyoblasts. In the botryoid variant of embryonal rhabdomyosarcoma, subepithelial aggregates of neoplastic cells can be observed (cambium layer). The diagnosis is supported by the immunostaining for desmin, myoD1, and myogenin (MYF4). However, these tumors may be positive for cytokeratins and neuroendocrine markers, causing diagnostic confusion (see below for discussion). The alveolar variant is characterized by a recurrent chromosomal translocation t(2;13)(q35;q14) or less frequently t(1;3)(p36;q14), with PAX3-FOXO1A and PAX7-FOXO1A rearrangements [34].

Sinonasal Ewing sarcoma occurs over a wide age range, although most patients are in the pediatric age or young adults [35]. Histologically, the lesion consists of a uniform population of round to oval cells, with scant clear or eosinophilic cytoplasm. Pseudorosette formation can be present in tumors showing neuroectodermal differentiation. Immunohistochemically, neoplastic cells are diffusely and intensely positive for CD99 at the cell membrane level. Focal positivity for cytokeratins may be detected in up to 30 % of cases [36, 37]. Recently, examples of Ewing sarcoma showing prominent squamous epithelial

differentiation, which have been designated “adamantinoma-like” Ewing Family Tumor, have also been reported in the sinonasal tract [38]. These tumors are positive for CD99, pancytokeratin, and p40, and therefore need to be distinguished from sinonasal epithelial and myoepithelial neoplasms. Detection of EWSR1 and FLI1 rearrangements is necessary to confirm the diagnosis. Identification of EWS rearrangement alone, however, is not sufficient to exclude a myoepithelial carcinoma [38].

Poorly differentiated synovial sarcoma presents with a round cell Ewing-like morphology. Immunohistochemical detection of cytokeratin positivity, which is usually focal, may not be sufficient, and demonstration of the SS18 gene rearrangement is necessary to confirm the diagnosis.

The diagnosis of mesenchymal chondrosarcoma and small cell osteosarcoma requires the recognition of matrix production by neoplastic cells, and it may be particularly challenging in absence of foci of cartilaginous or osteoid matrix formation. Positivity for SOX9 has been reported as a specific marker, and more recently, a recurrent HEY1-NCOA2 fusion transcript has been detected in mesenchymal chondrosarcoma, a finding with potential diagnostic utility [39]. Small cell osteosarcoma does not present EWSR1 and FUS gene rearrangements and it is positive for the osteoblastic marker SATB1 [40].

Olfactory Neuroblastoma

Olfactory neuroblastoma (ONB) is defined as a malignant tumor composed of neuroblasts derived from the olfactory membrane [41]. ONB represents approximately 6 % of all sinonasal malignancies and its incidence has increased significantly over the last decades [42], although this may be a consequence of an improvement in the histopathological diagnosis. ONB has a bimodal age distribution with

peaks in the 2nd and 6th decade [43]. ONB takes origin from the olfactory mucosa that lines the upper part of the nasal cavity, and grows in the sinonasal region and/or the anterior cranial fossa [44]. Only exceptionally ONB does not involve the olfactory membrane (so called “ectopic” olfactory neuroblastoma), but this diagnosis requires the careful exclusion of all other small round cell malignancies. Histologically, ONB typically shows a multinodular or lobular growth pattern, and is formed by a uniform population of round cells set in a fibrillary background. Homer Wright or Flexner-Wintersteiner type rosettes can be seen with variable frequency. ONB can be categorized in four grades according to the Hyams system, which is mainly based on the tumor architecture, presence of neurofibrillary matrix and rosettes, mitotic activity, and presence of necrosis [45]. High grade (Hyams III and IV) lesions (Fig. 6) show a solid growth, lack the fibrillary background, present brisk mitotic activity and areas of necrosis, thus requiring careful differential diagnosis with other round cell sinonasal malignancies.

Immunohistochemically, ONB is diffusely positive for synaptophysin, chromogranin A, neurofilaments, and CD56 [46]. In tumors with a lobular pattern, S100 protein is positive in sustentacular cells. CK is generally negative, although in rare cases ONB may exhibit focal staining for low molecular weight CK. Entrapped normal residual

epithelia are positive for CKs and this may be a source of confusion. P63 is negative or only focally positive, and EMA is negative [8]. A recently introduced useful marker for the diagnosis of ONB is calretinin, which is also expressed by olfactory neurons [47].

The differential diagnosis of ONB is broad and includes epithelial malignancies, such as SNUC, neuroendocrine carcinoma (NEC), lymphoepithelial-like carcinoma, basaloid squamous cell carcinoma, and NUT-midline carcinoma, which can be distinguished on the basis of diffuse positivity for cytokeratins of different molecular weights. Another epithelial neoplasm that may be entered in the differential diagnosis of ONB is ectopic pituitary adenoma [48]. This, however, usually involves the sphenoid, and it is positive for pan-cytokeratins.

Among non-epithelial neoplasms, the distinction of ONB from melanoma is based on the diffuse positivity for S100 protein, HMB45, and Melan-A of the latter. Ewing’s sarcoma/PNET is positive for CD99, while ONB is negative, and if necessary, the EWS gene rearrangement can be searched for by FISH or PCR. Rhabdomyosarcoma can be distinguished based on the positivity for desmin and myogenin. Sinonasal lymphomas and plasmacytoma may be difficult to separate from ONB on pure morphological grounds in small biopsies, but their immunoprofiles do not overlap (except for CD56 positivity), and therefore the

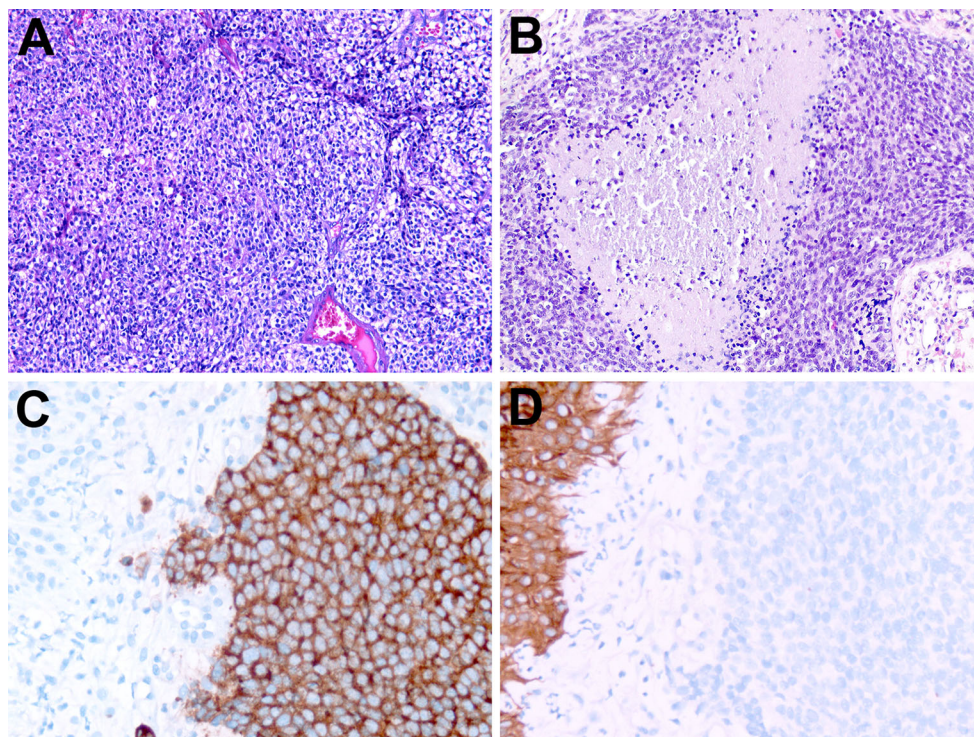


Fig. 6 High grade olfactory neuroblastoma is characterized by a solid growth pattern, moderate pleomorphism (a), and presence of necrosis (b). The tumor is positive for synaptophysin (c), while cytokeratin AE1/AE3 is negative (d). Positive non-neoplastic epithelium is visible on the left

Table 3 Summary of the salient diagnostic features of sinonasal hematolymphoid malignancies

	Histologic features	Immunophenotype	EBV
Diffuse large B cell lymphoma	Rather uniform population of large pleomorphic cells infiltrating the mucosa and the underlying bone	CD45, CD20, CD79a	Negative (rare cases EBER+)
Nasal natural killer/T-cell lymphoma	Angiocentric/angiodestructive pleomorphic neoplastic infiltrate, composed of small, medium-sized, large or anaplastic cells; Admixture of inflammatory cells (histiocytes, lymphocytes, granulocytes); necrosis	CD2, cytoplasmic CD3 (CD3 ϵ), and CD56; often positive for granzyme B, TIA-1, and perforin	Positive
Extramedullary plasmacytoma	Diffuse mucosal infiltration of neoplastic plasma cells	CD38, CD138, kappa and lambda light chains	Negative (rare cases EBER+)
Granulocytic sarcoma	Diffuse mucosal infiltration of primitive myeloid cells	Chloroacetate esterase, myeloperoxidase, lysozyme, CD43, CD79a, CD3	Negative
Histiocytic sarcoma	Infiltrate of large and pleomorphic neoplastic cells; accompanying inflammatory infiltrate, most often of neutrophils or lymphocytes	CD45, CD45RO, CD4, CD68, lysozyme, CD31	Negative

differential diagnosis is usually straightforward. Teratocarcinosarcoma may also be entered in the differential diagnosis of ONB for the presence of areas with neural differentiation, which may closely resemble the histological appearance of ONB. However, epithelial and mesenchymal elements can be recognized with a thorough sampling of the lesion. Melanotic neuroectodermal tumor of infancy arises predominantly in the facial bones, but it may also present with involvement of the paranasal sinuses [49], and therefore it can be considered in the differential diagnosis of ONB in the pediatric age group. Histologically, it shows a characteristic nested architecture, and it is composed of two populations of cells, including small neuroblast like elements surrounded by a large, epithelioid melanin containing cells, arranged in solid trabeculae or in gland-like structures. The neoplastic populations are embedded within a dense, well-vascularized fibrous stroma.

Haematolymphoid Neoplasms

The sinonasal tract is involved by a wide range of haematolymphoid malignancies, including lymphomas of B and T cell lineage, extramedullary plasmacytoma, granulocytic sarcoma, and histiocytic sarcoma. Their clinico-pathological features are fully discussed in another article of this issue [50]. Here, their salient histological features and immunohistochemical markers are reported in Table 3.

Conclusions

In recent years, the spectrum of differential diagnoses of round cell sinonasal malignancies has expanded significantly, due to the recognition of new entities and the

characterization of new markers. Immunohistochemistry remains the main tool for the diagnosis of sinonasal undifferentiated round cell malignancies. However, immunohistochemical markers are not entirely specific and sensitive, and should therefore be used in panels, having in mind a list of differential diagnoses guided by the clinical presentation and morphologic findings. A panel including cytokeratins, synaptophysin, S100 protein, desmin and CD45 may allow the classification of most sinonasal undifferentiated lesions or may help to narrow the list of differential diagnoses. Further refinement of the diagnosis requires second level markers, including the search for genetic alterations, which are becoming increasingly important in this group of tumors.

The recognition of the existence of overlaps in the immunophenotype of tumors with different histogenesis is essential in order to avoid misdiagnoses. The expression of cytokeratin in round cell undifferentiated sinonasal malignancies is not restricted to epithelial neoplasms, but it may also be detected in rhabdomyosarcoma, particularly in the alveolar variant, in the Ewing sarcoma family tumors, and in melanoma [51]. Neuroectodermal markers can be positive in several malignancies of different lineage. A relevant percentage of alveolar rhabdomyosarcomas are positive for CD56, chromogranin, and synaptophysin, with significant implications for the differential diagnosis with ONB and neuroendocrine carcinoma. Similarly, sinonasal melanoma may express neurofilament and synaptophysin, as well as other intermediate filament proteins [52, 53]. The expression of CD56 is not restricted to hematopoietic cell neoplasms, but it is frequently found in other small round cell tumors, including lesions arising in the nasal cavities. In their analysis of nine cases of CD56+ small round cell tumors of the nasal cavities unrelated to nasal NK/T cell lymphoma, Liu et al. [54] identified six examples of non-

haematological malignancies, including one case of olfactory neuroblastoma, two of primitive neuroectodermal tumor (PNET) and three rhabdomyosarcomas. Another possible source of diagnostic confusion when testing immunohistochemical markers is represented by sinonasal neoplasms with multiple lines of differentiation, such as teratocarcinoma and teratomas, or presenting heterologous elements. For example, areas with rhabdomyoblastic differentiation may be identified in peripheral nerve sheath tumors, in melanoma and in olfactory neuroblastoma [55].

Molecular techniques, including cytogenetics, FISH and RT-PCR, are becoming increasingly important for the diagnosis of sinonasal round cell undifferentiated tumors and they may be particularly useful in the more difficult cases. FISH has several advantages, including the possibility to perform the study on formalin-fixed paraffin-embedded material and to directly search for the alteration in neoplastic cells. On the other hand, demonstration of rearrangement of a single gene by FISH may not be sufficient to prove a diagnosis, as is the case of EWS gene, which may have different fusion partners in different tumor types. Therefore, data need to be complemented by morphology and immunohistochemistry, or by demonstration of rearrangement of the partner gene.

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