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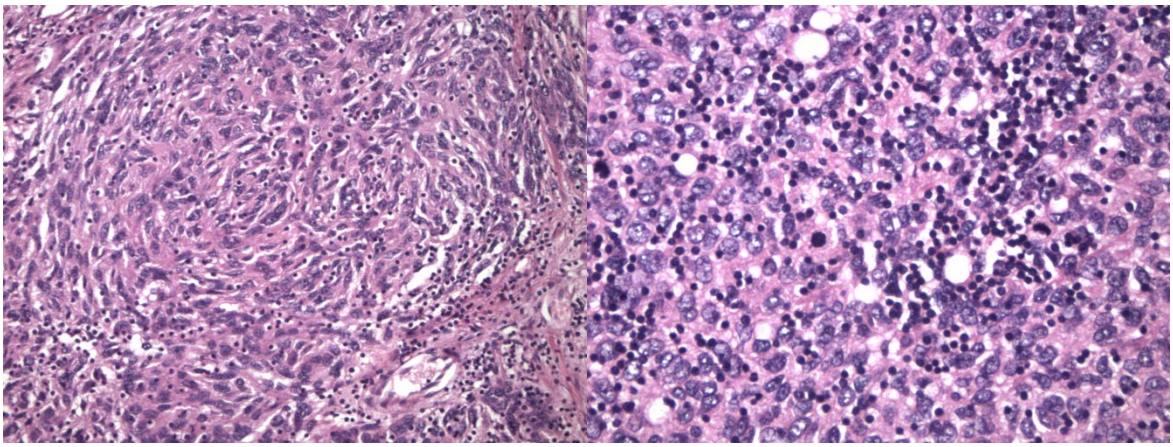
Case 1
Diagnosis: Follicular Dendritic Cell Sarcoma

Case History: A 53 year old smoker presented with painless left neck tumour just below the submandibular gland. Past medical history included hypertension, coronary artery disease treated with catheterization, H. pylori gastritis cured with antibiotics, dyslipidemia, alcoholism, cataracts, and appendectomy. Computed tomography showed necrotic 4 cm mass in the left lateral neck at the level of the hyoid bone, abutting and displacing but not infiltrating the left submandibular gland, most in keeping with a left level III nodal metastasis. There was no significant lymphadenopathy elsewhere and no obvious head and neck primary.

Workup: Fine needle aspirate showed cohesive epithelioid cells in a lymphocytic background, with no obvious keratinization, but significant nuclear atypia and mitoses. Diagnosis of high-grade malignancy was made and a differential diagnosis of poorly differentiated carcinoma or sarcoma was offered. The case was discussed at the Multidisciplinary Conference where it was decided to obtain a core biopsy, perform p16 IHC, and to perform panendoscopy with random biopsies from tonsils, base of tongue and piriform sinuses. The panendoscopy was negative for any other malignancy. The biopsy showed a high grade malignancy with necrosis and mitoses composed of epithelioid and spindle cells with lymphocytic background. Immunohistochemistry was negative for all epithelial and melanocytic markers, and most sarcoma markers. Tumour cells were positive D2-40, SMA, and weakly and focally for p63. Diagnosis was left as high grade malignancy with a differential diagnosis of sarcoma vs sarcomatoid carcinoma.

Resection: Left neck levels I-V dissection was performed and showed 79 uninvolved lymph nodes and one white well-circumscribed tumour in level III measuring 3.5 cm. There was no evidence of a pre-existing lymph node. Tumour showed solid sheets of epithelioid and spindle cells with ample eosinophilic cytoplasm, frequent vacuoles, inconspicuous cell membranes, large pleomorphic, vesicular nuclei, small nucleoli, interspaced lymphocytes, focal necrosis, and occasional swirled architecture. Mitoses reached 5 per 10 high power fields.



A. H&E100x B. H&E200x (notice multiple mitoses)

Immunohistochemistry: Immunohistochemistry showed the tumour cells were positive for CD21, CD23, D2-40, SMA, and vimentin, and were negative for all keratins, EMA, CEA, S100, Melan-A, HMB45, desmin, CD31, CD34, Erg, Fli1, Bcl2, CD56, synaptophysin, chromogranin A, calretinin, GFAP.

Diagnosis: Follicular dendritic cell sarcoma

Discussion:

Follicular dendritic cell sarcoma is a rare and under-recognized neoplasm first described in 1986 by Juan Rosai. The tumour presents as a painless, slow growing mass with head and neck being a predominant site. Of the cases published so far, approximately 2/3 occurred in head and neck, with the sites including tonsils, palate, nasopharynx, sinuses, parotid gland, thyroid, and neck. Patients show wide spectrum of ages from 20s to 80s and an equal sex distribution. The tumour can occur in lymph nodes or in soft tissues. The name reflects the fact that the tumour shows a phenotype of follicular dendritic cells (FDCs). FDCs are mesenchymal cells residing in lymph node follicles. Among their many functions, they organize lymphoid microarchitecture, remove cellular debris, help in memory B-cell selection, and have a possible role in prevention of autoimmunity.

FDCS shows solid and insular architecture composed of a mix of epithelioid and spindle cells sometimes arranged in swirling fascicles. The cells have ample eosinophilic cytoplasm, indistinct cell borders, nuclear pleomorphism and atypia, vesicular chromatin with small nucleoli, and frequent mitoses, including atypical ones. There is a background of intratumoral lymphocytes. Due to similarities in morphology, FDCS is often misdiagnosed as meningioma. In fact, its similarity to meningioma is a clue to the diagnosis! Immunohistochemistry is often frustrating as most markers are negative. In fact, it is often after one thinks of the diagnosis that the immunohistochemistry becomes useful. The tumour is positive for at least one, but often more FDC markers: CD21, CD23, CD35, clusterin, fascin, podoplanin, D2-40, CNA42. The also stain for CD99, vimentin, somatostatin receptor type 2A, and SMA. P63 can be weakly and focally positive, which may be wrongly interpreted as supportive of the diagnosis of spindle / sarcomatoid squamous cell carcinoma. Please remember that while it is true that spindle cell squamous

cell carcinoma can be negative for keratins and p63, this is not true of SCC that retains epithelioid cytology!

Malignant masses in the neck are most commonly nodal metastases of head and neck carcinomas or melanomas, with metastatic SCC being most common. Oropharyngeal SCCs are especially known for metastasizing early and presenting as a metastatic neck carcinoma of unknown primary. Nevertheless, there are number of lesions that may present as a neck primary that clinically simulates metastatic carcinoma. A differential diagnosis of unusual non-carcinoma, non-melanoma neck tumours includes:

- follicular dendritic cell sarcoma
- Interdigitating dendritic cell sarcoma
- histiocytic sarcoma
- ectopic meningioma
- ectopic hamartomatous thymoma
- solitary fibrous tumour
- rhabdomyoma
- PEComa
- paraganglioma
- germ cell tumours

Treatment of FDCS is surgical resection with clear margins. Adjuvant radiation treatment may be beneficial in cases with residual or unresectable disease. Five-year survival rates of nodal cases are about 70-85%; however, extranodal cases behave worse. With long-term follow up about half of cases recur locally, a quarter eventually have distal metastases, and death occurs in 1/6 of cases.

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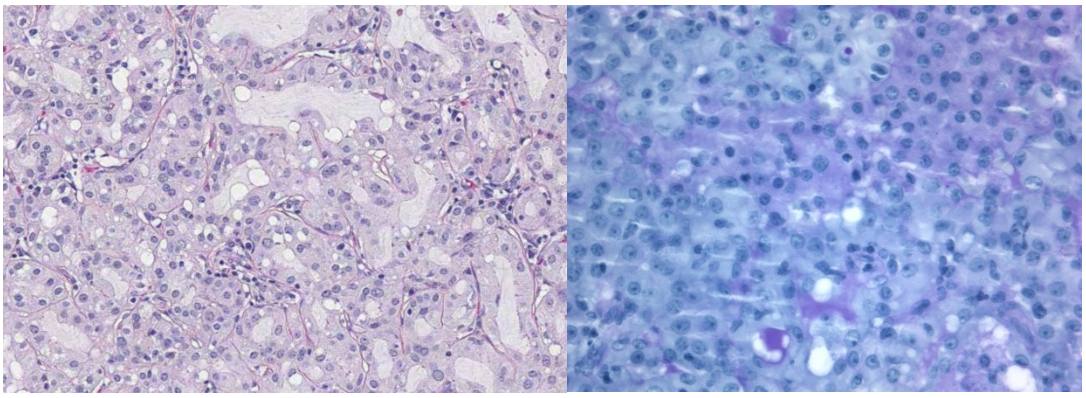
Case 2
Diagnosis: Secretory Carcinoma and mucocele

Case History: A 43 year old woman noticed fullness in her left cheek lasting about 4 months. Past medical history included goitre and three uncomplicated pregnancies. On exam she had a palpable, submucosal, firm, mobile mass in the left cheek, below linea alba. No other oral lesions were noted and there was no neck lymphadenopathy. Clinical diagnosis of mucocele was made and excisional biopsy was undertaken.

Pathology: The gross specimen consisted of a round tissue measuring 1.2 x 1.1 x 1.0 cm and showing partially cystic cross-section. Microscopically, the cystic area corresponded to a mucocele pseudocyst filled with pale secretions and lined with mucin-laden macrophages. There was associated mucin extravasation and hemorrhage. Next to the mucocele there was an epithelial tumour with microcystic architecture and focal areas of papillary and follicular patterns. The cystic spaces contained pale mucinous secretions. Cells showed vacuoles and had eosinophilic and finely granular cytoplasm, but no zymogen granules on PAS + diastase staining. The tumour was unencapsulated but was surrounded by a dense connective tissue. Mitoses were scarce.



A. H&E200x B. PAS with diastase200x

Diagnosis: Secretory carcinoma of the oral minor salivary glands and a mucocele.

Discussion: Secretory carcinoma (SC) is a low grade malignancy of the minor and major salivary glands described for the first time as a separate entity by Dr Alena Skalova in 2010[1]. Dr Skalova named the tumour Mammary Analog Secretory Carcinoma (MASC); however in 2017 the 4th edition of the WHO Classification of Head and Neck Tumours has shortened the name to Secretory Carcinoma to reflect the fact that the salivary gland secretory carcinomas are more common than the breast carcinomas[2]. Secretory carcinoma was first described in the breast by McDivitt and Stewart in 1966 under the name "juvenile breast carcinoma", and was subsequently shown to harbor a gene fusion between the ETV6 gene on chromosome 12 and the NTRK3 gene on chromosome 15 in the majority of cases.[3,4] This ETV6-

NTRK3 fusion oncogene functions as a constitutively active tyrosine kinase with potent transforming activity.

Published cases occurred in patients from second to the eight decade of life[5]. There is a bimodal distribution with most tumours occurring in the 2nd and 3rd decade and in the 5th and 6th decade (unpublished data). There is a slight male predominance. About half of the tumours occur in minor salivary glands of the oral cavity and the remainder mostly in parotid glands. Rare cases of SC have also been described in skin[6], thyroid[7], esophagus, lung, lacrimal gland, and sinonasal cavity. The typical clinical history is a slowly enlarging mass accompanied occasionally by pain or tenderness. Most tumours are single well-circumscribed nodules, often partially cystic on imaging. The cut surface may be brown to red, firm to soft, and solid to cystic.

Microscopically these tumours tend to have a mix of papillary and microcystic architecture; however solid and macrocystic patterns are also possible. The cystic tumours show good circumscription but tumour island infiltration into and beyond the capsule is common. The cells show eosinophilic cytoplasm, unlike the basophilic look of the acinic cell carcinoma. Hobnailing is common as are intracytoplasmic vacuoles. The cystic spaces are filled with eosinophilic fluid. The secretions are PAS and PASD-positive as are some of the intracytoplasmic vacuoles but zymogen granules are absent. The nuclei show low grade atypia and often prominent nucleoli, but mitoses are scarce. Electron microscopy shows lipid and mucin droplets and microvilli without rootlets.

Immunohistochemical profile is distinct and is helpful in making the diagnosis. The tumour cells are positive for S100 (usually diffusely), mammaglobin (at least focally), GCDFP15 (focal or diffuse), GATA3 (nuclear), and STAT5a, and are typically negative for p63 and high molecular weight keratins.

SC harbors a translocation of ETV6 on chromosome 12. In approximately 80% of cases the translocation partner is NTRK3, while the remaining 20% of cases have RET translocations[8]. The ETV6 break apart FISH is commonly used to demonstrate the rearrangement. ETV-NTRK3 fusions are not specific for SC and are also seen in malignancies such as congenital mesoblastic nephroma, infantile fibrosarcoma, and a subset of sinonasal adenocarcinomas. Therefore, demonstration of ETV6 rearrangement help to separate SC from other primary salivary gland malignancies, but metastatic lesions harboring the same translocation must still be excluded.

Previously similar lesions in salivary glands have been variously diagnosed as zymogen-poor papillary microcystic subtype of acinic cell carcinoma, or less frequently as adenocarcinoma NOS, cystadenocarcinoma, mucoepidermoid carcinoma, or papillary carcinoma. The main differential diagnosis is that of acinic cell carcinoma. The presence of zymogen granules usually clinches this diagnosis as most cases of zymogen granule-poor acinic cell carcinomas have now been shown to be SCs. In addition, acinic cell carcinomas of the minor salivary gland are extremely rare and most have by now been re-diagnosed as SC. While papillary-cystic morphology is characteristic of SC, some cases may show solid architecture. The differential diagnosis then includes intermediate grade mucoepidermoid

carcinoma, adenocarcinoma NOS, myoepithelial carcinoma, polymorphous adenocarcinoma, and metastatic carcinoma. Cystic SCs have a differential diagnosis of cystadenocarcinoma.

The information accumulated so far from the published cases of salivary SC suggests it is a low grade malignancy with a small chance of high grade transformation[9]. Initial case series showed the tumours had similar mortality to acinic cell carcinoma with some tumours showing aggressive course[5]. Population based study in British Columbia showed that this likely reflected referral bias. Local recurrence can occur, especially if the tumour was incompletely resected. Metastases are rare as is death from this carcinoma.

Surgical resection is the main treatment and offers high chance of cure. Adjuvant radiotherapy is indicated for incompletely resected tumours. The recurrent and metastatic tumours harboring the NTRK and RET translocations may be treated with tyrosine kinase inhibitors targeting those proteins.

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Case 3
Diagnosis: Central giant cell lesion with Neurofibromatosis 1

Central giant cell lesions (CGCL) of the jaws represent a heterogeneous group of lesions that can be reactive, neoplastic, or associated with syndromes. They are often histologically indistinguishable. Since most patients with these lesions seem to be in the first two decades of life and the lesions are generally found in areas of the jaw that has previously held deciduous teeth, the lesions may be derived from the odontoclasts.

The **differential diagnoses** of CGCLs include sporadic CGCLs, brown tumor of hyperparathyroidism (von Recklinghausen's disease of bone), and those associated with syndromes, including Cherubism (*SH3BP2* (4p16.3) mutation), Noonan Syndrome (*PTPN11* mutation with short stature, ocular hypertelorism, pulmonic stenosis, congenital heart defects and webbed neck), Jaffe-Campanacci Syndrome (café-au-lait spots, multiple non-ossifying fibromas of the long bones and jaw, and pathological fractures), Ramon Syndrome (gingival fibromatosis, hypertrichosis, epilepsy, mental and somatic retardation, and cherubism-like lesions), and Neurofibromatosis type 1 (NF1, chromosome 17q).

Brown tumor of hyperparathyroidism or von Recklinghausen's disease of bone is due to hyperparathyroidism (HPT). Primary hyperparathyroidism is a generalized disorder of calcium, phosphate, and bone metabolism due to excessive secretion of PTH. The main cause of PHPT is adenoma in about 80% cases. Hypercalcemia and hypophosphatemia are the most common presentation in laboratory tests. Secondary hyperparathyroidism is caused by impaired phosphate excretion and failure to activate vitamin D, which can be triggered by renal osteodystrophy. Elevated phosphate level, decreased ionized calcium level, and reduced serum calcitriol lead to continuous stimulation of the parathyroid glands that causes increased PTH release. Pelvis, ribs, clavicle, mandible and the extremities are the most commonly affected bones in brown tumor, whereas maxillary involvement is rare (0.1%).

Neurofibromatosis or Von Recklinghausen's disease is a genetic disorder characterized by the growth of tumors on the nerves. The disease can also affect the skin and cause bone deformities. There are 3 forms: neurofibromatosis type 1 (NF1), neurofibromatosis type 2 (NF2), and schwannomatosis, which is a variant of NF2. The difference between NF1, the most common form, and NF2 are the genetic alterations involving the disease process. NF 1 is caused by *NF1* (chromosome 17q) mutation and involves a protein called neurofibromin, which relates to cell growth and cell division. NF2 is caused by a mutation on chromosome 22 and involves a protein called merlin, which is thought to be involved in cellular shape and structure. Most complications of NF2 involve problems in vision, hearing, and balance; numbness or weakness in the face, arms or legs may also occur. However, there is no evidence that NF2 causes intellectual and learning disabilities which are very common in individuals with NF1.

NF1 is one of the most common hereditary autosomal dominant genetic disorders (1 in 3000 - 3500 newborns). This condition can be sporadic or hereditary.

The criteria of diagnosis (2 or more of the following):

1. **Six or more** café-au-lait macules over 5 mm in greatest diameter in prepubertal individuals and over 15 mm in greatest diameter in postpubertal individuals.
2. **Two or more** neurofibromas of any type or one plexiform neurofibroma - Subcutaneous or cutaneous neurofibromas are seen **rarely in young children,** but appear over time in older children, adolescents, and adults.
3. Freckling in the axillary or inguinal region.
4. Optic glioma.
5. Two or more Lisch nodules (iris hamartomas).
6. A distinctive osseous lesion such as sphenoid dysplasia or thinning of long bone cortex with or without pseudarthrosis.
7. A first-degree relative (parent, sibling, or offspring) with NF1 by the above criteria.

NF1 shows loss of neurofibromin, a negative regulator of Ras, which plays an important role in the mitogen signal transduction pathways that play a crucial role in the development of NF1 associated tumors. Most of the NF1 patients develop neoplasias, such as benign neurofibromas, malignant peripheral nerve sheath tumors, optic gliomas, or pheochromocytomas. These patients need to have long term follow-up under a team of specialists to manage symptoms or complications.

There are only a handful of case reports showing the co-existence of NF1 and CGCL. Due to its rarity, the prognosis is not clear. However, recurrence after surgical procedure has been reported. The underlying pathophysiological mechanism is not clear and the co-existence can be coincidental. Therefore, further study is required.

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Case 4
Diagnosis: Low-grade intraductal carcinoma

In 2017 February, WHO published the 4th edition of the WHO Head and Neck Tumors book. There are emerging entities of salivary glands tumors, including secretory carcinoma (mammary analogue secretory carcinoma or MASC), hyalinizing clear cell carcinoma, and intraductal carcinoma. To better understand the classification of the salivary gland tumors, one has to be familiar with the types of epithelial cells composing the salivary gland, including ductal (excretory, intercalated, striated), acinar, basal and myoepithelial cells.

Low-grade intraductal carcinoma (LG-IDC), previously known as low-grade cribriform cystadenocarcinoma, Intraductal adenocarcinoma, and low-grade salivary duct carcinoma, is a rare neoplasm. It is characterized by predominant intraductal growth, luminal ductal proliferation with bland microscopic features, and favorable clinical behavior with an appearance reminiscent of florid or atypical ductal hyperplasia to low-grade intraductal breast carcinoma.

Clinical: The majority of LG-IDC occurred in older patients with a slight female predominance. It mainly involves the parotid gland, but rare submandibular gland and minor salivary gland involvement have been reported. The tumor is not encapsulated with varying size from 1 to 4 cm.

Microscopic: The tumor is unencapsulated and composed of single or multiple cysts with an intraductal proliferation. The nests show both solid and cribriform architecture with “sieve-like spaces” similar to breast proliferations. Most of the tumor is ‘intraductal’; however, small areas of invasion may be present. The cystic spaces show varying architecture including a single cell lining, multilayered cellular proliferations with micropapillary structures and “Roman-bridges”. The cysts contain eosinophilic debris with macrophages, but no tumor necrosis is present.

Most of the tumor cells are small to medium sized with pale eosinophilic cytoplasm, and round to oval nuclei, which may contain finely dispersed or dark condensed chromatin with inconspicuous nucleoli and no pleomorphism. Foci of intermediate to high grade atypia, or invasive carcinoma or micro-invasion have been reported in up to ~20 % of cases. No mitotic activity is identified.

There are no areas showing infiltrative pattern, desmoplasia or other stromal reactions.

Ancillary testing: The tumor is diffusely positive for S100 and the tumor nests are surrounded by a thin non-neoplastic myoepithelial layer that is stained with p63, CK14, SMA and calponin. These markers do not stain the tumor cells. In addition, the Ki67 index is often low, under 5%. There is no specific genetic event associated with this entity.

Prognosis: LG-IDCs have an excellent prognosis with no cases of mortality reported so far. According to the clinically indolent behavior, most of the cases are treated with parotidectomy without radiotherapy.

Differential diagnoses: Due to the architectural similarities, the differential diagnoses include: cystadenocarcinoma, cystadenoma, salivary duct carcinoma in situ/high-grade intraductal carcinoma, papillary-cystic variant of acinic cell carcinoma, mammary analog secretory carcinoma, adenoid cystic carcinoma, and mucoepidermoid carcinoma (see Table 1).

Cystadenocarcinoma, a rare salivary gland tumor, is characterized by prominent cystic appearance-often with complex papillary architecture and lack of cribriform architecture—infiltrative growth, and absence of myoepithelial cells. **Cystadenoma** is often well-circumscribed and composed of variable-sized cystic spaces. There is no cellular atypia, solid growth, necrosis or mitoses and the tumor cells stain negative for S100 protein. **Salivary Duct Carcinoma *in situ*/High-Grade Intraductal Carcinoma (HG-IDC)** is composed of neoplastic ductal cells showing high nucleocytoplasmic ratio, large pleomorphic nuclei with prominent nucleoli, occasional to frequent mitoses, and foci of necrosis. HG-IDC and LG-IDC share many architectural features. The differences between LG-IDC and HG-IDC are nuclear grade and the presence of necrosis. The expression of S100 protein may help to separate these two lesions since HG-IDCs have been either negative or only partially positive for S100 protein. Similar to the conventional salivary duct carcinoma, HG-IDC shows positive for AR and HER-2. **Acinic cell carcinoma, papillary cystic pattern** should contain tumor cells with serous granules consistent with acinic cell differentiation. Immunohistochemically, acinic cell carcinoma is negative for S100 protein. **Mammary analog secretory carcinoma (MASC) or secretory carcinoma**, resembling its breast counterpart, contains the t(12;15)(p13;q25) translocation, which leads to a ETV6-NTRK3 fusion gene. With a lack of cribriform structures, the tumor cells have pink or vacuolated cytoplasm, vesicular nuclei and distinct nucleoli. The tumor is positive for mammaglobin, S100, vimentin, CK19, CK8, CK18, MUC1, MUC4, HMWK and focally with GCDFP-15, but negative for p63 and calponin. **Adenoid cystic carcinoma** shares cribriform architecture, but no true ductal/glandular formation. The tumor is positive for CD117, S100 and p63. **Mucoepidermoid carcinoma** may share some architectural similarities with LG-IDC but it is composed of intermediate/squamoid cells and goblet cells which are negative for S100 but positive for p63. **Basal cell adenocarcinoma** shares cribriform and solid architecture, and bland nuclear features with the LG-IDC and adenoid cystic carcinoma. Its solid nests show palisading of nuclei along the tumor/stromal interface and the tumor cells are CK5/6 positive but S100 negative.

Table 1. Summary of the characteristics of LG-IDC and its differentials

	Cystic	Microcystic	Cribriform	Solid	Cell	IHC	Molecular change
Low-grade intraductal carcinoma (LG-ICD)	+	+	True glandular	+	Bland; some apocrine	S100 + with a rim of p63, SMA, p40 cells; SOX10+, GATA-3, HER-2 –	-
Cystadenocarcinoma	+	+	-	-	Atypia	S100 –	
Salivary duct carcinoma *in situ*	-	+	True glandular	+	Cellular atypia/ oncocytic/apocrine change	S100 –, SOX10 – AR+, HER-2+, GATA-3 +	
Acinic cell carcinoma	-	+, papillary pattern	-	+	Zymogenic granules	S100-, p63- SOX10+	
Secretory Carcinoma (MASC)	+	+, papillary pattern	-	+/-	Increase cytoplasm with no granular appearance	S100+, SOX10+, GATA-3+ Mammaglobin+ p63-, SMA -	ETV6–NTRK3 Gene Fusion
Adenoid cystic carcinoma	-	-	+	+	Bland, can be clear cells or biphasic appearance	S100+, CD117++, p63 +	50% with MYB-NFIB Gene Fusion
Mucoepidermoid carcinoma	+	+	-	+	Goblet cells, intermediate cells, cellular atypia	S100 - , p63+ Mucin+ goblet cells	CRTC1–MAML2 or EWSR1–POU5F1 Gene Fusion
Basal cell adenocarcinoma	-	-	+/-	+	Bland, Palisading of nuclei along the stromal interface	S100-, p63 + CD117 focal +	

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Case 5
Diagnosis: Spindle cell-Sclerosing rhabdomyosarcoma

Background

The spindle cell variant of rhabdomyosarcoma (S-RMS) was first described by Cavazzana et al in 1992[1] from cases retrieved from the German-Italian Cooperative Soft Tissue Study. The 21 cases reported shared similar histopathological features and were located mostly at paratesticular and head and neck regions. One of the important features noted at the original description was the high degree of skeletal muscle differentiation determined by the expression of late differentiation markers and the overall better prognosis compared to other variants of RMS. 8 years later, Mentzel and Katempkamp [2, 3] reported 3 cases of rhabdomyosarcoma in adults that had prominent hyaline sclerosis and a pseudovascular pattern. These tumors were later identified as Sclerosing RMS (Sc-RMS) and similarly to the previous reports of S-RMS, these cases showed strong positivity for striated muscle differentiation markers (Myogenin, MyoD1) although the prognosis was guarded compared to the described cases of S-RMS. Since then, multiple publications have debated the classification of spindle cell and sclerosing RMS and reiterated the significant similarities between the two entities. The first prognostically relevant classification system for RMS was published by the International classification of rhabdomyosarcoma in 1995[4] and divided RMS into superior prognosis (Botryoid and spindle cell), intermediate prognosis (Embryonal) and poor prognosis (Alveolar). In 2013, the World Health Organization included sclerosing RMS as part of spindle cell RMS and separate from the embryonal RMS[18] In the next sections I will discuss the epidemiology, clinical features, histology and molecular/genetic profile of spindle cell/sclerosing RMS (S-Sc RMS).

Epidemiology and Clinical Features

Rhabdomyosarcomas are rare soft tissue malignancies with a reported incidence of 0.041 cases per 100,000 in the head and neck region based on a review of cases from the NCI Surveillance, Epidemiology, and End results (SEER) database [5]. RMS represents the largest subset of soft tissue sarcomas in infants and children (approximately 40% of cases) and most head and neck tumors are diagnosed between the age of 0-19 years [5, 6]. For completeness of this short review, the features described here include both earlier reports of S-RMS and Sc-RMS as well as recent reports that use the most recent S-Sc RMS nomenclature. S-Sc RMS is a rare variant of RMS that may affect both children and adults and has a significant male predilection. The most commonly affected areas are the head and neck region and the paratesticular region in children and the prognosis is heavily influenced by location and population (pediatric vs adults)[7]. In 2015, Rudzinski et al. evaluated the current 2013 WHO classification in 9 consecutive Children's oncology group clinical trials to determine the applicability of the WHO classification to pediatric RMS. They reported that in children, Spindle cell/Sclerosing (S-Sc RMS) have a similar prognosis compared to typical embryonal RMS when compared with same site [8].

Limited case reports and case series are available describing oral cases of S-Sc RMS[7, 9-12]. Owosho et al reported the different clinical and molecular features of 13 cases of head and neck S-Sc RMS which included 2 tumors of the tongue and 2 tumors of the mandible. All cases were Stage 1 and showed an

age range from 14-41 years old. Tumor size, histological features (spindle/sclerosing) and prognosis varied significantly but all cases were negative for MyoD1 mutations. A recent report by Smith et al, described 3 oral cavity cases of S-Sc RMS [11] with an age range of 22-39 years old and male predilection. An earlier report by Nascimento and Fletcher included 4 cases of oral cavity S-RMS located at buccal mucosa (2), gingiva and mandible. In this case series, all cases presented as masses causing tooth mobility and pain around teeth. Similar to the described gender distribution, 3 cases occurred in males (31-38) and only 1 female (38)[7]. Individual case reports describing diagnostically challenging lesions are also seen, as in a recent case report of a S-RMS presenting as a periapical radiolucent lesion in a 25-year-old male [10] .

Histopathology, immunophenotype and differential approach

S-Sc rhabdomyosarcomas are characterized by an infiltrative, non-encapsulated proliferation of spindle to ovoid cells arranged in a fascicular or storiform pattern. The tumor stroma is collagenous and may show marked hyalinization, which is the characteristic feature of sclerosing RMS. Cellular atypia including hyperchromatism, nuclear atypia and mitotic figures are commonly seen but variable. Occasionally, the hyalinised areas may show tumor cells arranged in nests, creating a pseudovascular pattern (see initial description by Mentzel and Katenkamp). Rhabdomyoblasts or strap cells may be only focally seen or completely absent in some cases as in the case presented here. In the present case, the cells are organized in a fascicular pattern with areas of marked palisading. Stromal hyalinization is seen but a pseudovascular pattern is not present. Typical rhabdomyoblasts and strap cells are not present in this case but are helpful when present.

Desmin is a muscle specific intermediate filament that is essential for muscle structure and function [13]. S-Sc RMS are diffusely positive for desmin and show variable expression of SMA and MSA. Myogenin or myogenic factor 4 (MYF4) is a transcription factor, part of the family of MyoD family of transcription factors. Animal models have shown that myogenin is required for the terminal differentiation of myoblasts and therefore is expressed in later stages of differentiation. In the context of S-Sc RMS, desmin and Myf-4 are the most reliable markers[14]. Some cases of sclerosing RMS may show limited expression of desmin and myogenin but usually retain positivity to myoD1 (Parham, 2013). Focal positivity for S100 and keratins have been reported but these markers are negative in the majority of the tumors.

The key differential diagnoses include leiomyosarcoma (LMS), malignant peripheral nerve sheath tumor (MPNST) and other potential spindle cell malignancies including spindle cell carcinoma (sSCC), melanoma and monophasic synovial sarcoma. Histologically, there is significant overlap between S-Sc RMS, LMS and MPNST. Leiomyosarcoma also has a fascicular growth and may show focal palisading pattern, variable atypia and cellularity. The typical neoplastic cells have blunt-ended nuclei, eosinophilic cytoplasm and vacuolation. Many reports show rhabdomyosarcomatous areas within leiomyosarcomas with variable positivity for desmin which can represent a major diagnostic challenge for S-Sc RMS. In this context, MYF4 is an important diagnostic maker since it is negative in LMS.

MPNST usually presents in adults and is associated with a nerve. Histologically, the tumor is organized in tightly packed bundles with occasional myxoid and whirled patterns. Nuclear palisading is seen but is usually focal and the neoplastic cells usually have a wavy pattern with tapered nuclei. Heterologus rhabdomyoblastic differentiation has been reported and represents a major diagnostic

challenge and MPNST with significant skeletal muscle differentiation (malignant Triton tumor) can occur in the context of Neurofibromatosis I[15]. The rhabdomyoblastic areas in MPNST can also be positive for desmin, Myf4 and MyoD1 but are usually focal. S100 is positive in less than 50% of MPNST (WHO) and may be helpful in differentiating MPNST and S-Sc RMS. Careful IHC evaluation would rule out cases of sSCC which are usually p63 and keratin positive. Melanomas are S100, melanin and HMB45 positive while Synovial Sarcomas are positive for keratins, CD99, EMA which are all negative in RMS.

Molecular and genetic profile

Numerous groups have investigated the genetic/molecular signatures of RMS and identified the presence of recurrent mutations and fusions in S-Sc RMS. Next generation sequencing and Sanger analysis have identified recurrent MyoD1 (L122R) mutations in both adult and pediatric populations and this mutation leads to "MYC-like" activation of MyoD1 leading to an unfavourable prognosis [16]. An analysis of 13 head and neck cases of Sc RMS shows that MyoD1 and PI3KCA mutations are also associated with a poor prognosis [12]. Alaggio et a performed complex molecular evaluation of 26 cases of S-Sc RMS to identify novel VGLL2 fusions. They report novel VGLL2 rearrangements in 63% of cases of infantile S-RMS while 27% harbored previously reported NCOA2 changes (TEAD1 and SRF-NCOA2 fusions). 67% of the patients over 1 year old had L122R MYOD1 mutations [17] and a poor prognosis.

Future directions

The functional characterization of specific fusion/mutations (L122R MYOD1, NCOA2, TEAD1 fusions) is still ongoing but will be at the center of new targeted therapies for S-Sc RMS. Certainly, the classification and prognostication of S-Sc RMS will also be affected by the discovery of new genetic changes that are characteristic to this RMS. Considering the ongoing movement to categorize diseases based on clinically relevant molecular/genetic signatures, it is possible that the classification of S-Sc RMS will continue to be updated to accommodate these new findings.

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Case 6

Post -transplant EBV + Lymphoproliferative Disorder (PTLD) Monomorphic PTLD Specify Subtype: Diffuse Large B Cell Lymphoma Non-germinal Center

Post-transplant lymphoproliferative disorders (PTLD) are among the most serious and most common potentially fatal complications of transplantation that are characterized by lymphoid and/or plasmacytic proliferations as a result of immunosuppression. The overall incidence is approximately 1 percent at 10 years, 30 to 50 times higher than in the general population, with a recent trend toward increases in frequency [1]. The majority (>80 percent) occurs in the first-year post-transplant. The principal risk factors underlying the development of PTLD are the degree of T cell immunosuppression and the Epstein-Barr virus (EBV) serostatus of the recipient. While the majority appears to be related to the presence of Epstein-Barr virus (EBV), EBV-negative disease does occur [2]. A number of EBV-encoded proteins such as latent membrane protein 1 (LMP-1), latent membrane protein 2A (LMP-2A), EBNA-2 and EBNA-LP drive signaling events that directly contribute to B cell growth and survival [3].

Three general types of PTLD have been described in transplant recipients:

- **Benign polyclonal lymphoproliferation (Early lesions)**: Early PTLD lesions are composed of plasmacytic hyperplasia and infectious mononucleosis-like PTLD [4]. The B lymphoid cells in these lesions are polyclonal and the architecture of the involved tissue is preserved. Histopathology may be consistent with reactive follicular hyperplasia. In such cases, discrete follicles of varying sizes and shapes are separated from one another by interfollicular regions rich in T cells. Tingible body (debris-laden) macrophages may be prominent. The polyclonal B cell proliferation has normal cytogenetics.
- **Polymorphic PTLD** – Polymorphic PTLD are polyclonal or monoclonal lymphoid infiltrates that demonstrate evidence of malignant transformation but do not meet all of the criteria for one of the B cell or T/NK cell lymphomas recognized in immunocompetent patients[5]. The infiltrate is composed of polyclonal or monoclonal cells all along the spectrum of B cell maturation and includes immunoblasts, plasma cells, and small- and intermediate-sized lymphoid cells. The tumor cells infiltrate and disrupt the underlying tissue and may undergo geographic necrosis. Immunophenotypically, the B cells may demonstrate kappa or lambda light chain class restriction, and upon genetic testing, the tumors have clonal immunoglobulin gene rearrangements. In addition, the tumor cells in polymorphic PTLD usually contain Epstein-Barr virus (EBV), as demonstrated by in situ hybridization for EBV encoded small nuclear RNAs (EBERs) [5].
- **Monomorphic PTLD**: Monomorphic PTLD is a heterogeneous group of tumors composed of monoclonal malignant cells of B cell or T cell origin. Histopathology demonstrates effacement of the normal tissue architecture by a lymphoid infiltrate. Monomorphic PTLD is further classified according to the subtype of lymphoma. The vast majority of these tumors are B cell lymphomas, most commonly diffuse large B cell lymphoma (DLBCL) and less commonly Burkitt lymphoma

(BL) or a plasma cell neoplasm (eg, myeloma or extramedullary plasmacytoma). Lymphomas of T cell or NK cell origin are uncommon, but, when seen, are usually classified as peripheral T cell lymphoma, not otherwise specified (PTCL, NOS), or EBV+ T/NK cell lymphoma [5]

Optimally, neoplastic forms of EBV-positive PTLD should have the following characteristics:

- Disruption of underlying tissue architecture by a lymphoid proliferation
- Presence of mono- or oligoclonal lymphoid cell populations as determined by immunoglobulin light chain expression and antigen receptor gene rearrangements
- EBV infection of many cells

If any two of these three features are present in combination with a lymphoid tumor, the diagnosis of a polymorphic, monomorphic, or classical Hodgkin lymphoma PTLD is made.

Differential Diagnosis

Epstein-Barr virus-positive mucocutaneous ulcer (EBV-MUC), a recently described lesion in 2016 WHO, is a localized EBV-associated lymphoproliferative disorder characterized by a well-circumscribed ulcer involving skin, and oral or gastrointestinal mucosa in immunocompromised patients [6]. Microscopically, EBV-MUC is characterized by a polymorphous infiltrate and atypical large B cells with Hodgkin/Reed Sternberg (HRS) cell-like morphology with abundant T cells. The tumor cells are positive for EBV by in situ hybridization[7]. They are positive for CD30, CD15, PAX5 (weak), MUM1 and EBERs, and negative immunohistochemistry for CD20, CD3, CD79 and CD45.

Although, these pathological, immunophenotypical, and molecular features might overlap with lymphoma, most EBV-MUC cases show an indolent course and require only conservative treatment. Most of the cases are self-limited.

It has been postulated that EBV-MUC patients lacked EBV DNA in blood, in contrast to frequently elevated EBV DNA levels in the more aggressive systemic PTLD. It has been hypothesized that EBV-MUC is a localized type of PTLD and lack of EBV DNA in blood might be a useful feature for distinguishing it from the more aggressive forms of PTL[8]. It is important to distinguish EBV-MUC from the more aggressive systemic PTLD and lymphomas, especially Classical Hodgkin Lymphoma, EBV-positive diffuse large B-cell lymphoma, plasmablastic lymphoma, and anaplastic large cell lymphoma. Thorough clinicopathological investigation and awareness of this rare entity are important for avoiding erroneous diagnosis and overtreatment.

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Case 7
Diagnosis: Sebaceous carcinoma

Sebaceous carcinoma is an uncommon malignancy of the sebaceous glands, accounting for less than 1% of all skin cancers.[1] The median age of diagnosis is 73 years, with a slight male predilection.[2] Sebaceous carcinoma can occur in any skin containing sebaceous glands and therefore areas with a high density of sebaceous glands (eyelids, face, scalp and neck) show a higher incidence. The most frequent site of disease is the eyelid; rare cases of sebaceous carcinomas have been reported in other anatomic sites, including the major salivary glands and oral cavity.[3-7]

Sebaceous carcinomas may be associated with Muir-Torre Syndrome, organoid nevi (nevus sebaceus), organ transplant recipients and rhinophyma. Lesions associated with Muir-Torre syndrome frequently demonstrate mutations in genes encoding DNA mismatch repair proteins resulting in microsatellite instability.[8]

Sebaceous carcinomas are traditionally segregated into periorbital and extraorbital categories. The majority of periorbital sebaceous carcinomas arise from ocular adnexa (Meibomian glands and glands of Zeiss). Periocular sebaceous carcinomas are the third most common eyelid malignancies after basal cell carcinoma and squamous cell carcinoma.[8] Malignant sebaceous tumours in the periorbital region are less often associated with Muir-Torre syndrome than extraorbital neoplasms.[8] Extraorbital tumours are less common than their periocular counterparts and usually occur on the head and neck of elderly patients.

Aside from the association with Muir-Torre syndrome, little is known of the etiology or pathogenesis of sebaceous carcinomas. A history of irradiation, immunosuppression or familial retinoblastoma appear to be risk factors.[9]

The tumours clinically present as an erythematous or yellow nodule, with or without ulceration.

On histology sebaceous carcinomas are infiltrative and composed of lobules or sheets of cells separated by fibrovascular stroma. The cells show variable sebaceous differentiation with finely vacuolated or foamy and clear cytoplasm. Smaller basaloid cells may also be present. The nuclei are large, with large nucleoli. Mitoses and focal necrosis may be seen. On frozen section the vacuolated cells show abundant lipid with oil red O or Sudan black. Identification of unequivocal sebaceous differentiation on routine H&E is usually sufficient for diagnosis. A morphologic clue to sebaceous differentiation is a centrally located nucleus indented by numerous lipid vacuoles.

Immunohistochemical markers of sebaceous differentiation include adipophilin and perilipin for formalin fixed tissue.[9] Adipophilin is a protein associated with intracytoplasmic lipid vesicles. Because androgen hormones play a role in activation of sebaceous glands, the tumour is frequently AR+. The cells are EMA+, variably D240+, but CEA-, S100-, GCFDP-15-. EMA can be helpful in identifying intracytoplasmic vesicles. Nuclear staining for AR is more reliable than EMA staining which may be absent in poorly differentiated tumours.

Immunostains for lipid droplet-associated proteins may be helpful, and include ABHD5 ($\alpha\beta$ hydrolase domain protein 5) which shows cytoplasmic punctate or vesicular staining and PGRMC1 (progesterone receptor membrane component-1) and SQS (squalene synthase), both of which show vesicular and membranous staining.[10] The basal/germinative cells will be CK15+ as sebaceous cells originate from CK15+ stem cells at the infundibular bulge.

All sebaceous tumours should be screened for the presence of the gene products of MSH-2, MSH-6, MLH-1, and PMS-2. Muir-Torre Syndrome is suspected when nuclear staining is absent in the tumours and should be followed by microsatellite instability testing.

The differential diagnosis includes other malignant and some benign clear cell tumours of skin, such as squamous cell carcinoma with clear cell features, basal cell carcinoma with sebaceous differentiation, clear cell basal cell carcinoma, trichilemmal carcinoma, balloon cell melanoma, clear cell sarcoma extending into the dermis, and metastatic clear cell carcinomas from other sites, especially metastatic renal cell carcinoma.[1,11] The major differential diagnosis is squamous cell carcinoma and basal cell carcinoma with sebaceous differentiation. Squamous cell carcinoma with clear cell features show areas of typical squamous cell carcinoma and PAS/PASD stains will confirm glycogen as the cause of cytoplasmic clarity. Given the rarity of basal cell carcinoma with sebaceous differentiation, the possibility of a genuine sebaceous neoplasm should be considered first when a basaloid neoplasm with focal sebaceous differentiation is encountered.[11]

Immunohistochemistry is helpful in distinguishing between SCC, BCC and sebaceous carcinoma.

	EMA	Ber-EP4	CK7 (ocular)	AR	ABHD5, PGRMC1 and SQS
Sebaceous carcinoma	+	-/+	+	+	+
Squamous cell carcinoma	+	-	-/+	-	-
Basal cell carcinoma	-	+	-	+/-	-

Sebaceous carcinomas lack several protein products identified in sweat gland tumours or metastatic carcinomas, such as CEA, S100 protein, GCDFP-15, CA-125, CA19-9 or the renal cell carcinoma marker. Note that adipophilin may stain renal cell carcinoma as well as sebaceous carcinoma, however, the staining pattern in non-sebaceous tumours is granular rather than membranous-vesicular.

Wide local excision is the standard of care. Mohs micrographic surgery may be useful in intraoperative assessment of surgical margins. Sentinel lymph node biopsy has been proposed but has not been adopted. As defects in expression of retinoic acid receptors has been reported, a possible potential treatment is retinoid therapy.[1] The use of radiation treatment is restricted to recurrent lesions, metastatic disease or palliative treatment in patients who are not candidates for surgical excision.[1]

The most common sites of metastasis are the regional lymph nodes. Periorbital tumours are associated with a higher rate of regional metastasis (4.4% of patients) versus extraorbital tumours (1.4%).[2] Distant metastasis is less frequent, and includes sites such as lung, liver, bone and brain. 5- and 10-year survival rates are ~92 and 79%, with no difference in outcome between periorbital and extraorbital cases,[2]

however, sebaceous carcinoma is the cause of death in 31% of cases and is a significant cause of morbidity and mortality.

Muir-Torre Syndrome

A variant of hereditary non-polyposis colon syndrome (Lynch syndrome), Muir-Torre syndrome is characterized by sebaceous tumours or keratoacanthomas, often multiple, in association with visceral neoplasms, usually gastrointestinal carcinomas.[3] Muir-Torre syndrome represents 1-2% of cases of Lynch syndrome. Immunosuppression may unmask a latent Muir-Torre Syndrome phenotype, especially in transplant recipients. Muir-Torre syndrome is inherited as an autosomal dominant trait and is the result of mutations in one of the DNA mismatch repair genes MLH1, MSH2, and MSH6. Mutations in MSH2 account for 90% of the cases. PMS2 mutations found in Lynch syndrome have not been reported in Muir-Torre Syndrome.

The sebaceous tumours are usually sebaceous adenoma or sebaceoma, less commonly sebaceous carcinoma. Multiple sebaceous tumours occurring before age 50 are strong indicators of this syndrome. Sebaceous hyperplasia is not an indicator of Muir-Torre syndrome. Because of the strong association of sebaceous neoplasms with Muir-Torre syndrome, immunohistochemistry using antibodies against the mismatch repair proteins MSH2, MLH1 and MSH6 is recommended. A lack of nuclear immunoreactivity suggests Muir-Torre syndrome and should be followed by microsatellite instability testing as assessed by PCR and chromatographic or electrophoretic assessment of multiple defined microsatellite regions of DNA.[8,12,13,14]

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Case 8
Diagnosis: Primary intraoral angiosarcoma

Angiosarcoma is a rare vascular malignancy and in the head and neck most commonly involves the scalp. Oral angiosarcomas can be primary or metastatic and are exceedingly uncommon, representing only 2% of all angiosarcomas.

Clinical Features:
The clinical appearance of intraoral angiosarcomas reported in the literature is variable, with many reports of an expanding mass that is blue/purple/red in appearance with or without ulceration. Most lesions are painless, and some present with bleeding. Intrabony lesions present as ill-defined areas of bony destruction. As the clinical appearance is non-specific, the clinical differential diagnosis for angiosarcoma may encompass several benign and malignant vascular lesions, including: pyogenic granuloma, hemangioma, Kaposi sarcoma, melanoma, carcinoma and metastatic disease.

Microscopic Features:
By definition angiosarcomas are vasoformative, but are known to show variable histopathology. Well-formed, anastomosing vessels may be seen, or tumours may show solid growth of epithelioid or spindled cells. Papillary growth may also be seen. Morphologically, the spindled type is reported to be the most common intraoral and salivary gland pattern; however, up to 33% of primary oral angiosarcomas in a large case series were epithelioid.

Immunohistochemistry:
Angiosarcomas are positive for vascular markers including CD31, ERG, FLI-1, CD34 and occasionally D240. CD 31 and ERG have both proven to be sensitive markers of vascular differentiation. Angiosarcomas, particularly epithelioid subtypes, may co-express keratins.

Genetics:
Recurrent genetic alterations in *KDR/PTPRB/PLCG1, MYC/FLT4* or *CIC* have been reported in a subset of angiosarcomas. *MYC/FLT4* coamplification is primarily reported in angiosarcomas secondary to radiation or chronic lymphedema. *CIC* rearrangements have been reported in a small subset of cases demonstrating epithelioid morphology, and have been primarily associated with young adults and cases occurring outside of the breast. The molecular alterations reported are thought to characterize approximately 50% of angiosarcomas.

Differential Diagnosis:
Depending on histomorphology, angiosarcoma may show histologic and immunohistochemical overlap with several other lesions.

<u>Epithelioid Hemangioendothelioma (EHE)</u>
EHE is a malignant, angiocentric vascular tumour. EHE is a rare malignancy, and exhibits more indolent behaviour than angiosarcoma. Microscopically, EHE demonstrates cords or nests of rounded to spindled endothelial cells. Cells with intracellular lumens (blister cells) are characteristic. The stroma in EHE is often myxoid to hyaline. Tumours are CD 31 and CD 34 positive, and epithelial markers are expressed in 25-40% (EMA, keratins). *WWTR1-CAMTA1* fusion is specific and not found in angiosarcoma. A subset of tumours lacking *WWTR1-CAMTA1* show *YAP1-TFE3*.

Pseudomyogenic Hemagioendothelioma (Epithelioid Sarcoma-like Hemangioendothelioma)

Pseudomygogenic hemangioendothelioma is typically found in young men, affecting the limbs, with a multinodular clinical presentation. Distinction from angiosarcoma is important as pseudomyogenic hemangioendothelioma often demonstrates clinically indolent behaviour with a low risk of metastasis. Both angiosarcoma and pseudomyogenic hemangioendothelioma are known to co-express endothelial and epithelial markers. Genetic testing can be helpful in excluding angiosarcomas as a *SERPINE1-FOSB* gene fusion is reported in pseudomyogenic hemangioendothelioma, but not angiosarcoma.[18 18 1] Non-*SERPINE1, FOSB* rearrangements have also been identified in a small subset of cases.

Epithelioid Sarcoma (ES)

ES occurs rarely in the head and neck. Two types of ES are described, the classic (distal) type and the proximal type. Proximal ES generally shows a multinodular to sheet-like growth pattern. Cells are epithelioid with eosinophilic cytoplasm, but can be spindled or rhabdoid. ES may have an angiomatous appearance due to loss of cellular cohesion and secondary hemorrhage. Tumour cells show positivity for cytokeratins (HMWK, LMWK), EMA, CD34 (50%), and most also show a loss of INI-1. Cases of ES with ERG positivity have been reported.

Spindle Cell Carcinoma

Diagnosis of spindle cell carcinoma depends on confirmation of epithelial phenotype by demonstration of a typical carcinoma component (in situ or invasive) or immunohistochemistry for epithelial markers (AE1/AE3, EMA, p40, p63). Spindle cell carcinomas may show pseudoangiomatous change, mimicking angiosarcoma. In some cases, there may be no traditional SCC component and epithelial markers may be negative. Non-homogenous tumours and small biopsies can lead to diagnostic difficulty. Rare cases of angiosarcoma with p63 positivity have been reported, which could lead to further challenges in diagnosis.

Treatment and Prognosis:

Angiosarcoma is an aggressive neoplasm and is generally associated with a poor prognosis. There is some evidence suggesting that when compared to angiosarcomas of other sites, primary intraoral angiosarcomas may exhibit more favourable biological behaviour. Tumours are primarily treated surgically, though some studies (not specific to the head and neck), have shown associations of improved survival with multimodal treatment.

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Case 9
Diagnosis: Soft tissue myoepithelioma

Soft tissue myoepitheliomas (SME) are most common in the extremities, and in a large case series, approximately 15% arose in head and neck. SMEs show no gender predilection and occur over a wide age range, though up to 20% have been reported in pediatric patients. Reported clinical symptoms vary, but SME commonly presents a swelling or mass, with or without pain or paresthesia.

Microscopic Features:
SME show diverse morphologic features, similar to their salivary counterparts. Benign tumours are often well-circumscribed to encapsulated with a lobular architecture. Cells may be epithelioid, plasmacytoid, spindled, or clear with vacuolated cells demonstrating a 'physaliferous' appearance. The cells are situated in a myxoid, hyalinised or chondromyxoid stroma. Osseous metaplasia and ductal differentiation can also be seen. As many soft tissue myoepitheliomas which exhibit otherwise benign biological behaviour show infiltrative growth, the presence of moderate cytologic atypia, including vesicular nuclei, prominent nucleoli and pleomorphism, should prompt classification as myoepithelial carcinoma. Increased mitoses and necrosis are commonly seen in myoepithelial carcinomas, though these have not proven to be reliable predictors of biologic behaviour.

Immunohistochemistry:
By definition, myoepitheliomas must show positivity with keratin and/or EMA and S-100 or myogenic markers (calponin, SMA). GFAP staining is variable and a subset of cases show loss of INI1. Sox10 expression is also variable, but is reported in the majority of benign cases. PLAG1 is negative in myoepitheliomas without tubuloductal differentiation.

Genetics:
The majority of myoepitheliomas arising in skin, soft tissue and bone demonstrate *EWSR1* rearrangements. Several fusion partners have been described, including *POU5F1, PBX1, ZNF444, ATF1* and *PBX3.* Tumours with ductal differentiation (mixed tumours) show *PLAG1* rearrangement in 37-72% of cases. A subset of cases lacking *EWSR1* or *PLAG1* rearrangements demonstrate *FUS* rearrangements.

Differential Diagnosis:
Due to their diverse histomorphology, soft tissue myoepitheliomas show histologic and immunohistochemical overlap with several other tumours.

<u>Myoepithelioma/Myoepithelial Carcinoma of Salivary Gland</u>
SME show the same morphologic diversity that is present in salivary myoepitheliomas. Tumours presenting in the neck could create diagnostic difficulty, and careful clinicopathologic correlation and imaging may be required to definitively rule out a neoplasm of salivary origin. In the salivary glands, the presence of cytologic atypia, mitotic activity and extraglandular extension may help categorize a myoepithelial neoplasm as malignant. As it is known that tumours may be deceptively bland, invasive growth is considered to be a useful marker of aggressive behaviour. *EWSR1* rearrangements have been reported in a subset of clear cell salivary gland myoepitheliomas. A subset of SME with ductal differentiation show *PLAG1* gene rearrangements, a feature which is shared many pleomorphic adenomas and carcinoma ex pleomorphic adenomas.

Ossifying/Non-ossifying Fibromyxoid Tumour (OFMT)

Ossifying fibromyxoid tumours typically affect the extremities and are uncommon in head and neck locations. Like SME, they have a lobular or multinodular growth pattern. Tumour cells are round/ovoid/spindled and are situated in a myxoid, hyalinised or fibromyxoid stroma. Calcifications and/or mature cartilage or osteoid may be seen. In the majority of cases the tumour is surrounded by a peripheral shell of bone, but this feature may be absent in up to 20% of cases, comprising the so-called non-ossifying variant. OFMT shares immunohistochemical overlap with SME; however, there are some helpful differences. S100 is often positive in OFMT, but rarely diffuse. Expression of SMA is rare and epithelial markers (CK/EMA), if expressed, are usually focal. OFMTs express desmin in up to 66% of cases. Rearrangements of the *PHF1* gene have been reported in 50-85% of cases.

Epithelioid Nerve Sheath Tumours

Like SME, epithelioid schwannomas often demonstrate a myxoid to hyalinised stroma. SME may also show a predominantly epithelioid phenotype. Both tumours show diffuse S100 and Sox10 positivity; however, keratin positivity in epithelioid schwannoma is rare and usually focal, and EMA is typically negative (may highlight perineurial capsule). Loss of INI1 expression has been reported in both SME and epithelioid schwannoma, but tends to be more common in the latter (42%). Most cases of epithelioid MPNST show demonstrable cytologic atypia, necrosis and mitotic activity (median 5/10HPF). Epithelioid MPNST also shows loss of INI1 in the majority of cases (67%).

Extraskeletal Myxoid Chondrosarcoma (EMC)

Differentiating SME from EMC can be difficult and genetic testing may be required. EMC has been categorized by the WHO as a tumour of unknown differentiation. Morphologically, this tumour has a multinodular growth pattern with fibrous septations. Cells form trabeculae, cords and clustered arrangements and are situated in a hypovascular myxoid to chondromyxoid stroma. Tumour cells may be positive for S100 (20%), CD 117 (30%) and occasionally synaptophysin and chromogranin. Unlike SME, expression of epithelial and myogenic markers is rare in EMC. Sox10 is not expressed in EMC and therefore maybe useful in distinguishing EMC from SME. Both SME and EMC show *EWSR1* rearrangements; however fusion partners are distinct. In EMC, *NR4A3-EWSR1* rearrangements are reported in > 90% of cases, with *NR4A3-TAF15* also reported.

Chordoma

The morphologic overlap between SME and chordomas is underscored by the historical use of the term 'parachordoma,' to describe soft tissue tumours resembling chordomas, now thought to represent other soft tissue neoplasms, including EMC and myoepitheliomas. Soft tissue chordomas have been described and are considered to be a diagnosis of exclusion. From an immunohistochemical perspective, both tumour types express cytokeratin and S100. The notochordal marker brachyury is expressed by chordomas, but not by its histologic mimics.

Prognosis and Treatment:

Myoepitheliomas are primarily treated with surgery, and are rarely fatal. A large case series reported no correlation between margin status and recurrence. In cases with benign or low grade microscopic features, no metastases or deaths were reported, though a small proportion of cases did recur locally. In cases with malignant cytologic features, 13 cases recurred locally, 10 cases metastasized and 4 cases demonstrated multiple recurrences. Death due to metastatic disease was reported in 4 cases. Tumours with atypia/high grade cytologic features were associated with a greater rate of recurrence (64% versus 29% with low grade/benign features).

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Case 10
Diagnosis: Malignant calcifying epithelial odontogenic tumor

The calcifying epithelial odontogenic tumor (CEOT, Pindborg tumor) was described in 1958 by Pindborg as "an unusual odontogenic tumor that does not show the ordinary features of ameloblastomas". Three cases were presented with similar radiographic and histologic features. These were large (> 2 cm), mixed radiolucent-radiopaque lesions of the posterior mandible, each associated with an impacted tooth. Histologic examination showed "sheets of polyhedral epithelial cells with well-outlined cell borders" and "pronounced difference in the size of the nuclei". The tumor contained a "homogeneous substance" and calcified material with the pattern of Liesegang rings [1].

A review of 23 cases of CEOT from the files of the AFIP described the histomorphologic variations that were seen even within the relatively small collection of this uncommon tumor [2]. The typical CEOT consisted of polyhedral epithelial cells with eosinophilic cytoplasm and intercellular bridges. There was nuclear pleomorphism but otherwise no sign of malignancy. A clear cell variant was described, composed chiefly of cells with clear, vacuolated cytoplasm that was mucicarmine negative, but areas of typical CEOT with eosinophilic cells were also present. The amount and type of calcified material varied from small, round concretions with Liesegang rings to large, irregular and amorphous masses. The deposits of homogeneous, eosinophilic, amyloid-like material also varied in amount; in some cases this material formed the predominant component so that the epithelial cells only formed inconspicuous clusters.

The early reports of CEOT addressed the behavior of this tumor and the optimal treatment to ensure successful removal. CEOT was considered to be a benign odontogenic tumor, albeit with a tendency to extend into the adjacent cancellous bone and show locally invasive growth. The recurrence rate for CEOT was thought to be lower than for ameloblastoma but there was uncertainty about this because of the rarity of CEOT and the variability of treatments rendered [3].

CEOT is currently defined as "a benign epithelial odontogenic tumor that secretes an amyloid protein that tends to calcify" [4]. The tumor cells have well-defined cell borders and eosinophilic cytoplasm. Nuclear pleomorphism is a characteristic feature and there may be bizarre or giant nuclei, but other histologic features of malignancy are generally absent [4]. A recent review of the literature yielded 339 cases, including 264 central lesions and 24 peripheral lesions. The clear cell variant is the most commonly reported histologic variant [5]. It is composed predominantly of clear or vacuolated cells, mixed with smaller areas of more classical eosinophilic cells. The clear cells contain intracytoplasmic glycogen (PAS positive, diastase sensitive). Amyloid deposits and calcified material are seen in association with both clear cells and eosinophilic cells [6, 7]. There is insufficient evidence to link the clear cell variant with a more aggressive clinical behaviour [5, 7].

The differential diagnosis for intraosseous CEOT includes a variety of odontogenic and non-odontogenic tumors, corresponding to the variation in histomorphology that may be seen in CEOT. Cases with abundant fibrous stroma and relatively less prominent epithelial islands should be distinguished from central odontogenic fibroma. Cases with large islands or sheets of epithelium may have a similar histologic appearance as squamous odontogenic tumor or primary intraosseous carcinoma. The clear cell variant of CEOT should be distinguished from intraosseous mucoepidermoid carcinoma with predominance of clear cells, clear cell odontogenic carcinoma and metastatic carcinoma, especially renal

cell carcinoma. The combination of polyhedral cells with distinct cell borders, nuclear pleomorphism, rare or absent mitotic figures and production of amyloid-like material that undergoes calcification allow definitive diagnosis of CEOT. Histochemical staining for mucin and glycogen, immunohistochemical staining to rule out metastatic clear cell carcinoma and molecular studies for EWSR1 gene rearrangement are helpful in the differential diagnosis of the clear cell variant of CEOT from other intraosseous clear cell epithelial tumors [4, 8-11].

Intraosseous CEOT typically presents as a painless, slowly enlarging, bony expansion. However, there are reports of CEOT that show accelerated growth and destruction of cortical bone to form a large tumor mass [12], invasion into the paranasal sinuses, nasal cavity and orbit [13] and intracranial extension [14]. Histopathologic examination showed the characteristic appearance of CEOT and no histologic features of malignancy were noted in these cases. There are rare reports of CEOT with histologic features of malignancy: increased cellularity, invasion into blood vessels, perineural invasion, increased mitoses, abnormal mitotic figures and increased proliferative index by Ki-67 labeling [6, 15, 16]. The diagnosis of malignant CEOT was made when histologic features of malignancy were associated with extensive invasion, paresthesia, or metastases to regional lymph nodes or distant sites [15, 17-19]. In cases where the malignant CEOT represented the recurrence of a conventional CEOT or showed a component of conventional CEOT mixed with areas of odontogenic carcinoma, the findings were interpreted as suggestive of malignant transformation of a longstanding or incompletely removed CEOT associated with decrease or loss of amyloid deposits and calcification [17-19].

The mandibular tumor presented here showed at least two morphologically distinct components - a predominant component of odontogenic carcinoma with necrosis, areas of increased mitotic activity and Ki-67 labeling index and minimal production of amyloid, adjacent to a smaller component of typical CEOT. Awareness of the biological complexity of this rare odontogenic tumor, adequate sampling in the incisional biopsies and close monitoring of the clinical course could help to improve the outcome of treatment.

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