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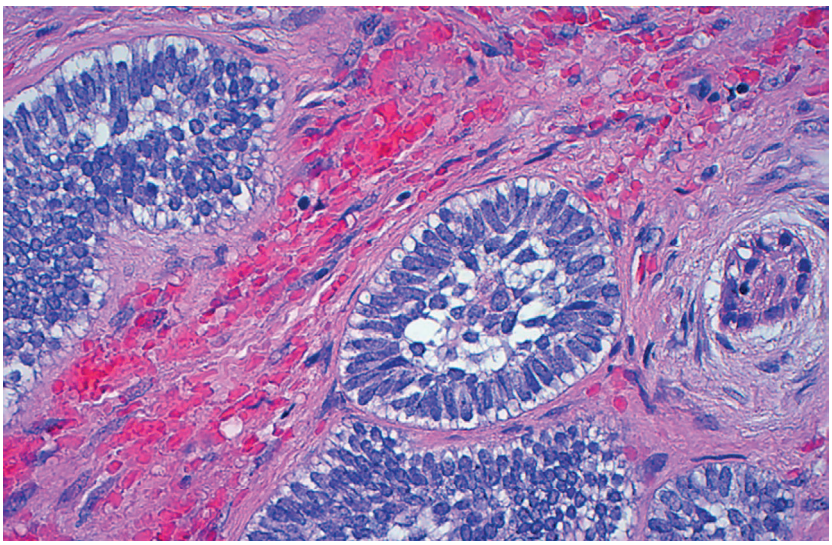
JETTA KELPPE

AMELOBLASTOMA



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DOCTORAL PROGRAMME IN ORAL SCIENCES
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Department of Pathology

Faculty of Medicine
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AMELOBLASTOMA

Jetta Kelppe

ACADEMIC DISSERTATION

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To my mother Raija, my father Seppo, and my aunt Riitta

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I. Kelppe J, Hagström J, Sorsa T, Suominen AL, Apajalahti S, Haglund C, Thorén H. Ameloblastoma: a retrospective single institute study of 34 subjects. *Acta Odontol Scand*. 2019 doi: 10.1080/00016357.2018.1532530.

II. Apajalahti S, Kelppe J, Kontio R, Hagström J. Imaging characteristics of ameloblastomas and diagnostic value of computed tomography and magnetic resonance imaging in a series of 26 patients. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2015 Aug;120(2):e118–30. doi: 10.1016/j.oooo.2015.05.002.

III. Kelppe J, Thorén H, Ristimäki A, Haglund C, Sorsa T, Hagström J. BRAF V600E expression in ameloblastomas: A 36-patient cohort from Helsinki University Hospital. *Oral Dis*. 2019 May;25(4):1169–1174. doi: 10.1111/odi.13072.

IV. Kelppe J, Thorén H, Haglund C, Sorsa T, Hagström J. MMP-7, MMP-8, MMP-9, E-cadherin, and beta-catenin expression in 34 ameloblastoma cases. Clin *Exp Dent Res*. 2020 Sept;1–7. https://doi.org/10.1002/cre2.331

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Abstract

Ameloblastoma is a benign albeit locally aggressive odontogenic tumor originating from remnants of the dental lamina, primarily affecting the mandible and potentially mutilating it if left untreated. Ameloblastomas are classified as ameloblastoma (conventional), unicystic ameloblastoma, and peripheral ameloblastoma. Annual incidence is estimated to be 0.5/1 million population. In the Helsinki University Hospital (HUS) district, approximately five ameloblastoma patients are treated each year. Etiology has yet to be elucidated, although new genetic findings have emerged relating to the mitogen-activated protein kinase (MAPK) pathway.

We surveyed the Q-pati system to identify all ameloblastoma patients (n = 64) treated at the Head and Neck Surgery Unit of HUS. A total of 30 to 36 patient records and the formalin-fixed paraffin-embedded tumor tissue samples were suitable for use from the Department of Pathology at HUS (HUSLAB) from 1985 through 2016. All patient reports were studied, and the parameters were collected using clinical data, Q-pati records, and the imaging reports. A total of 26 ameloblastoma patients' radiological findings were re-evaluated and studied. All tissue samples were revised microscopically, and representative paraffin blocks were chosen for immunohistochemistry with tested dilutions and protocol methods including positive and negative controls. BRAF, MMP-7, MMP-8, MMP-9, E-cadherin, and beta-catenin were of interest. For statistics, we used R studio, seeking correlations between parameters using the Fisher's exact test, z-test, t-test, χ^2, and logistic regression to determine statistical significance. We considered $p < 0.05$ significant.

Our results mostly coincide with previous knowledge with minor deviations and some notable differences to consider in future studies. Specifically, maxillary tumors occurred mostly in older, male patients. BRAF-positive tumors seemed to recur more often than BRAF-negative tumors in the mandible area. In addition, all maxillary tumors were BRAF-negative. Maxillary tumors are likely to recur easily, presumably along complex anatomical structures. Unlike previous studies, ameloblastoma cells did not express MMP-7, MMP-8, or MMP-9. MMP-9 positivity, however, was observed in inflammatory cells, macrophages, and osteoclasts. Beta-catenin expression appeared on

the cell membranes. E-cadherin expression varied, although maxillary tumors presented with a weak E-cadherin expression. Radiologic re-evaluation revealed that ameloblastomas eradicate cortical bone already during the early stages of tumor growth. Ultimately, we found that CT and MRI imaging remain essential in differential diagnostics, serving to protect the patient from radical surgery.

In conclusion, maxillary tumors might be reasonable to study separately from mandibular tumors because of their different protein proprieties. Our investigations among this Finnish ameloblastoma patient cohort has expanded our knowledge of a rare odontogenic tumor and further substantiated previous findings.

Abbreviations

AOT	Adenomatoid odontogenic tumor
BMP	Bone morphogenetic protein
CAM	Cell adhesion molecule
CT	Computer tomography
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EGFR	Epidermal growth factor receptor
ERK	Extracellular signal-regulated kinase
FGF	Fibroblast growth factor
fs	Fat suppression
FSE	Fast-spin echo
Gd-DTPA	Gadolinium-labeled diethylenetriamine penta-acetic acid
HE	Hematoxylin eosin
HUS	Helsinki University Hospital
HUSLAB	Department of Pathology, Helsinki University Hospital
IEE	Inner enamel epithelium
MAPK	Mitogen-activated protein kinase
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
MSCT	Multislice computer tomography
OEE	Outer enamel epithelium
PMN	Polymorphonuclear neutrophil
RAF	Rapidly accelerated fibrosarcoma
RAS	Rat sarcoma virus homolog
SHH	Sonic hedgehog
T1W	T1 weighted
TCF/LEF-1	T-cell factor/lymphoid enhancer–binding factor 1

TGFβ	Transforming growth factor beta
TNF	Tumor necrosis factor
WHO	World Health Organization
WNT	Wingless-related integration site

1. INTRODUCTION

Ameloblastoma is a rare epithelial odontogenic tumor affecting the jaw bones, primarily treated with radical surgery. Operations are often mutilating and, if left untreated, ameloblastoma can, despite its benign nature, cause severe facial deformation and even death. Early detection and correct primary diagnosis substantially reduce suffering resulting from operations, post-surgical prosthetic rehabilitation, and psychosocial impacts. Because of its low incidence, each study of ameloblastoma attempts to resolve questions related to the best post-surgical outcome and ameloblastoma patient wellbeing.

In the Helsinki University Hospital (HUS) district, roughly five patients are diagnosed with ameloblastoma annually. The tumor is often identified as an incidental radiologic finding. Through advanced imaging technologies, including magnetic resonance imaging (MRI) and computed tomography (CT), rather accurate preoperative estimations of the nature of the tumor can currently be determined. The etiology of this benign but locally aggressive tumor has not been completely established, although recent discoveries of the genetic background—particularly the mutation of the *BRAF V600E* and *SMO* genes—have provided an understanding of the biology regulating tumor progression, even leading to investigations of treatment modalities targeting these genes. Besides events occurring in the tumor itself, extracellular and inflammatory events have emerged as important factors impacting tumorigenesis.

Finnish ameloblastoma patients have not been widely studied. Therefore, this thesis focused on investigating a Finnish ameloblastoma patient cohort treated at HUS from 1986 through 2016, consisting of a total of 36 cases. We were interested in the demographic parameters characterizing Finnish ameloblastoma patients, BRAF expression via immunohistochemistry, and if our results correlated with previous studies and could possibly prove important in clinical practice. The imaging findings of ameloblastoma from CT and MRI were of particular interest. We were also interested in identifying different proteins (MMP-7, MMP-8, MMP-9, E-cadherin, and beta-catenin), which could be beneficial in ameloblastoma diagnostics and prognostics, and possibly explain the biological nature of this rare tumor.

In this thesis, the literature review consists of a broad overview of ameloblastoma research completed to date. I also present the results of our studies and conclude by discussing the implications of our findings.

2. LITERATURE REVIEW

2.1. Ameloblastoma

2.1.1. The origin of ameloblastoma is in tooth development

Events during tooth development explain the origin of ameloblastoma, a tumor consisting of ameloblast-like cells resembling those cells that form the tooth enamel in normal physiology. Tooth development begins during fetal development as the sequential and reciprocal interactions between neural crest-derived mesenchymal cells and stomodeal epithelial cells.[1] As these primordial cells condense, the tooth germ is formed containing the enamel organ, the dental papilla, and the dental sac, or, in other words, the tooth follicle.[2] The enamel organ forms an outer enamel epithelium, an inner enamel epithelium, a stellate reticulum, and a stratum intermedium (Figure 1).[3] Enamel-producing ameloblasts derive from these cells. A reduced enamel epithelium resides after the enamel has matured.[4] The sequential and reciprocal interactions between ectodermal and mesenchymal tissues regulate tooth morphogenesis. During tooth development, the cells of the inner enamel epithelium (IEE) elongate to form pre-ameloblasts. As dentin formation begins, marked by the deposition of type I collagen, the presecretory ameloblasts dramatically elongate, followed by a loss of the basement membrane, as indicated by an interruption to type IV collagen secretion.[5] At this transition from presecretory ameloblasts to secretory ameloblasts, the cells appear to come into physical contact with dentin matrix proteins.[5] The ameloblasts then shorten again and begin to secrete enamel matrix proteins.[5] Without these interactions, pre-ameloblasts do not develop into full secreting ameloblasts, resembling the cells of ameloblastoma.[3] Signal molecules of conserved families, such as transforming growth factor β (TGFβ), fibroblast growth factor (FGF), Hedgehog, and Wingless-related integration site (Wnt) families, mediate cell interaction throughout tooth development.[1] The genes regulated by these signal molecules differentiate the cells to react to new signals, to perform reciprocally, and, thereby, to enable and ensure communication between different cells and tissues.[1,3] Gene expression profiling analyses indicate that ameloblastoma associate with pathogenic mitogen-activated protein kinase (MAPK), sonic hedgehog (Shh), Wnt, and other pathways, which are normal regulators in tooth development. Ameloblastoma resembles

the enamel organ of a developing tooth with no intention of forming enamel or dentin since the properties of a dental mesenchyme are missing.[6]

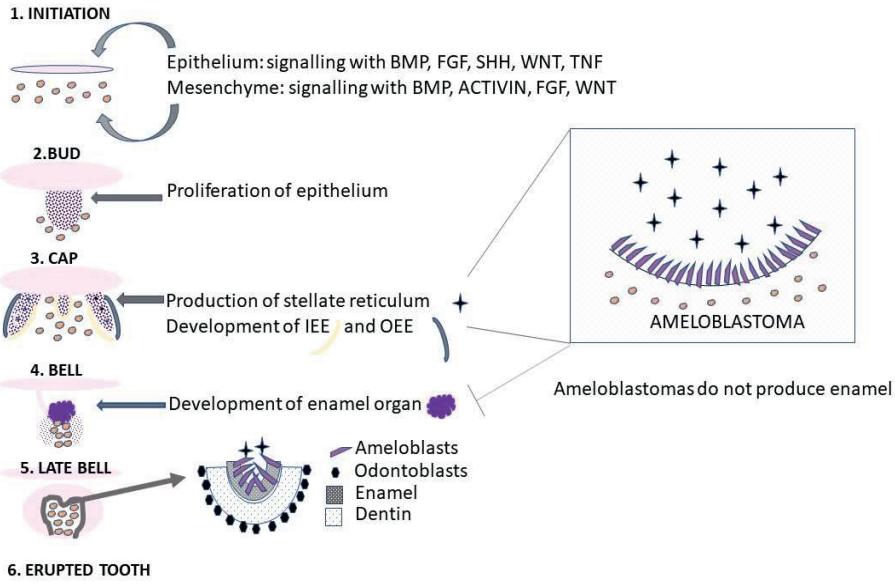

Figure 1. The five stages of tooth development and an example of ameloblastoma histopathological features that resemble, in part, the embryologic pattern seen in a developing tooth. The enamel organ and tooth development is roughly divided into the initiation stage, the bud stage, the cap stage, the bell stage, and maturation.[2] Initiation: epithelial thickening invaginates into the underlying mesenchyme to form a bud. Cap stage: the enamel knot and the enamel organ evolve, and between the inner and outer enamel epithelium a stellate reticulum is formed consisting of star-shaped epithelial cells. Bell stage: ameloblasts differentiate from the inner enamel epithelium and odontoblasts from the dental mesenchyme (dental papilla). Abbreviations: BMP, bone morphogenetic protein; FGF, Fibroblast growth factor; IEE, inner enamel epithelium; OEE, outer enamel epithelium; SHH, sonic hedgehog; TNF, tumor necrosis factor; WNT, Wingless-related integration site. Modified from illustrations from Thesleff (2003) and Diniz (2017).

2.1.2. Incidence and demographics

Ameloblastomas are benign, albeit locally aggressive odontogenic tumors with a high tendency to recur. Although it is the most common odontogenic tumor, ameloblastoma is rare, annually affecting approximately 0.5/1 million population.[7] It accounts for 1% of all jaw tumors and cysts, and 13% to 78% of odontogenic tumors.[8,9] Ameloblastomas primarily involve the jaw bones, but can also grow peripherally on the oral mucosa.

Ameloblastomas occur in patients of all ages. Two large meta-analyses with 10 123 ameloblastoma cases, showed a mean age of 34.3 to 35.9 years at the time of primary diagnosis. In Europe, North America (fourth or fifth decade of life), and Asian (fifth decade of life), ameloblastoma seemed to occur later in life when compared to patients in Africa and South America (third decade of life).[10,11]

The median age varies in different types of ameloblastomas. Unicystic ameloblastoma is diagnosed on average at the age of 30, often peaking during the first and second decades.[12–14] Maxillary ameloblastomas seem to occur primarily in the fifth decade.[11,15,16] Interestingly, female patients are typically younger than male patients at the time of diagnosis.[11] Only a slight male predominance emerged from these studies (male:female, 1.13:1).[10] Since ameloblastoma is a rare tumor, our studies contribute to knowledge of ameloblastoma and more precisely describe a northern European patient cohort, not vastly studied previously.

The World Health Organization (WHO) tumor classification (2017) divides ameloblastoma into conventional, unicystic, and peripheral ameloblastomas. Conventional ameloblastomas are solid, cystic, or multicystic lesions composed of islands of the odontogenic epithelium with peripheral columnar differentiation and a reversely polarizing nucleolus. The island centers resemble the stellate reticulum of the developing tooth, and cystic degradation is common.[17] In unicystic lesions, ameloblastoma growth may be intraluminal or intramural. Peripheral ameloblastomas grow on the oral mucosa, but histologically cannot be distinguished from their intraosseous counterparts (Figure 2). Our studies provided information on how the different types were distributed in a Finnish patient cohort.

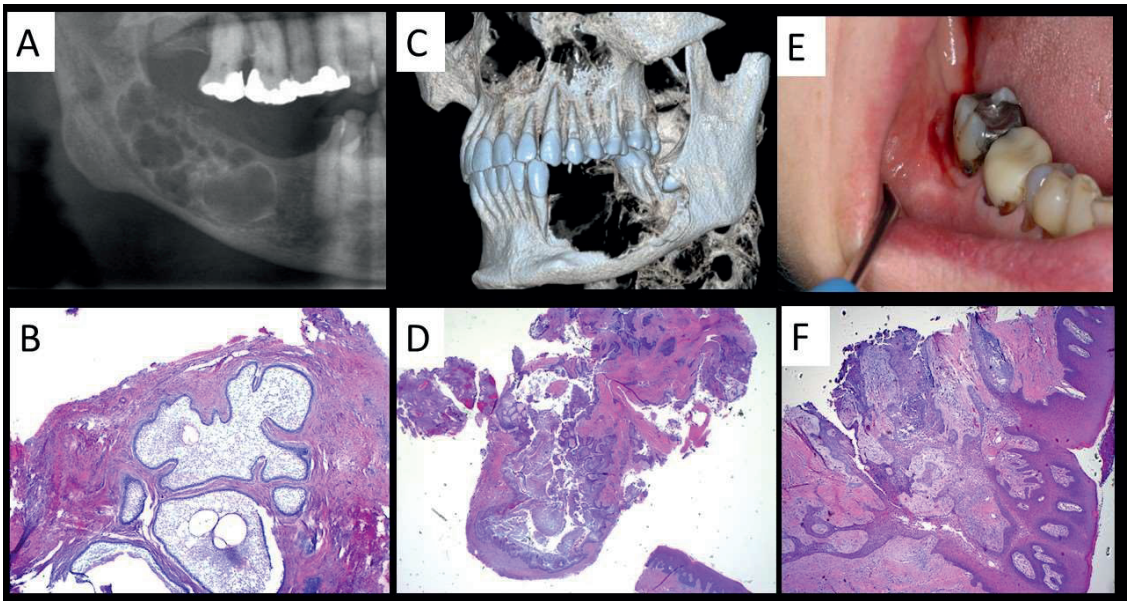

Figure 2. In radiology, a conventional ameloblastoma may present a multilocular radiolucency (A). In histology, this presents as islands of ameloblastoma with central cystic changes (B). An unicystic ameloblastoma occurs as a single cavity (C). In histology, the tumor growth can be mural, as seen in the stromal wall or luminal, growing into the cystic space (D). In the peripheral ameloblastoma, the tumor develops on the soft tissue of the oral cavity (E). In histology, a normal ameloblastoma morphology can be seen, often under a normal oral epithelium (F). Case courtesy of Assoc Prof Frank Gaillard, Radiopaedia.org, rID: 2577 (A), case courtesy of Dr Ian Bickle, Radiopaedia.org, rID (C): 23670, CC BY-SA 3.0 (D), PhD, DDS Jaana Willberg (E).

2.1.3. Histology

Ameloblastomas feature various histological growth patterns (Figure 3). Follicular ameloblastomas form tumor islands with stellate reticular centers and cystic degradation. Plexiform ameloblastomas consist of anastomozing long cords and sheets of odontogenic epithelial tumor growth within a loose, vascularized stroma. Unlike follicular ameloblastomas, the cystic changes rarely seen in plexiform ameloblastoma are typically caused by stromal degradation. If squamous cell metaplasia occurs, the term acanthomatous ameloblastoma is applied. Granular cells are seen in granular cell ameloblastomas. Basaloid cell nests with cuboidal rather than columnar cells around the nests are observed in basal cell ameloblastomas. Desmoid ameloblastomas grow as small islands and cords in a tight collagen-rich stroma.[17] The dominant variant determines the growth pattern in the majority of studies, although diversity is often ignored. Therefore, the variation in growth patterns came as a surprise when first reviewing the histology. We were particularly interested in the specific histology in our studies and wished to raise this matter for discussion.

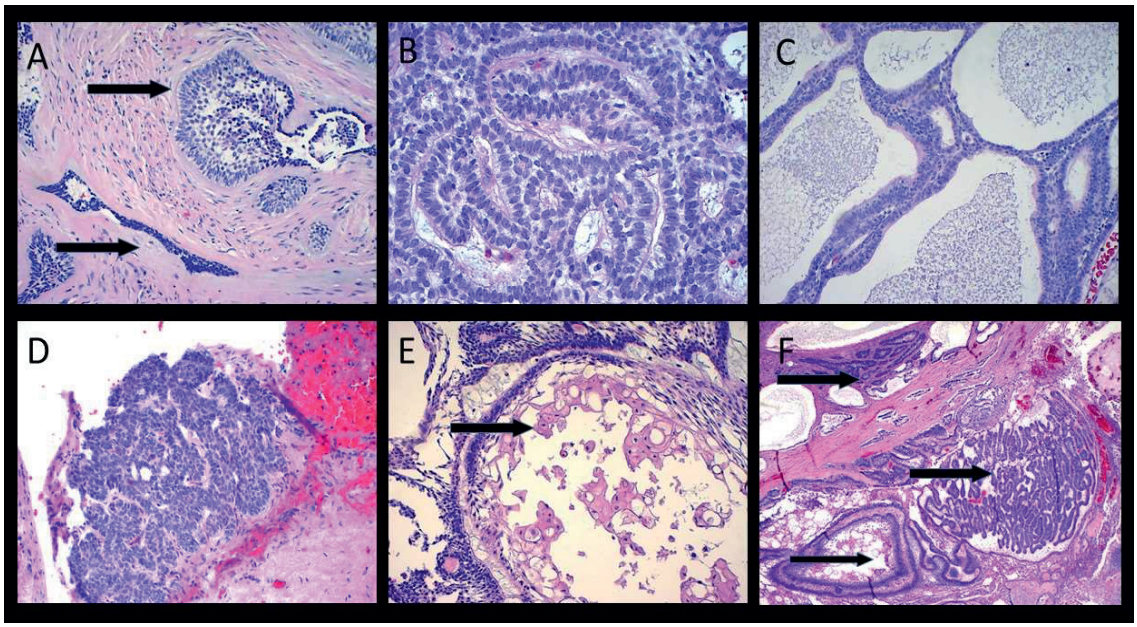

Figure 3. Different growth patterns in histology. Follicular ameloblastoma (A, upper arrow), desmoplastic ameloblastoma (A, lower arrow). Plexiform ameloblastoma (B and C). Basaloid ameloblastoma (D). Granular cell ameloblastoma (E). Ameloblastoma with various growth types (F): upper arrow, follicular ameloblastoma; middle arrow, plexiform ameloblastoma; and lower arrow, acanthomatous ameloblastoma.

2.1.4. Malignant ameloblastomas

2.1.4.1. Metastasizing ameloblastoma

Ameloblastoma can metastasize retaining its original benign histology,[7] although it is rare, with only 27 to 64 cases reported.[18–20] For instance, Van Dam et al.[18] described metastasizing ameloblastomas as late events, occurring on average 18 years after the initial surgery. The primary tumor is more frequently located in the mandible and often a multicystic or solid type.[7] A metastasizing ameloblastoma is associated with multiple recurrences and with insufficient primary excision margins.[21] Interestingly, histology appears not to predict the potential of ameloblastoma to metastasize, although Van Dam et al.[18] noted four cases with a granular cell histology[21]. Metastasis has been detected in the lungs, lymph nodes, and bone,[7] with the spread most likely hematogenous.[21] These patients might suffer from paraneoplastic hypercalcemia. Metastatic ameloblastomas with severe atypia represent ameloblastic carcinomas.[7] Furthermore, the 5-year survival rate reaches

approximately 70%, although that depends significantly on the site and surgical accessibility of the metastasis.[7] Adamantinoma, a tumor with an ameloblastoma-like histology, primarily occurs in the tibia and other small bones, and must not be mistaken as a metastasizing ameloblastoma[22]. Acknowledging the potential of this tumor to metastasize beyond frequent recurrences justifies radical surgery, as discussed below (Radical and conventional surgery).

2.1.4.2. Ameloblastic carcinoma

Ameloblastic carcinoma, the malignant counterpart to ameloblastoma, is rare and typically arises in the posterior mandible.[7] It may present as a primary tumor or develop from an existing ameloblastoma. [7] In radiology, ameloblastic carcinoma shows a radiolucency with poorly defined or irregular margins, cortical expansion, and perforation into the proximate structures.[7] In histology, a loss of an organized stratification, pleomorphism, a higher nuclear–cytoplasmic ratio, hyperchromatism, increased mitotic activity, atypical mitosis, and vascular or perineural invasion may be present.[7] Necrosis can help distinguish between a malignant and benign tumor. Differential diagnosis includes odontogenic carcinosarcoma or sarcoma if spindle cells are present. In addition, a BRAF mutation may be present. One-third of these patients develop lung metastasis while maxillary tumors accompany poorer outcomes.[23] In the USA, the overall incidence of metastasizing ameloblastoma and ameloblastic carcinoma reaches 1.79 cases per 10 million.[7] Ameloblastic carcinoma or other carcinomas (mucoepidermoid carcinoma or primary squamous cell carcinoma of the lung) must be suspected in the case of fast tumor growth and widespread metastasis.[18] Ameloblastic carcinomas require a more aggressive treatment protocol than a metastasizing ameloblastoma, which might have a rather indolent growth potential.[18] This thesis does not address malignant ameloblastomas, although this topic is important to briefly address on a general level. Since ameloblastic carcinoma may develop in an existing ameloblastoma, it is noteworthy that a biopsy identifying an ameloblastoma might leave out a component of an ameloblastic carcinoma.

2.1.5. Clinical features and challenges

Ameloblastoma initially presents as a harmless tumor and, often as an incidental radiological finding. As the tumor grows, the affected bone expands and causes intraoral or extraoral bulging. Tooth mobility, malocclusion, ulcerations, and secondary infections may appear. Pain is rarely mentioned and occurs in cases of secondary infection or nerve compression due to tumor pressure. Although ameloblastoma is a benign tumor, it might grow aggressively causing bone destruction. When surgery has been postponed for various reasons, tumors measuring up to 40 to 50 cm have been reported.[24] Ameloblastomas in the maxillary or sinonasal region might grow freely in the sinus cavities before resulting in any symptoms which can include, for example, nose congestion, epistaxis, or bone expansion. Only a few publications have described the subjective symptoms and objective signs of ameloblastomas. Thus, we sought to inventory the variety of symptoms and signs present in clinical investigations.

2.1.6. Current treatment modalities

After clinical examination and thorough radiological investigations, diagnosis is verified with a biopsy. An ideal sample includes sufficient tumor tissue for a correct diagnosis. In general, an insufficient biopsy is a waste of resources, money, and effort from the patient, clinician, technical staff, and the pathologist, while also, most importantly, delaying treatment. Choosing the right treatment modality depends on the tumor site, ameloblastoma type, and the patient's age. An ongoing debate exists on adequate surgical procedures. Ultimately, the goal is to achieve a tumor-free patient with as low recurrence potential as possible. Treatment modalities are always weighted individually based on the patient's age, condition, and the location of the tumor, while respecting the patient's wishes and expectations. Approximately 8.7% to 15% of ameloblastomas occur in children in Western countries and 14.6% to 25% in children from Asian or African countries.[25–29] When treating children, clinicians should carefully consider the ameloblastoma type, the cystic type subdivision, the tumor size and location, patient age, the child's wishes, compliance and

comprehension, projected recurrence, physical and mental impact, and the possibility of more advanced materials and surgical techniques available in the future.[30]

2.1.6.1. Radical and conventional surgery

According to the literature, unicystic ameloblastomas can be treated conservatively with curettage, enucleation, and cryosurgery. Ameloblastoma can reach the cancellous bone at a mean of 4.5 mm but up to 8 mm beyond the radiological boundary. Therefore, a radical approach to nonunicystic ameloblastomas through segmental or marginal resections with tumor-free margins is the preferred treatment modality to prevent recurrence.[31–34] An accurate description of the ameloblastoma type, growth pattern, and radiological diagnoses is needed to determine the best treatment option. The longer the tumor persists, the more frequently recurrence occurs; and the more conservative treatment procedures the patient has undergone, the greater the risk of malignant transformation.[35,36]

In cases of solid or multicystic ameloblastomas, radical surgery (marginal or segmental resection) is preferred, requiring a plate reconstruction or considerable reconstructive surgery.[37–39] Following mandibular reconstruction, oral function rehabilitation is facilitated using dental implants and restorative dentistry. Primary and recurrent peripheral ameloblastomas are excised through peripheral ostectomy. Peripheral ameloblastomas rarely recur following conservative surgery.[40]

2.1.6.2. Targeted therapy

ErbB-1 monoclonal antibodies (cetuximab and panitumumab) and erbB-1 tyrosine kinase inhibitors (erlotinib, gefitinib, and AG1478), which are MAPK targets, have been tested in ameloblastoma cells in vitro, resulting in tumor growth suppression, although resistance to erbB-1 inhibition occurred in BRAFV600E ameloblastoma cells.[41] Individual clinical reports have been published supporting BRAF or BRAF/MEK inhibitors to treat ameloblastoma patients harboring a BRAF mutation. These patients have either had multiple recurring tumors, lung metastasis,

comorbidities, or other reasons that excluded surgery as a treatment option. Tumor size reduction has thus far proved impressive, but further investigation is needed to follow-up regarding drug resistance and side effects.[31,39,42] One important long-term complication associated with these drugs includes an increased chance of developing squamous cell carcinomas.[43]

2.1.7. Recurrence

Ameloblastomas have a high tendency to recur. A meta-analysis by Hendra et al.[44] found a pooled recurrence rate of 8% for ameloblastomas treated radically and 41% when treated conservatively. The respective values for unicystic ameloblastoma recurrence were 3% and 21%.[44] They also found that conventional ameloblastomas recurred more often than unicystic ameloblastomas despite treatment modality, indicating a more aggressive behavior among ameloblastoma compared to unicystic ameloblastomas.[44] Current thinking indicates that a segmental resection with sufficient, healthy margins is the predominant choice for treatment regardless of type.[44]

2.2. Imaging

Radiology is crucial for tumor diagnosis, preoperative planning, postoperative assessment, and post-treatment follow-up. Modern imaging techniques provide information on the size, location, infiltration, specific characteristics of the tumor composition, and the sites of vital structures near the tumor, that is, the brain, large veins, or nerves that need to be handled carefully. Although ameloblastoma is the most common odontogenic tumor, other bone lesions with similar radiological features including a radicular cyst, dentigerous cyst, and keratocyst occur more frequently. Jawbone radiolucency is, therefore, usually primarily considered as one such cyst before more precise examinations with computer tomography (CT) or magnetic resonance imaging (MRI) are conducted, and the preoperative biopsy has proven otherwise. Dentigerous cysts do not reach the size of ameloblastomas or keratocysts, which are indistinguishable from each other when the lesion is unicystic in form.[45] Yet, a small dentigerous cyst is indistinguishable from a small unicystic ameloblastoma.

An ameloblastoma, as mentioned, is often detected during routine intraoral or panoramic radiographic examinations. Ameloblastoma typically appears as an unilocular or multilocular radiolucency with smooth or scalloped cortical borders. Thin, trabecular strands piercing the radiolucency might be seen. An impacted third molar, root, or tooth displacement, or sharp root resorption are typical findings in ameloblastoma. These features are nevertheless not pathognomonic for ameloblastoma. Using CT scanning, three-dimensional observation is possible and bone expansion, cortical thinning, and soft tissue involvement can be evaluated and malignancy ruled out.

MRI provides the means to evaluate tumor growth near the skull base, orbit, and paranasal sinuses. It is particularly useful in distinguishing between desmoplastic ameloblastoma and a fibro-osseous lesion, which might result in significantly different treatment modalities.[46] Prior to study II (see Original articles, Study II, p. 89) summarized here, no evaluations have examined the means of differentiating ameloblastoma using CT and MRI.

2.3. BRAF

Trauma, poor nutrition, inflammation or oral infections, and irritation from tooth extractions were previously considered triggers for ameloblastoma.[47] The discovery of remnants of the migrating epithelium at the cervical loop of the enamel organ directed ameloblastoma etiological investigations in a new direction.[17] These findings were further supported by the expression resemblance of cytokeratin and vimentin between developing tooth germ and ameloblastoma.[47]

To date, studies on ameloblastoma tissues, cell lines, and transgenic mice relied on the development of ameloblastoma to the dysregulation of the MAPK pathway (Figure 4). BRAF, KRAS, NRAS, HRAS, and FGFR2 mutations accompany most ameloblastomas, along with several non-MAPK mutations, including SMO, SMARCB1, CTNNB1, and PIK3CA.[47] Specifically, a mutation in the BRAF-gene transcript, a serine/threonine-protein kinase activating the MAPK/extracellular signal-regulated kinase (ERK) signaling pathway, exists in over 63% of ameloblastomas.[41,48,49] Mutations in non-MAPK signaling genes, such as smoothened (SMO), an effector component of the *SHH* signaling

pathway, is a developmental factor in maxillary ameloblastomas.[48,49] As scientific research techniques improve, novel insights on etiological factors are sure to emerge.

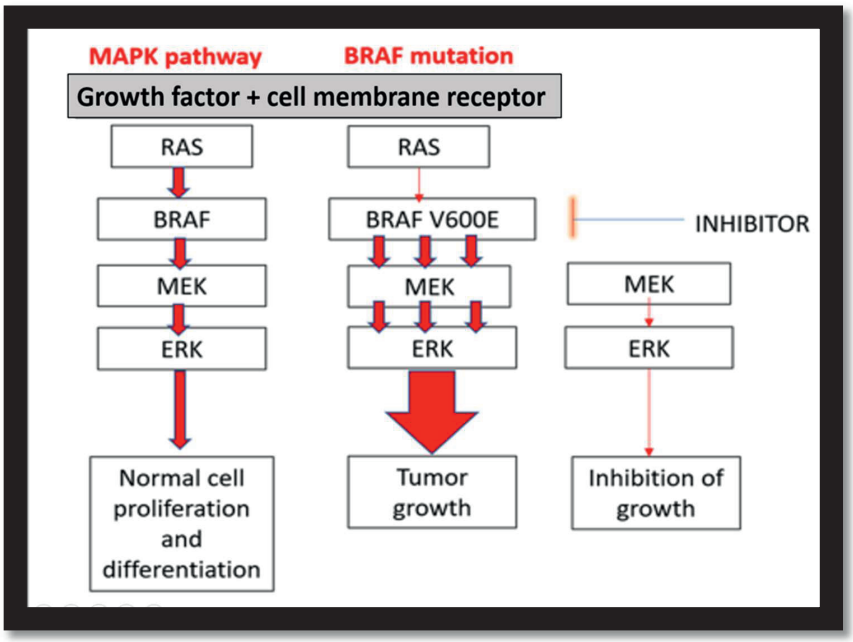

Figure 4. Normal MAPK pathway results in normal proliferation and differentiation. In mutations, BRAF no longer requires dimerization with RAS, causing a constant signal activation. Inhibition of the BRAF mutant pathway can be achieved, for example, using Vemurafenib, which causes the silencing of the downstream activation of the MAPK pathway and a decrease in the cell proliferation, inducing apoptosis. Abbreviations: ERK, extracellular signal–regulated kinase; MAPK, mitogen-activated protein kinase; RAF, rapidly accelerated fibrosarcoma; RAS, rat sarcoma virus homolog. Modified from an illustration in Swaika et al.[50]

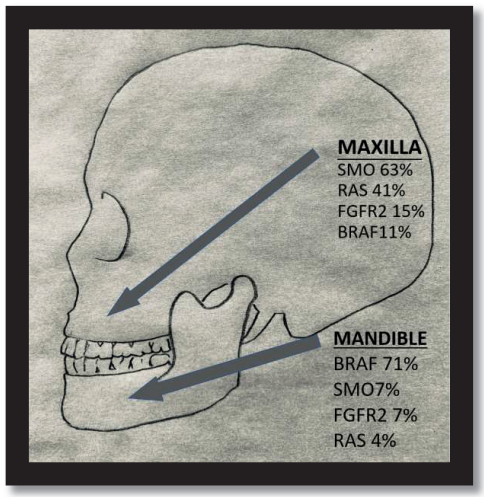

Figure 5. Most common mutations found in ameloblastomas. Modified from a figure in McClary et al. [35]

Brown et al. [48] showed that somatic *FGFR2–RAS–BRAF* mutations exist in most cases of ameloblastoma. Somatic mutations in *SMO*, *CTNNB1*, *PIK3CA*, and *SMARCB1* might operate as secondary mutations. [48] The most common mutation, *BRAF* V600E, associated with a younger age. [48] *BRAF* wild-type ameloblastomas arose more often in the maxilla and recurred earlier.[48] At approximately the same time, Sweeney et al.[49] found similar results to Brown et al. They discovered highly recurrent somatic mutations in the Hedgehog and MAPK pathways. Mutations in SMO (a seven-transmembrane Hedgehog signal transduction component; 10 encoding p.Leu412Phe and 1 encoding p.Trp535Leu) occurred in 39% (11/28) of ameloblastomas, whereby 46% (13/28) had BRAF mutations (12 encoding p.Val600Glu and 1 encoding p.Leu597Arg) and tended to be mutually exclusive suggesting two separate genetic etiologies. A mutation to BRAF was mutually exclusive with mutations in KRAS and FGFR2 in all but one case.[49] Figure 5 illustrates the most common mutations and locations (maxilla/mandible) found in ameloblastomas. Speculations exist on whether immunohistochemistry provides sufficient specificity on the mutational profile of the tumor. Since the Finnish population has been genetically isolated, it was interesting to see how BRAF expresses immunohistochemically in Finnish ameloblastomas.

2.4. Extracellular matrix and MMPs

Interest has increased in the ability of the extracellular matrix (ECM) to orchestrate tumor growth and integrity. Histologically, an ameloblastoma is often surrounded by a tight collagen-rich stroma, with fibroblasts and an alternating infiltration of inflammatory cells including lymphocytes, plasma cells, neutrophilic granulocytes, eosinophils, and sometimes multinuclear giant cells. Bone may be seen surrounding the tumor, but not between the tumor bulk. An ameloblastoma, in other words, destroys the bony structure in a “pushing border” manner, but does not invade the bone.

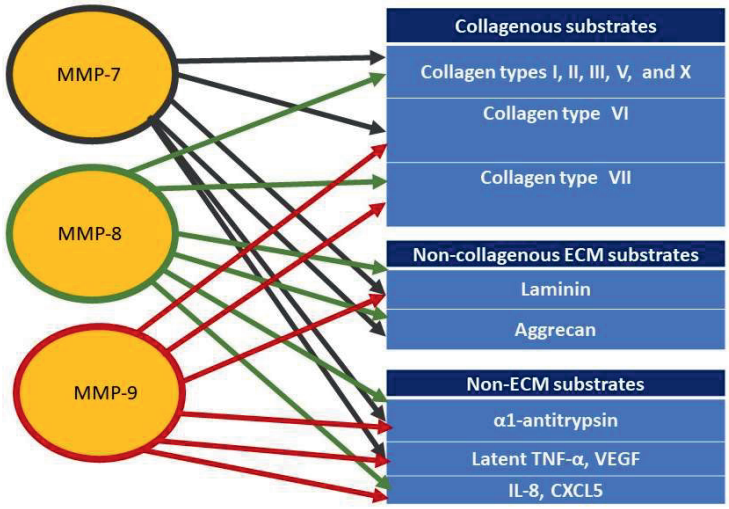

Table 1. demonstrating overlapping substrates of MMP-7, -8, and -9. Modified from a table in Djuric and Zivkovic (2017) [51]

The matrix metalloproteinase (MMP) family consists of 23 zinc-dependent proteinases that have a wide spectrum of overlapping biological substrates (Table 1) capable of disrupting various structures from the ECM. [52] The MMP family is divided into collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs, and other non-classified MMPs.[53]

MMPs are produced as pro-MMPs, or latent enzymes that inflammatory cells deposit. They can also be secreted and attach to cell membranes or membrane proteins or within the ECM. Pro-MMPs are proteolytically activated into MMPs, where a propeptide domain is released. Activation is regulated by endogenous tissue inhibitors. [53] Substrates for MMPs include proteinases, proteinase inhibitors, chemotactic agents, clotting factors, growth factor–binding proteins, latent growth factors, cell–cell and cell–matrix adhesion molecules, and cell surface receptors. [54] MMP function regulation is tightly controlled, occurs at multiple levels, and is associated with specific inflammatory, connective tissue, or epithelial cell types. [54] Normally, MMP transcripts are expressed at low levels, but these levels elevate quickly in situations like inflammation, wound healing, or cancer when tissues undergo remodeling. [55]

Inflammatory and stromal cells form MMPs in response to chemokines and cytokines that are likewise produced by inflammatory cells in tumor microenvironments. [55] MMPs can, in response, both stimulate or weaken tumor progression by releasing chemo-attractant, growth-promoting, and cytostatic signals. [56,57] MMPs might induce angiogenesis, but also fabricate basement-membrane collagen particles and plasminogens that inhibit angiogenesis and participate in apoptotic and anti-apoptotic actions. [56] In part, the actions of MMPs may be inhibited by anti-inflammatory actions, but trials testing MMP inhibitors have exhibited mixed results. [53,58,59] Some examples of synthetic inhibitors that underwent clinical trials include nonpeptidic molecules, synthetic peptides, bisphosphonates, and chemically modified tetracyclines. [52] The immunohistochemical expression of various MMPs have been studied on ameloblastomas, although results remain contradictory and, based on our estimation, the interpretation of results has varied.

2.4.1. MMP-7

MMP-7, also known as matrilysin, is a highly potent protease that degrades laminin, casein, fibronectin, type I/II/IV/V gelatins, collagen III/IV/V/IX/X/XI, proteoglycans, and elastin. [57,60] In normal tissue biology, MMP-7 is secreted by glandular epithelial cells, but overexpresses in various cancers. [61] Increased levels of MMP-7 mRNA appear to correlate with dedifferentiation and metastasis in colorectal cancers. [62] Decreased tumor incidence was observed in antisense RNA-mediated MMP-7 knockdown colorectal cancer cell lines and in MMP-7 knockout mice. [63-65] Only a few studies have examined MMP-7 expression in ameloblastomas. [66]

2.4.2. MMP-8

MMP-8, also known as collagenase-2 or neutrophil collagenase, is an important mediator in inflammation. It is released from polymorphonuclear neutrophils (PMNs), macrophages, epithelial cells, and fibrocytes, and cleaves, for example, triple-helical type I–III collagen, many ECM and non-ECM substrates, basement membrane proteins, and a1-antitrypsin. [67] Gingivitis, periodontitis, and other inflammatory conditions have increased active MMP-8 levels. Yet, MMP-8 functions to

suppress inflammation, for example, in osteoarthritis and neuro-inflammation. [68,69] In cancer, a high level of MMP-8 predicted a better outcome in tongue and some breast cancer patients, but a worse prognosis in, for example, ovarian and hepatocellular cancers. In colorectal cancer, survival seemed to vary considerably in relation to MMP-8 levels. [70] In skin and breast cancers, MMP-8 slows the metastatic process both in vivo and in vitro, explaining why patients benefit from MMP-8. [70] Many cancer treatments affect MMP-8 levels, which may cause changes in systemic inflammation. [70] In most cancers, the estimation of dangerous reactions to MMP-8 treatments remains unexamined, although it might be useful in cancers in which MMP-8 has been shown to play a role. The use of MMP-8 as an adjuvant in cancer drug treatment should be further investigated, according to Juurikka et al. [70] To our knowledge, the expression of MMP-8 in ameloblastomas and its possible participation in its locally aggressive nature has not been studied.

2.4.3. MMP-9

MMP-9, also known as gelatinase B or 92 kDa type IV collagenase, plays a general role in the degradation of ECM within a wide scale of physiological and pathophysiological events requiring tissue remodeling. MMP-9 is primarily known for the degradation of IV collagen, the basement membrane, and gelatin, [71] playing a crucial role in immune cell function. [72] MMP-9 is upregulated during development and wound healing and in pathophysiological conditions such as arthritis, diabetes, and cancer. [73] Solid-tumor expansion activates a wound healing response, where stromal fibroblasts differentiate into smooth muscle actin–expressing myofibroblasts partly in response to transforming growth factor β (TGFβ).[74] Syamala et al. [75] suggest, based on their study of myofibroblasts in odontogenic cysts and tumor environments, that when the amount of myofibroblast increases in the stroma, more aggressive behavior from the lesion can be anticipated. Specifically, they found that ameloblastomas and keratocysts presented with significantly more stromal myofibroblasts than dentigerous cysts, which are less aggressive in nature.[53,75] Dayer and Stamenkovic [74] suggest that the presence of MMP-9 also represents a prominent factor activating fibroblast differentiation in myofibroblasts. In pathological conditions, MMP-9 proteolytic actions

cause the immune response to initiate pathogenesis and intensify disease progression. [71] MMP-9 is also expressed in tooth germ mesenchymal cells and believed to control basement membrane remodeling during tooth development and participate in dentin mineralization and odontoblast differentiation.[76,77]

2.5. Cell adhesion molecules (CAMs)

Cell adhesion molecules (CAMs) are cell surface structures permitting dynamic action throughout tissue morphogenesis, development, and maintenance of adult epithelial tissues.[78] CAMs take part in cell regeneration and mobility, and are essential in cell-to-cell junctions and cell-to-ECM interactions.[79] These structures function in signaling, signal transduction processes, and cell division, migration, and differentiation as well as in dentinogenesis.[80] Signaling pathways regulate the expression of CAMs and the duration of adhesive contacts, and, therefore, control tissue integrity and stability.[78] Dysregulation may lead to tumors, and aid in tumor progression, recurrence, invasion, and metastasis[78] through the loss of cell adhesion, ECM degradation, increasing cell motility, and the ability to invade tissue.[81]

2.5.1. Beta-catenin

Beta-catenin is a component of the cell-to-cell adhesion structure regulating adhesion proprieties. As such, beta-catenin plays a role in the Wnt canonical pathway, and, thus, can regulate important operations such as cell proliferation, cell polarity, and cell fate determination in embryonic development and later in normal tissue homeostasis.[82] When dysregulated, beta-catenin might contribute to tumorigenesis and tumor progression.[83] Various studies of ameloblastomas indicate that beta-catenin expresses in the cytoplasm, cell membrane, and the stellate reticulum resembling cells.[78] However, the nuclear expression of beta-catenin was observed primarily in the solid ameloblastomas and odontogenic carcinomas.[83–85] The nuclear expression or nuclear accumulation of beta-catenin indicates an abnormal Wnt signaling and might relate to tumorigenesis and cell proliferation in ameloblastomas as well.[78,83,85] It remains unclear if the adhesive and signaling

functions of beta-catenin are interrelated. Presumably, newly synthesized beta-catenin primarily saturates the adhesion junction pool, which is unavailable for signaling.[86] Remaining free cytoplasmic beta-catenin protein might then be effectively degraded by an adenomatous polyposis coli (APC) complex.[86] It might be that this highly unstable pool is subject to regulation via Wnt signals.[86] These two functions of beta-catenin are presumed to independently act through two separate beta-catenin homologs in C. elegance.[87] The use of beta-catenin in providing information related to prognosis in ameloblastomas has not, to our knowledge, been examined, and we were interested in whether nuclear positivity could provide a correlation to the course of the disease, and if so, could these results be beneficial in diagnostics.

2.5.2. E-cadherin

Cadherins are cell membrane molecules that form cell-to-cell adherens junctions and communicate with different intracellular processes.[88] Cadherins are classified in E (epithelial), P (placenta), M (muscle), N (nerve), B (brain), and R (retina) cadherins, and combine with α-, β-, and γ-catenins.[88] The presence of cadherins in odontogenesis has promoted the investigation of cadherins in ameloblastomas.[84,89] Although the hypermethylation of an E-cadherin promoter represents an important factor in malignant transformations in carcinomas,[90] this process might not be associated with tumor progression in ameloblastomas.[91,92] Yet, the loss of E-cadherin expression could be associated with tumor advancement in ameloblastoma.[93] E-cadherin expression in well-differentiated tumors can be interpreted as the conservation of adhesion between tumor cells and the tissue architecture, associating with a better patient prognosis. However, in poorly differentiated tumors, E-cadherin expression was diminished, suggesting a loss of adhesions between the tumor-forming cells. Still, in a study by Kumamoto et al.,[93] one case of malignant ameloblastoma preserved E-cadherin and a-catenin expressions, while another case showed an obvious reduced expression. This suggests that the reduction of E-cadherin expression may be associated with malignant progression or the metastatic potential in some epithelial odontogenic tumors.[93,90] Yet, this study did not describe any intensity variations or differences between clinical parameters in ameloblastomas.

3. AIMS

This study aimed to investigate the clinical, radiological, and pathological features of ameloblastoma patients treated at Helsinki University Hospital (HUS).

The specific aims were as follows:

- To study the demographic characteristics of Finnish ameloblastoma patients and compare the results to patient cohorts from previous studies carried out in different countries (Study I).
- To determine whether CT, contrast CT, or MRI provide substantial benefits for the differential diagnostics in imaging vis-à-vis ameloblastoma (Study II).
- To examine the BRAF immunohistochemistry expression status in our HUS ameloblastoma patients and compare the results to clinical parameters (Study III).
- To determine if MMPs could be used as predictive prognostic tools in clinical practice and if beta-catenin or E-cadherin could predict outcomes (Study IV).

4. MATERIALS

We used the HUSLAB pathology department's Q-pati database system to identify all patients diagnosed with an ameloblastoma and treated at HUS during the period from 1985 through 2016, identifying at total of 64 patients (Figure 6). We retrieved all clinical data available. Patient cases missing histological, formalin-fixed paraffin-embedded blocks were removed from our sample, leaving us with a final cohort of 34 patients. In studies III and IV, we included six cases of recurrent ameloblastoma, treated primarily elsewhere.

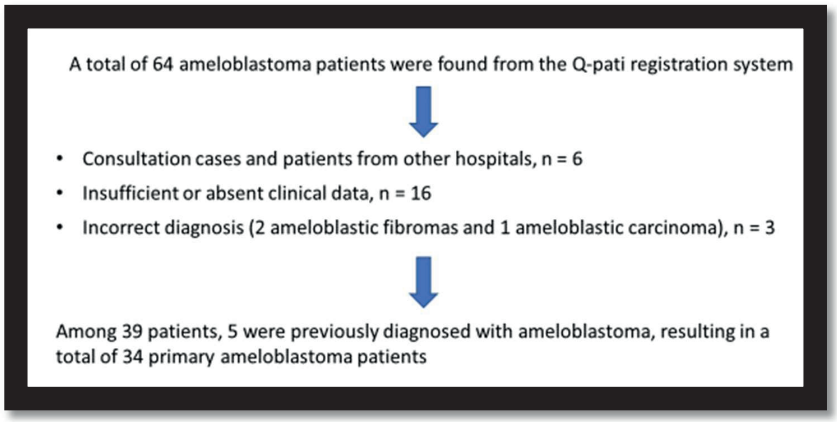

Figure 6. illustrates the inclusion criteria of patient cases in our studies.

We recorded the following demographic and clinical parameters: gender, age, subjective symptoms (i.e., experienced by the patient), preoperative clinical signs (i.e., observed by the clinician), size, and location of the tumor (maxillary or mandibular). Further classification included the following subgroups: region of incisors or canines (anterior), premolar, molar (posterior), or gingival. Potential recurrences were also recorded.

CT and MRI images were obtained from the HUS Medical Imaging Center archives for 27 patients and examined by an experienced oral radiologist on a Dome E2 grayscale display with a resolution of 1200 x 1600 pixels.

All ameloblastomas were primarily treated radically at HUS if the biopsy was proven to be an ameloblastoma.

5. METHODS

Tissue samples were microscopically re-evaluated for the tumor type and growth pattern. Ameloblastoma type was classified as solid/multicystic, unicystic, or peripheral. The growth pattern classification included follicular, plexiform, or mixed (i.e., variations of follicular, plexiform, acanthomatous, desmoplastic, and granular cell patterns). Information concerning the tumor size (mm) was collected from radiographic statements and, if unavailable, from the recorded pathological diagnosis. Representative samples were chosen for immunohistochemical staining. A new set of hematoxyline-eosine (HE) stained tissue samples were prepared.

5.1. Immunohistochemistry

For study III, formalin-fixed, paraffin-embedded, 3-µm-thick tissue sections were immunostained using a Ventana BenchMark XT immunostainer (Ventana Medical Systems, Tucson, Az, USA) with Ms Anti-BRAF V600E (VE1) Mab, Spring Bioscience diluted to 1:1500 and visualized using OptiView DAB IHCv3 (Ventana) with amplification. The specimens were counterstained with hematoxylin.

For study IV, paraffin-embedded tissue sections of 3 µm were fixed on glass at 60°C for 1 to 2 h. Xylene dissolved the paraffin and a graded-alcohol series ending with water rehydrated the tissue. A heated buffer (Dako ENvision Flex) specific to each antibody functioned as a heat-induced epitope retrieval. Autostainer 480 (Labvision UK Ltd., Suffolk, UK) with the Dako REAL EnVision Detection System, peroxidase, rabbit/mouse (Dako, Glostrup, Denmark) was used for staining. The primary incubation of each antibody was for 1 h at +4°C. A secondary horseradish peroxidase (HRP) -coupled antibody was incubated for 30 min. A chromogen, either3,3'-diaminobenzidine (DAB) if brown or magenta if pink, was used. Hematoxylin incubation for 2 min counterstained the nuclei. A graded-alcohol series dehydrated the tissue. The samples were finally mounted. Primary antibodies consisted of anti-MMP-2 (1:1000, Bioss Antibodies Inc.Woburn, Massachusetts, USA), anti-MMP-7 (1:1000, EMD Millipore Corporation, Temecula, CA USA), anti-MMP-8 (1:400), anti-MMP-9 (1:1000, NeoMarkers, Fremont CA and Calbiochem Inc., San Diego, CA, USA), and anti-beta-catenin

(1:400, Thermo Fisher Scientific, MA USA). Colon and oral squamous cell cancer tissues were used as the positive controls. In the negative control, the primary antibody was not added.

5.2. Scoring

BRAF immunoexpression was considered positive when observed without addressing the intensity. An inner control and a control without the antibody were used. Beta-catenin staining intensity was evaluated as weak, strong, or intense. MMP-8 and MMP-9 stains were graded as weak or strong. Positive and negative controls were incremented. An expression covering 10% or less of the cells of interest was scored as weak. Two oral pathologists, Jaana Hagström and Jetta Kelppe, performed the scoring independently and, in cases of disagreement, consensus was reached.

5.3. Imaging

Scanora, Soredex, Finland or OP 200 Instrumentarium Imaging devices for panoramic radiography were used on 25 patients before further imaging. Multislice computer tomography (MSCT) Light Speed Plus or Bright Speed (GE Healthcare, Milwaukee, WI) using either a 4- or 16-slice scanner provided high-resolution, helical CT images using bone and soft-tissue algorithms for all 27 patients. A contrast medium of either 300-mgl/ml Ultravist (Schering, Germany) or 350-mgl/ml Omnipaque (GE Healthcare, Milwaukee, WI) was used on 17 patients. The slice thickness used was 1.25 mm with a slice interval of 0.63 mm. Finally, 2.0- or 2.5-mm-thick axial, sagittal, and coronal images were reformatted from the data.

A 1.5 T unit MRI device (Magnetom Vision, Siemens, Erlangen, Germany) provided axial and coronal T2-weighted fast-spin echo (FSE) images with fat suppression (fs) and axial T1-weighted (T1W) FSE images (slice thickness 3 mm) with or without fs. Axial and coronal T1W fs images (slice thickness 3 mm) were acquired after intravenous administration of gadolinium (0.5 mmol/mL Magnevist; Schering, Germany).[91] The radiologic findings were compared with the histopathological diagnoses.

5.4. Statistical analyses

In study I, the outcome variables consisted of tumor location, type, growth pattern, and tumor size, while gender and age served as explanatory variables. Patients were classified into age groups: <50 years and ≥50 years. We calculated associations using the student's t-test for continuous variables and the χ^2 test for categorical variables. To determine the significance of the growth patterns in recurring tumors compared to non-recurring tumors, we used the Fisher's exact test. In study III, we calculated the risk ratios for BRAF-positive tumors and earlier age at onset and for BRAF-positive tumors and tumor site. The χ^2 tests and, where appropriate, 2-by-2 table functions were used to determine associations between BRAF positivity and recurrence, BRAF positivity and growth patterns, and BRAF positivity and ameloblastoma types. In study IV, correlations between MMP-7, MMP-8, MMP-9, beta-catenin, E-cadherin, age, gender, location, and recurrence were calculated. We also calculated the odds ratios for MMP-8 and MMP-9, MMP-8/MMP-9/beta-catenin and gender, recurrence and location, MMP8/beta-catenin and age. We relabeled scoring results 0 or 1 as mild (0), and 2 or 3 as strong (1). Logistic regression, χ^2 tests, and, when relevant, 2-by-2 table functions were used. We conducted the analyses using R 3.4.2 (R Core Team, 2017) and RStudio 1.1.383, considering $p \leq 0.05$ statistically significant.

5.5. Ethical considerations

The Ethics Committee of Surgery and HUS's Internal Review Board approved our study protocols (Dnro 151/13/03/02/2015), which follow the guidelines and ethical principles of the Helsinki Declaration. Patients were assigned unrecognizable case numbers.

6. RESULTS

6.1. Study I

The first study examines the demographic and clinical information from patients (n = 34) treated for primary ameloblastoma.

6.1.1. Patient characteristics

Our cohort consisted of 21 male and 13 female patients yielding a male:female ratio of 1.6:1. The mean age of these patients was 48.2 years, 55 years among men and 37 years among women. As expected, most ameloblastomas were located in the mandible, particularly in the molar and posterior region of the jawbone. The tumor size varied from 10 to 110 mm, with a mean of 42.5 mm.

6.1.2. Symptoms and clinical signs

Most patients experienced some sort of pain or a vague sensation around the tumor area (19/34). Six patients were asymptomatic. Among clinical findings, a jaw enlargement was reported in 29 cases. One patient had no clinical signs, but experienced pain at the tumor location. All reported signs and symptoms appear in Figures 7 and 8.

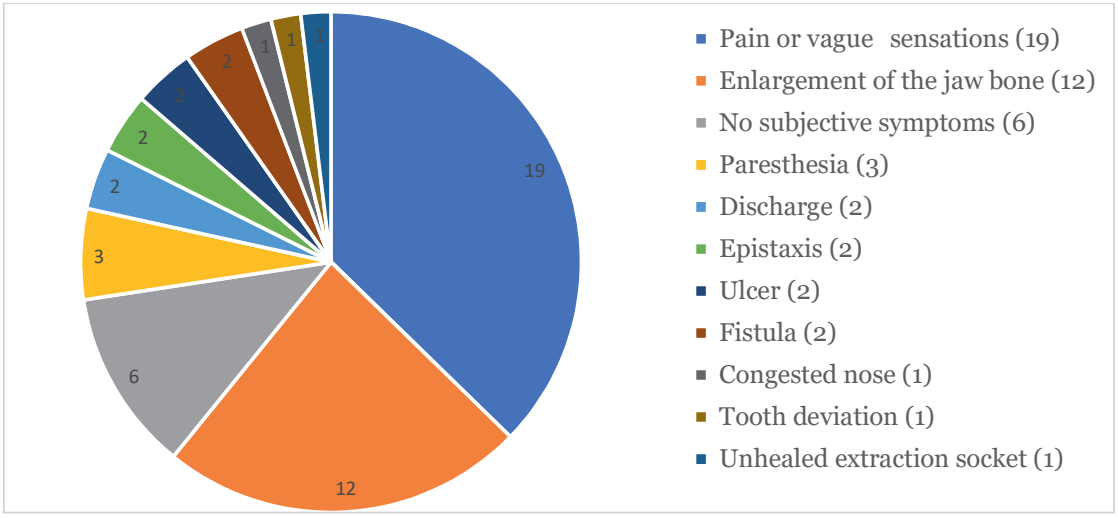

Figure 7. Proportion of subjective signs experienced by patients. Modified from Kelppe et al. (2019) with the permission of Taylor and Francis (see study I).

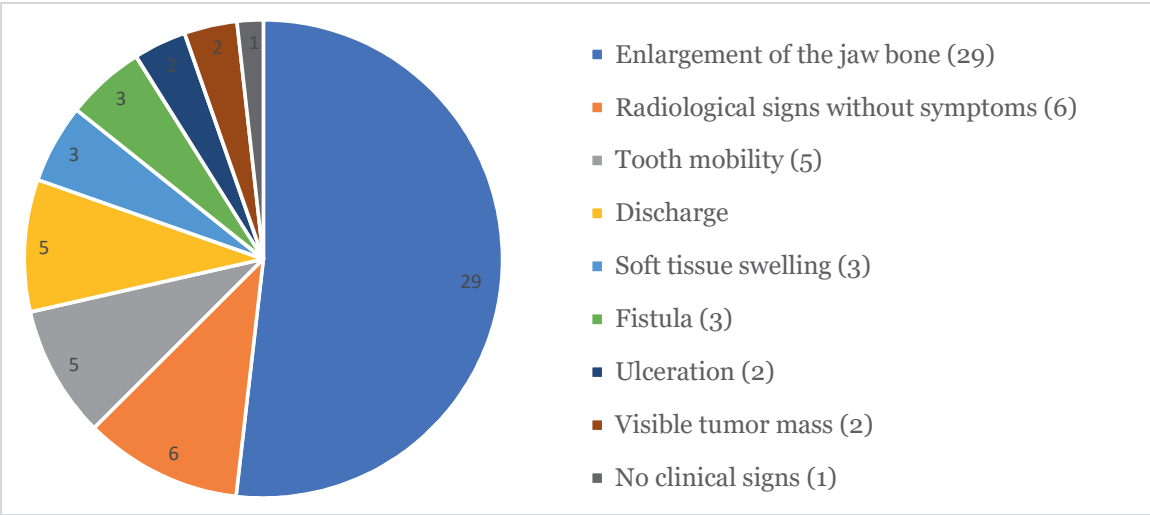

Figure 8. Proportion of clinical signs reported by clinicians. Modified from Kelppe et al. (2019) with the permission of Taylor and Francis (see study I).

Maxillary tumors occurred more often in male patients (p = 0.034) and in the older (≥50) age group (p = 0.007). As expected, men presented with larger tumors than women, on average 47.5 mm compared to 34.1 mm, respectively. Despite the 13.4-mm difference in size, this difference was not statistically significant (p = 0.083). Figures 9 and 10 compare results between men and women and between the two age groups (<50 and ≥50 years).

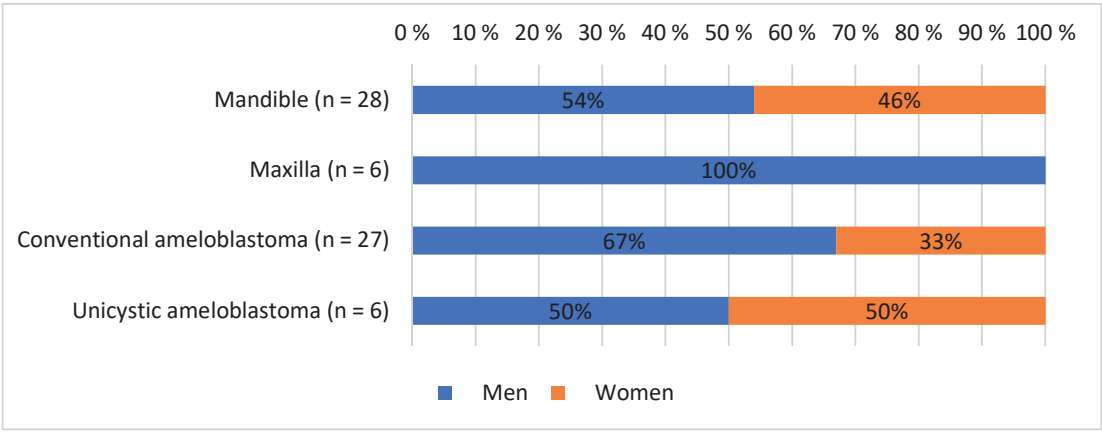

Figure 9. Comparison of proportions of location (mandible/maxilla) and ameloblastoma types (conventional and unicystic ameloblastoma) for men and women. Modified from Kelppe et al. (2019) with the permission of Taylor and Francis (see study I).

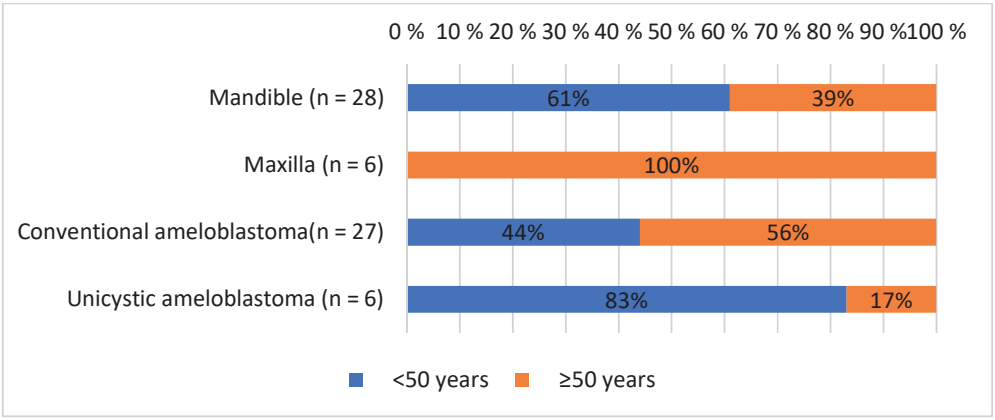

Figure 10. Comparison of proportions of location (mandible/maxilla) and ameloblastoma types (conventional and unicystic ameloblastoma) between age groups. Modified from Kelppe et al. (2019) with the permission of Taylor and Francis (see study I).

Among 34 tumors, 11 recurred. Figure 11 illustrates the growth patterns for different ameloblastoma types and non-recurring and recurring tumors. Recurrence in our material does not appear to depend on the growth pattern (p = 0.5773). Unexpectedly, the growth pattern was more varied than expected. We observed a uniform growth pattern in only 7 ameloblastomas.

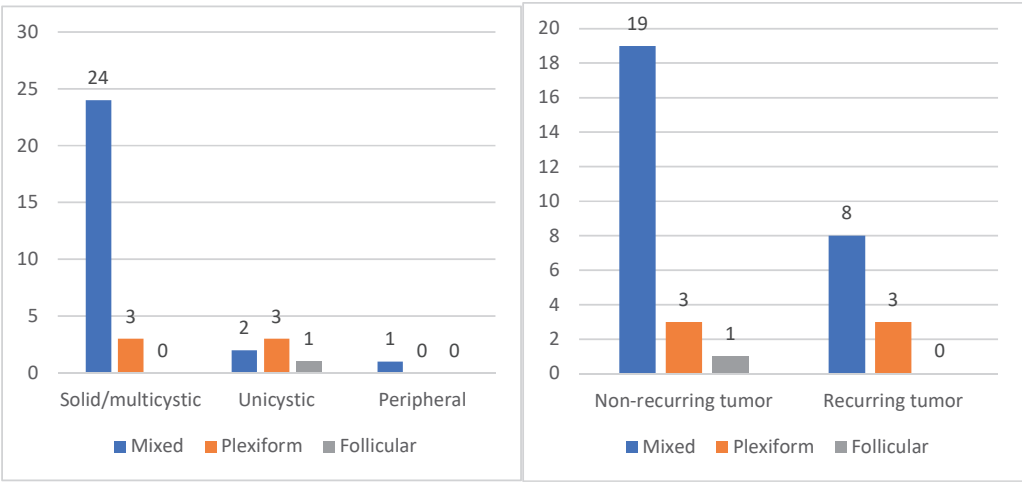

Figure 11. The occurrence of ameloblastoma growth patterns in ameloblastoma, unicystic ameloblastoma, and peripheral ameloblastoma compared to the most usual growth patterns, shown in the chart on the right. A mixed-growth pattern includes all reported growth patterns which were follicular, plexiform, basal cell, acanthomatous, granular cell, and desmoplastic ameloblastomas. Growth patterns compared to recurring and non-recurring tumors. Kelppe et al. (2019), with the permission of Taylor and Francis (see study I).

6.2. Study II

In our second study, in which the CT and MRI scans of ameloblastoma patients were retrospectively investigated, 16 cases involved the posterior body, angel, and/or the mandibular ramus. Most mandibular ameloblastomas (12/16) were of a nonunicystic type and presented multilocularity in panoramic imaging. However, the internal architecture presented more clearly in CT scans. A honeycomb or "soap bubble" pattern formed by numerous bent septa were seen in 6 of 16 mandibular cases.

Contrast-enhanced CT or MRI revealed a mixed cystic and solid pattern in 14/20 nonunicystic ameloblastomas. In noncontrast CT, three cases showed content that appeared as mixed isodense and hypodense to the muscle. Two small ameloblastomas had a solid content.

One nonunicystic ameloblastoma presented a thick-rim enhancement in an otherwise cystic lesion through contrast-enhanced CT. Histologically, this tumor appeared to have transformed from a dentigerous cyst.

All re-evaluated nonunicystic ameloblastomas revealed a homogeneous intermediate signal in MRI with T1-weighted images (T1WIs). Thick-rim enhancement and a homogeneous bright high signal in the cystic area appeared in T2-weighted images (T2WIs). A solid component presented with a heterogeneous or homogeneous high signal on T2WIs and a heterogeneous or homogeneous enhancement.

Five ameloblastomas were situated in the maxilla and involved the sinus. Tumors affecting the nasal fossa or the ethmoid were seen in three patients. Maxillary tumors did not exhibit multilocularity. Three of five of the sinonasal ameloblastomas showed a heterogeneous enhancement pattern. They differed from mandibular ameloblastomas in that mandibular tumors showed separate solid and cystic compartments.

Three of six unicystic ameloblastomas had a multilocular appearance. In two of these, a cystic lesion with an irregularly thick-rim enhancement or a contrast-enhancing solid component was observed. One unicystic ameloblastoma showed a cystic content via noncontrast CT. The remaining

ameloblastomas presented as circumscribed radiolucencies that surrounded the crown of an unerupted mandibular third molar, thus mimicking a dentigerous cyst on the panoramic radiograph, while differing from the dentigerous cysts because of their multilocular appearance and their unusual positioning in the mandibular body. Contrast-enhanced CT showed a cystic lesion with a mixed cystic or a solid pattern and intraseptal enhancement.

Expansion and thinning of the mandibular cortical plates or the maxillary sinus wall appeared in all CT-scanned ameloblastomas. Panoramic radiography could not be used to indicate the expansion of the buccal and lingual cortical plates. Severe expansion appeared in large ameloblastomas. In most of the ameloblastomas (n = 20), perforation was reported, already in early-stage, small ameloblastomas. Root resorption of the adjacent teeth occurred in 8 of 11 ameloblastomas involving tooth-bearing areas and dislocation in 4 cases.

6.3. Study III

In study III, the male-to-female ratio was 1.25:1, with a mean age among men of 55.9 years and 35.8 years among women. Recurrence occurred or the tumor was identified as recurrence beginning in 14 of 36 cases, among which 6 were men and 8 were women. The mean patient age among those with a BRAF-positive tumor was 46.8 years and 65.2 years among those with a BRAF-negative tumor.

An ameloblastoma was found in the mandible in 29 of 36 cases, among which 26 were BRAF-positive. Only 9 of 29 of mandibular ameloblastomas recurred, all of which were BRAF-positive. We observed no recurrence of BRAF-negative mandibular tumors ($n = 3$). We identified 7 maxillary ameloblastomas, all BRAF-negative. Recurrence occurred in 5 of these cases, 4 among men and 1 among a woman (see study III, Table 1).

BRAF positivity was two times more common in solid/multicystic ameloblastomas than BRAF negativity. All unicystic ameloblastomas were BRAF-positive. BRAF-negative tumors appeared to present with a more uniform growth pattern. BRAF-positive tumors often exhibited two or three

growth patterns. In addition, BRAF-negative tumors showed no acanthomatous differentiation. In BRAF-positive tumors, the positivity disappeared in desmoplastic ameloblastomas.

According to our results, it appears that BRAF-positive tumors occur earlier in life ($p = 0.015$) typically situated in the mandible ($p < 0.001$). We found no correlation between recurrence and BRAF status. Figures 12 and 13 illustrate the BRAF-negative and BRAF-positive expressions.

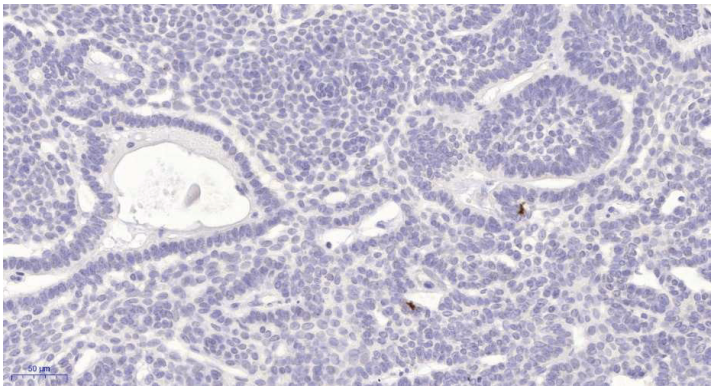

Figure 12. BRAF-negative expression.

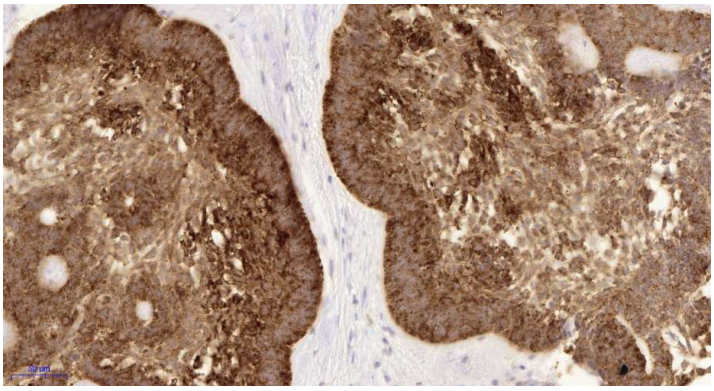

Figure 13. BRAF-positive expression.

6.4. Study IV

The cohort used in study IV consisted of 34 patients, among whom 19 were men and 15 women. The age distribution among men ranged from 13 to 87 years and 18 to 71 years among women. Of 34 tumors, 24 were located in the mandible and 10 in the maxilla or sinonasal area, with tumor sizes ranging from 7 mm to 110 mm.

6.4.1. MMP-7

MMP-7 was expressed in single apoptotic or mitotic cells in the basal layer area of ameloblastomas. Otherwise, the tumor tissue was negative or expressed only a mild membrane positivity. In addition, the ECM remained negative. Single neutrophilic granulocytes expressed positivity. Figure 14 illustrates the MMP-7 expression.

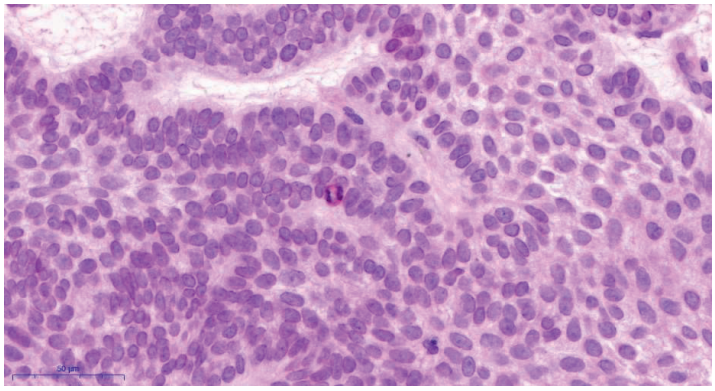

Figure 14. MMP-7 expression in a mitotic cell.

6.4.2. MMP-8

Neutrophilic granulocytes and plasma cells expressed MMP-8 positivity, although the ameloblastoma remained negative. MMP-8 positivity correlated with MMP-9 expression. Figure 15 provides an example of the MMP-8 expression.

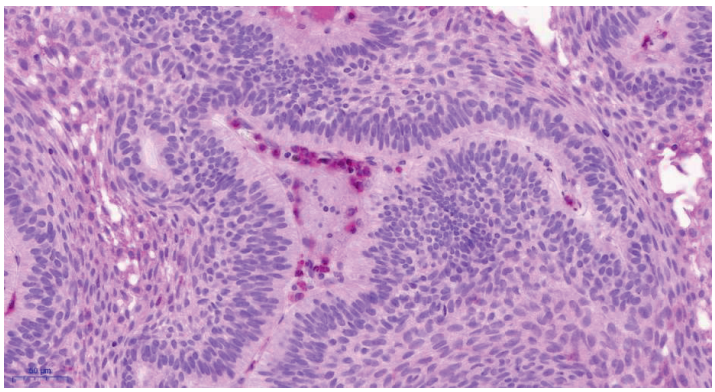

Figure 15. MMP-8 expression in neutrophilic granulocytes.

6.4.3. MMP-9

MMP-9 was expressed in inflammatory cells, multinuclear giant cells within inflammatory infiltration, and bone-lining osteoclasts. Tumor cells, however, remained negative. In logistic regression, a stronger MMP-9 positivity correlated with a stronger MMP-8 expression positivity ($p = 0.015$), with a wide confidence interval (OR = 8, CI 95% 1.5–42.4). Figures 16–18 illustrate the MMP-9 expression.

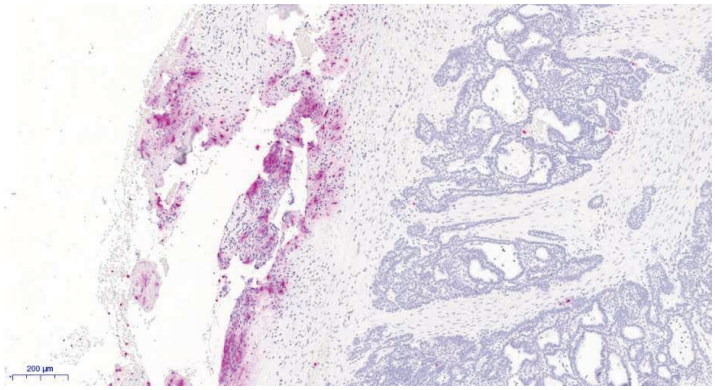

Figure 16. MMP-9 expression along the tumor front inflammatory cells and osteoclasts.

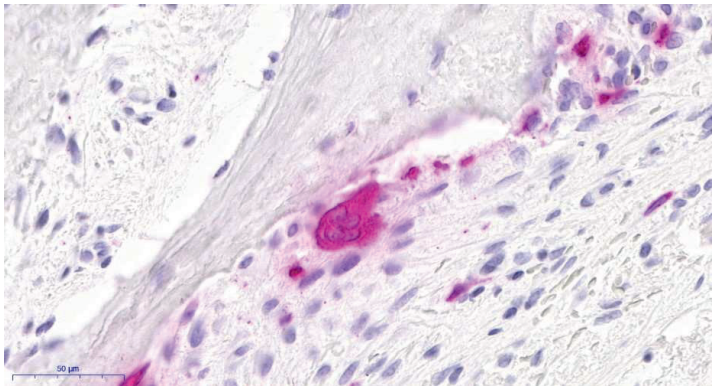

Figure 17. MMP-9 expression in an osteoclast.

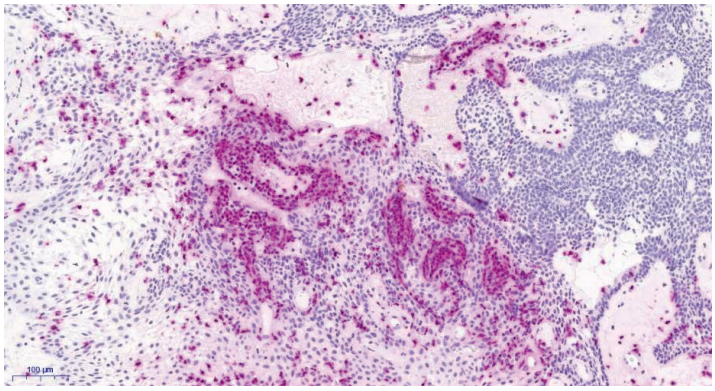

Figure 18. MMP-9 expression in neutrophilic granulocytes.

6.4.4. Beta-catenin

We observed beta-catenin expression in ameloblastoma cell membranes (100%). We did not, however, detect nuclear expression. Beta-catenin expression in ameloblastomas correlated with gender ($p = 0.015$). Male patients exhibited a stronger expression than female patients, although the confidence interval was rather wide (OR = 6, CI 95% 1.3–26.7). Figure 19 illustrates the beta-catenin expression.

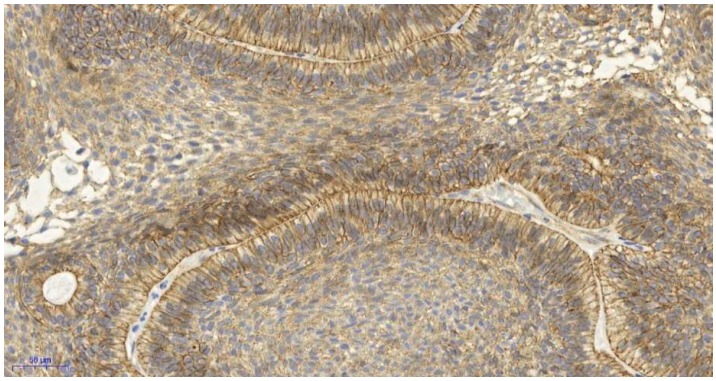

Figure 19. Beta-catenin expression in ameloblastoma cell membranes.

6.4.5. E-cadherin

Only 30 samples remained for this study. Ameloblastoma expressed E-cadherin in most of the mandibular tumors (20/30), particularly in stellate reticulum-like areas. In peripheral columnar cells, expression was pale or nonexistent. Most maxillary tumors (6/10) remained E-cadherin-negative or expression was weak. Figures 20-21 illustrate the strong and weak expressions of E-cadherin. We observed both positive and negative expression in desmoplastic ameloblastomas. A Fisher's exact test and cross-tabulations revealed a correlation between mandibular ameloblastomas and a stronger E-cadherin expression (OR = 0.167; CI 95% 0.031–0.889; p = 0.036). In logistic regression, E-cadherin correlated positively with beta-catenin (OR = 5.4; CI 95% 1.04–28.5; p = 0.045). We found both positive and negative expressions of E-cadherin in desmoplastic ameloblastomas. Recurring maxillary tumors expressed E-cadherin weakly in 4 of 6 cases, with 1 of 5 mandibular tumors expressing E-cadherin, although this finding was not significant.

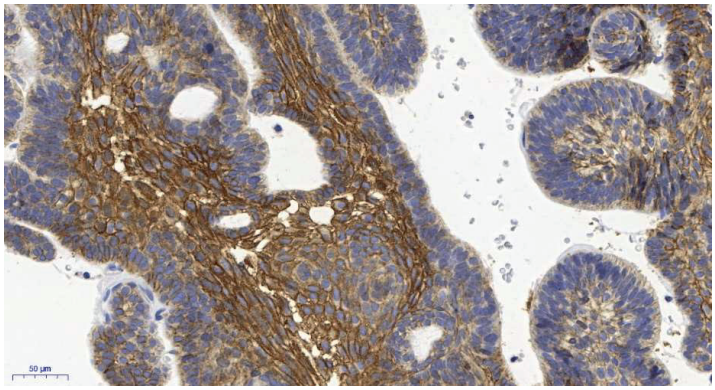

Figure 20. Positive E-cadherin expression in an ameloblastoma.

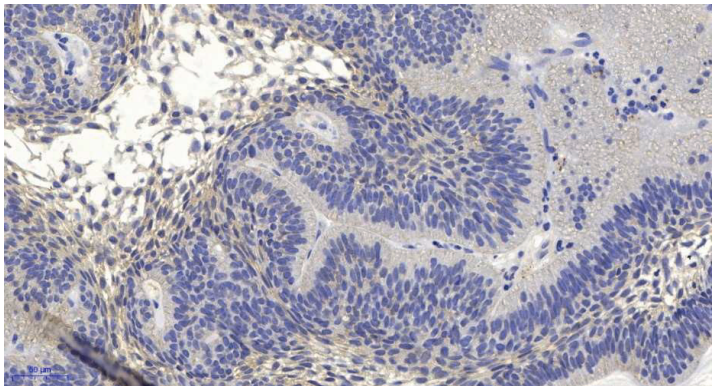

Figure 21. Weak E-cadherin expression in an ameloblastoma.

7. DISCUSSION

This thesis provides an overview of ameloblastoma patients treated at HUS across three decades, summarizing the subject retrospectively from the clinical, radiological, and pathological points of view. Rather expectedly, the findings indicate that Finnish ameloblastoma patients do not differ substantially from other ameloblastoma patients studied globally. This research, however, was able to specify the signs and symptoms noted by clinicians and experienced by patients, identify more detailed histological interpretations of ameloblastoma, pinpoint imaging details to facilitate differential diagnosis, and fortify previous assumptions regarding immunohistochemical findings. The data suggest that ameloblastoma is pathologically a diverse tumor with confirmed differences between tumors located in the mandible and the maxilla, an observation that should be taken into careful consideration when planning the treatment of ameloblastoma patients and designing future studies. Since ameloblastoma is a benign tumor with an aggressive behavior and carries the potential to metastasize and become malignant, it can provide clues of the processes involved in the transition from a benign to a metastasizing or malignant tumor. Yet, unfortunately, the rarity of this tumor sets limits to exploring these issues more extensively.

7.1. Gender and age at onset

The major findings here vis-à-vis demographic and clinical results coincide with previous reports. The majority of ameloblastoma studies reported relatively consistent cases between male and female patients with a slight male predominance. In a meta-analysis consisting of over 3000 ameloblastoma patients, Hendra et al.[10] found a predominance among men at a rate of 1.14:1 (male–female), an observation coinciding with Reichard et al.[11] For unknown reasons, likely associated with the small cohort size, our male predominance was stronger, at 1.6:1. The overall worldwide peak incidence was estimated in a meta-analysis as occurring during the third decade of life. [10] Yet, Hendra et al.[10] reported that European and North American patients were typically in their fifth and sixth decades, while Africans were identified during their third decade of life at disease onset. In our study, male patients were older (55 years) at onset than female patients (37 years), so logically the average age of

the cohort here exceeded the average age observed in previous studies, but largely agreed with age estimates for European and North American patients.[10,11] Among Asian patients, the age at onset varied more from the third to the sixth decades of life. Unicystic ameloblastomas occurred among younger patients,[94] similar to our results indicating that 5 of 6 unicystic ameloblastomas presented in the younger age cohort (<50). In addition, most unicystic ameloblastomas exhibited BRAF positivity. Since BRAF positivity appears to exist in younger age groups, this is only reasonable.[12] It has been argued that ameloblastomas would initially present as unicystic tumors and mature to increasingly develop additional cysts and solid tumor areas. This would explain why unicystic ameloblastomas are more common in younger patients.[29] Solid ameloblastomas that do not present with cysts also exists, suggesting that this theory may be only partially feasible.

7.2. Size

Ameloblastomas often grow by expanding the bone, unlike a keratocyst or a dentigerous cyst which primarily grow along the bone medulla. Small ameloblastomas mostly appear as incidental radiological findings. In the mandibular ramus or the sinus areas, tumors might grow freely without limits, for example, from the bordering bone structures, reaching substantial sizes before presenting symptoms. The range in sizes within our cohort of ameloblastomas was 10 to 110 mm, with men typically having larger tumors compared to women. This we associate with the difference in the size of the bone structure between the two genders, speculating that men, particularly older men, might postpone seeking health care, thus giving the tumor time to grow. In developing countries, ameloblastomas might grow to extreme sizes measuring up to 40 cm if the patient for some reason delays seeking medical help. Instituting policies by the government and efforts among nongovernmental organizations to promote health awareness, training practitioners to recognize symptoms early on, and raising awareness among people to eliminate fear of treatment and cultural beliefs would all facilitate those in more rural areas or in developing countries to seek treatment earlier.[95]

7.3. Clinical signs and symptoms

The clinical data revealed a spectrum of signs and symptoms described by clinicians and experienced by patients. Only a few studies have reported these in detail, with the most often mentioned consisting of the presence of bone expansion with only a minority reporting any sign of pain or sensations.[96–98] For instance, Milman et al.[99] found that 16% of patients experienced pain. In our material, one in two patients experienced pain or at least some sensation near the tumor area. Thus, pain as a symptom seems to be overexpressed in this Finnish patient material. Only 6 of 34 cases represented complete incidental radiological findings. In 5 cases, tooth mobility and discharge were present. Surprisingly, a visible tumor mass was reported in only 2 cases. The proper reporting of clinical signs and subjective symptoms provides crucial information for other clinicians, radiologists, and pathologists to aid in identifying suspected ameloblastoma.

7.4. Location

Our results agree with previous findings indicating that ameloblastomas occur primarily in the mandible (28/34 patients). In 6 patients, the tumor was located in the maxillary area. In our study, we did not attempt to distinguish between gnathic bone-bourn or sinonasal track-bourn ameloblastomas, which, in retrospect, would have been interesting. Still, in our estimation, when a tumor growth features projections extending to the radiological boundaries, determining the origin becomes nearly impossible. During development, rests of ameloblastic epithelium might remain in various locations in the maxillofacial area. There seems to be an ongoing debate regarding from which cells ameloblastomas originate when not connected to tooth-bearing areas or gnathic bones. Interestingly, there have been cases in which an ameloblastoma developed on the buccal mucosa with no connection to the tooth-bearing areas. For instance, one researcher presenting a case study speculated that a likely explanation of its origin lie in the pluripotent cells in the basal cell layer of the mucosal epithelium, although the more unlikely ectopic glands of Serres cannot be ruled out.[100] We must also acknowledge that tissue samples from tooth-bearing areas, particularly tissue near the periodontal ligament, often presents with islands of odontogenic epithelium (rests of Malassez),

likely the primal resource of odontogenic tumors. A study by Schafer et al.[101] reported a higher age of onset (59.7 years) and a male predominance for sinonasal track ameloblastomas. Their findings agree with our cohort of maxillary tumors, which occurred only among male patients all over 50 years of age. Like us, Schafer et al. also reflected on the reasons behind this phenomenon: a sinus or nasal tract space allowing tumor growth before symptoms appeared and, thus, delaying the time of diagnosis. In contrast to observations in our cohort, these tumors tended not to recur during an average follow-up period of 9.2 years.[32] Speculating that maxillary ameloblastomas were somewhat different from mandibular ameloblastomas, Sweeney et al.[49] reported maxillary tumors presenting more often with plexiform growth patterns, a finding in agreement with both Schafer et al.[101] and our findings. In addition, Sweeney et al.[49] described the plexiform ameloblastomas as carrying an SMO mutation, with 9 of 11 maxillary tumors exhibiting SMO mutations. They also demonstrated that SMO-mutated ameloblastomas recurred earlier than BRAF-mutant ameloblastomas.[49] This and other findings suggest that maxillary tumors appear to carry specific features differentiating them from mandibular tumors. In our estimation, it is reasonable to inspect mandibular and maxillary tumors separately in future studies.

7.5. Imaging

Imaging is crucial in planning ameloblastoma treatment. Currently, CT and MRI are extensively used to further obtain information of gnathic lesions primarily detected via intraoral or panoramic radiology. For example, keratocysts and ameloblastomas are, because of their similar location and multicysticity, at times impossible to differentiate from each other in intraoral or panoramic radiology. Cone-beam CT is not useful in tumor diagnostics because of its poor soft-tissue detection capacity. In study II, we demonstrated that primarily CT and MRI imaging provide information on multilocularity, cortical bone expansion, and the perforation of ameloblastomas already during the early stages of disease. The benefit of multiscan CT, particularly with a bone algorithm, is the possibility of examining the fine bony structures and septa formations, which can be observed as honeycomb or soup bubble patterns. In noncontrast CT, the attenuation of soft tissue can be

compared to muscle attenuation. Moreover, CT density values are clearly less dense compared to the muscle, corresponding to a cystic lesion not a tumor. The various desquamated combinations of solid and cystic content were observed in 70% of nonunicystic ameloblastomas when examined with either contrast-enhanced CT or MRI. That is, ameloblastomas exhibited a solid content and keratocysts exhibited a thin-rim enhancement and no solid content as in previous observations. In three of six unicystic ameloblastomas, panoramic radiology implied a dentigerous cyst, but in contrast-enhanced CT rim enhancement was stronger and an intraluminal solid component was noted. None of the unicystic ameloblastomas were examined with MRI, which performs better in soft-tissue differentiation. Nevertheless, when the resolution is sufficient and examination is thorough, the soft-tissue content will be identified. Desmoplastic ameloblastomas present with honeycomb-like lesions with well-defined margins, bone expansion, an anterior location, an internal texture generally described as mixed radiolucent and radiopaque, with calcified foci at the periphery, and with a trabecula-like high attenuation in radiographs and CT scans.[102–104] The differential diagnoses associated with these findings include odontogenic and nonodontogenic lesions, such as odontogenic keratocyst, odontogenic myxomas, ameloblastic fibromas, fibromyxomas, other fibro-osseous lesions, metastatic tumors, giant cell tumors, and aneurysmal bone cysts.[102] MRI is, thus, essential to attaining a more precise diagnosis. According to findings from Baba et al.[105], in MRIs desmoplastic ameloblastomas exhibit well-defined lesion borders, heterogeneous low-signal intensities on T2-weighted images, a heterogeneous intermediate signal intensity on T1W images, and small cystic high-signal intensities on T2-weighted images. In part, they observed a linear prominent low-signal intensity on T1- and T2-weighted images in Gadolinium-labeled diethylenetriamine pentaacetic acid (Gd-DTPA)-enhanced MRI with moderate enhancement and dynamic-enhanced MRI with persistent enhancement.[105]

7.6. Ameloblastoma types

When comparing ameloblastoma types, in most reports, ameloblastomas (solid/multicystic) are the most common, followed by unicystic and peripheral, respectively. Our results agree with this. In our

study, recurrence occurred in nine conventional ameloblastomas. Among six unicystic ameloblastomas, only one recurred. The only peripheral ameloblastoma recurred, presumably because the lesion was excised for biopsy without clean margins, and no re-excision was performed until the tumor recurred.

7.7. Ameloblastoma growth patterns

To our surprise, the growth pattern variety was more versatile than anticipated, with 27 of 34 tumors exhibiting a mixed growth pattern and only 7 of 34 exhibiting a uniform pattern. This may reflect the typical way in which ameloblastoma growth patterns are categorized by the predominant growth patter the literature. More precise characterization might be facilitated with digital pathology and as artificial intelligence techniques become available. Growth patterns do not reflect recurrence, although Hong et al.[37] argue a locally aggressive behavior occurs in follicular, granular, and acanthomatous variants of ameloblastomas. Maxillary tumors appear to have more acanthomatous metaplasia, with an increased cellularity presenting with a less palisading appearance.[106] We did not focus on these factors in our study, but reported a tendency towards a simpler growth pattern in maxillary tumors. Ameloblastomas with the most aggressive behavior appear to be granular cell ameloblastomas, present only in 3.5% of ameloblastomas.[107] These tumors also have a high rate of recurrence and are related to metastasizing ameloblastomas.[107]

7.8. BRAF mutations in ameloblastomas

Studies indicate that BRAF wild-type ameloblastomas exist, which could be treated with epidermal growth factor receptor (EGFR) inhibitors, and that ameloblastomas harboring BRAF mutations exist, which could be treated with BRAF-targeted therapies.[12] For some time now, the BRAF V600E mutation in ameloblastoma has been of interest as a target for therapy in cases involving very large tumors or among patients not suitable for surgery. BRAF might act, as Brown et al.[47] suggest, as a prognostic marker predicting recurrence, given that BRAF wild-type tumors appear to recur more

often. Immunohistochemistry on undecalcified tissue appears to be 100% sensitive and specific in colorectal cancer and 100% sensitive and 96.8% specific in melanoma, thus aiding clinical decisions.[108,109] The majority of our mandibular tumors expressed BRAF in immunohistochemical staining.

Debate continues on the association between the BRAF mutation affecting tumor aggressiveness. Brown et al.[48] and Sweeney et al.[49] found that BRAF-positive tumors associated with a longer disease-free survival, while Fregnani et al.[110] reported a significant association between BRAF positivity and an earlier recurrence and shorter disease-free survival compared to BRAF-negative tumors. In our study, none of the mandibular BRAF-negative tumors recurred, which agrees with results from Fregnani et al.[110] Yet, they failed, as did Diniz et al.[43], to find an association between BRAF mutation and tumor location.[43,110] In our study, none of the maxillary tumors expressed BRAF positivity and five of seven recurred. Because of the small number of cases in our study, we can only assume that this large percentage of recurring maxillary ameloblastomas are genuinely associated with BRAF negativity or that maxillary tumors are per se difficult to operate on. We identified no significant correlations.

Patients with a BRAF-positive tumor were on average 46.8 years and patients with a BRAF-negative tumor were 65.2 years. Brown et al.[47] found corresponding ages of 34.5 years and 53.6 years, respectively. We can only speculate whether this stems from the BRAF status or from the fact that maxillary/sinusoidal tumors freely grow before diagnosis. In a study on Iranian ameloblastoma patients, however, results contradicted our findings. That is, patients harboring the mutation were an average age of 43.6 years versus those without the mutation who were 38.6 years.[111]

When we compared ameloblastoma types, the solid/multicystic ameloblastomas were BRAF-positive two times more often than BRAF-negative, while unicystic ameloblastomas were all BRAF-positive, a finding that agrees with previous findings.[12,112] Heikinheimo et al.[12] found that 94% of mandibular and 33% of maxillary unicystic ameloblastomas were BRAF-positive. One mandibular unicystic ameloblastoma harbored an SMO mutation. In a cohort consisting of eight unicystic ameloblastomas, Pereira et al.[112] found that all were BRAF-positive via immunostaining, which

appeared along the entire length of the epithelium and in the odontogenic islands in the lesion capsule. They argued that BRAF cannot be used to distinguish ameloblastomas from other odontogenic tumors with an ameloblastic morphology from, for example, ameloblastic fibromas, ameloblastic fibro-odontomas, and ameloblastic carcinomas since these may also present with a BRAF mutation. Brunner et al.[113] also reported BRAF negativity in all investigated follicular cysts, dental follicles, and keratocysts.

For some reason, desmoplastic ameloblastomas did not show BRAF positivity although other growth patterns in the same tumors presented with BRAF positivity. Previous observations and the explanation for this finding have yet to be offered.

In our estimation, maxillary ameloblastomas exhibit simpler growth patterns than mandibular tumors, which might present with two or more growth patterns in one tumor. BRAF-positive tumors showed more variation in growth patterns. Acanthomatosis was present in almost half (17/36) of the ameloblastomas to at least some extent, although acanthomatosis did not occur in BRAF-negative tumors. Heikinheimo et al.[12] speculate that the differences in the mandible and maxillary tumors likely stem from the different expressions of homeobox genes, such as DLX and MSX, which regulate the patterning of developing teeth and which differ in the upper and lower jaws.[114, 124]

We can also speculate that acanthomatosis might represent metaplasia occurring inside ameloblastoma, as is the case in desmoplasia.

7.9. MMPs

Tooth development involves MMPs at various stages: MMP-1,- 2, -7, and -9 in angiogenesis; MMP-1, -2, -3, and-7 in cell migration; MMP-2, -3, -9, and -14 in cell growth; and MMP-7, -9, and -11 in apoptosis.[115] In this respect, MMPs occurring in an ameloblastoma and its ECM seems natural. We studied the immunohistochemical expression of MMP-7, -8, and -9, and compared our results with previous reports.

7.9.1. MMP-7

MMP-7 (matrilysin-1) participates in proliferation, apoptosis, pathogenesis during invasion, and metastasis.[62,116,117] MMP-7 expression remains low in the epithelial cells in normal physiology. Research has demonstrated its expression and importance through participation in tumorigenesis within odontogenic keratocysts and ameloblastomas, but has not been associated specifically with aggressiveness,[118,119] a role we also could not confirm. Reports on MMP-7 and odontogenic tumors remain rarer. For instance, the expression of MMP-7 and -26 in Gorlin syndrome keratocysts was stronger compared to the expression in incidental keratocysts, an observation associated with the more aggressive nature of keratocysts associated with this syndrome[119] Guimarães et al.[66] demonstrated that the expression of MMP-7 in solid and cystic ameloblastomas appeared in less than half the cells, in 60% of studied ameloblastomas in peripheral cells, and occasionally appeared in the central areas of tumor growth. We detected MMP-7 only in the apoptotic cells and could not associate expression with other factors. Thus, our results do not support previous findings. MMP-7 participates in tissue modeling by braking proteins, such as fibronectin, laminin, nidogen, type IV collagen, and proteoglycan core proteins along with other MMPs.[120,121] Since ameloblastoma expands and must cause stromal modifications, it seems odd that our results did not show extracellular MMP-7 expression. MMPs are often expressed in tumors and the extracellular tissue surrounding the tumor.[122,123] In malignant tumors, MMP-7 expression increases in apoptotic cells in the neighboring tissue and prevents apoptosis in cancer cells during tumor progression and invasion.[123,124] Because ameloblastoma is a benign tumor, it might reflect a milder MMP-7 expression. In addition, MMP-7 is thought to affect cell adhesion and enhance tumor proliferation and invasion.[123] Our findings related to MMP-7 expression were mild and positivity was only randomly identified. A connection to E-cadherin, a cell adhesion molecule, could not be found. Guimaraes et al.[66] reported a higher WNT5A correlating with a high MMP-7 expression in odontogenic tumors compared to radicular cysts, speculating that this was related to epithelial proliferation. WNT5A, a noncanonical WNT family member, is even thought to activate the β-

catenin-independent pathway. Specifically, Souza et al.[125] found that 60% of studied ameloblastomas expressed MMP-7 positivity. They identified positivity also in the epithelial cells, fibroblasts, endothelial cells, and in the inflammatory cells surrounding ameloblastomas. We did not find this in our study. Surprisingly, MMP-7 was not detected in the inflammatory cells around an adenomatoid odontogenic tumor (AOT), which might reflect the less aggressive behavior of AOTs.[125] They suggest that matrilysins, both MMP-7 and MMP-26, participate in tissue modeling processes possibly indirectly through other MMPs such as MMP-2 and MMP-9, which apparently contribute to a greater extent to the aggressiveness of ameloblastomas.[125]

7.9.2. MMP-8 and MMP-9

Neutrophil-derived MMP-8 is the predominant collagenase present in normally healing wounds, and the overexpression and activation of this collagenase may be involved in the pathogenesis of nonhealing chronic ulcers.[126] The surrounding area of an expanding tumor is in a constant state of healing or remodeling. MMP-8 immunohistochemical expression has previously been found in plasma cells as well as in the epithelial cells in keratocysts and follicular cyst tissue samples.[127,128] Otherwise, reports of MMP-8 expression in odontogenic lesions are sparse. MMP-8, however, has been shown to either suppress or promote tumor growth in different types of tumors.[7270] We found no correlation between tumor size or recurrence. MMP-8 correlated positively only with MMP-9 expression in inflammatory cells. This might reflect the overall inflammation surrounding the tumor and the fact that inflammatory cells overlap with proteolytic functions or relate to the same mediators. MMP-2 and MMP-9 likely play a crucial role in tumor development by participating in epithelial–mesenchymal interactions. Previous studies established MMP-9 expression in ameloblastomas and odontogenic myxomas, suggesting that these proteinases are involved in ECM degradation and might play a role in the local aggressiveness typical for ameloblastomas, similar to changes in the expression of vascular endothelial growth factor and E-cadherin.[129] In our cohort, MMP-9 appeared in the osteoclasts lining the bone and multinucleated giant cells in the inflamed areas, indicating that MMP-9 participates in bone resorption along the tumor front perhaps in a

more indirect manner since ameloblastomas did not express MMP-9. This could be explained by the tumor causing the surrounding tissue to adapt to the changing environment and providing more space for the tumor to grow. For instance, Yang et al.[130] demonstrated that an elevated hydrostatic pressure promoted cell motility and invasiveness and upregulated MMP-2 and MMP-9 expression via the Wnt/beta-catenin pathway. Kumamoto et al.[131] studied MMP-1, -2, and -9 in components of dental follicles, dental papillae, and the stromal cells of ameloblastomas and detected strong expression in the mesenchymal components. They argue that these molecules might play a role in regulating tumor progression in ameloblastomas and regulating developmental processes in tooth germs.[131] Kumamotos et al.'s[131] study agrees with our results, although they also did not report MMP-9 positivity in tumor cells. We do not know the reason for these contradictory results, but suspect there might be differences in procedures, clones, tissue preparation, and even interpretation of results.

7.10. Beta-catenin and E-cadherin

Beta-catenin and E-cadherin are important structures in cell-to-cell adherence junctions, which participate in controlling proliferation and maintaining tissue integrity. Alterations in the junction structures and Wnt signaling interactions can lead to proliferation and tumor progression.[81] Beta-catenin, a member of the Wnt signaling pathway, plays an important role in dental development. Alterations in beta-catenin degradation can cause beta-catenin to accumulate in the nucleus and bind to T-cell factor/lymphoid enhancer–binding factor 1 (TCF/LEF-1), which serves as a transcription factor in regulating the expression of target genes, such as cyclin-D1 and Myc. Many studies on beta-catenin expression report only weak immunoexpression in ameloblastomas. Kim et al.[132] compared beta-catenin immunohistochemistry in peripheral ameloblastoma and oral basal cell carcinoma, concluding that the peripheral ameloblastoma remained negative, while positivity was strong in oral basal cell carcinoma, although they do not discuss this finding in their report.[142] Neither was there a strong expression in a giant granular cell ameloblastoma, granular cells, and, based on the histological image, the peripheral ameloblastoma cells.[133] Similarly, Martínez–Martínez

et al.[134] reported only weak or negative beta-catenin expression. Yet, we easily detected membrane beta-catenin immunopositivity in our cases. Because ameloblastoma is a benign epithelial tumor, it is reasonable that the cell membrane expresses beta-catenin. The intensity between cases varied, however. Hao et al.[135] observed a moderate beta-catenin expression in the normal mucosa epithelium, but the expression diminished in the cell membrane of ameloblastomas, while they also observed increased cytoplasm and/or nucleic expression. Likewise, Sekine et al.[136] demonstrated ameloblastomas expressing cytoplasmic and nuclear beta-catenin. They also detected an ameloblastoma with beta-catenin mutation *(*CTNNB1), as did Brown et al.[48] in their gene studies. Could it be that the ameloblastomas with a CTNNB1 mutation have beta-catenin accumulating excessively in the nucleus? Most calcifying odontogenic cysts (9/10), which somewhat histologically resemble ameloblastomas, harbored a CTNNB1 mutation. Cytoplasmic and nucleus expressions were not present in our ameloblastomas. In some malignancies, the nuclear accumulation of beta-catenin associated with a more aggressive course of disease.[137,138]

In many epithelial cancer types, the loss of E-cadherin expression indicates malignant progression. It has been demonstrated that a reduced E-cadherin function promotes cell migration and invasion. E-cadherin expression can restore epithelial morphology and prevent tumor invasion and metastasis.[139–141] A diminished E-cadherin expression has also been linked to earlier recurrence.[142] In ovarian cancer, Yoshida et al.[143] demonstrated that the epithelial cells gained migratory abilities and could invade the stromal tissue. They reported that inhibition of cell adhesion when epithelial to mesenchymal transition (EMT)-related proteins Slug and Snail bind to the E-cadherin promoter.[143] The expression of transcription factors Slug, Snail, and Twist have been investigated in ameloblastomas as well, apparently associating with local invasiveness and recurrence.[144–146] Kurioka et al.[146] reported a strong expression of Slug and TGFβ in follicular and plexiform ameloblastomas. The expression of these proteins was present in areas where E-cadherin expression diminished. They also detected strong Snail and Slug expressions in four of their recurrent cases.[146] Feng et al.[145] observed a stronger Twist expression in ameloblastomas compared with unicystic ameloblastomas, related to the involvement of Twist in the local invasiveness of ameloblastoma. They also detected cases rich in stromal cells, which exhibited a larger amount of Twist-positive cells,

implicating the possible regulatory role of the stromal cells in the tumor environment. Siar et al.[144] observed Snail positivity in 94% of ameloblastomas, while stromal fibroblasts and vascular endothelium near the immunoreactive tumor sites often stained positive. Interestingly, the shift from E-cadherin to N-cadherin expression in ameloblastoma, causing a transition in the tumor epithelial cells to a neuroectodermal phenotype has also been reported.[147]

Turning to E-cadherin, in our cohort, we observed a statistically significant correlation between maxillary tumors and a weaker E-cadherin expression, possibly indicating a role in earlier recurrences of maxillary tumors. It would be interesting to study how these EMT-related transcription factors express in maxillary tumors with weak or negative E-cadherin expressions. Sung et al.[148] studied the phenotypes of esophageal squamous cell carcinoma cells, concluding that tumors with a prevalent mesenchymal phenotype exhibited a stronger Snail expression and a worse prognostic outcome. They also noted that the loss of E-cadherin itself without the mesenchymal phenotype might not be linked to invasiveness. Then, again, tumor cells with a mesenchymal phenotype despite E-cadherin expression demonstrated an aggressive course of disease in esophageal squamous cell carcinoma.[148] Ameloblastoma has no invasive abilities and, therefore, cannot be directly compared to cancer in such cases. Yet, some ameloblastoma cells have a spindle-like morphology, the tumor is locally aggressive and possesses, although extremely seldomly, the capability of metastasizing. Considering these factors, it would be interesting to study the epithelial–mesenchymal transition further in future studies, especially in maxillary ameloblastomas as well as desmoplastic ameloblastoma because of their unique morphology. Since EMT is also involved in wound healing, we must keep in mind that the EMT proteins expressed in previous ameloblastoma studies might be related to the constant turnover of the extracellular matrix attempting to maintain consistency. The recurring maxillary tumors in our cohort expressed E-cadherin weakly in four of six cases, while in the mandibular tumors, the proportion fell to one in five, although no significant outcomes emerged. This result requires further investigation. We also examined the relationship between E-cadherin and BRAF negativity, finding no association.

8. LIMITATIONS

This study carries several limitations. We were able to collect only 30 to 36 tissue samples and clinical data related to ameloblastomas. Decalcified tissue samples could not be used in immunohistochemistry. This is important to consider when preparing samples for examination. Samples containing bone should be softened in both decalcification and ethylenediaminetetraacetic acid (EDTA), the former for the prompt diagnosis by the clinician and the latter for further immunohistochemical investigations and for possible research use in future. In ameloblastoma and other rare tumor cases, these are important procedures to consider, since the bone surface lining, especially in ameloblastoma, represents an interesting and a primarily unexplored territory. A sufficient amount of such samples would have been beneficial in these studies as well.

Furthermore, we could have expanded our cohort of ameloblastoma cases by including other university hospitals in Finland. Because of the retrospective nature of our study, some samples were already old and immunohistochemistry probably would have provided more accurate expression results on tissue samples of a similar age. Immunohistochemistry could have been completed more extensively, but the tissue material amount available was limited. Western blotting or the use of ameloblastoma cell lines used in various studies could have provided additional support for our results in immunohistochemistry. We failed to provide proper answers to questions related to recurrence.

9. CONCLUSIONS

In our studies, we were interested in patients treated for ameloblastoma at HUS, which serves a population of 1.5 million inhabitants, covering about 27% of the Finnish population. We examined this topic primarily from a demographic, pathological, or immunohistochemical point of view, but also included a radiological perspective.

This thesis reinforces previous knowledge about the demographic and clinical aspects of ameloblastoma. Yet, we highlight that, in our studies, maxillary tumors occurred in older male patients. Although ameloblastoma is a rare tumor—even rarer in the maxillary or sinonasal areas—it is important to keep in mind the possibility of ameloblastomas when examining older male patients with rhinology symptoms. Histologically, the growth types varied more than anticipated.

Radiological re-evaluation revealed that ameloblastomas eradicate cortical bone already during the early stages of tumor growth, justifying CT and MRI imaging as essential tools in the differential diagnosis, protecting the patient from radical surgery when not needed.

We argue that a BRAF-immunohistochemical investigation of ameloblastoma samples provides a predictive tool for clinicians when planning treatment and during follow-up, since BRAF-positive tumors seemed to recur more often than BRAF-negative tumors in the mandible. Maxillary tumors, which are usually BRAF-negative, are likely to recur easily, presumably in complex anatomical structures of the maxillary and sinonasal areas. Furthermore, a weaker E-cadherin expression, linked to more infiltrative tumor growth in epithelial malignancies, was also seen in maxillary ameloblastomas. Considering recurrence and subsequent re-excisions, these findings suggest that maxillary ameloblastoma patients could benefit from radical primary surgery.

Unlike previous studies, ameloblastoma cells did not express MMP-7, MMP-8, or MMP-9 in our studies. Yet, MMP-9 positivity was found in inflammatory cells, macrophages, and osteoclasts, but failed to provide a benefit to clinical diagnostics at present. MMP-8 correlated positively with MMP-9 expression in the inflammatory cells, reflecting the inflammatory tumor ECM environment that promotes tumor growth in ameloblastoma. MMP-9 expression in the osteoclasts lining the bone and multinucleated giant cells indicated that MMP-9 participates in bone resorption at the tumor front, perhaps more in an indirect manner since ameloblastomas cells did not express MMP-9. Beta-

catenin expression was observed on cell membranes, reflecting its benign nature. But this requires further investigation in, for example, metastatic ameloblastomas.

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