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Diagnostic difficulties in lesions of the minor salivary glands

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Abstract

A wide range of lesions arise from the intra-oral salivary glands, and often present a diagnostic challenge to specialists and generalists alike. Of the salivary neoplasms, pleomorphic adenoma is the most common, but its morphological diversity may bring several other entities to mind, notably polymorphous adenocarcinoma, particularly in a small incisional biopsy. Polymorphous adenocarcinoma in turn shares features with adenoid cystic carcinoma. Immunohistochemistry and molecular cytogenetic studies can assist diagnosis in the face of overlapping morphology. The other salivary neoplasms most likely to be encountered in the oral cavity are canalicular adenoma, mucoepidermoid carcinoma, secretory carcinoma and acinic cell carcinoma. Of the non-neoplastic conditions, necrotising sialometaplasia is most likely to be misdiagnosed as neoplastic on both clinical and histological grounds. However, careful consideration of the clinicopathological features of an adequate tissue specimen will enable correct diagnosis.

Key words

Salivary gland diseases; mouth neoplasms, immunohistochemistry, cytogenetics
Introduction

The histopathology of the salivary glands, and particularly of salivary gland tumours, is complex and may be problematic for even the most experienced diagnostic pathologist. Since this topic was last reviewed [1] a number of new entities have been described and terminology has been altered to more accurately reflect the behaviour and nature of some lesions. In addition, there have been advances in molecular techniques enabling more accurate diagnosis of some lesions. These changes have been incorporated into the latest World Health Organization (WHO) Classification of Head and Neck Tumours [2]. The classification contains over 40 named neoplasms, many of which show significant morphological diversity. As a result, there are overlapping features, which make differentiation between tumour types difficult. Added to this, there is also a range of non-neoplastic lesions that may resemble tumours both clinically and histologically. The purpose of this review is to provide an update on current terminology and new diagnostic techniques, and to highlight areas of diagnostic difficulty or controversy.

Tumours of the minor salivary glands

The oral cavity contains between 400 and 800 small minor salivary glands located throughout the connective tissues of the lips, cheeks, retromolar trigone, palate (including the uvula), tongue and floor of mouth. All are predominantly of mucous type, the exception being the serous glands (of von Ebner) associated with the circumvallate papillae at the interface of the anterior two-thirds and posterior third of the tongue. Theoretically, the intra-oral salivary glands can be affected by any of the salivary neoplasms recognised by the WHO, but in practice even specialist centres see only a few of these types with any frequency, thus this article will concentrate on the most commonly encountered entities and the diagnostic difficulties they pose. They are listed in Table 1.
**Table 1-** Salivary gland tumours most likely to be encountered in the minor salivary glands, in approximate descending order of frequency.

<table>
<thead>
<tr>
<th>Benign</th>
</tr>
</thead>
<tbody>
<tr>
<td>▪ Pleomorphic adenoma, occasional examples of which are solid enough to merit being termed a myoepithelioma</td>
</tr>
<tr>
<td>▪ Canalicular adenoma</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Malignant</th>
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<tbody>
<tr>
<td>▪ Mucoepidermoid carcinoma</td>
</tr>
<tr>
<td>▪ Adenoid cystic carcinoma</td>
</tr>
<tr>
<td>▪ Polymorphous adenocarcinoma</td>
</tr>
<tr>
<td>▪ Cribriform adenocarcinoma of minor salivary glands</td>
</tr>
<tr>
<td>▪ Secretory carcinoma of salivary glands (MASC)</td>
</tr>
<tr>
<td>▪ Acinic cell carcinoma</td>
</tr>
<tr>
<td>▪ Adenocarcinoma NOS</td>
</tr>
</tbody>
</table>

Overall most intra-oral salivary neoplasms affect the palate, but canalicular and basal cell adenomas characteristically affect the upper lip. Indeed, whereas most swellings of the lower lip are mucocoeles, most swellings of the upper lip are neoplasms and a diagnosis of mucocoele at this site should be issued with caution. It is likely that a retromolar tumour will prove to be a mucoepidermoid carcinoma. Most diagnostic difficulties are the result of the morphological diversity of the neoplasms and the bland cytology of all but high grade lesions, which makes evidence of infiltration a critical feature in the diagnosis of malignancy. These problems are often compounded by the submission of small biopsies. For major salivary glands, FNA has been popular but should now be superseded by ultrasound guided core biopsy, which gives superior results [3]. FNA should never be used for intra-oral lesions and even core biopsies may
yield insufficient tissue for accurate diagnosis. For diagnosis of minor salivary gland lesions therefore we always recommend an adequate scalpel biopsy. Palatal lesions are most common and here, the biopsy should be taken down to bone. Shallow biopsies may only yield the superficial aspect of the lesion and may not be representative (Figure 1).

Figure 1. An incisional biopsy of a palatal tumour, only the most superficial aspect of which is present. The tumour has a pleomorphic pattern, with evidence of local infiltration. This tumour was originally diagnosed as a pleomorphic adenoma; the correct diagnosis of polymorphous adenocarcinoma was only made after complete excision.

**Pleomorphic adenoma**

This is the most common salivary gland tumour, comprising about 70% of parotid lesions and 50% of minor gland tumours. Intra-orally, the most common site is the junction of the hard and soft palate. Because pleomorphic adenoma is the most frequently encountered tumour the features are well described, but its relative frequency may lead to over-diagnosis. Morphological diversity is characteristic of pleomorphic adenoma, but this is also a particular problem with small biopsies where only one histological pattern
may be seen. Some of the typical features are listed in the left column of Table 2. When seen in isolation in small biopsies, these features may lead to confusion with the lesion in the right column; the consequences of a misdiagnosis are clearly apparent. The pathologist must take into account the clinical history and the site of the lesion, but even so it may be necessary to issue a differential diagnosis, with or without a favoured option. The true nature of the tumour may not become apparent until the lesion has been completely excised and examined in its entirety.

**Table 2** Characteristic features of pleomorphic adenoma and lesions with which it may be confused.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Resemblance</th>
</tr>
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<tbody>
<tr>
<td>Morphological diversity</td>
<td>Polymorphous adenocarcinoma</td>
</tr>
<tr>
<td>Bilayered ducts and cribriform pattern</td>
<td>Adenoid cystic carcinoma; Cribriform adenocarcinoma</td>
</tr>
<tr>
<td>Bilayered ducts with clear outer cells</td>
<td>Epithelial-myoepithelial carcinoma; adenoid cystic carcinoma</td>
</tr>
<tr>
<td>Sheets of basaloid cells</td>
<td>Basal cell adenoma or adenocarcinoma</td>
</tr>
<tr>
<td>Lymphoid infiltration at periphery of tumour</td>
<td>Acinic cell carcinoma; mucoepidermoid carcinoma</td>
</tr>
<tr>
<td>Myxoid stroma</td>
<td>Myxoma, neural tumours</td>
</tr>
<tr>
<td>Chondroid stroma</td>
<td>Chondrosarcoma</td>
</tr>
<tr>
<td>Plasmacytoid cells</td>
<td>Plasmacytoma</td>
</tr>
<tr>
<td>Spindled myoepithelial cells</td>
<td>Schwannoma, other benign soft tissue tumour, or sarcoma</td>
</tr>
<tr>
<td>Squamous metaplasia</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>Oncocytic metaplasia</td>
<td>Oncocytoma</td>
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</table>

Pleomorphic adenomas are usually well demarcated, but minor gland tumours are not necessarily encapsulated. There may be no evidence of infiltration of the adjacent structures, but the lobular shape of the lesion may lead to the phenomenon of “pseudocapsular invasion”, where tumour apparently extends into, and beyond, the
capsule (Figure 2). This is not uncommon and should not be misinterpreted as a sign of malignancy. Carcinoma arising in pleomorphic adenoma, a well recognised entity, is more likely to complicate a major rather than a minor salivary gland lesion.

Figure 2 - Pleomorphic adenoma of the upper lip, showing an apparently detached satellite of tumour in the tissue surrounding the main nodule. However, serial sectioning proved the tumour to comprise a single large nodule.

The key histopathological features of pleomorphic adenoma are a variable pattern of epithelium in a loosely fibrous matrix, which may be myxoid, mucoid or chondroid. Although present in the tumour shown in Figure 2, chondroid areas are less common in minor than major gland tumours. The epithelium is usually arranged in sheets, strands and ductal structures, often bilayered, from which spindled or stellate cells that characteristically “melt away” into the matrix (Figure 3). In the minor glands, lesions are often more solid or cellular than those seen in the major glands, and “altered” polygonal myoepithelial cells with pale, eosinophilic cytoplasm, the so called “plasmacytoid” or “hyaline” cells (Figure 3) are so typical as to be almost pathognomonic; their presence in small biopsies is an especially helpful feature.
Occasional lesions may be composed entirely of myoepithelial cells. If ductal structures are not apparent the lesion may be diagnosed as myoepithelioma. Immunocytochemistry is not usually needed for the diagnosis of pleomorphic adenoma, but in small biopsies cytokeratins are positive and PLAG1 or glial fibrillary acidic peptide (GFAP) expression can be useful and, in context, are almost specific for pleomorphic adenoma. Myoepithelial cell markers can also be useful and most will stain for cytokeratin 14, α smooth muscle actin (αSMA) or calponin. S100 may also be positive, but is not specific. Note that in pleomorphic adenomas plasmacytoid myoepithelial cells are often negative for all myoepithelial markers, but are positive for pan-cytokeratin antibodies and usually cytokeratin 5/6 and cytokeratin 7.

**Canalicular adenoma**

These are quite common benign tumours that are only found in minor salivary glands, with over 80% arising in the upper lip. They are well circumscribed, often encapsulated lesions with monomorphic histological features comprising characteristic branching
ribbons and tubules lined by a single layer of basophilic columnar cells lying in a sparse, highly vascular matrix (Figure 4).

Figure 4- Canalicular adenoma. A typical example with ductal structures and tubules (“canals”) lined by a single layer of basophilic columnar cells. The stroma is loose vascular and haemorrhagic.

There are two principal diagnostic difficulties with these lesions. The first is differentiation from malignant basal cell or basaloid lesions in small or fragmented biopsies which do not include the margins. In such cases the single layer of columnar cells is characteristic and the clinical history is helpful, but if necessary a second biopsy should be sought. The second problem is that canalicular adenomas may be multifocal, with small satellite tumours affecting glands adjacent to or more distant from, the site of the main tumour [4]. Such multifocality should not be confused with invasion or malignancy. Each small tumour is usually discrete and circumscribed, if not encapsulated. Occasional lesions show cystic change and some may contain more solid areas resembling basal cell adenoma.

**Polymorphous adenocarcinoma**

This was formally called *polymorphous low grade adenocarcinoma*, but in 2002 we
suggested the omission of the “low grade” nomenclature for this tumour, since the behaviour may be unpredictable and some do not behave in low grade fashion [5]. The latest WHO classification has now dropped “low grade” and the new terminology should be polymorphous adenocarcinoma [1].

Polymorphous adenocarcinoma is almost exclusively a tumour of minor salivary glands, with over 60% arising in the palate. The remainder usually occur in the buccal mucosa or upper lip. It was first described in 1983, but prior to that most lesions were probably diagnosed as pleomorphic adenoma or adenoid cystic carcinoma. This reflects two key features of this neoplasm – morphological diversity (polymorphism), and the frequent finding of cribriform or multicystic areas (Figure 5).

Figure 5- Polymorphous adenocarcinoma. The tumour has a monotonous pale staining pattern and shows polymorphism with lobules, ductal structures and trabeculae. At the left side, the tumour is clearly infiltrative into muscle and shows a streaming pattern of single cell filing. There is also very typical perineural infiltration with a whorling or targetoid pattern.

On occasions even an experienced specialist pathologist may be unable to distinguish polymorphous adenocarcinoma, pleomorphic adenoma and adenoid cystic carcinoma in a small incisional biopsy, particularly if there is no evidence of infiltration of the adjacent tissues. An infiltrative pattern is typical and occasional cases infiltrate widely, with widespread destruction at first presentation. However, polymorphous adenocarcinoma
also exhibits some characteristic microscopic appearances. These include a “washed out” or pale staining pattern, particularly in solid areas, with nuclei, which are pale and open with a finely dispersed chromatin pattern. There are diverse morphological patterns, with sheets, lobules and cords of cells, usually in a matrix with a basophilic hue. Lobular areas may show peripheral palisading, which resembles that seen in basal cell adenoma or adenocarcinoma. Single cell filing reminiscent of that seen in lobular carcinoma of breast is typically seen at the periphery of the tumour, and is a very useful diagnostic feature. Perineural infiltration is as common in polymorphous adenocarcinoma as it is in adenoid cystic carcinoma. However, as well as obvious nerve involvement, there may also be areas where whorls of cells appear to swirl around an unseen nerve (Figure 5). Cystic, and papillary cystic, change is also common. Lesions that are predominantly papillary cystic are thought to have a worse prognosis, but alternative diagnoses such as papillary cystadenocarcinoma [6] and cribriform adenocarcinoma (see below) should be considered if this feature is particularly prominent. As well as extensive papillary cystic change, any evidence of cytological atypia or necrosis is a worrying histological feature.

In small biopsies immunohistochemistry for CD117 (c-kit), GFAP and myoepithelial markers (e.g. calponin, αSMA, p63, SOX10) may be helpful if pleomorphic adenoma, polymorphous adenocarcinoma and adenoid cystic carcinoma are all in the differential diagnosis. Most adenoid cystic carcinomas are positive for CD117 in the inner epithelial cells and most pleomorphic adenomas are GFAP-positive; both of these tumours also shows an “epithelial-myoeptithelial” morphology with bilayered ductal areas and widespread myoepithelial cell staining. Polymorphous adenocarcinoma is usually negative for GFAP, and only occasional cases are positive for myoepithelial markers (calponin, α-SMA) or CD117 (c-kit) [6]. p63 however is usually positive, but p40 is consistently negative. This p63+/p40- immunophenotype is very unusual and helps distinguish it from both adenoid cystic carcinoma and pleomorphic adenoma [7, 8]. Adenoid cystic carcinomas also show a high proliferative index and Ki-67 (Mib-1) staining uniformly shows a high number of positive cells (>10%). In our own studies, 30% of cells in adenoid cystic carcinoma were positive for the proliferation marker minichromosome maintenance protein-2 (Mcm-2). Adenoid cystic carcinomas always
showed more than 10% positivity, whereas polymorphous adenocarcinoma and pleomorphic adenomas were 5% and 7% positive respectively and rarely had more than 10% positive cells [9]. Gene fusions involving the transcription factors PLAG1 (on 8q12) and HMGA (on 12q14-15) have been consistently and specifically shown in pleomorphic adenomas and can help differentiate it from other salivary tumours [10]. However, immunohistochemistry and fluorescence in situ hybridisation (FISH) for these markers is not widely available in routine practice.

Whilst many polymorphous adenocarcinomas are indeed low grade, the behaviour is unpredictable and can be comparable to, or worse than, mucoepidermoid carcinoma. Evans and Luna showed that 15% of cases had cervical metastases, 7.5% had distant metastases and 12.5% of patients died of disease [11]. Thus polymorphous adenocarcinoma should be managed in the same way as other malignant salivary gland neoplasms, based on clinical stage and a careful consideration of the histological features. Hopefully omission of the term “low grade” will reduce inappropriately conservative management of high grade variants. However, it has been suggested that many aggressive polymorphous adenocarcinomas are, in fact, cribriform adenocarcinomas (see below) [12].

**Cribriform adenocarcinoma of minor salivary glands (CAMS)**

CAMS was originally reported in the tongue [13], but is now known to involve other minor salivary gland sites. CAMS has been shown to harbour RAS mutations including alterations of the PRKD gene family (PRKD 1-3) with reports of more aggressive behaviour and higher frequency of metastases [14]. At present, it is still classified as a variant of polymorphous adenocarcinoma [2], but as more cases are reported it is likely to become a separate entity. The lesion is composed of bland appearing epithelial cells forming mostly solid islands, but with a prominent cribriform pattern (Figure 6). The nuclei are pale and open and may be clear, resembling the characteristic nuclei of papillary thyroid carcinoma. However, although psammoma bodies are occasionally encountered TTF-1 and thyroglobulin are always negative [15]. The tumour islands may have a palisaded outer layer, and as a result basal cell adenoma and basal cell
adenocarcinoma may enter the differential diagnosis, as well as polymorphous adenocarcinoma.

![Figure 6- Cribriform adenocarcinoma of minor salivary glands, shows a similar pale staining pattern to polymorphous adenocarcinoma. Multiple ductal structures impart a cribriform pattern.](image)

**Adenoid cystic carcinoma**

There are few difficulties associated with a histological diagnosis of a typical adenoid cystic carcinoma, except when small biopsies are encountered as discussed above. Adenoid cystic carcinoma may have a long and apparently indolent clinical history, and pathologists and surgeons may at first be lulled into a false sense of security regarding diagnosis and management. However, they are aggressive, widely infiltrative tumours with a poor long term prognosis. The pathologist therefore needs to pay particular attention to early and accurate diagnosis. In the case of intra-oral tumours this sometimes requires determined requests for second and more adequate biopsies. For palatal lesions in particular, small incisional biopsies, punch or core biopsies and fine needle aspirates should be discouraged and all biopsies should be full thickness down to periosteum.

Histological grading of adenoid cystic carcinoma is useful and usually straightforward, because it depends on a simple analysis of the morphological pattern of the tumour, i.e.
tubular (low grade), cribriform (intermediate grade) and solid (high grade). Some high grade tumours also show “dedifferentiation”. The tubular and cribriform patterns predominate in about 80% of lesions (Figure 7), but it has recently been suggested that a tumour should be regarded as high grade if any solid areas are present [16] and, anyway, histological grading has limited overall value. Less than 10% of patients survive ten years with solid and “dedifferentiated” tumours, but whilst lower grade tumours may do better at five years survival is still less than 50% at 10 years [17].

![Figure 7](image)

Figure 7- An adenoid cystic carcinoma demonstrating the characteristic ‘Swiss cheese’ cribriform pattern. The branching, hyperchromatic epithelium lies in a matrix of basophilic basement membrane-like material.

The differential diagnosis of adenoid cystic carcinoma and the value of immunocytochemistry have been discussed above. In addition, over 80% of adenoid cystic carcinomas show a translocation involving the MYB oncogene and the transcription factor gene NFIB [18]. However, the MYB-NFIB rearrangement may not be evident in solid or dedifferentiated lesions. The translocation can be detected by FISH and an antibody to the MYB-NFIB fusion protein has also been developed, but neither of these techniques is widely available as yet.
**Mucoepidermoid carcinoma**

In most studies, mucoepidermoid carcinoma is the most common malignant salivary gland tumour, representing approximately 10% of all salivary gland tumours and 25% of malignant lesions. Although most common in the parotid gland, mucoepidermoid carcinoma is frequently encountered in the oral cavity in the palate, cheeks, lips, tongue and retromolar region.

Importantly, mucoepidermoid carcinoma is the most common salivary malignancy in children. Incisional biopsies of mucoepidermoid carcinomas are occasionally misdiagnosed as mucocoeles since they are frequently cystic. It is re-emphasised that mucocoeles, especially in children, are almost always encountered in the lower lip or floor of mouth (ranulae). Biopsies from the palate or retromolar region which show a cystic lesion should therefore be considered carefully before a diagnosis is made.

Histological diagnosis of mucoepidermoid carcinoma is based on the identification of an admixture of epidermoid cells and mucous cells. The most frequent pattern is a multicystic tumour with cystic spaces lined by pale staining, duct-like or epidermoid cells, and mucous cells. In almost all cases more solid areas containing cytologically bland, so-called “intermediate cells” are also seen (Figure 8).

![Figure 8 - A low grade, extensively cystic mucoepidermoid carcinoma of the tongue, showing intermediate (arrow), mucous and clear cells in the cyst lining.](image-url)
Clear cell change may be extensive. Mucoepidermoid carcinomas are infiltrative and are often described as having a “tatty” histological appearance at low power. The cystic pattern is typical of low grade lesions and is seen in over 70% of cases. Intermediate and high grade lesions show more solid areas and an increased proportion of epidermoid cells. High grade lesions may also show cytological atypia, perineural infiltration, necrosis and a more invasive growth pattern. Focal keratinisation and squamous carcinoma-like areas are additional findings in some cases. Tumour-associated lymphoid infiltrates are more commonly found in parotid than intra-oral neoplasms.

Histological grading of mucoepidermoid carcinoma is a useful prognostic indicator and the most popular scheme is that of Brandwein et al. [19]. This is based on the above mentioned features and grades lesions into low, intermediate and high grade. Using this scheme it was shown that all patients with low grade lesions were disease free at 10 years, compared to 70% with intermediate grade and 40% with high grade lesions [19].

Mucoepidermoid carcinomas do not have a characteristic immunohistochemical profile, but most lesions do show a characteristic t(11;19)(q21;p13) translocation and CRAL1-MAML2 gene fusion [20]. This can be detected by FISH and is a useful marker in difficult cases and in small indeterminate biopsies. Tumours with the translocation were thought to be low to intermediate grade and have a better prognosis in terms of recurrence rates, metastases and tumour-related deaths [20,21]. However, high grade lesions may also harbour the translocation and therefore MAML2 alterations cannot be relied on for grading or prediction of outcome [22].

Clear cells are frequently seen in mucoepidermoid carcinomas, but careful examination for the presence of mucous, epidermoid and/or intermediate cells should establish the correct diagnosis. Abundant clear cells may prompt consideration of other clear cell lesions such as clear cell carcinoma and metastatic renal cell carcinoma. FISH for EWSR1 rearrangements [23] and immunohistochemistry for renal cell markers can help differentiate between these lesions if required. In the context of salivary gland tumours, EWSR1 translocations are specific for clear cell carcinoma, but they are also seen in odontogenic clear cell carcinomas, so the clinical history and site of the lesion must be
considered. This is especially the case in palatal lesions, which may have an origin in bone.

**Acinic cell carcinoma**

The recognition of the secretory carcinoma of salivary glands (see below) has, at a stroke, rendered much of the published data on acinic cell carcinoma invalid. Henceforth, the characteristic sheets of basophilic, granular cells resembling the serous acini of the parotid are essential for a diagnosis of acinic cell carcinoma, but until 2010 [24] they were not. Never a common tumour, in future unequivocal examples of acinic cell carcinomas of the minor salivary glands may be quite rare. Both of the minor gland “acinic cell carcinomas” illustrated in the original version of this paper [1] have subsequently been proven to contain the genetic translocation that is the hallmark of the secretory carcinoma. Given the serous appearance of the granular cells of acinic cell carcinomas, it is not surprising they predominate in the parotid. In our studies, a retrospective review of all our acinic cell carcinomas showed that about 15% harboured the *ETV6* translocation and were therefore secretory carcinoma [25].

Acinic cell carcinoma is characterised by sheets of basophilic cells with a typical cystic or microcystic, sieve-like appearance (Figure 9).
Figure 9- Acinic cell carcinoma. a) typical a sieve-like, microcystic pattern. Note focal accumulations of lymphoid tissue within the tumor. b) PAS (with diastase) e shows positive staining of zymogen granules in the acinar cells.

The follicular pattern resembling thyroid gland is another useful diagnostic feature (Figure 10). Papillary cystic change is also common, but should form only a minor part of the lesion. If it is extensive, then polymorphous adenocarcinoma or papillary cystadenocarcinoma should be considered.

Figure 10- An acinic cell carcinoma of the cheek mucosa showing typical sheets of basophilic cells, but also containing a follicular area resembling thyroid gland (arrow).
Most of these morphological features may also be seen in secretory carcinoma, but the key characteristic feature of acinic cell carcinoma is that the acinar differentiation is easily demonstrated by highlighting the fine, diastase-resistant periodic acid-Schiff (PAS)-positive cytoplasmic zymogen granules (Figure 9b). If this granular PAS-positive staining is absent then secretory carcinoma should be considered. Immunocytochemistry may also be useful, in differentiating lesions from secretory carcinoma (see below). Acinic cell carcinomas are low grade tumours and may be circumscribed and even encapsulated. High grade or dedifferentiated variants have been described but are rare. However, all lesions require complete excision if troublesome recurrence is to be avoided.

**Secretory carcinoma**

“Mammary analogue secretory carcinoma” of the salivary glands was first described as a variant of acinic cell carcinoma, but it is now regarded as a distinct entity and has been re-designated as “secretory carcinoma” [2, 24]. It shows a resemblance to secretory carcinoma of the breast and harbours the same t(12;15) (p13;q25) translocation, with the resulting $ETV6-NTRK3$ gene fusion [24]. As noted above, secretory carcinoma has many overlapping histological features with acinic cell carcinoma, but crucially lacks the basophilic acinic cells containing PAS-positive zymogen granules. It shows a lobular growth pattern, often well demarcated, with a microcystic, solid, follicular, clear cell or papillary cystic morphology (Figure 11). Indeed, it has been suggested that a papillary cystic pattern is more common in secretory carcinoma than in acinic cell carcinoma. PAS (with diastase) staining is the single most useful diagnostic aid; in secretory carcinoma the pale eosinophilic cells are PAS-negative (Figure 11b). Conversely, secretory carcinoma shows a very characteristic globular, “bubbly” PAS staining within cystic spaces and in intercellular spaces (Figure 11b). FISH for $ETV6$ rearrangement is regarded as the gold standard for diagnosis, but immunocytochemistry shows a characteristic phenotype [25]. Lesions are strongly and diffusely positive for S100 (Figure 11c) (acinic cell carcinomas are negative or only patchy) and mammaglobin, but are largely negative for DOG-1 [24, 25].
Figure 11 - Secretory carcinoma. a) The tumour is pale staining, but has a microcystic pattern similar to acinic cell carcinoma. b) PAS (with diastase) is negative in the cells, with no evidence of zymogen granules. However a globular intraductal and intercellular positivity is typical. c) Secretory carcinomas are strongly and diffusely positive for S100 protein.

Acinic cell carcinomas show diffuse strong positivity for DOG-1 and for SOX10 on the luminal aspect of acini and small (intercalated) ducts [25]. Most lesions can therefore be diagnosed without recourse to molecular techniques on the basis of a characteristic pattern of PAS positivity, diffuse staining for S100 and mammaglobin, and negativity for DOG-1 [25, 26].

**Non-neoplastic lesions**

There are a number of non-neoplastic conditions which may mimic a neoplasm clinically
or histologically, as well as rare entities that may not be recognised [27]. The pathologist must always pay special attention to the clinical history and the age of the patient, especially in distinguishing benign cystic lesions from cystic neoplasms, and determining the correct diagnosis in necrotising sialometaplasia.

**Sclerosing polycystic adenosis**

This is a rare disorder of the salivary glands that is thought to be inflammatory in origin, and resembles fibrocystic disease of the breast. Almost all cases have been reported in the parotid gland, but occasional cases have been seen in the submandibular gland and intra-oral minor glands. The lesions are usually well demarcated, lobular areas of densely collagenous, relatively acellular fibrous tissue containing multiple, cystically dilated ductal structures and residual acini (Figure 12). Cribriform and papillary cystic patterns may be seen, as may mucous cells, sebaceous cells and squamous metaplasia. There is usually an associated chronic inflammatory cell infiltrate. Diagnosis is complicated by apparent cytological atypia at times suggestive of carcinoma *in situ* [28]. Recently, the presence of X-chromosome inactivation detected by the human androgen receptor assay (HUMARA) has been reported in sclerosing polycystic adenosis suggesting a monoclonal nature, and leading to suggestions that it may be neoplastic in nature (“sclerosing polycystic adenoma”) [12,29]. However the new WHO classification still regards the lesion as reactive in nature [2]. Sclerosing polycystic adenosis may resemble a range of salivary tumours, including mucoepidermoid carcinoma, adenoid cystic carcinoma and cystadenocarcinoma. However, there are no reports of malignancy arising in this condition and the significance of these findings remains uncertain. Careful review and follow-up is nevertheless advisable.
Figure 12 - Sclerosing polycystic adenosis shows focal accumulations of cystically dilated ducts in a sclerotic fibrous stroma. Areas with a cribriform or papillary pattern can be seen.

**Necrotising sialometaplasia**

This entity, which resembles malignancy both clinically and histologically, is well described yet continues to cause diagnostic confusion; misdiagnosis has obvious unfortunate consequences. The cause is unknown, but the lesion appears to be a reactive process and infarction has been suggested as the primary aetiological event. Diagnostic errors are compounded by the fact that necrotising sialometaplasia may frequently be found at the surgical margins of a previous excision, making it very difficult to differentiate the lesion from a recurrence of the previous malignancy. Necrotising sialometaplasia most commonly affects the palatal glands, presenting as a firm nodule that usually ulcerates, producing the appearance of malignancy. If left untreated the lesions usually heal spontaneously in 4-6 weeks. Histologically, the typical appearance is of islands of squamous epithelium in heavily inflamed, sometimes fibrinopurulent fibrous connective tissue. Alone, this appearance may be very difficult to distinguish from a carcinoma, but necrotising sialometaplasia also shows areas of necrotic, “ghostly” salivary tissue (Figure 13) and areas of transition from normal ducts to squamous metaplasia will be evident. Cytologically the epithelium is bland, the
squamous islands are typically rounded and the lobular architecture of the gland is usually retained.

Figure 13- Necrotising sialometaplasia. Islands of squamous epithelium appear to infiltrate the connective tissues. However the islands are well demarcated and the cytology is bland. Areas of transition from normal ducts can be seen. Sheets of necrotic “ghostly” salivary acini are seen in the centre of the picture.

**Adenomatoid hyperplasia**

This sessile, tumour-like swelling of the minor glands is most often encountered on the palate due to hyperplasia of the mucous acini, but clinically it resembles pleomorphic adenoma. If the biopsy is adequate, histological diagnosis is straightforward. Typically there are lobules of enlarged mucous acini in which the cells are filled with secretory granules and the nuclei are squeezed to the basal portions. Most cases are associated with focal inflammation and (paradoxically) atrophy, ductal dilatation and a history of trauma is often elicited [30]. There is a need for some caution, particularly in a small biopsy, because the diagnosis is to some extent one of exclusion, and occasional lesions are associated with an adjacent salivary gland tumour. The pathologist and clinician must therefore be confident that the sample is representative of the clinical lesion.
**Intercalated duct hyperplasia (Adenomatous ductal hyperplasia)**

Very few cases of this lesion have been reported but it is regarded as an entity [2, 31]. It presents as a well demarcated nodular accumulation of well-formed bilayered ducts (Figure 14). The condition most often affects the parotid gland and is usually an incidental finding at the margin of an unrelated salivary gland tumour. It is rare in the minor glands. Some lesions are poorly demarcated and permeate adjacent normal tissues. This should not be confused with malignancy. Confusion with epithelial-myoeipthelial carcinoma is the greatest diagnostic risk, but in intercalated duct hyperplasia, the ducts are well formed, clearly demarcated, regular in appearance and uniformly distributed through the lesion.

![Intercalated duct hyperplasia](image)

Figure 14- Intercalated duct hyperplasia, shows focal accumulations of well-formed bilayered ducts. The ducts are small and evenly distributed in a monotonous pattern through the lesion.

**Salivary gland cysts**

Mucocoeles are the most common cystic lesions of the salivary glands and rarely present any diagnostic difficulties. Almost 90% of mucocoeles are of the extravasation (rather than retention) variety and are most frequently encountered in children and in the lower lip. Retention mucocoeles are less common, but are most often found in patients over 40 years and may present in the floor of the mouth, palate or buccal mucosa. As already mentioned, it is important to remember that mucocoeles are very rare in the
upper lip or intra-orally in children (with the exception of ranulae in the floor of the mouth). The possibility of a cystic neoplasm should always be considered when apparent extravasation cysts arising in unusual sites or circumstances present for histological analysis.

Occasionally mucous extravasation cysts may arise in the superficial lamina propria and present as a small raised blister [32]. These superficial mucocoeles are often multiple and appear to be more common in women. They are often confused with an autoimmune vesiculo-bullous disorder, particularly mucous membrane pemphigoid. Histologically a simple mucin stain (PAS) will clarify the nature of the lesion.

**Summary and conclusions**

A wide range of lesions arise in the intra-oral salivary glands. They may present a challenge to even the most experienced diagnostic pathologist. Both the pleomorphic adenoma and polymorphous adenocarcinoma are characterised by morphological diversity, which can result in errors if non-representative samples are examined in small biopsies. Particular attention should therefore be given to the examination of an adequate tissue specimen and small biopsies and punch biopsies, especially of palatal lesions, should be discouraged. The differential diagnosis of pleomorphic adenoma, polymorphous adenocarcinoma and adenoid cystic carcinoma may be particularly problematic, but proliferation markers and the expression of GFAP by pleomorphic adenomas, and CD117 (c-kit) by adenoid cystic carcinomas, are useful immunohistochemical methods for distinguishing these tumours. The advent of molecular pathology now helps in the diagnosis of adenoid cystic carcinoma, mucoepidermoid and secretory carcinoma and discovery of new fusion proteins and translocations in salivary tumours may yet identify further new entities, which will challenge and undermine the current demographic data on this diverse group of neoplasms.
Practice points

- Beware small incisional biopsies; if distinguishing features are absent issue a differential diagnosis and request a further sample.
- Infiltration of adjacent tissues by a salivary gland tumour might be the only clue to a malignant process; cytological atypia is only a feature of high grade neoplasms.
- Mucocoeles are rare at any site other than the lower lip, with the exception of mucous extravasation cysts of the floor of the mouth (ranulae).
- Swellings of the upper lip are likely to be neoplastic.
- “Plasmacytoid” or “hyaline” cells are virtually pathognomonic of pleomorphic adenoma.
- “Single cell filing” is unusual in pleomorphic adenoma and adenoid cystic carcinoma, but typical of polymorphous adenocarcinoma.
- Perineural infiltration is characteristic of polymorphous adenocarcinoma and adenoid cystic carcinoma.
- Immunohistochemistry for CD117, GFAP and myoepithelial markers can help distinguish pleomorphic adenoma, polymorphous adenocarcinoma and adenoid cystic carcinoma.
- Immunohistochemistry for S100, mammaglobin, DOG1 and SOX10 can help differentiate between acinic cell carcinoma and secretory carcinoma.
- Only make a diagnosis of acinic cell carcinoma if the characteristic acinar cells containing diastase-resistant, PAS-positive zymogen granules are present; if the tumour otherwise resembles a “pre-2010” acinic cell carcinoma, consider a diagnosis of secretory carcinoma.
- FISH for MAML2, ETV6, EWSR1 and MYB rearrangements can help identify mucoepidermoid carcinoma, secretory carcinoma, clear cell carcinoma and adenoid cystic carcinoma respectively.
References


7. Rooper L, Sharma R, Bishop JA. Polymorphous low grade adenocarcinoma has a consistent p63+/p40− immunophenotype that helps distinguish it from adenoid cystic carcinoma and cellular pleomorphic adenoma. Head Neck Pathol 2015; 9: 79–84.


