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# Keratocystic Odontogenic Tumors – Clinical and Molecular Features

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Additional information is available at the end of the chapter

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## 1. Introduction

Keratocystic odontogenic tumors (KCOTs) are certainly among the most studied lesions in oral pathology, which is not a surprise considering their perplexing clinical behavior and complicated mechanism of pathogenesis. In fact, the specific KCOT features are the reason for numerous discussions regarding the true nature and classification of these lesions, which are still debated in the scientific community. Until recently these lesions were known as odontogenic keratocysts (OKCs), a term first used by Philipsen in 1956. In the beginning, the term was used to describe any jaw cyst in which keratin was formed. However, it became obvious that some other types of jaw cysts, such as radicular and residual cysts, may exhibit keratinization as well, leading to the conclusion that specific histological features of OKCs and not solely the presence of keratin, should be used to distinguish these lesions from other cysts of the jaws [1]. Researchers soon realized that OKCs show aggressive clinical behavior and high recurrence rates, features which are not typical for other odontogenic cysts [2]. Besides that, it has been noted that OKCs are among the most prominent feature of Nevoid Basal Cell Carcinoma Syndrome (NBCCS), also known as Gorlin-Goltz syndrome. Finally, numerous studies have shown that genetic factors are predominant in etiology of these lesions and that some mechanisms of pathogenesis, typical for neoplastic lesions, are also involved in formation of OKCs [3]. Therefore, in 2005 these lesions were reclassified as Keratocystic Odontogenic Tumors (KCOTs) and defined as benign, odontogenic, uni- or multicystic intraosseous tumors, with characteristic parakeratinized squamous epithelium lining, having a potential for aggressive and infiltrative growth [4]. However, since KCOTs also exhibit some cysts-like features, including response to decompression [5], the tumoral

nature of this lesion remains the subject of debate among investigators. Herein, we present the diagnosis and treatment modalities of this lesion.

## 2. Prevalence

In a broad range of scientific publications it has been stated that KCOTs represent about 10% of all jaw cysts [6]. As a matter of fact, scientific data on incidence of KCOTs are very heterogeneous, which actually reflect differences in diagnostic criteria and sample selection in individual studies. For example, in some studies distinction between ortho- and parakeratinized lesions has not been made, which is an important issue, since nowadays it is believed that orthokeratinized cysts do not exhibit features of KCOTs and should not be considered as a part of the KCOTs spectrum [6,7]. According to data from South Africa, the annual incidence in the Caucasian population is 4.86 per million for men and 3.5 per million for women [8].

Around 40% to 60% of all KCOTs are diagnosed in patients in their 2<sup>nd</sup> and 3<sup>rd</sup> decade of life. In some studies, bimodal age distribution has been noted, with highest number of cases in patients aging from 10 to 19 and from 20 to 29 years, just to be followed by another rise in a group of those from 50 to 64 years of life [6]. In an attempt to explain such data, it has been suggested that older population is more susceptible to two independent mutations which are necessary for KCOTs development [9], a view supported by the fact that in NBCC patients KCOTs occur in much younger age than in sporadic cases [10]. The youngest reported patient in the literature was a one and a half year-old girl in whom, during the observation period, no criteria for NBCCS diagnosis had been met [11].

KCOTs are more frequent in men compared to women (1.7:1) [6]. However, in NBCCS, the majority of patients are female (55%), compared to 38% in sporadic cases [10, 12]. Although KCOTs can occur in any part of the jaws, the vast majority of the lesions are located in the mandible, from 69% to 83% of all diagnosed cases [13, 14]. In addition, about half of all KCOTs arise in the region of mandibular angle [6]. However, in patients aging more than 50 years there is a tendency for growing number of KCOTs involving the upper jaw [15]. Also, sporadic lesions are more common in the angle of the mandible (60% of sporadic and 44% of syndromic lesions), while in the posterior parts of the upper jaw the majority of lesions are related to NBCCS (21% of syndromic vs. 11% of sporadic KCOTs) [10, 12].

## 3. Etiology and pathogenesis

It is widely accepted that KCOTs originate from odontogenic epithelium. Remnants of dental lamina, and also proliferations of the basal cell layer of oral epithelium, are considered as possible sources of epithelial cells which may proliferate to form a KCOT [6]. In a recent study on keratin profiling in KCOTs, it was demonstrated that similar keratins (17 and 19) are expressed both in KCOTs epithelial cells and in the cells of dental lamina in rats, sup-

porting the theory that KCOTs arise from its remnants [16]. On the other hand, there are opinions that the main source of epithelial cells required for KCOT formation is derived from basal cells of oral epithelium, which proliferate into the deeper tissues and form microcysts, suggesting that KCOTs should be considered as hamartomas [17]. Results of studies, showing that the highest number of microcysts and epithelial islands are located in parts of KCOTs walls which are in direct contact with oral mucosa are in agreement with such an opinion [18]. Also, proliferations of basal epithelial cells of oral mucosa into the subepithelial mucosal layer were identified in NBCCS patients, further supporting this possibility [17]. Still, since both types of epithelial cells share a common embryogenic origin and are subject to common inductive influences, it has been suggested that these two theories should not exclude one another [19].

### 3.1. Genetic factors in pathogenesis of KCOTs

Regardless of the source of epithelial cells, the etiology of KCOTs is strongly related to genetic factors, in particular to mutation of tumor-suppressor PTCH gene, which is an important part of Sonic hedgehog (SHH) signaling pathway. The PTCH gene encodes PTCH transmembrane protein, which, together with SMO (smoothened), forms a receptor for SHH ligands and suppresses SMO mediated transcription of cellular proliferation genes. Therefore, lack of PTCH function results in increased transcription of genes responsible for cell proliferation and, ultimately, in tumor formation.

Evidence of PTCH gene mutations in KCOTs came from studies of genetic basis of NBCC syndrome. Levanat and co-workers showed that frequency of allelic loss in 9q22 chromosome (where PTCH gene has been mapped) is significantly higher in syndromic compared to sporadic lesions and concluded that inactivation of NBCC syndrome gene represents an important step in pathogenesis of KCOTs [20]. However, mutations of PTCH gene were identified in samples of KCOTs from both syndromic and sporadic cases. In an analysis of expression of SHH pathway components in KCOTs, mutations of PTCH gene were detected in 3 out of 5 sporadic and 4 out of 4 recurrent KCOTs [21]. It is of interest that, in the study from Baretto and co-workers, mutation identified in sporadic KCOTs has not been present in constitutional DNA of the affected individual [22]. Such results supported opinions of Lench and colleagues that in NBCCS patients one mutation is already present in germ line and only one more mutation in somatic cells is required to cause homozygous inactivation of PTCH gene and KCOTs formation. In contrast to this, in sporadic cases two independent mutations in somatic cells are required [23]. Nevertheless, in subsequent studies it was suggested that KCOTs may also occur in cases of PTCH gene haploinsufficiency i.e. loss of only one allele. As a matter of fact, in samples of KCOTs in whom PTCH gene mutations were detected, immunohistochemical analysis revealed expression of PTCH protein, despite the fact that antibody used in this study could not detect mutant forms of this protein [24].

Once mutations in PTCH gene have occurred, KCOTs may become targets of additional genetic alterations, facilitating tumor progression. In an analysis of loss of heterozygosity (LOH) for several tumor-suppressor genes in sporadic KCOTs, frequency of allelic loss was 66% for p53 and 60% for PTCH gene. It is of particular interest that relationship between

these mutations and presence of satellite microcysts in KCOT walls was established [25]. In a similar fashion activation of *H-ras* oncogene in KCOTs was demonstrated [26]. Also, recent study indicated that alterations in *BIRC5* gene, encoding antiapoptotic protein survivin, may contribute to the pathogenesis of KCOTs. It was shown that GG homozygotes of 31G/C survivin promoter gene polymorphism are at significantly higher risk for development of KCOT compared to other genotypes of this polymorphism [27]. At this point, it may be appropriate to notice that the growing body of evidence for strong involvement of genetic defects in pathogenesis of KCOTs support the opinion of the tumoral nature of these lesions. According to Barreto and colleagues, as loss of function of tumor-suppressor gene (PTCH gene) is by definition characteristic of a neoplasm, KCOTs should be considered benign cystic tumors [22].

### 3.2. Cell proliferation and apoptosis

Besides genetic factors, numerous studies suggest that dysregulation of cell cycle and proliferation may be important for KCOT pathogenesis. It is believed that KCOTs show increased cell proliferation rates and that such a phenomenon may be related to its aggressive growth.

PCNA (*Proliferating Cell Nuclear Antigen*) is a protein which is expressed in the nucleus of replicating cells. It is considered to be a marker of cell replication, but also may be expressed during DNA repair process and under the influence of several growth factors [28]. In a sample of 11 OKCs and 10 periapical and dentigerous cysts, the highest number of PCNA positive cells was identified in the suprabasal epithelial layer of KCOTs, suggesting that these lesions have higher proliferative activity compared to periapical and dentigerous cysts [29]. In addition, it was demonstrated that PCNA expression was more pronounced in syndromic compared to sporadic KCOTs [28].

Similar to this, in the study of Li and colleagues, Ki-67, another marker of cell replication, was significantly more expressed in KCOTs compared to other types of odontogenic cysts, and again, its expression was stronger in syndromic vs. sporadic lesions [30]. It is of interest that correlation of PCNA and Ki-67 expression was observed, in particular regarding localization of positive cells and confirming that suprabasal epithelial layer contains the highest number of actively proliferating cells.

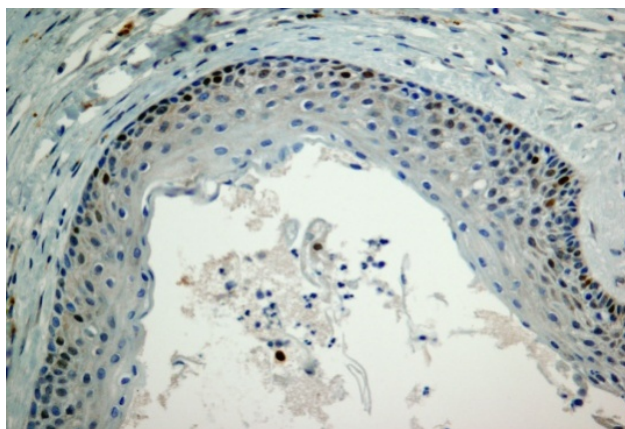
Another process, which plays a crucial role in maintaining tissue homeostasis, is apoptosis or “programmed cell death”. This process is critically important for embryogenic development and aging, but also acts as a defense mechanism, once irreparable damage to the cell has occurred. Lack of apoptosis is a common feature of many tumors [31]. Therefore, it is not surprising that numerous studies investigated whether dysregulation of apoptosis may be implicated in pathogenesis of KCOTs.

Extensive research has focused on protein p53 and its role in these lesions (Figure 1).

It is a product of TP53 tumor-suppressor gene and is capable to arrest cell cycle and induce apoptosis. Mutations of TP53 gene were identified in more than a half of all human malignancies. Also, over expression of p53 protein is very typical for malignant tumors. As a matter of fact, increased p53 expression in KCOTs, compared to other jaw cysts, was



demonstrated in several studies [32-35], but also, it was shown that level of expression in KCOTs was lower than in squamous cell carcinomas of the oral cavity [32, 35]. Since they were unable to identify mutations of TP53 gene in their sample, Li and co-workers concluded that over expression of p53 in KCOTs is not a result of mutation, but overproduction and stabilization of “normal” p53 [32]. In contrast to this, in another study, loss of heterozygosity for TP53 was detected in 66% of KCOTs [25]. Also, using Pab 244 antibody, which selectively recognizes mutant p53 protein, p53 reactivity was detected in 12 out of 78 KCOTs (15.4%), suggesting that mutations of TP53 gene may be important for pathogenesis of KCOTs [36]. In the same study, correlation between expression of mutant p53 and epithelial dysplasia was established.

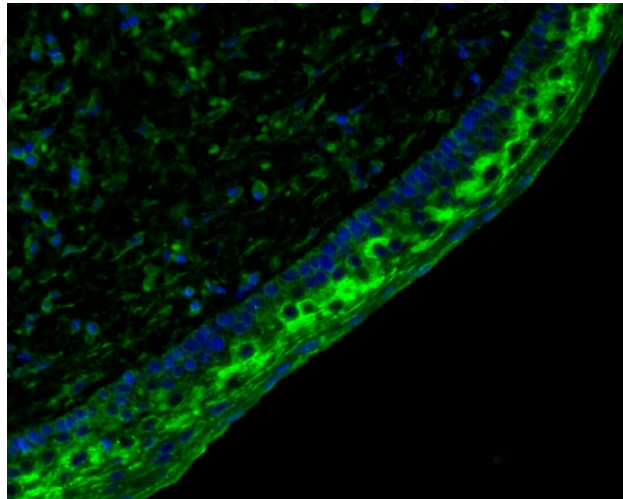


**Figure 1.** Immunohistochemical staining of KCOT wall for p53. Nuclear expression of p53, predominantly in the basal epithelial layer.

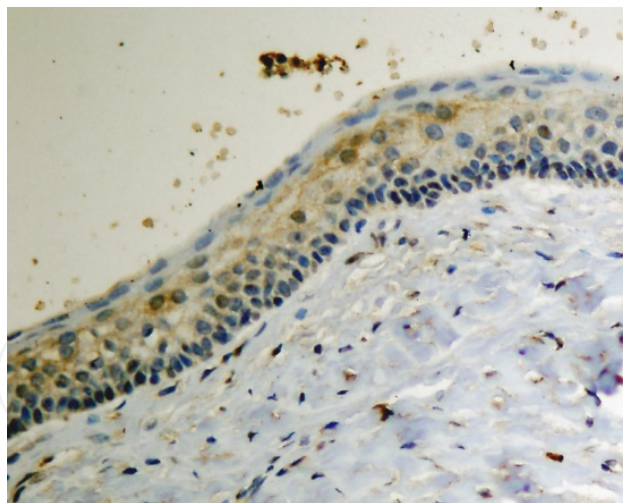
Antiapoptotic protein bcl-2, commonly detected in malignant tumors of the oral cavity [37], was also detected in KCOTs. Its expression was demonstrated in several studies, mostly in the cells of basal epithelial layers, indicating that inhibition of apoptosis may be implicated in development of KCOTs [38, 39]. In contrast to this, epithelial cells of dentigerous cysts have not showed bcl-2 positivity [40]. In the same study, expression of p53, Ki-67, bcl-2 and presence of TUNEL positive cells was investigated. TUNEL (*Terminal deoxynucleotidyl transferase dUTP nick end labeling*) positivity is typical for cells in which apoptotic process was initiated and may be considered as a marker of apoptotic activity. In agreement with previous studies, bcl-2 was expressed in basal cells of epithelial layers; suprabasal cells showed expression of p53 and Ki-67 and TUNEL positive cells were detected in superficial epithelial cells. Based on these results, authors concluded that there is relative balance between cell proliferation and apoptosis in KCOTs, which is the reason why they are formed as cystic instead of solid lesions, despite high proliferative activity of their cells [40]. Recently, expression of another apoptotic protein, survivin, was demonstrated in KCOTs [41, 42] (Figures 2 and 3).

This protein, product of *BIRC5* gene, acts as an inhibitor of apoptosis, but also stimulates cell proliferation and angiogenesis. Its over-expression is a common feature of many malignant neoplasms. It was shown that survivin expression is much more pronounced in KCOTs

compared to periapical cysts [41], but also that highest numbers of survivin-positive cells were detected in suprabasal epithelial layers [42], which may be expected, having in mind that this part of KCOTs epithelium shows the highest cell proliferation activity. Based on these findings, the authors suggested that inhibition of apoptosis may be important for KCOTs pathogenesis, reinforcing opinions on the tumoral nature of these lesions.



**Figure 2.** Confocal microscopy of KCOT specimen, exhibiting survivin immunoreactivity in suprabasal epithelial cells.



**Figure 3.** Immunohistochemical staining shows that survivin is expressed in the cytoplasm of suprabasal epithelial cells.

From available literature, it appears that several mechanisms of pathogenesis, otherwise typical for tumor development, are also implicated in formation of KCOTs, supporting re-classification of these lesions into benign odontogenic tumors. The etiology of some other types of odontogenic cystic lesions of the jaws, such as periapical cysts, is closely related to

infective agents [43, 44] and inflammatory stimuli, including production of pro-inflammatory cytokines, TNF- $\alpha$  and presence of inflammatory cells within the cystic wall [45]. In contrast, KCOTs show lower concentrations of pro-inflammatory cytokines, including TNF- $\alpha$  [46], which suggests that these types of reactions are not crucial for the development of KCOT.

#### 4. Clinical features

KCOTs are benign but locally aggressive lesions with high propensity to recur following surgical treatment. Aggressive growth within the jaws, tendency to invade surrounding anatomical structures and occasional malignant alteration are features which distinguish KCOTs from other types of odontogenic tumors. Yet, the majority of KCOTs are asymptomatic until they reach a significant size. If symptoms are present, most of the patients will complain on swelling, pain and discharge of cystic fluid into the mouth (Figures 4 and 5).



**Figure 4.** Painless swelling of the left mandible in a patient with KCOT.



**Figure 5.** Panoramic radiograph of the same patient. Multilocular radiolucency of the left mandibular body.



Occasionally, involvement of the inferior alveolar nerve may result in paresthesia of the lower lip. Secondary infection of the lesion will result in signs of acute inflammation.

KCOTs tend to grow relatively fast within medullary bone, while bony expansion becomes clinically evident only when a lesion reaches large size, which is a fact that contributes to late diagnosis [47]. Still, aggressive growth of KCOTs is illustrated by numerous case reports of these lesions with unusual clinical presentation. Involvement of the maxillary sinus and floor of the orbit may result in proptosis as a first clinical sign indicating tumor presence [48, 49]. Also, penetration into surrounding soft tissues [50], orbit and infratemporal fossa [51, 52] and even involvement of the skull base [53] have been reported. In 7% to 12.5% of patients more than one KCOT are diagnosed [47]. Since multiple KCOTs are among the most constant features of NBCC syndrome, whether they occur in patients not affected by this syndrome remains the subject of debate. Woolgar and co-workers suggested that multiple OKCs should be considered as manifestation of the syndrome in which other features are so mild that diagnostic criteria cannot be met [10, 12].

While the vast majority of KCOTs occur within the jawbones, a peripheral variant of this lesion, occurring in gingiva, is a well recognized phenomenon. These lesions are termed *peripheral odontogenic keratocysts* [54]. Immunohistochemical analysis of peripheral OKCs linings showed same pattern of expression of cytokeratins, p53, PCNA and Ki-67 as in surrounding normal gingiva, but basal epithelial cells of cystic lining showed expression of antiapoptotic protein bcl-2 in contrast to healthy gingival tissue [55]. Although it is believed that peripheral OKCs do not show aggressive clinical behavior typical for central lesions, recurrent cases have been reported in the literature [56]. In addition, two cases of cystic lesions of the buccal mucosa, exhibiting histological features of OKCs, were recently reported, but their odontogenic origin has been questioned, having in mind atypical localization of the lesions. Again, immunohistochemical analysis of obtained samples showed the same pattern of expression of cytokeratins, bcl-2 and Ki-67 as in central and peripheral OKCs, indicating that all these lesions exhibit similar immunophenotypes [57].

Finally, reports on intraosseous solid lesions, exhibiting histological features of KCOTs but devoid of cystic cavity, added a new entity to the spectrum of KCOTs – *solid keratocystic odontogenic tumors*. First reports on this new entity were published in 2002 and 2004, describing a multilocular lesion of posterior maxilla which, on histological examination, revealed numerous microcysts with typical histological features of KCOTs, surrounded by supporting connective tissue [58, 59]. Even more intriguing was a report of an KCOTs which recurred several times, gradually changing its histological presentation from typical KCOTs to a solid tumoral lesion [60]. This kind of presentation completes the spectrum of KCOTs– from soft tissue lesions to cystic and solid intraosseous tumors, supporting opinions on its neoplastic nature, similar in fashion to dentinogenic ghost cell tumors and calcifying odontogenic cysts [61].

#### 4.1. Recurrence

Besides aggressive growth within the jawbones, another astonishing feature of KCOTs is a remarkably high incidence of recurrence following surgical treatment. Reported recurrence rates vary from 3% up to 62% [62, 63]. Such discrepancies in reported results may be contributed to

different duration of follow-up periods and wide range of surgical techniques used to treat these patients. In a classic study from Browne, in a sample of 85 OKCs, recurrence occurred in 25%, most of them within five years following cyst removal [14]. The importance of adequate follow-up was demonstrated by Forssell and colleagues, by the fact that only 3% of KCOTs recurred within the first postoperative year, but after three years recurrence rate rose to 37% [64]. In another study from Korea, out of 132 lesions, treated by enucleation alone, recurrences were diagnosed in 58.3% of cases, including 11.7% multiple recurrences [65].

The fact is that the exact reason for this phenomenon is not completely understood. The most obvious explanation is that during enucleation parts of KCOTs lining are left in place, which may be expected for lesions with thin and vulnerable walls. As an argument for such a hypothesis, it was shown that recurrences are more common in KCOTs which are removed in several pieces, but also in multilocular lesions and lesions which had perforated the cortical bone [64]. Still, well-documented reports of recurrences occurring sixteen or even twenty years after the initial surgery [14, 18] suggest that this cannot be accepted as the only explanation for this phenomenon. Therefore, three possible mechanisms responsible for KCOTs recurrences were proposed: Incomplete removal of the lesions during the surgery, formation of satellite microcysts within the cystic lining and development of new lesions from epithelial off-shoots of the basal layer of the oral epithelium [62]. Several studies supported this opinion. In an analysis of 72 primary, 11 recurrent and 9 syndromic OKCs, proliferations of basal cells of cystic epithelium were recorded in 45% of recurrent and 44% of syndromic lesions, in contrast to only 8% of primary KCOTs. Satellite microcysts were noted in 78% of syndromic, 18% of recurrent and 4% of primary lesions [66]. Similarly, it has been shown that occurrence of microcysts and basal cells proliferations is significantly more common in syndromic (51%) and recurrent (53%), compared to primary KCOTs (17%) [67]. The same fact was pointed out by Myoung and co-workers, who found that occurrence of so-called “daughter cysts” is significantly more common in recurrent KCOTs [65].

It is also possible that high recurrence rates are related to KCOTs mechanisms of pathogenesis and that formation of lesions *de novo*, from other remnants of dental lamina, may give rise to development of recurrences [6]. Also, continued proliferation of basal cells of oral epithelium may contribute to recurrence formation, even if entire tumoral lining was removed at initial surgery [17]. Indirect evidence to support this possibility was published describing recurrence of an KCOT in an autogenous bone graft used to reconstruct the mandible after removal of the lesion, indicating that the source of the recurrence was located not in the bone, but in the soft tissues covering the graft [68].

#### 4.2. Malignant transformation

Despite aggressive growth and high recurrence rates, KCOTs are benign lesions. However, cases of malignant transformation and subsequent development of squamous cell carcinomas are documented in the literature [69-71]. These tumors are known as *primary intraosseous odontogenic carcinomas* (PIOC), referring to squamous cell carcinomas arising within the jaws, probably from remnants of odontogenic epithelium. To establish diagnosis of PIOC two principal criteria should be met: absence of initial connection with the overlying mucosa

or skin and exclusion of metastasis from a distant primary tumor during at least a 6-months follow-up period. The most widely used classification of PIOC is the one from Waldron and Mustoe (Table 1).

|        |  |
|--------|--|
| Type 1 | PIOC arising from odontogenic cyst   |
| Type 2 | 2A - Malignant ameloblastomas<br>2B - Ameloblastic carcinoma                   |
| Type 3 | PIOC arising <i>de novo</i><br>a) Keratinizing type<br>b) Nonkeratinizing type |
| Type 4 | Intraosseous mucoepidermoid carcinoma  |

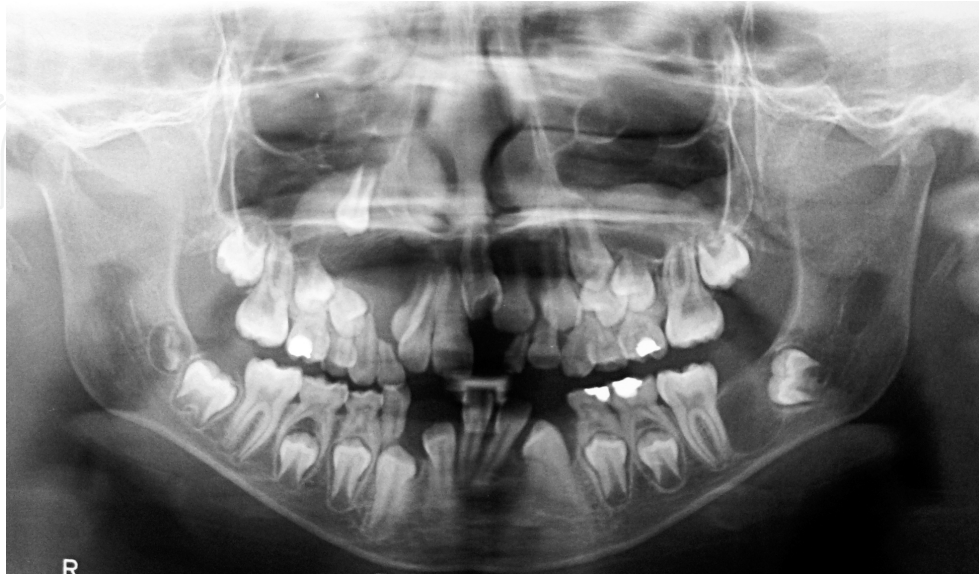
**Table 1.** Waldron and Mustoe’s classification of odontogenic carcinoma.

Although several other types of odontogenic carcinomas were reported, including clear cell odontogenic carcinoma, odontogenic ghost cell carcinoma and the malignant variant of calcifying epithelial odontogenic tumor, term PIOC is most commonly used for carcinomas arising *de novo* or from odontogenic cysts. Following the recent reclassification of OKCs into odontogenic tumors, in a 2005 WHO classification of head and neck tumors a new entity was included – primary intraosseous squamous cell carcinoma derived from KCOT [4]. Still, from available data it does not seem that, compared to other cystic lesions of the jaws, KCOTs have pronounced tendency to malignant alteration. As a matter of fact, in a recent literature review it was demonstrated that majority of PIOC were related to residual inflammatory cysts. Out of 134 PIOC cases, 82 of type 1 (*ex odontogenic cysts*) and 52 of type III (PIOC *de novo*) were identified. Regarding type 1, as already mentioned, majority of cases arose from residual cysts, followed by dentigerous cysts and KCOTs being in third place [70]. Therefore, although clinicians should be aware of the possibility of malignant transformation of KCOTs, there is no evidence that these lesions should be considered as premalignant.

5. Nevoid basal cell carcinoma syndrome

This syndrome, also known as Gorlin or Gorlin-Goltz syndrome is an autosomal dominant inherited condition which exhibits high penetrance and variable expressivity. The principal genetic defect is mutation in the PTCH gene, which has been mapped to chromosome 9q22.3-q31. As already mentioned, this is a tumor-suppressor gene, which explains why occurrence of different types of tumors is the main clinical feature of this syndrome. This syndrome is diagnosed in 1 out of 60.000 newborns, but data from several studies suggest substantial geographic and demographic differences, with prevalence ranging from 1:56000 to 1:256000 [72]. Gender predilection has not been noted. The most prominent clinical manifestations of NBCCS are occurrence of multiple basal cell carcinomas (BCCs) and KCOTs.

These lesions tend to occur at a much younger age compared to patients with sporadic tumors. Therefore, it is not unusual to see patients with BCCs in their 2<sup>nd</sup> or 3<sup>rd</sup> decades or to diagnose multiple KCOTs in children under the age of ten (Figure 6).



**Figure 6.** Multiple KCOTs in the maxilla and mandible in a 9-year old girl with NBCC syndrome.

Besides these tumors, other common features of the syndrome are ectopic calcifications (e.g. those of falx cerebri), skeletal anomalies (most commonly of the ribs), and typical palmar and/or plantar pits. Some of the patients have characteristic facial features, with enlarged head circumference, frontal and temporal bossing and hypertelorism (Figure 7).



**Figure 7.** Hypertelorism (left) and frontal bossing (right) are typical facial features of NBCCS.



Diagnosis is based on so-called major and minor diagnostic criteria (Table 2).

| Major  | Minor  |
|--|--|
| Multiple BCCs or one under the age of 20     | Macrocephaly   |
| KCOT (histological verification required)    | Cleft lip or palate  |
| Palmar or plantar pits                       | Frontal bossing  |
| Bilamellar calcification of the falx cerebri | Hypertelorism  |
| Bifid, fused or markedly splayed ribs        | Pectus excavatum / carinatum   |
| First degree relative with NBCCS             | Syndactyly of the digits   |
|  | Radiological abnormalities: bridging of the sella turcica, vertebral anomalies |
|  | Ovarian fibromas   |
|  | Medulloblastomas   |

**Table 2.** Diagnostic criteria for NBCC syndrome (adapted from ref. [73]).

It is believed that diagnosis of NBCCS may be established if two major or one major and two minor criteria are met. Since most of the lesions associated with the syndrome are not life-threatening prognosis is generally favorable. However, medulloblastomas, malignant tumors of posterior fossa, may occur in about 1% to 2% of the patients, typically during the first two years of life, again in an age significantly younger compared to cases not associated to NBCCS [72]. Although these tumors are usually of desmoplastic type, which is related to better outcomes, early deaths from this kind of malignancy are still possible. NBCCS patients are particularly sensitive to ionizing and UV radiation, so judicious usage of radiographic imaging techniques and constant UV protection of the skin are useful in reducing number of BCCs.

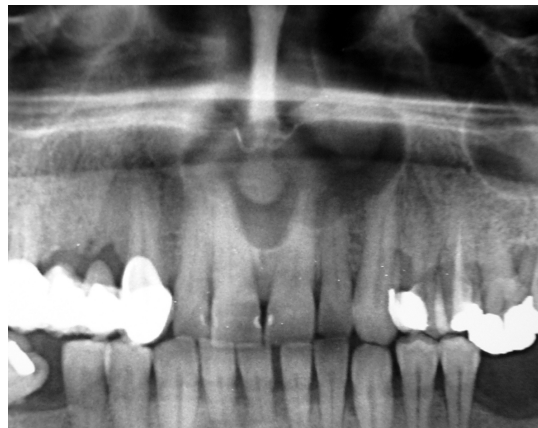
Multiple KCOTs are present in as much as 92% of NBCCS patients [72]. Although it was shown that syndromic KCOTs exhibit a higher number of epithelial proliferations and satellite microcysts within the fibrous wall [66, 12]; it is not possible to reliably differentiate syndromic from sporadic lesions on histological examination. Also, it is not clear whether higher recurrence rates of syndromic *vs.* sporadic KCOTs are truly related to their biological features or simply represent occurrence of new lesions in affected patients. As related to this, some data from the literature suggest that KCOTs in NBCCS patients may exhibit more aggressive phenotype than their sporadic counterparts. In an analysis of PCNA, bcl-2, p53 and bcl-1 (cyclin D1) in syndromic and sporadic KCOTs, Lo Muzio and colleagues observed that PCNA and bcl-2 were equally expressed in both groups, but p53 and bcl-1 expression was restricted solely to syndromic lesions. Based on these findings authors pointed out that KCOTs aggressive clinical behavior could be due to dysregulation of the expression of cyclin D1 and p53 proteins, involved in a check-point control of cellular proliferation [74]. Also, using several cell-cycle and apoptosis-related markers (cyclin D1, p16, Fas, Fas-L, single stranded ss DNA-positive nuclei) Kimi and co-workers concluded that NBCCS-associated KCOTs may be a distinguishable entity from solitary KCOTs [75]. In a similar fashion, it was demonstrated that mast cells values presented by syndromic KCOTs were significantly greater than those of the



sporadic lesions [76]. In conclusion, neither clinical nor histological criteria are reliable for differential diagnosis of syndromic and sporadic KCOTs. However, it was shown that KCOTs in NBCCS patients tend to occur more commonly in the upper jaw, in females and in younger age compared to sporadic cases [10, 12, 15], and diagnosis of KCOT in those groups should prompt the clinician to consider diagnosis of NBCC syndrome.

## 6. Radiographic features

KCOTs are typically presented as round or ovoid radiolucencies with smooth or scalloped margins. Therefore, three distinct radiographic types are recognized – unilocular, multilocular and multilobular lesions. It has been suggested that multilobular KCOTs with scalloped margins are a result of unequal growth activity in different parts of the tumoral wall [6], but this opinion requires further scientific support (Figure 8).

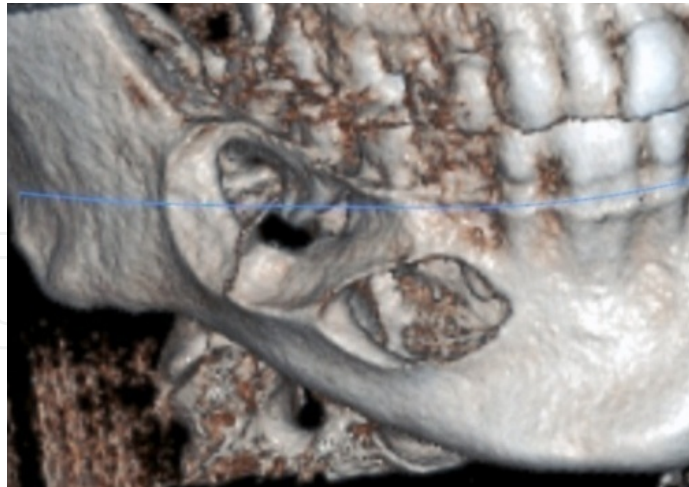


**Figure 8.** Multilobular KCOT of the anterior maxilla. Such presentation may be suggestive of a nasopalatine cyst.

About one quarter of all lesions exhibit multilocular appearance with bony septa within the lumen, the majority of them being located in the mandible (Figures 9 and 10).



**Figure 9.** Cone-beam CT of multilocular KCOT involving the right mandibular body and angle. Note lack of cortical expansion in the area of the base of the mandible.



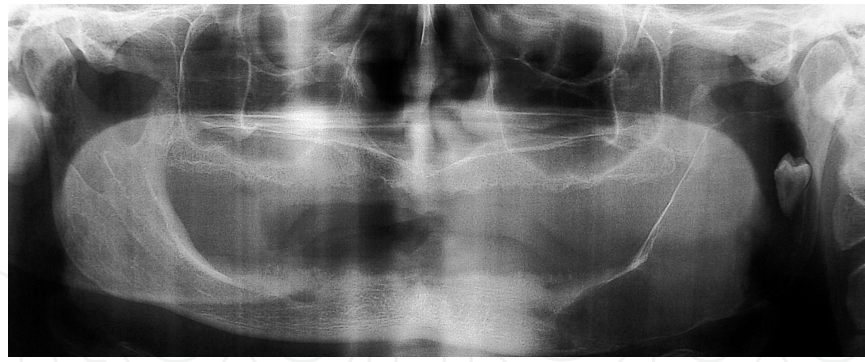
**Figure 10.** reconstruction of the same lesion in figure 9.

In a series of 135 KCOTs, 25% were of multilocular type [77], all of them in the lower jaw. Also, in 25 to 40% of cases, an impacted tooth is present within the KCOT lumen [7], and such lesions should be distinguished from dentigerous cysts. Actually, in many cases, impaction of neighboring teeth is a result of expansive growth of KCOT; a useful feature to clinically differentiate KCOT from a dentigerous cysts is whether radiolucency is attached to the cemento-enamel junction (dentigerous cysts) or encircles entire tooth (KCOT). However, in unilocular variants it may be practically impossible to distinguish between these two types of lesions (Figure 11).

As already mentioned, KCOTs located in the mandibular body rarely result in significant expansion of cortical bone. However, this phenomenon may be very pronounced in the region of the mandibular ramus (Figure 12).

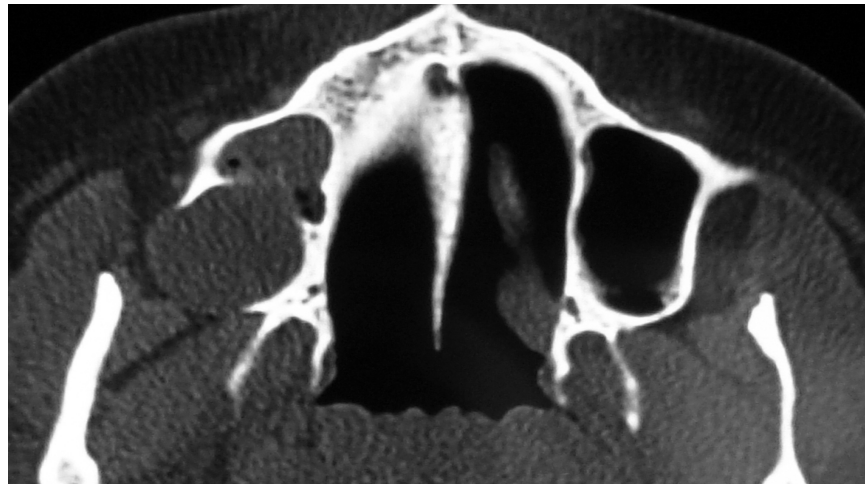


**Figure 11.** Unilocular radiolucency encircling the crown of impacted third molar. This lesion was believed to be a dentigerous cyst but histological examination revealed a typical KCOT.



**Figure 12.** Panoramic radiograph of a KCOT involving left mandibular angle and ramus. Note significant expansion of cortical bone in the ramus region.

Also, it is not uncommon to observe destruction of cortical bone and invasive growth of the lesion into the surrounding soft tissues (Figure 13).



**Figure 13.** Axial CT scan of a KCOT of the right maxilla. The lesion has occupied the entire maxillary sinus and penetrated the surrounding soft tissues.

Finally, resorption of teeth roots might be observed, although less frequently compared to dentigerous cysts (Figure 14).

Despite the fact that some of described radiographic features may be highly suggestive of KCOT, it was shown that radiographic and histological diagnosis are in agreement in only 25.2% of the cases [65]. However, some of more sophisticated radiographic techniques, including computerized tomography (CT) and magnetic resonance imaging (MRI), may be useful for this purpose. It was shown that in CT scans an area of increased attenuation may be observed in the lumen of KCOTs, being the result of keratin accumulation and having a potential role in diagnosis of these lesions [78]. Also, in an analysis of 21 KCOTs, using T2 MRI sequence, van Rensburg and co-workers were able to establish correct diagnosis in 85% of the cases [79]. In agreement with this, it was shown that MRI may be useful in differentiating KCOTs from ameloblastomas, since T2 relaxation times of cystic components were sig-

nificantly shorter in KCOTs compared to ameloblastomas, and no overlap of these values were observed for these two lesions [80].



**Figure 14.** KCOT of left mandibular body causing resorption of the canine root.

Regarding radiographic differential diagnosis, in most of the cases KCOTs should be distinguished from dentigerous cysts and ameloblastomas (Figure 15).



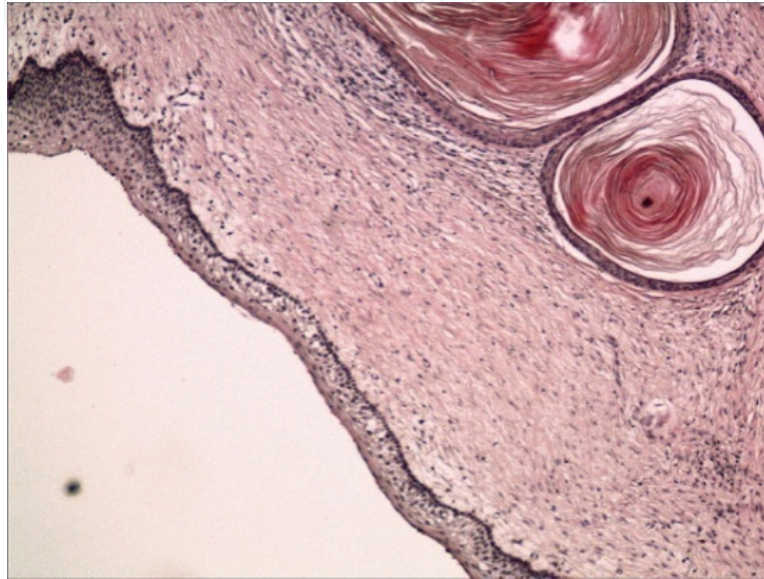
**Figure 15.** Multilocular radiolucency of the left mandibular body and angle. Significant cortical expansion and extensive resorption of the roots are not typical for KCOT. Histological analysis of biopsy specimen revealed a plexiform ameloblastoma.

## 7. Histology

Typical KCOT exhibits a uniform layer of parakeratotic, stratified squamous epithelium. The epithelial lining is relatively thin, usually consisting of up to eight cell layers, with characteristic flat connective tissue interface [81]. It is not uncommon to observe detachment of the epithelial layer from the supportive fibrous wall. The basal epithelial layer consists of



palisaded cuboidal or columnar cells, which are frequently hyperchromatic [7]. The superficial layer is usually corrugated, consisting of flattened, parakeratotic cells. It has been demonstrated that the mitotic index in KCOTs' epithelial layer is higher compared to periapical cysts [48]; higher mitotic activity was also observed in syndromic compared to sporadic lesions [12]. The fibrous layer is thin and typically without inflammatory infiltrate. Within this part of KCOTs wall, proliferations of odontogenic epithelium and formation of microcysts may be observed (Figure 16).



**Figure 16.** Photomicrograph of a KCOT specimen, exhibiting formation of satellite microcysts within the fibrous wall (courtesy of Prof. Zvezdana Tepavcevic).

Frequency of microcysts formation has been reported to be from 7% to 26% [7], although even higher values have been described. It is important that occurrence of microcysts is more common in syndromic and recurrent OKCs, compared to sporadic cases (78% *vs.* 18% and 4% of cases, respectively) [66]. Although parakeratosis is a hallmark of KCOTs, occasionally lesions exhibiting orthokeratotic epithelial layer are encountered. These lesions are termed *orthokeratinized odontogenic cysts* (OOCs) [82] and nowadays they are considered to be a separate entity and not the part of the KCOTs spectrum. It is believed that these lesions do not exhibit aggressive clinical features typical of KCOTs, an opinion which is based predominantly on observation that OOCs recur significantly less frequently compared to KCOTs. In a series of 24 OOCs only one recurrent case was recorded [83] and, in another study, no recurrences were noted in 42 analyzed lesions [84]. However, it seems that, apart from clinical behavior, these two types of lesions differ in some molecular features as well. For example, glycoprotein gp38, which is considered to be a marker of basal cell carcinomas, was identified in parakeratotic KCOTs but not in orthokeratinized ones [85]. Furthermore, in several studies it was demonstrated that cytokeratin profiles of these two types of lesions are substantially different [82, 86, 87], supporting the opinion that KCOTs and OOCs should be considered as distinct entities.



## 8. Diagnosis

Diagnosis of KCOTs is largely based on histological examination of specimens obtained during the surgery. In fact, histological features of KCOTs are so characteristic that differential diagnosis should be relatively easy in most of the cases. However, in some cases, particularly if the fibrous wall of the lesion shows inflammatory changes, those typical features might be changed up to the level which makes reliable diagnosis impossible. Inflammation of the fibrous wall usually results in significant changes of KCOTs histological features. Proliferation of epithelial cells and loss of parakeratosis and palisaded basal layer result in a histological appearance of nonspecific inflamed odontogenic cyst [7]. If these changes affect larger parts of KCOT wall it may be very difficult to establish a definitive diagnosis. In a series of 112 OKCs, inflammation of the fibrous wall was recorded in as much as 76% of cases. While loss of typical histological features was evident in affected parts of the lesions walls, it was noticed that in 10 cases (8.9%) the characteristic KCOTs appearance was preserved, despite inflammatory changes in the supporting connective tissues [88]. It was also shown that inflammation of KCOTs results in significant increase in numbers of PCNA, Ki-67 and Ag-NOR (*Argyrophilic Nucleolar Organizer Region*) positive cells, reflecting higher proliferative activity of epithelial cells compared to non-inflamed lesions [89].

It is still not clear whether these histological changes affect biological behavior of KCOTs. As one may expect that transformation of typical microscopic features of KCOT into those of a nonspecific odontogenic cyst may result in loss of aggressive behaviour, data from the literature suggest the contrary. As a matter of fact, there are some studies which indicate that inflamed KCOTs may be even more aggressive (as measured by frequency of recurrences) compared to non-inflamed KCOTs. Although a relationship between inflammation and recurrence rates was observed [64, 88], possible reasons for this phenomenon remain unresolved.

Additional diagnostic techniques may be used for KCOTs diagnosis in doubtful cases; specimens should be obtained in a minimally invasive fashion. In an attempt to achieve such goals, several studies investigated if analysis of material obtained by aspiration of KCOT lumen has diagnostic value. Using FNAB (*Fine Needle Aspiration Biopsy*), August and co-workers analyzed immunocytochemical expression of cytokeratin 10 in a sample of 10 KCOTs and 4 periapical and 4 dentigerous cysts. Cytokeratin 10 was expressed in samples obtained by aspiration of KCOTs walls, but not in the samples of periapical and dentigerous cysts [90]. Although promising, these results have not been tested in a larger sample. Moreover, in subsequent research, the same authors showed that after KCOTs decompression and loss of typical histological features, cytokeratin 10 positivity was also diminished [91]; this fact may affect diagnostic reliability of this technique. In a similar study, consistent immunostaining for pan-cytokeratin and cytokeratin 19 was observed in samples of KCOTs obtained by FNAC (*Fine Needle Aspiration Cytology*) [92]. Finally, it is possible that simultaneous analysis of several markers is needed for reliable diagnosis of KCOTs. In a mixed sample of KCOTs, several types of odontogenic cysts and unicystic ameloblastomas, a panel of five immunohistochemical markers, namely keratin 10 and 17, perlecan, proliferating cell nuclear antigen (PCNA) and UEA-I lectin binding (UEA), showed distinct expression pattern in all types of the lesions, providing an effective method for differential diagnosis [93]. An issue which is

not yet resolved is whether or not inflammation of the KCOT wall affects expression of these markers, in a similar fashion as it affects histological features of these lesions.

Since histological diagnosis of KCOTs may be doubtful in inflamed specimens (and particularly if the pathologist examines only a limited amount of tissue obtained during the biopsy) there is an objective risk that, in some cases, definitive diagnosis cannot be established. This will result in a dilemma regarding the most appropriate type of treatment which should be rendered in the particular case. Having in mind such a problem, Stoelinga proposed that, if clinical data suggest a possibility that the lesion in question may be an OKC, but histological diagnosis cannot confirm such assumption, decision on definitive treatment should be based on location of the lesion. In the parts of the jaws which are accessible for surgical treatment and in which possible recurrences are easily diagnosed and treated (e.g. frontal segment of the upper jaw and mandibular body), lesion should be treated by simple enucleation. In contrast to this, if the lesion is located in posterior parts of the upper jaw and in mandibular angle and ramus region, it should be treated as an OKC, using additional techniques to minimize risk of recurrence [94].

## 9. Treatment

Difficulties in removal of thin and fragile walls, occurrence of multilocular lesions and high propensity for recurrence after the surgery are factors which make surgical treatment of KCOTs considerably more complicated compared to other cystic lesions of the jaws. Still, being a benign lesion without significant tendency for malignant transformation, routine use of radical surgery (such as resection of involved jaw) is questionable, both from medical and ethical point of view. Therefore, it is not surprising that numerous adjunctive techniques have been developed for treatment of KCOTs. Establishing the balance between effective reduction of recurrence risk and selection of the least aggressive surgical procedure for each individual patient is a basic principle in treatment planning for these lesions [95].

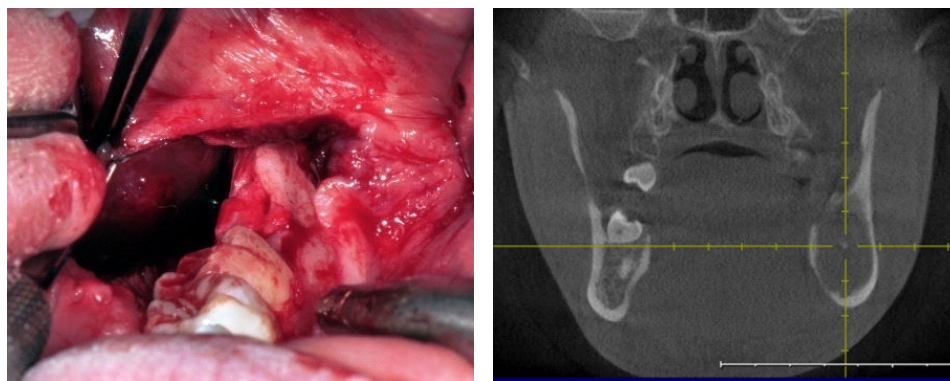
### 9.1. Enucleation

Bearing in mind the high recurrence rates, it is accepted that the standard procedure of enucleation is not adequate for KCOT treatment [96]. In order to improve results of enucleation, peripheral ostectomy was introduced, aiming to eliminate remnants of tumoral tissue or satellite microcysts from the periphery of the defect, particularly in multilobular and multilocular cases. Although it may be effective in reduction of recurrence risk, lack of ability to control the amount of removed bone is considered to be a major disadvantage of this procedure [96].

### 9.2. Resection

In contrast to enucleation, resection of the affected jaw has proved to be very effective in prevention of recurrences. Actually, it is the only technique for which case series without recurrences were reported [97–100]. Besides resection of bone, excision of soft tissues in contact with the lesion is another important concept aimed to reduce risk of recurrence. In a

series of 31 mandibular KCOTs, marginal resection of affected jaw in conjunction with soft tissue excision resulted in complete elimination of recurrences during the follow-up period of up to eight years [98]. Still, having in mind high morbidity associated with this kind of surgery and necessity for additional reconstructive interventions, it was suggested that such a procedure should be reserved only for specific situations – large, recurrent lesions, lesions involving condylar process, and lesions with malignant alteration or pathological fracture of the jaw [96]. However, the concept of soft tissue excision is not restricted solely to resection of the jawbones. An opinion that such a procedure may reduce risk of KCOTs recurrence is based on observations that lesions which have perforated cortical bone show higher recurrence rates compared to non-perforating ones [64]. In addition, Stoelinga and co-workers suggested that some of the recurrences may be attributed to continuing proliferation of basal cells of oral epithelium into the deeper tissues, even after removal of the original lesion [94]. They believed that such a situation is particularly common in the mandibular retromolar region (Figure 17) and therefore proposed that any soft tissues which are in direct contact with the lesion wall should be excised (Figure 18).



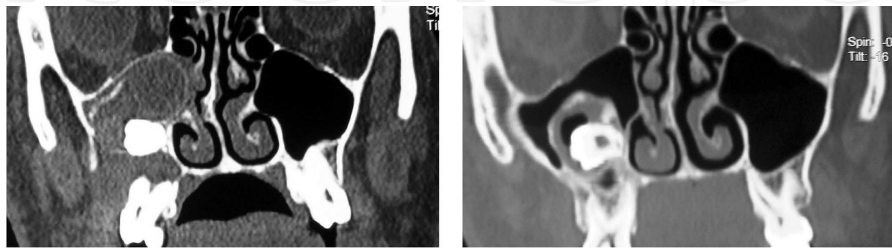
**Figure 17.** Direct contact of retromolar soft tissues with a KCOT of left mandibular angle. Cone beam CT of the same lesion demonstrates cortical perforation.



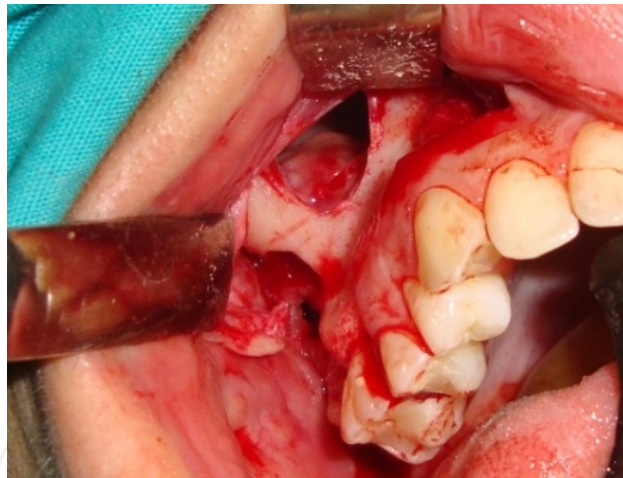
**Figure 18.** Excision of soft tissues after removal of the lesion.

### 9.3. Decompression

Another option, which is widely used for treatment of large cystic lesions of the jaws is decompression, followed by enucleation in the second surgery. The principal goal of such a procedure is to reduce the size of the original lesion, which facilitates complete removal at the second-stage surgery and reduces the risk of injury to surrounding anatomical structures (e.g. inferior alveolar nerve, teeth etc.). Besides this, when used for KCOTs treatment, decompression usually leads to thickening of lesion wall, which also makes enucleation of remaining tumoral tissue easier (Figures 19 and 20).



**Figure 19.** Coronal CT scans of KCOT of right maxilla, before and six months after the decompression. Significant reduction in size of the lesion is apparent.



**Figure 20.** Clinical photograph of the lesion in figure 19. A Caldwell-Luc approach was used for enucleation at the second stage surgery.

However, although it is clear that decompression facilitates enucleation of the lesion, it is more controversial whether such a procedure reduces risk of KCOTs recurrence. Brondum and Jensen reported no recurrences in a series of 12 KCOTs treated by decompression and subsequent enucleation in contrast to 8 out of 44 recurrent cases (18%) of KCOTs treated by one-stage enucleation [5]. In another study with follow-up period of up to sixteen years, no significant differences in recurrence rates were observed for KCOTs treated by decompression and enucleation compared to enucleation alone (26.1% *vs.* 20% of recurrent cases, respectively) [101]. Having in mind reclassification of KCOTs into odontogenic tumors, it is



particularly intriguing to seek which mechanisms are responsible for reduction of KCOT size after the decompression.

#### 9.4. Marsupialization

It was shown that marsupialization of KCOTs results in significant reduction of Ki-67 and IL-1 $\alpha$  mRNA expression in these lesions. As IL-1 $\alpha$  exerts osteolytic activity, the authors concluded that decreased expression of this interleukin may contribute to the effects of marsupialization [102]. Lower expression of IL-1 $\alpha$  receptor IL-1RI and KGF (keratinocyte growth factor) were also demonstrated in response to decompression [103, 104]. Finally, an experimental study showed that positive pressure of 80 mmHg enhanced the expression of IL-1 $\alpha$  mRNA and protein in KCOTs epithelial cells, and increased the secretion of MMP-1, MMP-2, MMP-3, and PGE2 in a co-culture of KCOTs fibroblasts and the epithelial cells. Based on this, the authors pointed out that increased intracystic pressure may play a crucial role in OKCs growth via stimulating the expression of IL-1 $\alpha$  in epithelial cells [105].

Similar to decompression, a fact that marsupialization may result in complete regression of KCOTs was used as an argument to question opinions on the tumoral nature of these lesions. Pogrel and Jordan presented ten cases of KCOTs which were treated by marsupialization as a definitive treatment method. In all cases complete resolution of the lesions, both clinically and radiographically, was observed. Histological examination revealed that in the area of previous KCOTs no remnants of cystic epithelium could be identified. Lack of immunohistochemical expression of bcl-2 in biopsy samples (a marker which is commonly detected in KCOTs epithelium) led to the same conclusion. Authors pointed out that the fate of the cystic epithelium remained unresolved. It may undergo metaplasia to normal mucosa, or a creeping substitution by normal mucosa from the edges of the lesion [106]. Shear commented that it is difficult to explain this unusual and important finding and raised a question what has happened to the KCOTs epithelium with all its potential for active and infiltrative growth [6]. However, in subsequent study, with increased number of cases (n=42) and with longer follow-up period (ranging from 1.5 to 4 years), same authors recorded 5 recurrences (12%) which resulted in partial retraction of previous papers [107]. A fact that recurrences were present despite apparently complete resolution of the lesions, reinforced opinions that KCOTs are actually benign odontogenic tumors, although responsive to decompression, a feature also demonstrated by unicystic ameloblastomas (which are widely accepted as tumoral lesions) [108].

In an attempt to overcome the problem of retention of parts of KCOTs lining and/or microcysts upon lesion removal, techniques of chemical and thermal fixation of surrounding tissues were developed. The most widely accepted is application of Carnoy's solution which acts as a chemical fixative and hemostatic agent. Several studies showed that usage of Carnoy's solution following enucleation significantly reduces number of recurrences compared to enucleation alone [62, 97]. Developing this method, Stoelinga and co-workers proposed a protocol of treatment consisting of enucleation, followed by application of Carnoy's solution and excision of soft tissues in contact with cystic lining. In this manner, frequency of recurrences was reduced to 6% compared to 18% in a group of lesions treated only by enucleation



[18]. Finally, for the same purpose of microcysts removal, liquid nitrogen has been used to treat surrounding tissues and it was shown that such a procedure may be useful even for recurrent cases [109].



**Figure 21.** Upon KCOT removal, Carnoy's solution was applied to the bony bed of the lesion and left in place for 3 minutes.



**Figure 22.** Surgical field after the application of Carnoy's solution.

Although all these techniques were assessed in numerous studies, currently available level of evidence is insufficient to recommend any of them as a standard procedure for KCOTs treatment. Until more prospective and randomized clinical trials are performed, selection of surgical treatment will be based on the surgeon's preference and institution-based protocols.

## 10. Conclusion

Due to their unique clinical and biological features, KCOTs still represent an important problem in oral and maxillofacial surgery and remain to be a subject of controversy among researchers and clinicians. Numerous aspects of KCOTs pathogenesis support opinions on their tumoral nature. However, response to decompression and importance of increased intracystic pressure for their growth indicate that the borderline between odontogenic tumors and cysts may not be as distinctive as we previously believed. As there is a consensus that

standard treatment options for cystic lesions of the jaws are not suitable for KCOTs, additional effort should be made to establish correct diagnosis in doubtful cases. Regarding selection of the most appropriate treatment modality, it is important to establish a balance between effective reduction of recurrence risk and selection of least aggressive surgical procedure for each individual patient. Finally, better understanding of KCOTs pathogenesis may provide clues for new treatment strategies, including use of survivin and Sonic hedgehog (Shh) signaling pathway inhibitors.

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