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Adenomatoid Odontogenic Tumor (AOT) of Maxillary Sinus: Case Report with Respect to Immunohistochemical Findings

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Abstract. *This report describes the surgical therapy, clinical course and morphological characteristics of an adenomatoid odontogenic tumor that developed in the maxilla of a 16-year-old patient. The cystic tumor filled the maxillary sinus and was removed with the retained tooth. Healing was uneventful and no local recurrence was observed during a two-year period of follow-up control. The tumor showed immunoreactivity for certain types of cytokeratins, vimentin and p63, and was focally immunoreactive for alpha smooth muscle actin and epithelial membrane antigen.*

Adenomatoid odontogenic tumor (AOT) is a benign tumor of odontogenic origin (1). The tumor was first described more than 100 years ago. However, the distinction of the entity from other odontogenic tumors was problematic for decades, in particular from ameloblastoma, as verified by the number of terms formerly used (2). It was not until 1971 that the WHO adopted the term "adenomatoid odontogenic tumor" (3) to describe this entity, as had been proposed by Philipsen and Birn (4). The current WHO classification of odontogenic tumors defines AOT as being composed of odontogenic epithelium in a variety of histoarchitectural patterns, embedded in a mature connective tissue stroma and characterized by slow but progressive growth (1). The differential diagnosis of AOT is crucial in terms of surgical management. Local excision is adequate to free the patient from AOT. The prevalence of AOT is not known. AOT is estimated to constitute about 2.2% to 7.1% of odontogenic tumors, as reported in a recent study (5). However, the range of AOT found in odontogenic tumors might be even wider than previously calculated (1). The increasing number of reports on AOT points to the fact that the tumor develops more frequently than formerly expected (5). This report adds a further description of AOT with particular reference to the clinical, radiological, histological and immunohistochemical findings.

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Case Report

A 16-year-old male patient was referred to the maxillofacial surgery clinic due to swelling of the left maxillary alveolar region and radiographic evidence of a retained permanent tooth within an expanding well-demarcated unilocular zone of radiolucency in the left maxillary sinus. The left deciduous canine was still in place. The oral swelling was decisive for the investigations. The facial appearance of the patient was inconspicuous and revealed no asymmetry. Intraorally, the left maxillary alveolar process was prominent distal to the incisors. The oral mucosa was apparently unaffected. All teeth proved to react sensitively to adequate stimuli.

Radiology. On the orthopantomogram taken at the time of the first investigation of the patient, the left canine was retained in the roof of the maxillary sinus (Figure 1). The tooth seemed to be fully developed and the sinus appeared slightly radiopaque, possibly due to soft tissue filling the cavity. Additionally, punctuate radiopacities were scattered around the tooth's crown projected into the region of the sinus. Further, the developmental stage of the roots of the premolars and molars seemed to be behind the roots of the corresponding teeth of the contralateral maxillary side. These shortened roots of the premolars and molars of the affected side protruded into the sinus. Their apices appeared to be rounded and directed to the tumor, hampering the differential diagnosis of root resorption *vs.* premature growth stop (5).

The initial tentative diagnosis was dentigerous cysts with a retained maxillary canine, based on the physical aspect and radiograph. However, thorough inspection of the radiograph disclosed patchy radiopacities in the surroundings of the retained tooth indicative of an odontogenic tumor (5).

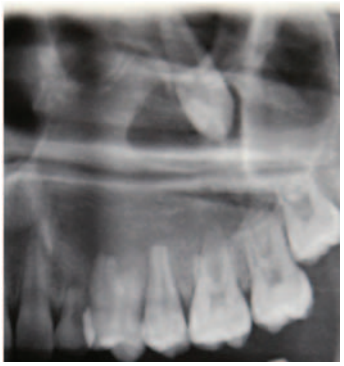

Figure 1. *Detail of panoramic radiograph showing the left side of the maxilla. The roots of the left premolars and molars are shortened with wide apical foramen (in particular first premolar). In the region of the sinus scattered radiopacities can be seen, some of them arranged in a wavy or twisted pattern. The deciduous left upper canine is still in place but the root is partly resorbed.*

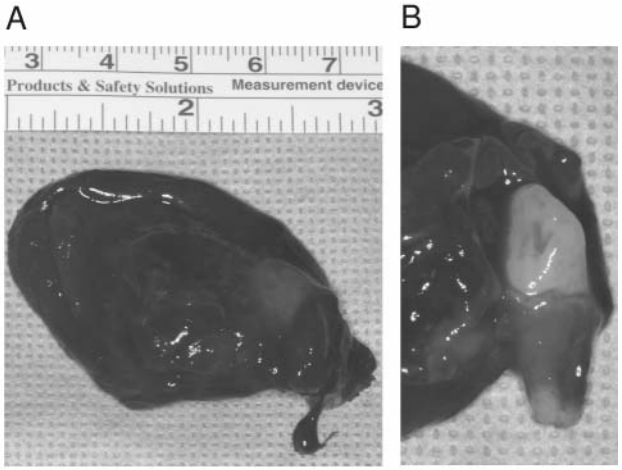

Figure 2. *Extirpated tumor completely encased the tooth (A). After incision of the cyst, the bag adhered circumferentially to the upper third of the root (B).*

Table I. *Antibody/target antigen, clone and supplier/dilution.*

Antibody/target antigen	Clone	Protocol
AE1 / AE3	M 3515	DAKO, 1:50
CK 20	M 7019	DAKO, 1:10
CK 7	M 7018	DAKO, 1:50
Calretinin	18-0211	Zymed, 1:100
CK 18 (DC10)	M 7β1β	DAKO, 1:25
CK 14	L-LL002	Novocastra, 1:100
CK 5/6	D5/16B4	Chemicon, 1:100
CK 19	M 0888	DAKO, 1:100
p63	M 7247	DAKO, 1:25
Vimentin	M 0725	DAKO, 1:1000
SMA	M 0851	DAKO, 1:400
EMA	M 0613	DAKO, 1:50

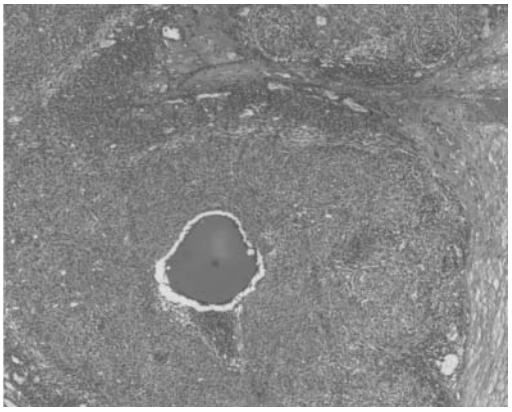

Figure 3. *Nodular epithelial odontogenic tumor with typical histoarchitectural pattern: small darker rounded epithelia forming cribriform and trabecular structures are located peripherally and between the nodules. A narrow zone of fusiform cells with bright cytoplasm can be seen superficially to the former. The broadest layer is formed of duct-like or rosette-like structures with focal luminal eosinophilic material. Most superficially, smaller darker epithelial cells with eosinophilic cytoplasm can be found. Dark eosinophilic acellular material or mineralized particles can be seen in the central lumen of neoplastic nodules (HE, magnification ×50).*

Surgery. The surgery was performed under general anesthesia. The left antrum of Highmore was exposed *via* a fenestration of the facial aspect of the maxilla. The bone appeared to be thinned to a pergament-like layer but was intact. Below the bone, a reddish, bulky layer of a cystic tumor became evident. The cyst filled the sinus completely and was consecutively excavated. There were no apparent infiltrations of the surrounding bones. The retained canine was completely embedded in the cyst (Figure 2). The oral defect was closed by primary intention. Healing was uneventful. The radiographic follow-up examination after two years revealed no local recurrence.

Histology. Tumor tissue was completely embedded in paraffin. Four-μm thick sections were cut and stained with hematoxylin-eosin, PAS (periodic acid Schiff), Giemsa, Elastica van Gieson and Congo Red. Pathological analysis revealed cystic formation with smooth solid nodular areas adjacent to the retained tooth. Histologically, solid appearing masses revealed typical finding of AOT (Figure 3) with peripheral narrow strands of smaller cells (Figure 4), which formed net-like proliferation and cribriform structures both at the base of nodules and between them. More superficially two-to four-cell layer forming broad zones of fusiform cells with oval nuclei and bright cytoplasm were detected in the

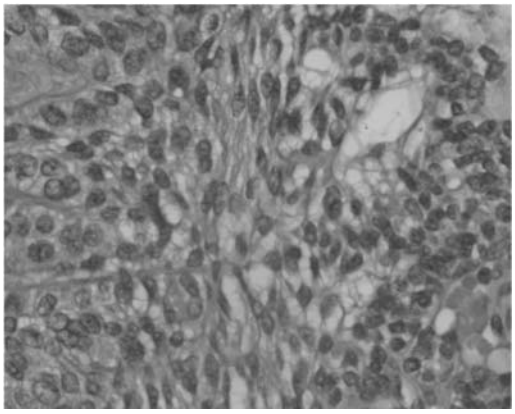

Figure 4. *Histological appearance of the neoplastic epithelium. On the left, small round cells with rounded dark nuclei and only a few cytoplasm. In the middle of the microphotograph, a vertical 2- to 4-cell layer broad narrow zone of fusiform cells with oval nuclei, bright and more abundant cytoplasm can be seen. On the right, basal layers of duct-like broad zone of polyedric to cubic epithelial cells with round bigger nuclei, small nucleoli and bright cytoplasm can be seen (HE, magnification ×400).*

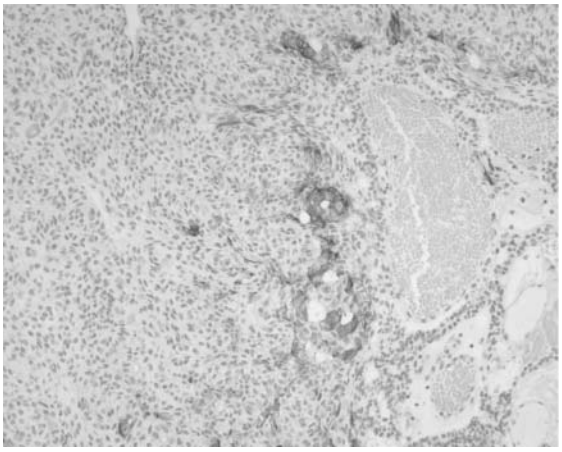

Figure 6. *A minority of cells of the basal duct-like structures and probably a few of the fusiform epithelial cells showing a positive reaction against α-smooth muscle actin. Positive reaction of the perivascular cells of two small arterioles can also be observed (mid right) (target antigen: α-SMA, magnification ×100).*

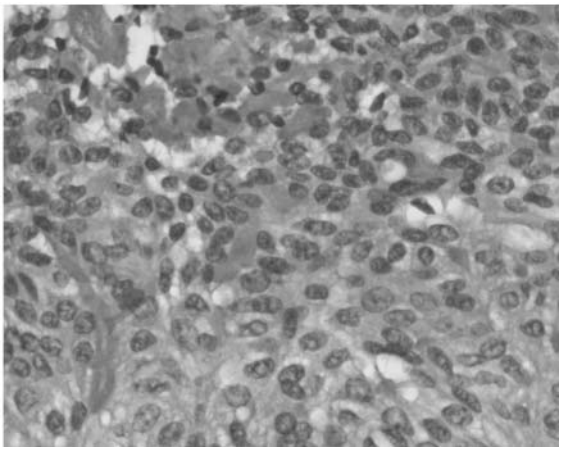

Figure 5. *Central areas of the neoplastic nodules with extracellular eosinophilic material and small round superficial tumor cells (upper half) in addition to the most superficial layers of the duct-like structures (lower half). Note that the intraluminal eosinophilic material within the duct-like structures is likely to be associated with central acellular masses (lower left) (HE, magnification ×400).*

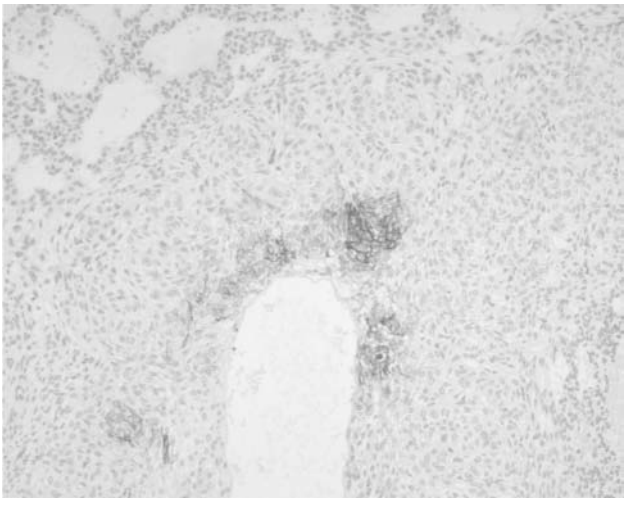

Figure 7. *Some neoplastic epithelia of superficial duct-like structures showing positive reaction against epithelial membrane antigen (target antigen: EMA, magnification ×100).*

majority of neoplastic nodal epithelial proliferations (Figure 4). The broadest zone was formed of multiple rosette-like or tubular structures of polyhedric to cuboid cells (Figure 5). In these areas central or intraluminal collagenous acellular material remaining cementicles were detected, rarely reaching the luminal surface of nodules. The latter were mostly rounded and empty; however, some of them contained dark eosinophilic acellular material remaining cement. In the center of a minority of neoplastic nodules the darker smaller oval to spindle-shaped cells with rounded nuclei and eosinophilic cytoplasm were found (Figure 5). Cellular atypia or hyperchromasia was observed. Rare mitotic figures were found (fewer than 1 per 10 high-power fields).

Loose connective tissue stroma was edematous with hyalinized collagen fibers in addition to multiple hyalinized blood vessel walls. Neither CEOT-like nor squamous

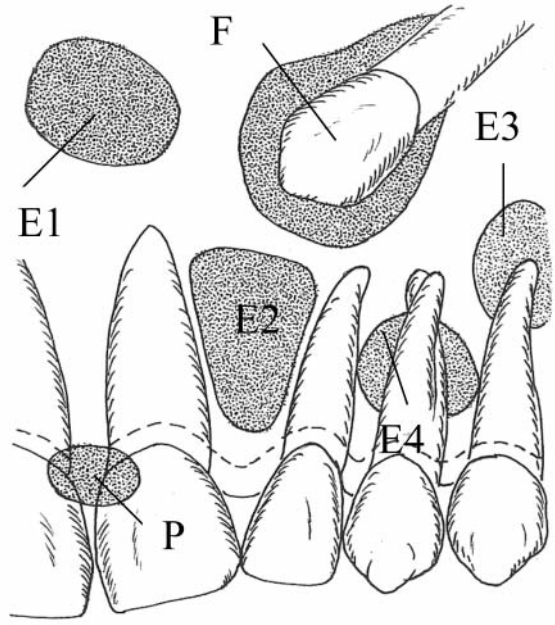

Figure 8. *Schematic drawing of AOT topography in relation to the teeth, as the lesion might appear on orthopantomograms (adapted from Reichart and Philipsen (5), with slight modifications). The intraosseous follicular variant (F) is the most frequent type of AOT. The extent of tumorous coverage of the root varies but does not exceed the coronal third of the root. On the contrary, the association of tumor and tooth root of the extrafollicular type is not as close: E1, There is no relation of tumor to tooth. E2, Interradicular localized tumor with expansive growth (divergence of roots). E3, Superimposition of tumor and the root apex. E4, Tumor superimposed on the midroot level. An extraosseous and peripheral type of AOT (P) appears like an epulis and might be associated with superficial erosion of the bony crest.*

differentiated areas were found. Furthermore, no amyloid depositions were detected with Congo Red staining by viewing under polarized light.

Immunohistochemistry. Immunohistochemical analyses were carried out in accordance with standard protocols (Table I). The immunoreactivity was evaluated in terms of location and intensity (Table II). To quantify the staining properties of tumor cells, intensity of the staining reaction was graded as negative (–), weak (+), moderate (++) and strongly (+++) positive. Negative controls of antibody reactions were performed using the same protocol but omitting the primary antibody (6). The AOT was immunoreactive for certain cytokeratin subtypes, vimentin and p63 (Table II). Focal areas of tumor cells were also stained after incubation with antibodies identifying α-smooth muscle actin (α-SMA) and epithelial membrane antigen (EMA) (Table II, Figures 6 and 7).

Table II. *Distribution of antigen–antibody reactions in defined regions of AOT.*

	Superficial	Duct-like	Basaloid net-like	Fusiform	Cyst basal	Cyst superficial
AE1/AE2	+	++	++	++	++	++
CK 20	–	–	–	–	–	–
CK7	–	–	–	–	–	–
Calretinin	–	–	–	–	–	–
CK 18	–	–	–	–	+	+
CK 14	–	+++	+++	+++	+++	+++
CK 5/6	–	+++	+++	+++	+++	+++
CK 19	+++	+++	+	–	+	+++
p63	–	+++	+++	+++	+++	–
Vimentin	–	–	+	+	–	–
SMA	–	++ (focal basal)	–	–	–	–
EMA	++ (focal)	++ (focal)	–	–	–	+++

Discussion

The adenomatoid odontogenic tumor (AOT) is an infrequent benign epithelial tumor, preferentially found in children and young adults (5). Both intraosseous and extraosseous forms are distinguished. The subtyping of AOT is based on clinical and radiological findings. The follicular (intraosseous) type is by far the most frequent growth type of AOT (5). In the case of follicular growth type, the tumor is localized around the crown of a retained tooth and additionally may cover the upper part of the dental root. In the current case, the cyst-like tumor covered the tooth completely, giving access to the hard tissue after intended incision of the resection specimen (Figure 2B). The gross macroscopic appearance of the tumor is in accordance with former definitions of the entity recommended by the WHO (3): the AOT may be "partly cystic and in some cases the solid lesion may be present only as masses in the wall of a large cyst", as in this case (5).

On radiographs, the intraosseous follicular variant of AOT shows a well-delineated, uni-locular radiolucency sourrounding the crown of a retained tooth, a picture indistinguishable from follicular cysts. Indeed, the radiological findings of AOT frequently share characteristics of other odontogenic lesions such as dentigerous cyst, calcifying odontogenic cyst or tumor, ameloblastoma, keratocystic odontogenic tumor, or periapical disease (7). Minute radiopacities around the retained tooth may be found in AOT and are considered a characteristic but not pathognomonic finding (5). About 2 out of 3 AOT show distinct radiopaque calcification on radiograms (8).

This presented report on the case of an AOT demonstrates immunohistochemical findings which are in accordance with

published analyses. It should be kept in mind that the histological diagnosis of AOT is still based on conventional staining. Immunohistochemistry is recommended for research purposes but not as a routine tool to establish diagnosis of odontogenic tumors, including AOT (8). An earlier report pointed out that AOT phenotype is characterized by a cytokeratin profile resembling follicular cysts and gingival epithelium (9). This comparison was based on the knowledge of AOT immunoreactivity for cytokeratin subtypes, which shows some zonal differences within neoplastic nodules. In the present case, immunoreactivity was found for cytokeratins and focal coexpression of vimentin and, surprisingly, also of SMA at the base of the broad duct-like zone. The coexpression of vimentin points to the neoplastic nature of the entity and is in accordance with earlier reports on AOT immunohistochemistry (1-13). Vimentin coexpression might be 69.2% and is restricted to tumor cells at the periphery of ductal, tubular and whorled structures. Mineralized and hyaline material does not show immunohistochemical reactivity for cytokeratin, as to be expected (12). *In vitro,* the coexpression of cytokeratin and vimentin of oral epithelia is well known (14).

The proliferation rate of AOT in terms of Ki-67-positive tumor cells is low in general (9, 12, 13) and counted for less than 2% of nuclei in the present case.

The detection of focal α-SMA-positive basal duct-like cells might point to myo-epithelial differentiation of a small population of cells within the variety of histoarchitectural patterns in AOT, characterized by positivity for p63, CK14 and α-SMA. In this case, focal loss of immunohistochemical reactivity was found for cytokeratin 5/6, cytokeratin 14 and p63 in the most superficial epithelial tumor cells. Interestingly, some of these cells reacted for EMA. Other studies are necessary to confirm this finding in a larger number of AOT cases. Tatemoto *et al*. (11) found all AOT to be desmin-negative. In the present case, no amyloid depositions were observed. These may occur in AOT with focal CEOT-like component (1).

A recent meta-analysis on AOT suggests the frequency of this entity among odontogenic tumors to be in the range of 0.6% to 38.5% (1). Approximately 88% of reported patients were in their second or third decade of life. The distribution by gender is about twice as frequent for females. The follicular variant (Figure 8) is found in about 71% of cases and a predominance for the maxilla is evident (64.3%). About 60% of follicular AOT are associated with a retained maxillary canine. AOT are extremely rarely seen associated with retained wisdom teeth (2.8%) (1). The survey of these epidemiological data discloses the reported case to represent the typical clinical and radiological prerequisites for the tentative diagnosis of AOT.

Conservative surgical intervention, such as enucleation or curettage, is the treatment of choice. Recurrence of AOT is exceptionally rare. Only a few reports deal with recurrent AOT and all are from Japan (5). Reports from Asia (15, 16) and Africa (1, 17) refer to possible regional differences in the prevalence of AOT.

Conclusion

Adenomatoid odontogenic tumor is a rare benign epithelial odontogenic tumor that can be treated by local excision. Expert morphological diagnosis is required to establish differential diagnosis, in particular from ameloblastoma, thus preventing extensive surgery. Immunohistochemical investigations validate cytoskeletal characteristics which this entity shares with odontogenic cysts. The identification of α-SMA in a small population of tumor cells points to a myoepithelial differentiation of a subset of the tumor.

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