UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

**FÁBIO RAMÔA PIRES**
***Cirurgião-dentista***

# ESTUDO IMUNOHISTOQUÍMICO E ANÁLISE MULTIVARIADA DE FATORES PROGNÓSTICOS DE CARCINOMAS MUCOEPIDERMÓIDES DE GLÂNDULAS SALIVARES

Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, para obtenção do Título de Doutor em Estomatopatologia, área de Estomatologia.



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**Orientador: Prof. Dr. Oslei Paes de Almeida**
**Co-Orientador: Prof. Dr. Luiz Paulo Kowalski**

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# FACULDADE DE ODONTOLOGIA DE PIRACICABA
# UNIVERSIDADE ESTADUAL DE CAMPINAS

A Comissão Julgadora dos trabalhos de Defesa de Tese de DOUTORADO, em sessão pública realizada em 30 de Agosto de 2002, considerou o candidato FÁBIO RAMÔA PIRES aprovado.

1. Prof. Dr. OSLEI PAES DE ALMEIDA

2. Prof. Dr. ADRIANO MOTA LOYOLA

3. Prof. Dr. MAURO KAZUO IKEDA

4. Prof. Dr. MARCIO AJUDARTE LOPES

5. Prof. Dr. JOSÉ MAGRIN

## DEDICATÓRIA

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**SUMÁRIO**

## RESUMO

O estadiamento clínico e a gradação histológica têm sido relatados como os principais fatores prognósticos em carcinomas mucoepidermóides (CME). O objetivo deste estudo foi avaliar parâmetros clínicos, histopatológicos e imunohistoquímicos como fatores prognósticos em CME. De 1953 a 1997, 173 CME de cabeça e pescoço foram avaliados dos registros do Hospital do Câncer A. C. Camargo, São Paulo, Brasil. Dados clínicos e histopatológicos foram obtidos dos registros dos pacientes e da revisão histopatológica dos casos. Reações imunohistoquímicas contra PCNA, ki-67, p53, c-erbB-2, CEA, bcl-2 e Mel-CAM, e análises de sobrevida univariada e multivariada foram realizadas. Parótidas foram afetadas em 61 casos (35,2%), TNM revelou 50,3% em estádios I ou II, e 45,2% e 36,3% eram CME de baixo e alto grau, respectivamente. Recorrência local e metástases regionais e a distância foram encontradas em 12,7%, 9,8% e 9,2% dos pacientes, respectivamente, e a sobrevida global em 5 anos foi de 70%. Análise univariada de sobrevida revelou que a idade dos pacientes (>40 anos) ($p<0.001$), gênero masculino ($p=0.005$), fixação dos tumores ($p=0.002$), invasão de estruturas adjacentes ($p=0.004$), T ($p<0.001$), N ($p<0.001$), estadiamento clínico ($p<0.001$) e grau histológico ($p<0.001$), mostraram-se relacionados a pior prognóstico. CME em submandibular mostrou pior taxa de sobrevida global. Expressão de PCNA ($p<0.001$), ki-67 ($p<0.001$) e p53 ($p<0.001$) mostrou correlação com pior prognóstico e, de forma contrária, a expressão de CEA ($p=0.012$) e bcl-2 ($p<0.001$) foi relacionada a melhor prognóstico. Análise multivariada revelou que a idade dos pacientes (>40 anos), fixação dos tumores, T, N e grau histológico mostraram-se fatores prognósticos independentes significantes. Expressão de Mel-CAM mostrou relação inversa com a recorrência local e com metástases regionais e a distância. Diversos achados clínicos e imunohistoquímicos mostraram correlação com prognóstico em CME e também devem ser considerados para o estabelecimento do tratamento.

## ABSTRACT

Clinical staging and histological grade have been reported as the main prognostic factors in mucoepidermoid carcinoma (MEC). The aim of this study was to report clinical, pathological and immunohistochemical features as prognostic factors in head and neck MEC. From 1953 to 1997, 173 cases of head and neck MEC were retrieved from the files of the A. C. Camargo Cancer Hospital, São Paulo, Brazil. Clinical and histopathological data were obtained from the patients records and from the histological review. Immunohistochemical reactions against PCNA, ki-67, p53, c-erbB-2, CEA, bcl-2 and Mel-CAM, and univariate and multivariate survival analysis were performed. Parotids were affected in 61 cases (35.2%), TNM revealed 50.3% stages I or II and histological grading revealed 45.2% and 36.3% low and high-grade MEC, respectively. Local recurrence, regional and distant metastasis were found in 12.7%, 9.8% and 9.2% of the patients, respectively, and five-year overall survival rate was 70%. Univariate survival analysis revealed that age of the patients (>40 years) ($p<0.001$), male gender ($p=0.005$), fixation of the tumors ($p=0.002$), invasion of the adjacent structures ($p=0.004$), T ($p<0.001$), N ($p<0.001$), clinical stage ($p<0.001$) and histological grade ($p<0.001$) were correlated to a worst prognosis. Submandibular cases had the worst survival rates. PCNA ($p<0.001$), ki-67 ($p<0.001$) and p53 ($p<0.001$) expression were also correlated to a worst prognosis and, on the contrary, expression of CEA ($p=0.012$) and bcl-2 ($p<0.001$) were correlated to a better prognosis. Multivariate survival analysis revealed that age of the patients (>40 years), fixation of the tumors, T, N and histological grade were independent significant prognostic factors. Mel-CAM expression demonstrated a inversely correlation to local recurrence, regional and distant metastasis. Several clinical and immunohistochemical features were correlated to prognosis in MEC and should be also considered when establishing patient management.

## INTRODUÇÃO

Carcinomas mucoepidermóides (CME) são os tumores malignos mais freqüentes de glândulas salivares, podendo representar 3 a 20% de todos os tumores de glândulas salivares e 12 a 40% dos malignos (Spiro et al., 1978; Nascimento et al., 1986; Auclair & Ellis, 1991; Loyola et al., 1995; Lopes et al., 1998; Plambeck et al., 1999; Brandwein et al., 2001). Sua origem parece ser a partir de células dos ductos excretores e intercalares (Dardick et al., 1984; Loyola et al., 1998).

CME pode acometer pacientes em qualquer faixa etária, mas apresenta pico de prevalência na 4ª e 5ª décadas, afetando ambos os sexos igualmente, e acometendo preferencialmente a parótida e as glândulas menores intra-orais (Spiro et al., 1978; Nascimento et al., 1986; Auclair & Ellis, 1991; Clode et al., 1991; Loyola et al., 1995; Brandwein et al., 2001). Nas glândulas salivares menores, o palato é o sítio de predileção, e apenas cerca de 5% dos casos afetam as glândulas submandibulares e sublinguais (Spiro et al., 1978; Nascimento et al., 1986; Clode et al., 1991; Loyola et al., 1995). Usualmente se apresentam como aumento de volume assintomático, eventualmente associado a ulceração superficial, dor e parestesia (Loyola et al., 1995).

Histologicamente, o CME é composto por diferentes tipos celulares. Células mucosas possuem citoplasma abundante, eosinofílico e claro, contendo mucina. As células intermediárias podem apresentar citoplasma eosinofílico, arranjarem-se de forma sincicial e usualmente apresentam aspecto basalóide. Células epidermóides têm citoplasma eosinofílico abundante, são arredondadas ou ovóides, podendo se apresentar como células escamosas maduras. Podem ser encontradas ainda células claras, poligonais, grandes, com citoplasma claro, e células colunares com núcleos pequenos e citoplasma eosinofílico (Auclair et al., 1992; Ellis & Auclair, 1996). Estes tipos celulares estão arranjados como misturas de estruturas císticas e glandulares (padrão cístico), em combinação com lençóis

e ilhas de células tumorais (padrão sólido), usualmente com algum grau de tecido colagenizado no estroma (Batsakis & Luna, 1990; Ellis & Auclair, 1996).

A gradação histológica do CME é dada pela avaliação dos achados cito/histológicos, principalmente considerando-se o grau de desenvolvimento cístico/sólido do tumor, a celularidade resultante da diferenciação das células intermediárias e epidermóides e o padrão de invasão tumoral (Batsakis & Luna, 1990; Ellis & Auclair, 1996). Nos tumores de baixo grau há presença de cistos, células mucosas e epidermóides, poucas células intermediárias, pleomorfismo discreto, raras mitoses, lagos de mucina extravasada, além de fibrose e inflamação crônica (Hicks et al., 1995; Ellis & Auclair, 1996). Nos de grau intermediário existem ninhos sólidos de células com poucos cistos, predomínio de células intermediárias com ou sem diferenciação epidermóide, poucas células mucosas, pleomorfismo brando a moderado, mitoses ocasionais, infiltração não circunscrita, e inflamação crônica na periferia do tumor (Hicks et al., 1995; Ellis & Auclair, 1996). Nos tumores de alto grau há predominância dos ninhos celulares e/ou microcistos, presença de células epidermóides e intermediárias pobremente diferenciadas, raras células mucosas, pleomorfismo marcante, nucléolos proeminentes, mitoses freqüentes, invasão de tecidos moles com ou sem invasão vascular ou perineural, desmoplasia acompanhando as células invasivas e inflamação crônica menos proeminente (Hicks et al., 1995; Ellis & Auclair, 1996).

A gradação usualmente inclui três graus de malignidade (baixo, intermediário e alto) e mostra correlação com o prognóstico dos tumores (Healey et al., 1970; Spiro et al., 1978; Hickman et al., 1984; Batsakis & Luna, 1990; Auclair et al., 1992; Hicks et al., 1994; Hicks et al., 1995; Goode et al., 1998). A sobrevida dos pacientes diminui significativamente com o aumento da gradação do tumor, de baixo para alto grau (O'brien et al., 1986; Clode et al., 1991; Hicks et al., 1995; Goode et al., 1998; Brandwein et al., 2001).

Além da gradação histológica, o estádio clínico do tumor, as margens tumorais comprometidas após a cirurgia, a presença de metástases regionais e à distância, presença de sintomatologia no momento do diagnóstico, a localização

do tumor nas glândulas submandibulares, na língua e no assoalho de boca, a idade avançada dos pacientes e a presença de invasão óssea também parecem modular o prognóstico do CME (Nascimento et al., 1986; O'brien et al., 1986; Auclair et al., 1992; Hicks et al., 1994; Plambeck et al., 1996; Goode et al., 1998; Brandwein et al., 2001).

Parece haver correlação entre o estádio clínico e a gradação histológica, ou seja, tumores em estádio I são freqüentemente de baixo grau e tumores em estádio III usualmente são de alto grau (Spiro et al., 1978; Brandwein et al., 2001). As taxas de recorrência local do CME usualmente situam-se próximas à 15%, variando de 10% para tumores de baixo grau a 75% para tumores de alto grau (Healey et al., 1970). As taxas de sobrevida de 5 e 10 anos, para todos os graus de malignidade, em geral situam-se em torno de 60 a 70% e 40 a 60%, respectivamente (Hickman et al., 1984; Clode et al., 1991; Suzuki et al., 1998; Okabe et al., 2001).

As metástases regionais e à distância do CME afetam cerca de 3 a 15% e 6 a 15% dos pacientes, respectivamente (Nascimento et al., 1986; Plambeck et al., 1999; Brandwein et al., 2001) e são influenciadas pelo grau histológico, estádio clínico e o local de origem dos tumores, sendo mais comuns em tumores de glândulas submandibulares (Spiro et al., 1978; Auclair et al., 1992). Os locais de predileção das metástases à distância são os pulmões, o esqueleto e o cérebro (Spiro et al., 1978; Auclair et al., 1992).

Alguns marcadores prognósticos de agressividade biológica têm sido demonstrados através de estudos imunohistoquímicos em CME, incluindo marcadores de proliferação celular, proteínas oncogênicas e supressoras de tumor, receptores de fatores de crescimento, marcadores de diferenciação celular e proteínas anti-apoptóticas (Skalova et al., 1994; Angelov et al., 1996; Kamio, 1996; Suzuki et al., 1998; Cardoso et al., 2000; Yin et al., 2000; Okabe et al., 2001).

**Antígeno nuclear de proliferação celular (PCNA)**

O antígeno nuclear de proliferação celular (PCNA) é uma proteína nuclear não-histônica de 36 kDa que funciona como uma proteína auxiliar para a DNA-polimerase, sendo um requisito para a síntese de DNA. PCNA é freqüentemente expresso em CME, e seu índice positivo, o padrão de sua distribuição e a intensidade de expressão celular podem servir para avaliar o grau de diferenciação do tumor (Frankenthaler et al., 1994; Cardoso et al., 2000). O aumento da expressão de PCNA correlaciona-se positivamente com piora na sobrevida, mostrando que sua medição parece ser útil na avaliação do prognóstico de pacientes com CME (Frankenthaler et al., 1994; Cardoso et al., 2000).

**Ki-67**

O Ki-67 é uma proteína nuclear cuja expressão aumentada relaciona-se com aumento na proliferação celular, com pico de expressão na fase M. A expressão aumentada deste antígeno em CME e outros tumores de glândulas salivares correlaciona-se com comportamento mais agressivo e pior prognóstico, sugerindo sua utilização como marcador útil para aumentar a sensibilidade da gradação histológica convencional (Murakami et al., 1992; Skalova et al., 1994; Okabe et al., 2001).

**p53**

O gene Tp53 está localizado no cromossomo 17 e o produto da sua expressão - a proteína p53 - funciona como inibidor da divisão celular na fase G1 do ciclo celular, induzindo apoptose. A expressão da proteína p53 mutante tem sido relacionada ao desenvolvimento de neoplasias malignas e a expressão de p53 é encontrada em 40 a 67% dos CME, mas esta positividade tem mostrado correlação variável com a recorrência local, doença metastática ou com a sobrevida dos pacientes (Kamio, 1996; Karja et al., 1997; Yin et al., 2000).

**C-erbB-2**

O oncogene c-erbB-2 codifica um receptor de fator de crescimento transmembrana de 185 kDa, que desempenha importante papel na diferenciação, desenvolvimento e sinalização mitogênica em células normais. A expressão aumentada de c-erbB-2 é encontrada em até 30% dos CME, especialmente nos tumores de alto grau (Kamio, 1996; Cho et al., 1997; Suzuki et al., 1998). Entretanto, outros estudos têm demonstrado índices inferiores de expressão da oncoproteína c-erbB-2, não evidenciando relação entre sua expressão e o prognóstico dos CME (Kernohan et al., 1991; Shrestha et al., 1992).

**Antígeno carcinoembrionário (CEA)**

O antígeno carcinoembrionário (CEA) é uma glicoproteína da superfície celular, cuja função parece ser de molécula de adesão intercelular. Sua expressão aumentada em células que ainda apresentam capacidade proliferativa parece interferir com outras interações da superfície celular, levando à distorção da arquitetura tissular e a um bloqueio na diferenciação celular terminal. A expressão imunohistoquímica do CEA pode estar aumentada no CME, sugerindo que sua expressão é importante no desenvolvimento destes tumores (Angelov et al., 1996).

**Bcl-2**

O oncogene bcl-2 codifica uma proteína de 26 kDa de mesmo nome que inibe a apoptose, favorecendo a sobrevida prolongada tanto de células normais quanto de células neoplásicas. O aumento da expressão imunohistoquímica de bcl-2 tem sido demonstrada com freqüência maior em CME de baixo grau de malignidade e parece ser fator prognóstico favorável nestes tumores (Yin et al., 2000).

**Mel-CAM (CD146)**

Mel-CAM (CD146 ou MUC18) é uma glicoproteína de adesão célula-célula de 113kDa, pertencente a superfamíla das imunoglobulinas. Sua expressão é

encontrada em vários tecidos normais, como endotélio, músculo liso, células de Schwann e células ductais e mioepiteliais de glândulas salivares (Shih et al., 1998). Esta molécula tem sido relacionada a adesão, organização do citoesqueleto, interações intercelulares, manutenção da forma da célula e controle da migração e proliferação celular (Shih, 1999). Mel-CAM também é expresso em tecidos tumorais, estando relacionado ao tamanho e progressão dos tumores, potencial metastático e agressividade (Shih et al., 1998). Sua expressão em CME foi recentemente descrita na literatura, entretanto não existem trabalhos avaliando seu potencial prognóstico nestes tumores (Shih et al., 1998).

## OBJETIVO

O objetivo deste estudo foi avaliar os achados clínicos, histológicos e a expressão imunohistoquímica das proteínas PCNA, ki-67, p53, c-erbB-2, CEA, bcl-2 e Mel-CAM em carcinomas mucoepidermóides de glândulas salivares maiores e menores, correlacionando os achados com a sobrevida dos pacientes e identificando seu significado prognóstico através de análises univariada e multivariada.

## CAPÍTULO 1

# CARCINOMA MUCOEPIDERMÓIDE DE CABEÇA E PESCOÇO: ESTUDO CLÍNICO-PATOLÓGICO DE 173 CASOS

# HEAD AND NECK MUCOEPIDERMOID CARCINOMA: CLINICOPATHOLOGIC STUDY OF 173 CASES

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**RESUMO**

**Introdução:** Carcinoma mucoepidermóide (CME) é o tumor maligno mais comum de glândulas salivares, entretanto poucos estudos em populações brasileiras têm sido relatados na literatura. **Objetivo:** Reportar os dados clínico-patológicos de 173 CME de cabeça e pescoço do Centro de Tratamento e Pesquisa Hospital do Câncer A. C. Camargo em São Paulo. **Material e métodos:** Cento e setenta e três casos de CME tratados entre 1953 e 1997, obtidos dos arquivos do Centro de Tratamento e Pesquisa Hospital do Câncer A. C. Camargo foram utilizados no estudo. Os dados foram obtidos a partir dos prontuários e da revisão histológica de todos os casos. **Resultados:** A idade média dos pacientes foi de 44 anos e 93 (53,8%) eram homens. Parótida foi acometida em 61 casos (35,2%) e as glândulas salivares menores intra-orais em 75 (43,4%), TNM revelou 50,3% dos casos em estádios I e II, e a gradação histológica revelou 45,2%, 18,5% e 36,3% tumores de baixo grau, grau intermediário e alto grau de malignidade, respectivamente. Tratamento cirúrgico foi utilizado em 80,3% dos casos, complementado por esvaziamento cervical em 52 casos (30,1%) e radioterapia em 73 (42,2%). Recidiva local e metástases regionais e a distância foram encontradas em 12,7%, 9,8% e 9,2% dos pacientes, respectivamente, e a sobrevida global dos pacientes em 5 e 10 anos foi de 70% e 60%, respectivamente. **Conclusões:** A avaliação dos 173 casos de CME de cabeça e pescoço mostrou que estes tumores ocorreram preferencialmente na glândula parótida e no palato de indivíduos adultos, sem predileção por sexo. Metade dos casos encontravam-se em estádios clínicos iniciais e 64% dos tumores eram de grau baixo ou intermediário de malignidade. O tratamento de escolha foi cirúrgico e o prognóstico dos pacientes revelou sobrevida global em 5 anos de 70%.

## SUMMARY

**Introduction:** Mucoepidermoid carcinoma (MEC) is the most common malignant salivary gland tumor, however few studies have been reported in Brazilian populations. **Objective:** To report clinical and pathologic data from 173 head and neck MEC treated in the Centro de Tratamento e Pesquisa Hospital do Câncer A. C. Camargo in São Paulo. **Material and Methods:** From 1953 to 1997, 173 cases of MEC were found in the files of the Centro de Tratamento e Pesquisa Hospital do Câncer A. C. Camargo. Data were obtained from the patients' records and histological review of all cases. **Results:** The mean age of the patients was 44 years and 93 (53,8%) were men; parotid glands were affected in 61 cases (35,2%) and intra-oral minor salivary glands in 75 (43,4%). TNM revealed 50,3% of the cases in stages I and II, and histological grading revealed 45,2%, 18,5% and 36,3% low-grade, intermediate-grade and high-grade tumors, respectively. Surgical treatment was employed in 80,3% of the cases, with neck dissection in 52 cases (30,1%), and radiotherapy in 73 (42,2%). Local recurrence, regional and distant metastasis were found in 12,7%, 9,8% and 9,2% of the patients, respectively; 5-year and 10-year overall survival rates were 70% and 60%, respectively. **Conclusions:** MEC affected mainly the parotid gland and the palate of adults, without gender predilection. Half of the cases were diagnosed in initial clinical stages and 64% of the tumors were low or intermediate-grade lesions. Surgery was the treatment of choice and overall 5-year survival was 70%.

## PALAVRAS-CHAVE

Glândulas salivares; cabeça e pescoço; parótida; palato; carcinoma mucoepidermóide.

## KEY-WORDS

Salivary glands; head and neck; parotid; palate; mucoepidermoid carcinoma.

## INTRODUÇÃO

Carcinomas mucoepidermóides (CME) são tumores malignos originados de ductos excretores de estruturas glandulares, que acometem as glândulas salivares maiores e menores intra-orais em mais de 90% dos casos[5,6]. CME também pode ocorrer nas glândulas de revestimento dos seios maxilares, glândulas lacrimais, orofaringe, nasofaringe, laringe, pregas vocais, traquéia e pulmões[6]. Histologicamente, os CME têm sido classificados em três graus de malignidade (baixo, intermediário e alto grau), e esta subdivisão tem se mostrado útil no estabelecimento da terapêutica e no prognóstico destes tumores[1,3,9]. Poucos trabalhos na literatura analisaram os dados clínicos e patológicos de grandes séries de CME de diferentes sítios[2,7,16,17,21]. Na maioria dos relatos, os CME são considerados junto com outros tumores benignos e/ou malignos da parótida ou glândulas menores intra-bucais[4,8,12,13,14,18,19,20].

O objetivo deste trabalho é apresentar e discutir os dados clínicos e histológicos de 173 casos de CME tratados em uma única instituição e compará-los aos dados da literatura mundial.

## MATERIAL E MÉTODOS

Os arquivos do Centro de Tratamento e Pesquisa Hospital do Câncer A. C. Camargo, São Paulo/SP foram revisados, e todos os casos com diagnóstico de carcinoma mucoepidermóide, adenocarcinoma, carcinoma glandular sólido e carcinoma mucinoso da região de cabeça e pescoço tratados até o ano de 1997 foram selecionados para revisão. O diagnóstico histopatológico de todos os casos foi confirmado avaliando-se cortes corados com hematoxilina & eosina, ácido periódico de Schiff (PAS) e mucicarmin, e os casos com diagnóstico final de carcinoma mucoepidermóide foram incluídos neste estudo.

Dados clínicos, sócio-demográficos, estadiamento, localização, tratamento, tempo de acompanhamento e situação de proservação atual dos pacientes foram obtidos a partir de seus prontuários. O estadiamento TNM foi realizado de acordo com a localização específica de cada tumor. A gradação dos tumores em baixo

grau, grau intermediário e alto grau de malignidade foi realizada utilizando-se os critérios sugeridos por Ellis & Auclair[5] (Tabela 1).

## RESULTADOS

Entre os anos de 1953 e 1997, 173 casos de CME do Centro de Tratamento e Pesquisa Hospital do Câncer A. C. Camargo foram confirmados após a revisão clínica e histológica. A relação homem:mulher foi de 1,2:1, com 93 casos (53,8%) afetando homens. A idade dos pacientes variou de 6 a 96 anos, com média de 44 anos. A média de idade foi maior em homens (média de 47,3 anos – variação de 6 a 79 anos) do que em mulheres (média de 40 anos – variação de 6 a 96 anos), sendo que 55,5% dos casos afetaram pacientes entre a 4ª e 6ª décadas de vida. O tempo de queixa dos pacientes variou de 1 a 480 meses, com média de 38 meses, e os sintomas mais freqüentes foram aumento de volume no local em 162 casos (93,6%), dor em 69 casos (39,9%) e parestesia em 11 casos (6,4%).

Oitenta casos (46,2%) afetaram as glândulas salivares maiores, sendo 61 nas parótidas, 17 nas submandibulares e 2 nas glândulas sublinguais. Dos 93 casos (53,8%) afetando glândulas salivares menores, 75 acometeram glândulas intra-orais e 18 extra-orais. A distribuição dos 173 casos de acordo com a localização está mostrada na Tabela 2. O maior diâmetro dos tumores variou de acordo com o local das lesões, sendo maior nas glândulas salivares menores extra-orais (média de 5,5cm - variação de 2 a 9cm), seguido das glândulas maiores (média de 4,6cm - variação de 1 a 16cm) e das glândulas menores intra-orais (média de 3,6cm - variação de 1 a 8cm). Quanto a mobilidade, 74,3% dos casos apresentavam-se fixos ou semi-fixos aos tecidos adjacentes, 25,7% apresentavam-se móveis, e a invasão de uma ou mais estruturas adjacentes estava presente em 70,5% dos casos.

Oitenta e sete casos (50,3%) foram classificados como T1 ou T2; 31 (17,9%) e 3 (1,7%) pacientes apresentavam metástases regionais e à distância, respectivamente, no momento do diagnóstico. O estadiamento TNM revelou que 50,3% dos casos encontravam-se nos estádios I e II (Tabela 3).

Os critérios de gradação mostraram que 73 casos (42,2%) tinham componente intra-cístico menor que 20% do tumor, 15 casos (8,7%) invasão neural, 38 casos (22,0%) presença de necrose, 31 casos (17,9%) 4 ou mais mitoses por 10 campos de grande aumento e 87 casos (50,3%) anaplasia. Ao final da gradação, 61 casos (35,3%) foram classificados como de baixo grau de malignidade, 25 (14,5%) como de grau intermediário, 49 (28,3%) como de alto grau e 38 casos (22,0%) não sofreram gradação em virtude da pequena amostra tumoral disponível. Considerando apenas os 135 casos que sofreram gradação histológica, estes valores foram de 45,2% (baixo grau), 18,5% (grau intermediário) e 36,3% (alto grau).

Com relação ao tratamento, 45 casos (26,0%) já haviam sido submetidos a tratamento oncológico cirúrgico e/ou radioterápico prévio em outras Instituições. No Centro de Tratamento e Pesquisa Hospital do Câncer A. C. Camargo, 151 pacientes (87,3%) foram submetidos a tratamento, incluindo cirurgia com ressecção local em 139 casos (92,1%), esvaziamento cervical em 52 (34,4%), radioterapia em 72 (47,7%) e quimioterapia em 10 (6,6%). Vinte e dois casos (12,7%) não foram submetidos a tratamento no Hospital. Os quatro tipos de tratamento mais utilizados incluíram cirurgia local isolada ou associada a radioterapia e/ou esvaziamento cervical. A Tabela 4 mostra a distribuição dos pacientes quanto ao tipo de tratamento efetuado no Hospital A. C. Camargo.

As cirurgias locais com margem (43,9%) e as parotidectomias parciais ou totais (40,3%) foram os tipos de cirurgia mais utilizados nos 139 casos submetidos a procedimento cirúrgico (Tabela 5). Com relação aos 52 pacientes submetidos a esvaziamento cervical, o esvaziamento cervical radical unilateral (53,8%) e o cervical supra-omo-hióideo unilateral (40,4%) foram as duas formas mais freqüentemente utilizadas (Tabela 5). Dos 52 casos submetidos a esvaziamento cervical, 22 (42,3%) apresentavam linfonodos metastáticos após avaliação histológica.

A remoção cirúrgica dos tumores foi associada a ressecção de estruturas adjacentes em 69 casos (49,6%). Somente uma estrutura foi ressecada em 55

casos (79,7%) e nos demais casos mais de uma estrutura foi ressecada. A Tabela 6 mostra a distribuição das estruturas ressecadas nos 69 pacientes. Complicações pós-cirúrgicas como paralisia do nervo facial, seroma e infecções estiveram presentes em 52 (37,4%) dos 139 pacientes submetidos a procedimentos cirúrgicos no Hospital. Quarenta e um pacientes (78,8%) apresentaram somente uma complicação e 11 (21,2%) mais de uma complicação pós-cirúrgica (Tabela 7).

Após o tratamento, 22 pacientes (12,7%) apresentaram recidiva local. Metástase regional foi encontrada em 17 pacientes (9,8%), sendo homolateral em 10 casos (5,8%), contralateral em 5 casos (2,9%) e bilateral em 2 casos (1,1%). Metástase à distância acometeu 16 pacientes (9,2%), sendo mais freqüente nos pulmões (12 casos – 6,9%) e nos ossos (5 casos – 2,9%). Tratamento de recidivas e metástase foi realizado em 35 casos (20,2%) e incluiu ressecção local, esvaziamento cervical, radioterapia e quimioterapia. O tempo de acompanhamento dos pacientes variou de 1 a 389 meses (média de 98 meses) e revelou 83 pacientes (48,0%) vivos sem doença, 6 pacientes (3,5%) vivos com doença, 45 pacientes (26,0%) mortos pela doença, 16 pacientes (9,2%) mortos por outras causas. Vinte e três pacientes (13,3%) foram perdidos de seguimento. A sobrevida atuarial global dos pacientes foi de 70% e 60%, respectivamente para 5 e 10 anos (Figura 1).

## DISCUSSÃO

CME usualmente afeta glândulas salivares maiores e menores intra-orais, entretanto, tem sido encontrado em associação a uma grande variedade de outros epitélios glandulares na região de cabeça e pescoço, tais como do revestimento dos seios maxilares, nasofaringe, orofaringe, pregas vocais, laringe, traquéia, glândulas lacrimais e tireóide[6,22]. A maioria dos estudos restringem a avaliação dos CME a sítios específicos, dificultando as estimativas reais de incidência destes tumores nas diversas localizações[9,13,14]. Nossos resultados confirmaram que as glândulas salivares maiores e menores intra-orais são os sítios de

predileção dos CME, mas realçam que outras glândulas menores podem ser acometidas em até 20% dos casos[2,21,22].

Os CME afetam especialmente adultos jovens sem predileção por sexo, e usualmente se manifestam como aumento de volume de evolução lenta, normalmente assintomáticos, mas que eventualmente podem estar associados a ulceração superficial, dor e parestesia[1,5,14,21]. Em nossos casos, cerca de 40% dos pacientes apresentavam dor no momento do diagnóstico. Nossos resultados mostraram ainda que os tumores parecem afetar os homens em faixas etárias mais elevadas que as mulheres[6,12].

A localização dos tumores mostrou que a parótida foi o sítio de acometimento mais freqüente, seguida do palato e da glândula submandibular. Consideradas em conjunto, a incidência nas glândulas maiores e nas glândulas menores intra-orais foi semelhante, em concordância com a literatura[2,4,5,6,16,17]. Com relação ao tamanho dos tumores, os CME afetando glândulas salivares menores extra-orais apresentaram a maior média de diâmetro, em especial os tumores de antro maxilar, que podem atingir grandes dimensões antes de causar sintomatologia aos pacientes.

Quanto ao estadiamento, 50,3% dos casos apresentavam-se na consulta inicial em estádios clínicos TNM I e II, valores inferiores aos relatados na literatura mundial[2,17,21], mas semelhantes aos achados em outras populações brasileiras[16]. No entanto, 74,3% dos tumores apresentavam-se fixos ou semi-fixos, o que pode justificar o prognóstico desfavorável de alguns casos diagnosticados mesmo em estádios iniciais, por possível subestadiamento. As glândulas menores apresentaram tumores em estádios mais avançados que as glândulas maiores, como já relatado na literatura[17]. No geral, metade dos casos apresentavam-se em estádios T iniciais (T1 e T2), 17,9% apresentavam comprometimento regional e 1,7% metástases à distância, valores próximos aos encontrados na literatura[2,17].

O diagnóstico histopatológico dos CME se baseia na evidenciação de três tipos celulares (células mucosas, intermediárias e escamosas) organizados em ilhas, lençóis e formações císticas[5]. A positividade citoplasmática para PAS e

mucicarmin ou Alcian blue é importante para confirmar a natureza mucóide do material celular armazenado e secretado pelas células tumorais. A gradação histológica dos CME usualmente considera o arranjo tumoral, o grau de diferenciação e anaplasia das células tumorais, e a inter-relação entre os três tipos celulares[9]. Diversas classificações já foram propostas, e a gradação usualmente separa os tumores em baixo grau, grau intermediário e alto grau de malignidade[1,2,7,11,16]. Os critérios sugeridos por Auclair et al.[1], Ellis & Auclair[5], Ellis et al.[6] e Goode et al.[9] para gradação de CME de glândulas salivares maiores e menores baseiam-se em cinco parâmetros histológicos de fácil observação em microscopia óptica, facilitando a distinção entre os três tipos histológicos. No entanto, tanto esta como outras classificações ainda conferem limitações quanto a reprodutibilidade de certos parâmetros, visto que incluem potencial subjetivo de avaliação, em especial o grau de anaplasia do tumor[1]. Nossos resultados mostraram que, à semelhança dos achados da literatura[2,6,10,15,16,17,21], a maioria dos tumores apresenta baixo grau ou grau intermediário de malignidade, o que contribui para seu prognóstico favorável.

Ressecção local ampla, associada ou não a esvaziamento cervical, e eventualmente a radioterapia pós-operatória, é o tratamento de escolha para os CME[2,5,9,10,15,17,21]. A ressecção de estruturas adjacentes está indicada em casos onde exista comprometimento detectado previamente ou durante a cirurgia. Houve necessidade de ressecções ampliadas na metade dos nossos casos tratados por cirrugia. Esvaziamento cervical está indicado em casos onde existam evidências clínicas de metástase regional, estadiamento clínico avançado ou alto grau histológico de malignidade[2,5,8,21]. Em cerca da metade dos casos nos quais foi realizado esvaziamento cervical foi demonstrado comprometimento neoplásico histologicamente.

As taxas de recidiva local (12,7%) e metástase regional (9,8%) e à distância (9,2%) encontradas em nossos casos, encontram-se entre os valores descritos na literatura[1,2,6,7,9,15,16,17,21]. A situação final dos pacientes revelou que a sobrevida global em 5 anos foi de 70%, realçando o comportamento relativamente favorável

e o bom prognóstico dos CME, quando comparados a outros tumores de glândulas salivares[10,16,17].

## CONCLUSÕES

Carcinomas mucoepidermóides de cabeça e pescoço afetaram principalmente adultos na 4ª a 6ª décadas de vida, sem predileção por sexo. A incidência foi semelhante nas glândulas salivares maiores e menores, envolvendo principalmente a parótida e o palato. A maioria dos tumores era de baixo grau ou grau intermediário de malignidade e tratamento cirúrgico local foi utilizado em cerca de 80% dos casos, complementado por esvaziamento cervical e radioterapia em 35% e 48% dos casos, respectivamente. A sobrevida global foi de 70% e 60%, respectivamente para 5 e 10 anos, confirmando o bom prognóstico geral dos carcinomas mucoepidermóides.

## COMITÊ DE ÉTICA

O protocolo de pesquisa que incluiu este trabalho foi aprovado pelo Comitê de Ética em Pesquisa do Centro de Tratamento e Pesquisa Hospital do Câncer A. C. Camargo, São Paulo/SP.

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## TABELAS

Tabela 1. Critérios de gradação histológica dos carcinomas mucoepidermóides (segundo Ellis & Auclair[5]).

| ***Parâmetro Histológico*** | ***Pontuação*** |
|---|---|
| Componente intra-cístico < 20% | +2 |
| Presença de invasão neural | +2 |
| Presença de necrose | +3 |
| Mitoses (4 ou + por 10 campos de grande aumento) | +3 |
| Presença de anaplasia | +4 |
| ***Grau Histológico*** | ***Pontuação*** |
| Baixo grau de malignidade | 0 a 4 |
| Grau intermediário de malignidade | 5 a 6 |
| Alto grau de malignidade | 7 a 14 |

Tabela 2. Distribuição dos 173 casos de carcinomas mucoepidermóides de cabeça e pescoço de acordo com sua localização.

| Localização | n | % por grupo | % do total |
|---|---|---|---|
| *Glândulas salivares maiores* | 80 | 100 | 46,2 |
| Parótida | 61 | 76,3 | 35,2 |
| Submandibular | 17 | 21,3 | 9,8 |
| Sublingual | 2 | 2,4 | 1,2 |
| *Glândulas salivares menores intra-orais* | 75 | 100 | 43,4 |
| Palato | 41 | 54,7 | 23,7 |
| Mucosa jugal | 7 | 9,3 | 4,0 |
| Área retromolar | 7 | 9,3 | 4,0 |
| Assoalho | 6 | 8,0 | 3,5 |
| Língua | 6 | 8,0 | 3,5 |
| Rebordo alveolar | 4 | 5,4 | 2,3 |
| Pilar amigdaliano | 3 | 4,0 | 1,7 |
| Lábio superior | 1 | 1,3 | 0,6 |
| *Glândulas salivares menores extra-orais* | 18 | 100 | 10,4 |
| Antro maxilar | 7 | 38,8 | 4,0 |
| Mandíbula (intra-ósseo) | 4 | 22,2 | 2,3 |
| Supraglote | 2 | 11,1 | 1,2 |
| Fossa nasal | 2 | 11,1 | 1,2 |
| Valécula | 1 | 5,6 | 0,6 |
| Rinofaringe | 1 | 5,6 | 0,6 |
| Cordas vocais | 1 | 5,6 | 0,6 |
| ***Total*** | **173** | - | **100** |

Tabela 3. Estadiamento TNM dos 173 casos de carcinomas mucoepidermóides.

| **Critérios de Estadiamento TNM** | **n (%)** |
|---|---|
| *Tamanho do tumor (T)* | |
| T1 | 31 (17,9%) |
| T2 | 56 (32,4%) |
| T3 | 32 (18,5%) |
| T4 | 29 (16,8%) |
| Tx | 25 (14,5%) |
| *Presença de Metástases Regionais (N)* | |
| Sem metástase | 142 (82,1%) |
| Com metástase | 31 (17,9%) |
| *Presença de Metástase a Distância (M)* | |
| Sem metástase | 170 (98,3%) |
| Com metástase | 3 (1,7%) |
| *Estadiamento Clínico* | |
| Estádio I | 53 (30,6%) |
| Estádio II | 34 (19,7%) |
| Estádio III | 16 (9,2%) |
| Estádio IV | 45 (26,0%) |
| Sem estadiamento | 25 (14,5%) |

Tabela 4. Distribuição dos 173 casos de carcinomas mucoepidermóides de cabeça e pescoço quanto ao tratamento efetuado.

| **Tipo de tratamento** * | **n** | **%**** |
|---|---|---|
| *Não tratados no Hospital A. C. Camargo* | *22* | *12,7* |
| *Tratamento no Hospital A. C. Camargo* | *151* | *87,3* |
| Cirurgia local | 55 | 36,4 |
| Cirurgia local + radioterapia | 31 | 20,5 |
| Cirurgia local + esvaziamento cervical | 21 | 13,9 |
| Cirurgia local + esvaziamento cervical + radioterapia | 27 | 17,9 |
| Cirurgia local + esvaziamento cervical + radioterapia + quimioterapia | 3 | 2,0 |
| Cirurgia local + esvaziamento cervical + Quimioterapia | 1 | 0,7 |
| Cirurgia local + Quimioterapia | 1 | 0,7 |
| Radioterapia | 7 | 4,6 |
| Radioterapia + quimioterapia | 4 | 2,6 |
| Quimioterapia | 1 | 0,7 |

* Quarenta e cinco pacientes (26,0%) já haviam sido submetidos a tratamento oncológico prévio fora do Hospital; ** A porcentagem de cada tipo de tratamento está expressa em relação aos 151 pacientes que foram submetidos a tratamento no Hospital.

Tabela 5. Tipo de cirurgia local e de esvaziamento cervical efetuado nos pacientes com carcinomas mucoepidermóides tratados cirurgicamente no Hospital A. C. Camargo (n=139).

| **Tipo de Cirugia Local e esvaziamento cervical** | **n** | **%** |
|---|---|---|
| *Tipo de Cirurgia Local (n=139)** | | |
| Cirurgia Local com margem | 61 | 43,9 |
| Parotidectomia total ou parcial | 56 | 40,3 |
| Submandibulectomia | 13 | 9,4 |
| Ressecção maxilar | 10 | 7,2 |
| Laringectomia | 1 | 0,7 |
| Outros tipos de cirurgia | 4 | 2,9 |
| *Tipo de esvaziamento cervical (n=52) *** | | |
| Radical unilateral | 28 | 53,8 |
| Supra-omo-hióideo unilateral | 21 | 40,4 |
| Radical bilateral | 1 | 1,9 |
| Outros tipos de esvaziamento cervical | 3 | 5,8 |

* Seis pacientes foram submetidos a mais de um tipo de cirurgia local; ** Um paciente foi submetido a mais de um tipo de esvaziamento cervical.

Tabela 6. Distribuição da freqüência de cada estrutura ressecada adjacente aos tumores em relação ao seu número total (n=69) e ao total de pacientes submetidos a procedimentos cirúrgicos no Hospital A. C. Camargo (n=139).

| **Estrutura ressecada*** | **n** | **% (n=69)** | **% do total (n=139)** |
|---|---|---|---|
| Maxila | 22 | 31,9 | 15,8 |
| Pele | 20 | 29,0 | 14,4 |
| Mandíbula | 15 | 21,7 | 10,8 |
| Nervo | 9 | 13,0 | 6,5 |
| Músculo | 4 | 5,8 | 2,9 |
| Outra estruturas | 18 | 26,1 | 12,9 |

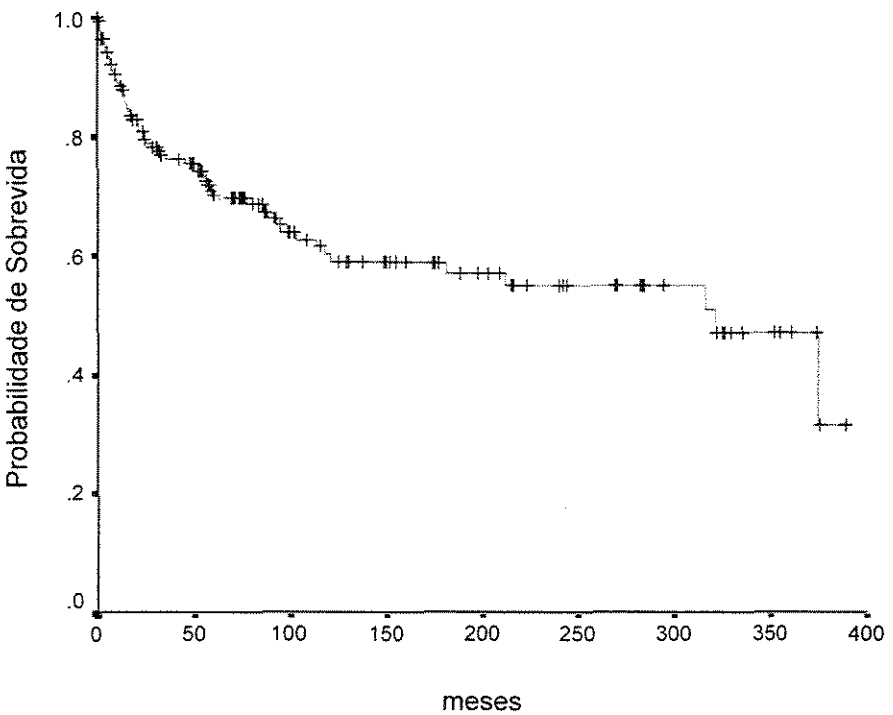
* Em 14 dos 69 casos (20,3%), mais de uma estrutura adjacente foi ressecada.

Tabela 7. Distribuição da freqüência das complicações pós-cirúrgicas em relação ao seu total (n=52) e em relação ao total de pacientes submetidos a procedimentos cirúrgicos no Hospital (n=139).

| **Complicações Pós-Cirúrgicas** | **n** | **% (n=52)** | **% do total (n=139)** |
|---|---|---|---|
| *Sem complicações pós-cirúrgicas* | *87* | - | *62,6* |
| *Com complicações pós-cirúrgicas* | *52* | - | *37,4* |
| Paralisia facial | 20 | 38,5 | 14,4 |
| Infecção | 12 | 23,1 | 8,6 |
| Seroma | 8 | 15,4 | 5,8 |
| Necrose do retalho | 5 | 9,6 | 3,6 |
| Síndrome de Frei | 5 | 9,6 | 3,6 |
| Óbito pós-cirúrgico | 3 | 5,8 | 2,2 |
| Outras complicações | 12 | 23,1 | 8,6 |

## FIGURAS

Figura 1. Curva de sobrevida global (método de Kaplan-Meier) dos 173 casos de carcinomas mucoepidermóides tratados no Hospital A. C. Camargo.



## CAPÍTULO 2

# CLINICO-PATHOLOGICAL AND IMMUNOHISTOCHEMICAL PROGNOSTIC FACTORS IN HEAD AND NECK MUCOEPIDERMOID CARCINOMA

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**KEY-WORDS:** Salivary gland tumors; mucoepidermoid carcinoma; prognostic factors; survival analysis.

**RUNNING TITLE:** Mucoepidermoid carcinoma.

## ABSTRACT

**Background:** Clinical staging and histological grade are the main prognostic factors in mucoepidermoid carcinoma (MEC). **Methods:** Clinical, histopathological and immunohistochemical data from 173 cases of MEC were

analysed through univariate and multivariate survival analysis. **Results:** TNM revealed 50.3% stages I or II, 45.2% were low grade MEC and five-year overall survival was 70%. Univariate survival analysis revealed that age (>40 years) ($p<0.001$), male gender ($p=0.005$), fixed tumors ($p=0.002$), invasion of the adjacent structures ($p=0.004$), submandibular tumors ($p=0.001$), T ($p<0.001$), N ($p<0.001$), clinical stage ($p<0.001$), histological grade ($p<0.001$), and PCNA ($p<0.001$), ki-67 ($p<0.001$) and p53 ($p<0.001$) expression were correlated to a worst prognosis. Expression of CEA ($p=0.012$) and bcl-2 ($p<0.001$) were correlated to a better prognosis. Age (>40 years), fixation of the tumors, T, N and histological grade were independent significant prognostic factors after multivariate survival analysis. **Conclusions:** Clinical, histological and immunohistochemical data were correlated to prognosis in MEC and should be also considered when establishing patient management.

## INTRODUCTION

Mucoepidermoid carcinoma (MEC) is the most common malignant salivary gland tumor, accounting for about 3 to 15% of all salivary gland tumors and 12 to 40% of the malignant[1,2,3,4]. They are supposed to originate from pluripotential cells of the excretory ducts, explaining its variable histological features[5]. Although major and minor salivary glands, specially parotids and minor glands from the palate, are the affected sites in more than 90% of the MEC cases, extra-oral glands such as lacrimal glands and glands from the epithelium lining of the maxillary sinus, oropharynx, nasopharynx, larynx, trachea and bronchus can also be affected[1,4,5]. Histologically, MEC has been usually classified in three malignant grades (low, intermediate and high), according to cytological features, pattern of invasion and amount of each cellular type[6,7].

Several clinical and histological significant prognostic factors for MEC have been demonstrated in univariate and multivariate survival analysis, but the most consistent ones have proved to be clinical stage and histological grade of the tumors[1,2,3,4,5,7,8,9,10]. Expression of some proliferative markers, tumor supressor

proteins, membrane receptors and anti-apoptotic proteins have been also demonstrated as valuable prognostic factors in MEC[10,11,12,13,14,15,16,17]. However, few papers have demonstrated the influence of both clinical/histological and immunohistochemical parameters in large series of MEC from different sites[10,11,16,17].

The aim of this paper was to analyse clinical, pathological and immunohistochemical prognostic factors in a large series of MEC treated in a single institution in a 44-year interval.

## MATERIALS AND METHODS

The files of the Hospital do Câncer A. C. Camargo, São Paulo, Brazil, were reviewed and all cases diagnosed as MEC from 1953 to 1997 were retrieved for the study. All cases were histologically reviewed using hematoxilin & eosin, Schiff periodic acid and mucicarmine staining to confirm histological diagnosis. Clinical, epidemiological, treatment and follow-up data were obtained from the patients records. Histological grading of the tumors was performed according to Ellis & Auclair[7] in low, intermediate and high grade tumors, using the following criteria: amount of cystic component, necrosis, perineural invasion, mitosis and anaplasia.

Immunohistochemical reactions against PCNA, ki-67, p53, c-erbB-2, CEA and bcl-2 were performed as follows: 3μm histological sections were deparaffinyzed, hydrated in alcohol and washed in 10% hydrogeneous peroxyde for 30 minutes to inhibit endogenous peroxidase. Microwave antigen retrieval using citrate buffer and overnight incubation with the primary antibodies against proliferation cell nuclear antigen (PCNA, clone PC10, Dako A/S Denmark, dilution 1:16.000), ki-67 antigen (Clone ki-S5, Dako A/S Denmark, dilution 1:200), p53 protein (Clone DO-7, Dako A/S Denmark, dilution 1:200), c-erbB-2 protein (Dako A/S Denmark, dilution 1:200), carcinoembryonic antigen (CEA, Clone II-7, Dako A/S, Denmark, dilution 1:500) and bcl-2 protein (Clone 124, Dako A/S Denmark, dilution 1:50), were performed. Secondary antibodies conjugated to a streptavidin-biotin-peroxidase system (Strept ABComplex/HRP Duet, Mouse/Rabbit, Dako A/S,

Denmark) were used, followed by diaminobenzidine as the chromogen. Slides were counterstained with Carazzi hematoxyllin, mounted and analysed by the same author (FRP). Positive and negative controls were included in all reactions. Percentage of cells expressing each of the markers, after analysing 10 different high-power fields of each case, was classified as follows: PCNA (negative: <1% to 5% of positive cells; weak: 6% to 20% of positive cells; strong: >21% positive cells); ki-67, p53, c-erbB-2, CEA and bcl-2 (negative: <1% to 5% of positive cells; positive: >5% of positive cells).

Kaplan-Meier method and log-rank test were used for univariate survival analysis. Overall survival was calculated from the date of starting treatment (surgery or radiotherapy) or the date of first consultation to the last follow-up information. The cases were considered uncensured when death was the outcome independent of its cause. For multivariate survival analysis, all variables were analysed by the Cox regression model.

## RESULTS

### Clinical and Socio-demographic

Gender distribution of the 173 cases showed that the male:female ratio was 1.2:1, with 93 cases (53.8%) affecting males. Mean age of the patients was 44 years (range 6 - 96 years), and the mean age was higher for males (mean 47.3 years, range 6 - 79 years) than for females (mean 40 years, range 6 - 96 years). The majority of patients (55.5%) were in the $4^{th}$ to $6^{th}$ decade. Duration of complaints showed a mean of 38 months (range 1 - 480 months), and the most reported symptoms were swelling in the area (93.6%), pain (39.9%) and paresthesia (6.4%).

Site of the tumors included 80 cases (46.2%) affecting major salivary glands, being 61 parotid, 17 submandibular and 2 sublingual. From the 93 cases (53.8%) affecting minor salivary glands, 75 involved intra-oral and 18 extra-oral sites. Distribution of the 173 cases according to the involved site is shown in Table

1. The diameter of the tumors varied according to the site of the lesions, being greater in the extra-oral minor salivary glands (mean 5.5 cm, range 2 - 9 cm), followed by major glands (mean 4.6 cm, range 1 - 16 cm) and intra-oral minor salivary glands (mean 3.6 cm, range 1 - 8 cm). Mobility revealed 74.3% fixed or partially fixed tumors and invasion of the adjacent structures was present in 70.5% of the cases. TNM clinical stage showed that 87 cases (50.3%) were T1 or T2, and that 31 patients (17.9%) and 3 patients (1.7%) showed regional and distant metastasis at the first evaluation. Final TNM staging revealed that 50.3% of the cases were in stages I and II (Table 2).

**Histology**

Histological grading criteria showed that 73 cases (42.2%) revealed intra-cystic component lower than 20% of the tumor, 15 (8.7%) peri-neural invasion, 38 (22.0%) necrosis, 31 (17.9%) 4 or more mitosis in 10 high-power fields and 87 (50.3%) anaplasia. Final histological grade showed 61 (35.3%) low-grade, 25 (14.5%) intermediate-grade and 49 (28.3%) high-grade tumors, and 38 (22.0%) were not submitted to histological grading due to the limited available amount of tumoral tissue. Considering only the 135 cases that were histologically graded, low-grade, intermediate-grade and high-grade tumors represented 45.2%, 18.5% and 36.3%, respectively.

**Immunohistochemistry**

Immunohistochemical reactions revealed that PCNA was expressed by 92.9% of the tumors, being weak in 45.7% and strong in 47.2%. Expression of ki-67, p53, c-erbB-2, CEA and bcl-2 was found in 34.1%, 16.4%, 80.0%, 68.6% and 63.2% of the tumors, respectively.

**Treatment**

Previous oncologic treatment was reported in 45 cases (26.0%), and in the A. C. Camargo Hospital, surgical procedures were employed in 151 patients

(87.3%), including local resection in 139 cases (92.1%) and neck dissection in 52 cases (34.4%). Radiotherapy and chemotherapy were employed in 72 (47.7%) and 10 cases (6.6%), respectively, either as adjuntive measures or isolated. Twenty-two cases (12.7%) were not submitted to any treatment in the Hospital. Local resection alone (36.4%), local resection and postoperative radiotherapy (20.5%), local resection, neck dissection and postoperative radiotherapy (17.9%) and local resection and neck dissection (13.9%) were the most common treatment forms. From the 52 cases submitted to neck dissection, 22 (42.3%) showed metastatic lymph nodes after histological evaluation. Surgical removal of the tumors was associated to resection of adjacent structures, such as maxillary bones, skin, mandible or nerve trunks, in 69 cases (49.6%).

**Local recurrence, regional and distant metastasis**

Twenty-two patients (12.7%) showed local recurrence, with a mean interval of 41.7 months (ranging from 2 to 207 months). Regional metastasis were found in 17 patients (9.8%) (mean interval of 29.3 months, ranging from 3 to 77 months), being homolateral in 10 cases (5.8%), contralateral in 5 cases (2.9%) and bilateral in 2 cases (1.1%). Distant metastasis affected 16 patients (9.2%) (mean interval 66.1 months, ranging from 3 to 370 months), being more frequent in lungs (12 cases - 6.9%) and in bones (5 cases - 2.9%).

**Follow-up and survival rates**

The mean follow-up interval was 98 months (range 1 - 389 months) and revealed 83 patients (48.0%) alive with no evidence of disease, 6 (3.5%) alive with disease, 45 (26.0%) dead by the disease, 16 (9.2%) dead during treatment or by other causes and 23 (13.3%) lost to follow-up. Overall survival rates were 70% and 60%, respectively, for 5 and 10 years.

**Univariate and multivariate survival analysis**

Univariate survival analysis of clinical and epidemiological parameters revealed that age of the patients >40 years ($p<0.001$), male gender ($p=0.005$), decreased mobility of the tumors ($p=0.002$), invasion of the adjacent structures ($p=0.004$), and higher T ($p<0.001$), N ($p<0.001$) and clinical stage ($p<0.001$) were statistically correlated to a worst prognosis (Table 3). The site of the tumors showed that parotid and submandibular cases had, respectively, the best and worst survival rates (Table 3). Increased final histological grade ($p<0.001$) and presence of all histological parameters analysed, except for perineural invasion, were also correlated with a worst prognosis (Table 4). Treatment parameters showed that previous oncologic treatment ($p=0.990$) and radiotherapy were not related to a worst prognosis, but patients submitted to surgery ($p=0.004$) and neck dissection ($p=0.007$) showed better prognosis. Increased PCNA ($p<0.001$), ki-67 ($p<0.001$) and p53 ($p<0.001$) immunohistochemical expression were also correlated to a worst prognosis and, on the contrary, higher expression of CEA ($p=0.012$) and bcl-2 ($p<0.001$) were correlated to a better prognosis (Table 5).

Multivariate survival analysis revealed that age (>40 years), fixation of the tumors, T, N and histological grade were independent significant prognostic factors (Table 6).

## DISCUSSION

MEC can affect patients in a wide age range from children to elderly, but has a predilection for adults in the $4^{th}$ to $6^{th}$ decade, without gender predilection[3,8,9,10,11,17,18,19,20]. In our patients, mean age of the affected males was older than affected females, as described in the literature[17]. MEC usually present clinically as long-lasting swellings, and presence of symptoms such as surface ulceration, pain and paresthesia have been commonly described in the literature and were found in about 40% of our cases[1,2,4,8,20]. As also shown by our cases, the literature reports that the time of complaint in mucoepidermoid carcinomas is large, having a mean of 4 to 27 months, ranging from 1 to 480 months[2,3,4,9,20].

Our results confirmed that MEC usually affects major and intraoral minor salivary glands, but also showed that it can arise in other different head and neck glands in about 10% of the cases, including the epithelium lining of the maxillary sinus, glands from the nasopharynx, nasal cavity, oropharynx, voccal cords, larynx and trachea and ectopic intraosseous glands, as already described in the literaure[1,4,5].

Site of our tumors showed that most cases affected the parotid, followed by the palate and the submandibular gland, in accordance with the literature[1,2,4,5,11]. As a group, intraoral minor salivary glands showed higher prevalence than the parotid, confirming the findings of other authors[2,3]. Intraorally, the palate is the most common affected site, representing 22 to 50% of the lesions, being followed by the buccal mucosa, tongue, alveolar mucosa and retromolar area[1,2,3,4,10,17,20]. Size of the tumors was different when comparing the three main groups in our cases (major salivary glands, intraoral minor salivary glands and extraoral minor salivary glands). Auclair et al[20] reported a mean size of 1.6 cm to intraoral minor salivary gland MEC, in contrast to our mean of 3.8 cm. In our cases, MEC affecting extraoral minor salivary glands showed greater size than cases in major and intraoral minor salivary glands. This characteristic was probably related to the presence of maxillary sinus MEC cases in the former group (38.8% of the cases), which can reach considerable size before being noticed by the patients.

Clinical staging revealed that 50.3% of our cases were TNM stages I and II, values that are similar or slightly lower than the literature reports[2,3,4,8,10]. Despite 50.3% of our cases were diagnosed in stages T1 and T2, 74.3% of the tumors showed at least partial fixation to the adjacent tissues and 70.5% invaded one or more adjacent structures, explaining the worst prognosis of some cases, even with limited size. Minor salivary glands showed tumors in more advanced TNM stages than major ones, as already described in the literature, and frequency of T1 and T2 tumors (50.3%), and N (17.9%) and M (1.7%) percentages in our cases were similar to the ones described in the literature[1,3,10].

Histological diagnosis of MEC is based on the presence of three cellular types (mucous, intermediate and squamous cells) organized in islands, sheets and cystic structures. PAS, mucicarmine and Alcian blue positive staining are important to confirm the mucoid nature of the cytoplasmic material in the tumoral cells[5,7]. Histological grading of MEC have included several parameters such as pattern and grade of invasion, vascular and lymphatic invasion, bone invasion, necrosis, presence of inflammtory infiltrate, cell differentiation, cellular anaplasia, proportion of mucous and epidermoid cells, solid or cystic growth pattern, and mitotic activity[1,2,4,6,7,9,18,20]. Several classifications have been proposed and grading usually divides tumors in low, intermediate and high malignant grade[2,4,5,6,9,18,20]. Literature shows that the proportion of low, intermediate and high-grade tumors can vary from 15 to 62%, 9 to 48% and 22 to 38%, respectively, ranges that include our findings[1,2,4,10,14,17,18,21].

Inclusion of many histological parameters can aid grading of MEC, but sometimes brings difficulties as some informations are not always available in all cases. Grading MEC should consider few easily observable parameters present in most cases, and its utility as prognostic factors should be evaluated. The five criteria suggested and evaluated by Ellis & Auclair[7], Goode et al[9] and Auclair et al[20] are based in easily observable histological parameters, allowing the distinction of the three MEC histological types, and show considerable prognostic significance. However, as other grading systems, there are some limitations regarding reproducibility of some, at least partially, subjective features, such as the grade of anaplasia and the pattern and grade of invasion of an individual tumor[20]. Both extreme lesions, namely low and high grade MEC, are usually easily distinguishble, but intermediate-grade tumors can show both low and high grade features. Although intermediate-grade MEC share more microscopical features with low-grade tumors, its clinical behavior can be variable and they usually show positive margins and local recurrence more frequently than low-grade MEC[9,18]. Some authors have indeed reported that intermediate-grade tumors should behave closely to high-grade lesions[10]. It seems important to establish this intermediate

group, but grading and prognostic studies should focus on separating good and poor prognosis intermediate-grade MEC[18,20].

Some authors have called attention that there seems to be a correlation of size and clinical stage of the tumors with their histological grade[1,4,8,21]. In fact it should be expected that low-grade MEC have low proliferative activity, in comparison to high-grade tumors, showing lower size and consequently lower clinical stages.

Wide surgical approach, eventually complemented by neck dissection and radiotherapy is the treatment of choice for MEC[1,3,4,8,9,14,19]. Resection of adjacent structures is indicated when there are previous or trans-surgical evidence of invasion, and was performed in about half of our cases managed by surgery. Neck dissection is indicated when there is clinical evidence of regional metastasis, high TNM stages, high histological grade, or tumor proximity to regional lymph nodes[1,4,18]. Half of our cases submitted to neck dissection proved to have metastatic tumoral tissue in the lymph nodes histologically. Post-surgical radiotherapy seems indicated where there are clinical or histological-proved positive margins, regional metastasis and in high-grade tumors[4,18].

Local recurrence (12.7%), regional (9.8%) and distant (9.2%) metastasis taxes of our cases were similar to the literature, that ranges from 7 to 26%, 3 to 16% and 6 to 15%, respectively[1,2,3,4,20]. Our five-year and ten-year overall survival rates of 70% and 60%, respectively, are also in accordance with their ranges in the literature, from 57 to 92% and from 28% to 90%, respectively[2,10,14,19], reinforcing the relatively good prognosis of MEC. Malignant salivary gland tumors are characterized by late recurrence and metastasis, sometimes 5 or 10 years after treatment, so patients should be routinely followed for long periods after treatment[18].

Univariate and multivariate prognostic studies of MEC have been reported in the literature, most of them including clinical and pathological parameters. Clinical parameters included male gender, increasing age, presence and duration of symptoms, localization of the tumors in the submandibular gland or intraorally in

the tongue of floor of the mouth, increased size of the tumor and presence of regional or distant metastasis at diagnosis[2,4,8,9,10,18,20,21]. Age of the patients seems to be an important prognostic factor, but, as most tumors in younger groups (1st and 2nd decades) are low-grade lesions, it is difficult to ascertain its real importance[2,19,21]. Hicks & Flaitz[16] reinforced that MEC affecting children and adolescents show predilection to major salivary glands and similar complaint intervals and symptoms than in adults, however, more than 90% of the cases are low or intemediate-grade lesions, which can be responsible for its overall excellent prognosis in this age group. In our patients, although most cases in youngsters were low-grade tumors, age of the patients >40 years was found to be a significant prognostic factor in both univariate and multivariate survival analysis. In a similar way, the real prognostic significance of the male gender would be explained by the fact that most high-grade tumors affect men and most low-grade tumors affect women[2,21]. Similarly to age of the patients, although in our cases intermediate or high grade tumors were more common in men, male gender proved to be an important prognostic factor in univariate survival analysis.

Mean size of the tumors seems also to be lower in females and younger patients, contributing to its better overall survival[1]. Our findings showed that size (T) and mobility of the tumors, invasion of adjacent structures and regional metastasis are important prognostic factors in MEC. Regarding site, from the major salivary glands, submandibular tumors seem to have a worst prognosis[1,8,20], although this concept is not universally accepted[4]. Our data showed that localization of the tumors in the submandibular gland was related to a worst prognosis, and some authors suggest that submandibular tumors should receive radical treatment, independently of its histological grade[9]. Although the presence of symptoms, such as pain and paresthesia, was not related to a worst prognosis in our cases, it has been reported as an important prognostic factor in other studies[1,8,9,20].

Histological grade has been described as one of the most important prognostic factors in MEC. Overall five-year survival rates show significant

correlation to histological grade of the tumors in the literature, varying from 92 to 100% in low-grade, 62 to 92% in intermediate-grade and 0 to 43% in high-grade tumors[1,2,8,17,19,21]. Histopathological parameters shown to be prognostically significant are decreased cystic component, perineural invasion, necrosis, increased mitosis, anaplasia and histological grade of the tumors[2,9,18,20,21]. Goode et al[9] and Auclair et al[20] demonstrated that their five suggested grading criteria showed correlation to survival of their patients. Our study confirmed that all these parameters, except for perineural invasion, are important prognostic factors in MEC, supporting their utility as components of grading systems. The importance of perineural invasion in MEC prognosis should be confirmed by other large series studies. In addition, our results agree with other reports that most MEC are low and intermediate grade lesions, contributing to its overall favorable prognosis[1,2,4,10,14,17,18,21].

Proliferating cell nuclear antigen (PCNA) and ki-67 antigen are cell cycle related proteins whose immunohistochemical expression has been correlated to cell proliferation, agressiveness and malignant behavior in many salivary gland tumors, including MEC[10,11,15]. Increased PCNA and ki-67 expression seem to be related to histological grade, from low-grade to high-grade tumors, and were proved to be important adjuvant prognostic factors in MEC[10,11,15,17]. Increasing in the number of proliferating cells can be also directly related to increasing mortality, decreasing survival rates and worst prognosis in MEC[11,16]. In addition, proliferative markers, such as PCNA and ki-67, can also be useful as an aid in distinguishing if intermediate-grade tumors would behave as low or high-grade lesions[16]. Our results showed that both PCNA and ki-67 immunohistochemical expression proved to be significant prognostic factors in MEC in univariate survival analysis.

Oncoproteins, tumor supressor proteins, membrane receptors and differentiation markers have been also immunohistochemically studied and described as important prognostic factors in salivary gland tumors such as MEC. The p53 protein has been variably expressed in MEC, ranging from 8 to 80% of the tumors, and some authors have reported its correlation to higher histological grade,

increased size of tumors, presence of regional metastasis and, consequently, a worst prognosis[13,17]. Immunohistochemical expression of p53 protein was found in 16.4% of our cases and was correlated to a worst prognosis. C-erbB-2 expression has been also variable in MEC, ranging from 0 to 24% of the tumors, and correlated to histological grade, size of tumors and presence of regional metastasis[13]. Expression of p53 and c-erbB-2 seems to be also correlated to the proliferative activity of MEC, measured by ki-67 immunohistochemical expression, and have prognostic significance[13]. C-erbB-2 increased expression in MEC, associated or not to high histological grade, seems to be a marker for tumoral agressiveness and also an important prognostic factor[14], a finding that was not not confirmed by our results. Conversely, increased expression of bcl-2 by MEC cells is more common in low-grade tumors and is correlated to less agressiveness, higher survival rates and a better prognosis[17], in accordance with our results. Although a higher immunohistochemical expression of bcl-2, an anti-apoptotic protein, can improve the ability of MEC cells in avoiding the apoptotic pathway, other mechanisms should control cell proliferation in these tumors. Carcinoembryonic antigen (CEA), have also been evaluated in MEC and in tumoral tissue its expression is higher than in normal glands, specially in high-differentiated ductal and cystic areas[12]. Although it seems to exist a correlation between CEA expression and a high proliferative activity in pleomorphic adenoma and its malignant transformation[12], its increased expression in our MEC cases was correlated to low-grade tumors and, consequently, a better prognosis.

Multivariate survival analysis in the literature has shown that histological grade, local recurrence, presence of symptoms at first diagnosis, size of the tumors, conservative surgery and clinical staging are important prognostic factors in MEC[8,10]. In addition to T, N and histological grade, our results of multivariate survival analysis revealed that age of the patients (>40 years) and fixation of the tumors are also important independent clinical prognostic factors in MEC.

Although the most frequently reported important prognostic factors in MEC are clinical stage and histological grade of the tumors[1,2,3,4,5,7,8,10], treatment should

not be guided by each one isolated[9]. Our results supported that other clinical, histological and immunohistochemical prognostic factors are important in univariate and multivariate survival analysis in MEC and should be evaluated by other comparative studies in large series of MEC.

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## TABLES

Table 1. Site distribution of 173 cases of head and neck mucoepidermoid carcinomas.

| Localization | n | % (each group) | % (total) |
|---|---|---|---|
| **Major salivary glands** | **80** | **100** | **46.2** |
| Parotid | 61 | 76.3 | 35.2 |
| Submandibular | 17 | 21.3 | 9.8 |
| Sublingual | 2 | 2.4 | 1.2 |
| **Intraoral minor salivary glands** | **75** | **100** | **43.4** |
| Palate | 41 | 54.7 | 23.7 |
| Buccal mucosa | 7 | 9.3 | 4.0 |
| Retromolar area | 7 | 9.3 | 4.0 |
| Floor of mouth | 6 | 8.0 | 3.5 |
| Tongue | 6 | 8.0 | 3.5 |
| Alveolar mucosa | 4 | 5.4 | 2.3 |
| Tonsil | 3 | 4.0 | 1.7 |
| Upper lip | 1 | 1.3 | 0.6 |
| **Extraoral minor salivary glands** | **18** | **100** | **10.4** |
| Maxillary antrum | 7 | 38.8 | 4.0 |
| Mandible | 4 | 22.2 | 2.3 |
| Supraglotis | 2 | 11.1 | 1.2 |
| Nasal cavity | 2 | 11.1 | 1.2 |
| Valecule | 1 | 5.6 | 0.6 |
| Nasopharynx | 1 | 5.6 | 0.6 |
| Voccal cords | 1 | 5.6 | 0.6 |
| **Total** | **173** | - | **100** |

Table 2. Clinical stage (TNM) of 173 head and neck mucoepidermoid carcinomas.

| TNM staging criteria | n (%) |
|---|---|
| **Size of tumor (T)** | |
| T1 | 31 (17.9) |
| T2 | 56 (32.4) |
| T3 | 32 (18.5) |
| T4 | 29 (16.8) |
| Tx | 25 (14.5) |
| **Regional metastasis (N)** | |
| With metastasis | 31 (17.9) |
| Without metastasis | 142 (82.1) |
| **Distant metastasis (M)** | |
| With metastasis | 3 (1.7) |
| Without metastasis | 170 (98.3) |
| **Clinical stage TNM** | |
| Stage I | 53 (30.6) |
| Stage II | 34 (19.7) |
| Stage III | 16 (9.2) |
| Stage IV | 45 (26.0) |
| Without staging | 25 (14.5) |

Table 3. Univariate survival analysis of clinical and epidemiological parameters of 173 cases of mucoepidermoid carcinomas.

| Parameter | n | 5-year survival | 10-year survival | P value |
|---|---|---|---|---|
| **Gender (n=173)** | | | | **0.005*** |
| Male | 93 | 59.8% | 48.8% | |
| Female | 80 | 81.3% | 72.4% | |
| **Age (n=173)** | | | | **<0.001*** |
| ≤ 40 years | 72 | 92.4% | 92.4% | |
| > 40 years | 101 | 54.3% | 36.2% | |
| **Site of the tumors (n=145)** | | | | **<0.001*** |
| Parotid | 48 | 75.6% | 71.6% | |
| Extraoral minor glands | 14 | 63.6% | 63.6% | |
| Intraoral minor glands | 67 | 76.1% | 56.4% | |
| Submandibular | 16 | 25.0% | 25.0% | |
| **Mobility of the tumor (n=151)** | | | | **0.002*** |
| Mobile | 39 | 92.1% | 79.6% | |
| Fixed/Partially fixed | 112 | 60.1% | 49.3% | |
| **Invasion of adjacent structures (n=165)** | | | | **0.004*** |
| Yes | 49 | 53.2% | 44.3% | |
| No | 116 | 76.0% | 65.3% | |
| **T (Size of the tumor) (n=147)** | | | | **<0.001*** |
| T1 + T2 | 86 | 87.4% | 73.2% | |
| T3 + T4 | 61 | 40.2% | 36.5% | |
| **N (Regional metastasis) (n=173)** | | | | **<0.001*** |
| Yes | 31 | 29.2% | 17.5% | |
| No | 142 | 78.6% | 69.1% | |
| **Clinical stage (TNM) (n=147)** | | | | **<0.001*** |
| I + II | 86 | 85.7% | 74.8% | |
| III + IV | 61 | 42.4% | 33.3% | |

* Statistically significant

Table 4. Univariate survival analysis of histological parameters of 134 cases of mucoepidermoid carcinomas submitted to histological grading.

| Parameter | n | 5-year survival | 10-year survival | P value |
|---|---|---|---|---|
| **Intra-cystic component < 20%** | | | | **<0.001*** |
| Yes | 72 | 50.5% | 39.2% | |
| No | 62 | 94.1% | 85.3% | |
| **Presence of perineural invasion** | | | | **0.213** |
| Yes | 15 | 52.5% | 39.4% | |
| No | 119 | 72.6% | 63.1% | |
| **Presence of necrosis** | | | | **<0.001*** |
| Yes | 38 | 38.1% | 27.2% | |
| No | 96 | 83.1% | 73.5% | |
| **4 or more mitosis in 10 high-power fields** | | | | **<0.001*** |
| Yes | 31 | 26.9% | 20.1% | |
| No | 103 | 83.0% | 72.4% | |
| **Presence of anaplasia** | | | | **<0.001*** |
| Yes | 86 | 55.4% | 44.8% | |
| No | 48 | 97.7% | 90.4% | |
| **Histological grade** | | | | **<0.001*** |
| Low grade | 61 | 94.0% | 88.4% | |
| Intermediate grade | 24 | 82.5% | 66.0% | |
| High grade | 49 | 34.4% | 22.9% | |

* Statistically significant

Table 5. Univariate survival analysis of immunohistochemical markers in mucoepidermoid carcinomas.

| Parameter | n | 5-year survival | 10-year survival | P value |
|---|---|---|---|---|
| **PCNA (n=140)** | | | | **<0.001*** |
| Strong | 66 | 51.9% | 47.5% | |
| Weak | 64 | 84.3% | 74.3% | |
| No | 10 | 100.0% | 60.0% | |
| **Ki-67 (n=123)** | | | | **0.001*** |
| Yes | 42 | 48.6% | 33.3% | |
| No | 81 | 78.7% | 69.7% | |
| **P53 (n=140)** | | | | **<0.001*** |
| Yes | 23 | 27.2% | 27.2% | |
| No | 117 | 78.4% | 67.0% | |
| **c-erbB-2 (n=140)** | | | | **0.279** |
| Yes | 112 | 70.6% | 60.8% | |
| No | 28 | 66.3% | 58.0% | |
| **CEA (n=140)** | | | | **0.012*** |
| Yes | 96 | 74.4% | 68.4% | |
| No | 44 | 59.1% | 41.0% | |
| **Bcl-2 (n=125)** | | | | **<0.001*** |
| Yes | 79 | 80.1% | 68.3% | |
| No | 46 | 44.6% | 39.7% | |

* Statistically significant

Table 6. Independent significant prognostic factors after multivariate survival analysis in 173 head and neck mucoepidermoid carcinomas.

| Variable | Categories | Relative risk | Confidence interval | P value |
|---|---|---|---|---|
| Age | > 40 years | 3.8 | 1.7 - 8.4 | < 0.001 |
| T | T3 + T4 | 3.1 | 1.7 - 5.6 | < 0.001 |
| N | N + | 2.4 | 1.3 - 4.4 | < 0.001 |
| Fixation of the tumor | Partially fixed/fixed | 2.2 | 1.0 - 4.8 | 0.044 |
| Histological grade | High grade | 2.6 | 1.3 - 5.5 | < 0.001 |

## CAPÍTULO 3

# SYNCHRONOUS MUCOEPIDERMOID CARCINOMA OF TONGUE AND PLEOMORPHIC ADENOMA OF SUBMANDIBULAR GLAND

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**ABSTRACT**

Synchronous salivary gland tumors are rare and include mainly Whartin's tumor and pleomorphic adenoma of the parotid, being extremely uncommon in minor salivary glands. The authors report a case of simultaneous mucoepidermoid carcinoma of the tongue and pleomorphic adenoma of the submandibular gland in

a 40-year-old woman. The submandibular mass was firstly interpreted as a regional lymph node metastasis, but it was shown to be an intra-glandular nodule during the surgical approach. This seems to be the first report of such association in the English-language literature.

**KEY-WORDS**

Mucoepidermoid carcinoma; pleomorphic adenoma; synchronous tumors; salivary gland tumors.

## INTRODUCTION

Multiple synchronous or metachronous salivary gland tumors are rare, accounting for about 0.4 to 1.3% of all salivary gland neoplasms[1]. The most common synchronous and metachronous salivary gland tumors are Whartin's tumor and pleomorphic adenoma, involving mainly the parotids[1,2]. Synchronous or metachronous tumors are extremely unusual in the minor salivary glands, with few cases reported in the English-language literature[3,4]. Shaw et al[4] reported a case of synchronous palatal low-grade mucoepidermoid carcinoma and parotid pleomorphic adenoma in a 29-year-old male. We did not find any report of simultaneous involvement of the submandibular gland and minor salivary glands of the tongue. Our purpose is to report a case of synchronic mucoepidermoid carcinoma of the tongue and pleomorphic adenoma of the submandibular gland in a 40-year-old woman.

## CASE REPORT

A 40-year-old white female was referred to the Department of Head and Neck Surgery and Otorhinolaryngology, AC Camargo Cancer Hospital, São Paulo, Brazil, in September 1996 complaining of an asymptomatic nodule on the right anterior border of the oral tongue lasting for about 4 years. Her previous and current medical histories were uneventfully.

Clinical examination revealed a non-ulcerated 3.0cm firm submerse nodule on the right anterior border of the oral tongue. Neck palpation showed a mobile, asymptomatic and fibroelastic nodule on the right submandibular area with 3.0 cm in its maximum dimension. Computed tomography showed the two well-defined distinct circumscribed lesions: one on the anterior tongue and another in the submandibular region. Chest X-rays and laboratory exams were normal. Clinical diagnosis was a salivary gland or a mesenchymal malignant tumor of the tongue with regional lymph node metastasis. Incisional biopsy of the tongue nodule was performed and the diagnosis was mucoepidermoid carcinoma. Clinical stage was established as III (T2N1M0) and the proposed treatment was partial glossectomy associated to right supraomohyoid neck dissection. During surgical approach the cervical nodule was found to be inside the right submandibular gland, and trans-surgical frozen diagnosis was of a benign salivary gland tumor, probably pleomorphic adenoma. Surgery was completed including the removal of the submandibular gland and associated lymph nodes. Post-surgical course was uneventfully.

Hematoxylin and eosin stained histological slides of the tongue lesion revealed a tumor composed of cystic structures lined by mucous and epidermoid cells (Fig. 1). Several areas showed mucous cells filled with mucoid mucicarmine-positive material. Other areas showed neoplastic cells infiltrating striated muscle, but neither necrosis nor perineural or perivascular tumoral infiltration were found. Final diagnosis was low-grade mucoepidermoid carcinoma. The submandibular tumor was well-circumscribed and encapsulated, showing areas of ductal and cellular proliferation, interspersed by loose, hyaline and myxoid matrix, rendering the diagnosis of pleomorphic adenoma (Figs. 2 and 3). Material from the neck dissection revealed 25 normal lymph nodes.

After follow-up of 20 months, the patient returned complaining of a nodule on the left submandibular area. Clinical examination and computed tomography revealed a firm, fibroelastic nodule of about 2.5cm, which was suggestive of a contralateral regional metastasis from the tongue mucoepidermoid carcinoma (Fig.

4). During supraomohyoid left neck dissection, frozen diagnosis was of mucoepidermoid carcinoma metastasis. Histological examination of the specimen confirmed metastasis of low-grade mucoepidermoid carcinoma in one submandibular lymph node of the 38 dissected nodes (Fig. 5). The patient was submitted to post-operative radiation therapy with a total dose of 5940 cGy. At present, after 37 months of follow-up, the patient does not present any signs of local recurrence and regional or distant metastasis.

## DISCUSSION

In multiple salivary gland tumors some characteristics considered include the affected glands (tumors in the same or in different glands), histology (same or different tumors), side distribution (unilateral or bilateral), and timing (metachronous or synchronous)[2]. Most of the cases seem to be metachronous and almost all reported cases include the parotid as one or the unique affected gland, with few cases involving submandibular or minor intraoral salivary glands[1,3,5]. Whartin's tumor, pleomorphic adenoma, oncocytoma and acinic cell carcinoma are the commonest histological subtypes associated with multiple salivary gland tumors[1,2,6].

Mucoepidermoid carcinoma is rarely associated with other synchronous or metachronous salivary gland tumors, with less than 15 cases reported in the English-language literature. It is mainly reported with Warthin's tumor and pleomorphic adenoma, and has rarely been associated with other benign or malignant salivary gland tumors[1,2,6,7,8,9,10,11].

In our case, the diagnosis of mucoepidermoid carcinoma of the tongue led to consider clinically and tomographically the submandibular nodule as a metastasis. Otherwise, other exams such as magnetic resonance image and fine-needle aspiration biopsy, could help to better define the submandibular nodule, and perhaps the diagnosis of synchronic pleomorphic adenoma would have been established before surgery. It is well known that inflammatory, benign and

malignant lesions can mimic metastatic disease and should be always included in differential diagnosis of cervical nodules.

Neck dissection in tongue T2 mucoepidermoid carcinoma is debatable, because some cases can develop regional metastasis. Our patient had a T2 low-grade mucoepidermoid carcinoma of the tongue, and presented a regional contralateral metastasis 20 months after surgery. It is also pertinent to consider that salivary gland lesions, such as pleomorphic adenoma, can eventually be found in ectopic glandular tissue inside submandibular lymph nodes, difficulting clinical and computed tomography interpretations[12].

The association of submandibular and minor salivary glands tumors is extremely uncommon, nevertheless it is important to consider this possibility since it can be relevant for the accurate diagnosis and treatment planning. To our knowledge, synchronous mucoepidermoid carcinoma of the tongue and pleomorphic adenoma affecting the submandibular gland has not been previously reported in the English-language literature.

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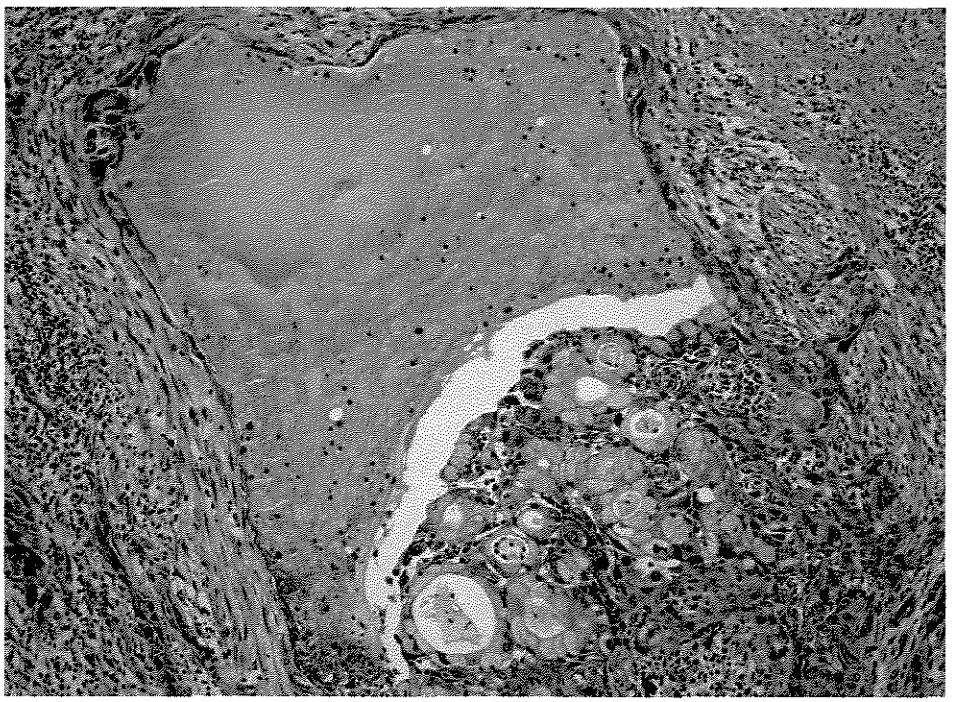

Figure 1. Low-grade mucoepidermoid carcinoma of the tongue showing cystic structures lined by mucous and epidermoid cells (H&E, 100x).

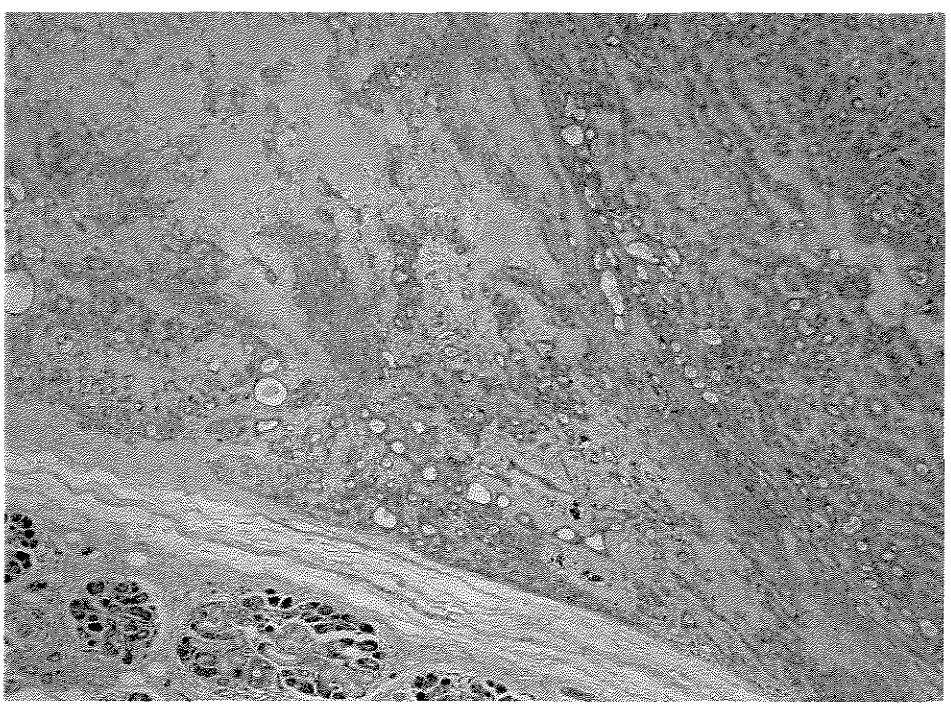

Figure 2. Well-circumscribed submandibular pleomorphic adenoma, showing areas of ductal and cellular proliferation and hyaline matrix. Normal submandibular gland at the left bottom of the figure (H&E, 20x).

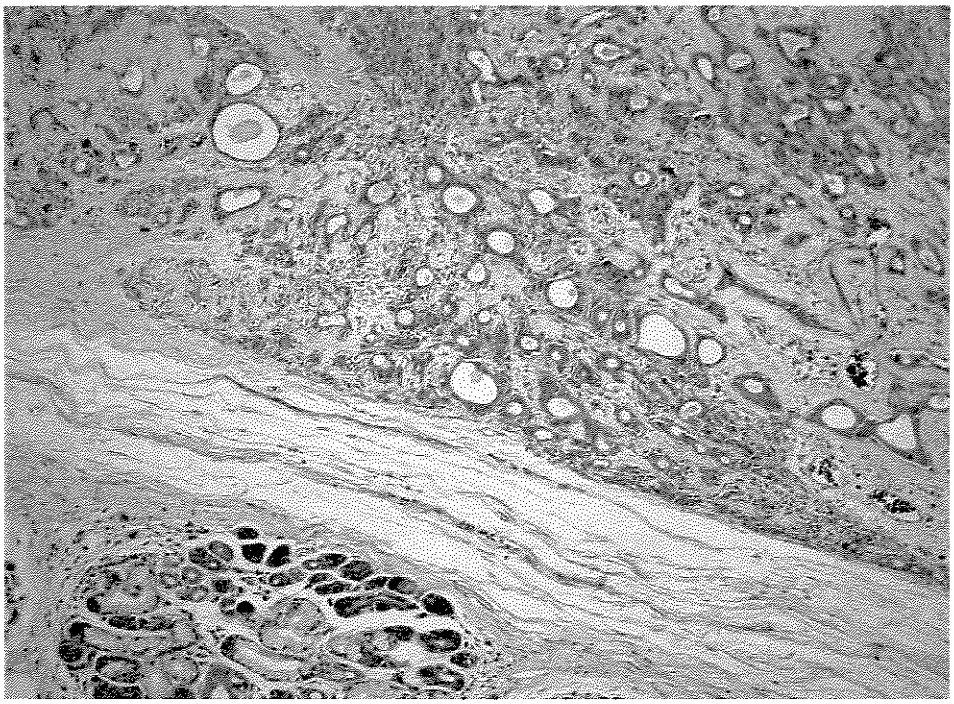

Figure 3. High-power view of the ductal proliferation in the submandibular pleomorphic adenoma (H&E, 60x).

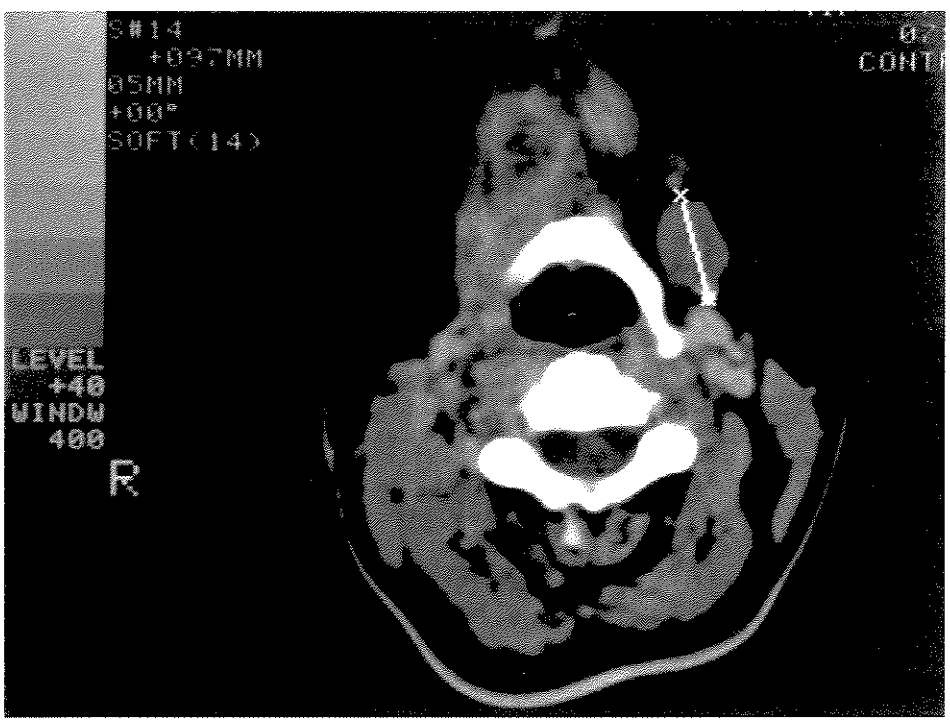


Figure 4. Computed tomography showing a left cervical 2.5cm nodule. It was confirmed as a contralateral regional metastasis, 20 months after surgical removal of the mucoepidermoid carcinoma of the tongue. Histology is shown in figure 5.

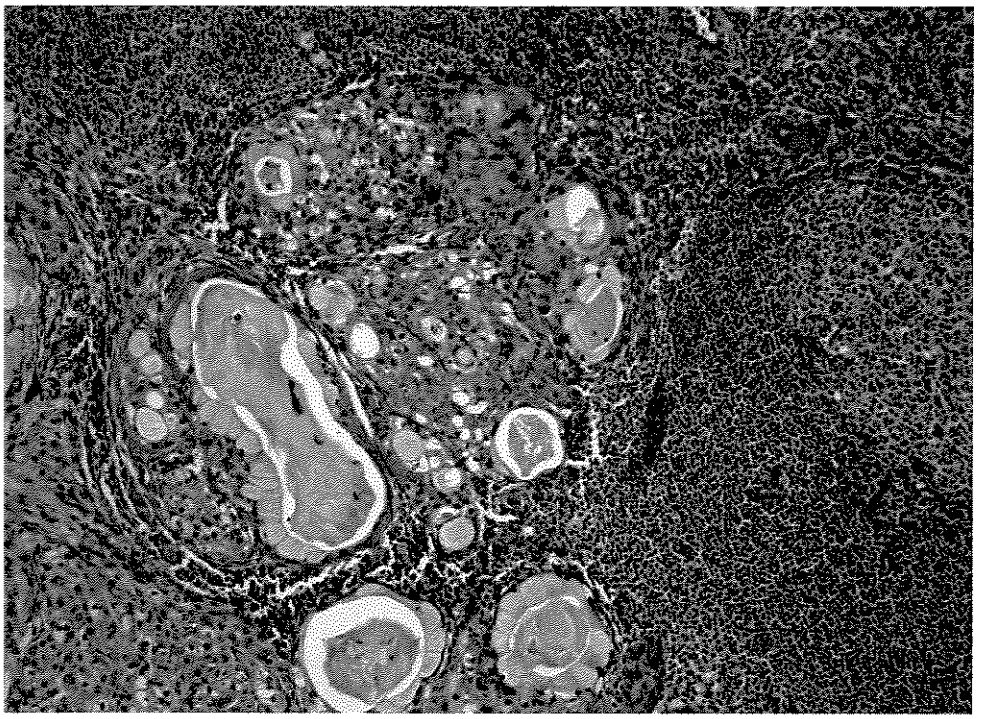

Figure 5. Lymph node cervical metastasis of low-grade mucoepidermoid carcinoma of the tongue, showing areas of intermediate, squamous and mucous cells (H&E, 100x).

## CAPÍTULO 4

# CENTRAL MUCOEPIDERMOID CARCINOMA OF THE MANDIBLE: REPORT OF FOUR CASES WITH LONG-TERM FOLLOW-UP.

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KEY-WORDS: **Mucoepidermoid carcinoma; central; intraosseous; mandible.**

SHORT TITLE: **Central mucoepidermoid carcinoma.**

## ABSTRACT

Central mucoepidermoid carcinomas are uncommon tumors, representing about 2 to 4% of all mucoepidermoid carcinomas. They are histologically low-grade cancers, usually affecting the mandible as uni or multilocular radiographic lesions. The authors report four cases of central mucoepidermoid carcinomas affecting the

mandible and discuss their clinical, radiographic and histological findings. Four females were affected, with a mean age of 42 years and all cases involved the posterior mandible. Treatment included surgery in three cases and surgery associated to neck dissection and radiotherapy in one case. Two patients showed no recurrence and were alive without signs of the disease after a mean follow-up of 78 months. The other two patients showed local recurrence and one was alive with disease with a follow-up of 384 months, and the other was followed-up for 324 months and died for other causes without signs of the tumor. Central mucoepidermoid carcinomas of the mandible are low-grade tumors, and effective surgical treatment involving wide local excision or en bloc resection allows patients to have a favorable prognosis after long-term follow-up.

**KEY-WORDS**

Mucoepidermoid carcinoma; central; intraosseous; mandible.

## INTRODUCTION

Mucoepidermoid carcinoma (MEC) is the most common malignant salivary gland tumor, affecting mainly the parotid and palatal minor salivary glands[5]. Central involvement of the mandible or maxilla is uncommon, representing only about 2 to 4.3% of the cases[2,6]. The origin of the intraosseous MEC is controversial and various possibilities have been considered, including: 1) metaplasia of odontogenic cysts epithelium; 2) entrapment of salivary tissues from the submandibular, sublingual or minor salivary glands, during embryonic development; 3) entrapment of minor salivary glands from the retromolar area; 4) maxillary sinus epithelium; 5) iatrogenic entrapment of minor salivary glands (e.g. chronic osteomielitis and sinusitis); 6) odontogenic remnants of the dental lamina[1,2,7]. More recently, intraosseous salivary tissue has been demonstrated in 0.3% of bone specimens from the jaws studied by Bouquot et al[2], providing new evidences for the origin of intraosseous salivary carcinomas. Nevertheless, although its etiology is uncertain, central MEC is a well-accepted entity.

The first description of a MEC affecting the mandible is attributed to Lepp in 1939 and, since then, about 110 well-reported cases of central MECs were reported in the English-language Literature[2,10]. However, most of the cases show very short follow-up information, difficulting prognosis comparison and establishment of the behavioral pattern of these tumors. The aim of this paper is to report four cases of central mucoepidermoid carcinoma of the mandible with long-term follow-up, discussing their clinical, radiographic and histologic characteristics, and analyzing their treatment and prognosis.

## MATERIAL AND METHODS

Cases diagnosed as MECs according to Ellis & Auclair[5] and Seifert[12] were reviewed from the files of the Department of Head and Neck Surgery and Otorhinolaryngology, AC Camargo Cancer Hospital, São Paulo, Brazil, from 1957 to 1997. Four out of 173 cases were unequivocally considered as originated centrally in the mandible. The criteria for selecting cases with central (intraosseous) origin was the proposed by Alexander et al[1] and modified by Browand & Waldron[4] and Brookstone & Huvos[3]. All cases were submitted to extensive histological analysis to exclude an odontogenic tumor and systemic evaluation to exclude the possibility of a metastatic tumor from the salivary glands or other sites. Clinical and follow-up data were reviewed from the patients records.

Histological slides were reviewed in hematoxillin and eosin, Schiff periodic acid and mucicarmine stainings. The tumors were graded according to Ellis & Auclair[5] in low, intermediate and high-grade tumors. Immunohistochemical reactions against PCNA, ki-67, p53, c-erbB-2, CEA and bcl-2 were performed as follows: slides were deparaffinyzed, hydrated in alcohol and washed in 10% hydrogeneous peroxyde for 30 min. to inhibit endogenous peroxydase. Microwave antigen retrieval using citrate buffer and overnight incubation with the primary antibodies against proliferation cell nuclear antigen (PCNA, clone PC10, Dako A/S Denmark, dilution 1:16.000), ki-67 antigen (Clone ki-S5, Dako A/S Denmark, dilution 1:200), p53 protein (Clone DO-7, Dako A/S Denmark, dilution 1:200), c-

erbB-2 protein (Dako A/S Denmark, dilution 1:200), carcinoembryonic antigen (CEA, Clone II-7, Dako A/S, Denmark, dilution 1:500) and bcl-2 protein (Clone 124, Dako A/S Denmark, dilution 1:50), were performed. Secondary antibodies conjugated to a streptavidin-biotin-peroxidase system (Strept ABComplex/HRP Duet, Mouse/Rabbit, Dako A/S, Denmark) were used, followed by diaminobenzidine as the chromogen. Slides were counterstained with Carazzi hematoxyllin, mounted and analysed by the same author (FRP). Expression indexes were defined as negative (0-5% of positive cells), weak (<20% of positive cells), moderate (20-50% positive cells) and strong (>50% positive cells), after analyzing ten high-power fields in light microscopy.

## RESULTS

A hundred and seventy-three cases of MEC were retrieved from the AC Camargo Cancer Hospital files, São Paulo, Brazil, and four cases (2.3%) were diagnosed as central MECs of the mandible. All patients were white females with ages varying from 24 to 59 years (mean 42.3 years). All patients complained of swelling in the region covered by normal oral mucosa, associated to pain (1 case) and trismus (1 case), and the mean time of complaint was 25.3 months (range from 5 to 60 months). All cases affected the posterior mandible, the mean size of the lesions was 5.0 cm (range from 3.0 to 8.0 cm) and two cases showed rupture of one of the cortical plates (cases 1 and 3). None of the cases showed evidences of regional or distant metastasis on first examination.

Radiographically, three cases showed a radiolucent multilocular image and in one case it was unilocular. All cases were submitted to incisional biopsies, with the diagnosis of MEC. Patients 1, 2 and 4 were treated by local wide resection, and patient 3 was treated by hemimandibulectomy, followed by suprahomohyoid neck disection on the same surgical procedure, and post-surgical radiotherapy. Patient 1 presented a local reccurrence 17 years after the initial treatment, that was managed by hemimandibulectomy. Patient 2 showed two local recurrences: the first one after 240 months of follow-up, that was treated by local excision; the

second one in november 2001, after 384 months of follow-up, and now the patient is being submitted to pre-surgical evaluation (Fig. 1). The other two patients (patients 3 and 4) did not show evidences of local reccurrence, regional or distant metastasis, and were alive without disease after a follow-up interval of 76 and 80 months, respectively. Patient 1 presented also a breast ductal carcinoma and a uterine squamous cell carcinoma, dying from distant metastasis from the breast carcinoma, without signs of local, regional or distant active disease from the mandible tumor after 324 months of follow-up. Table 1 shows a summary of clinical data from the four cases of central MECs of the mandible.

Three cases were classified as low grade (cases 1, 2 and 3) and case 4 was of intermediate grade (Fig. 2). Paraffin blocks were available for immunohistochemical studies in cases 1, 2 and 4 and the results are summarized in Table 2.

## DISCUSSION

Central salivary gland tumors are uncommon and MEC is the most frequent, representing 64 to 75% of the cases[2,3,16]. It is considered that there are about 110 well-reported cases in the literature since the first description by Lepp in 1939[2,10]. According to Ellis et al[6], 74 (4.3%) of the 1701 AFIP cases of MEC were of central origin, and Waldron et al[15] reported another 6 cases in an extensive review of minor salivary gland tumors, but both papers contained no detailed description of the cases. Most of the well-reported cases are single case reports and very few short series were published[4,8,11,14]. Central MEC represented 2.3% of all MEC diagnosed and treated in the AC Camargo Cancer Hospital of São Paulo, Brazil.

Criteria for diagnosing central MECs include: 1) presence of a radiographic distinct osteolytic lesion; 2) positive mucicarmine staining; 3) absence of rupture of one or more cortical plates; 4) clinical and histological exclusion of a metastasis or an odontogenic lesion; 5) exclusion of the origin from a soft tissue salivary gland; 6) histologic confirmation[1,3,4]. Two of our cases showed rupture of the cortical plates, but It has been regularly shown in the literature that intact cortical plates

should not be an essential feature for diagnosis of central MECs[8,16]. We are confident that our 4 cases fulfilled the acceptance criteria described above.

Our cases showed similar epidemiological and clinical features when compared to the published literature. Our four cases affected females, the mean age was 42 years and all the cases affected the posterior mandible. Swelling in the region was found in all cases and pain was reported by only one patient (25%). Large tumor size and long period of complaint are also common findings in central MEC[3,4,14,16].

Radiographically, central MECs can show an uni or multilocular radiolucent appearance, being associated to impacted teeth or cyst in almost 50% of the cases[4,7,8]. Three of our cases showed a multilocular radiolucent appearance and none of them was associated to an impacted tooth. These radiographic findings and the location of the tumors highly suggest the possibility of odontogenic cysts and tumors, and clinical-radiographic differential diagnosis should include ameloblastomas, keratocysts and dentigerous cysts[4,11,16]. However, cases with irregular radiographic or tomographic borders and expansion and rupture of one or more cortical plates should be interpreted with caution, and the possibility of a malignant lesion should be suspected[8]. It seems to exist a positive correlation regarding radiographic and tomographic findings of irregular margins, cortical destruction and invasion of central MEC, with their histological grade and prognosis[8].

About 60 to 85% of central MEC affect the mandible, specially the premolar-molar region, angle and ramus, above the mandibular channel[3,4,6,8,16]. Some authors have claimed that maxillary lesions are more common than mandibullary ones, but this is not well accepted[14]. It should be stressed that maxillary lesions should not include lesions originated from the mucous glands of the maxillary antrum lining. Extensive antral cases of MEC can rupture its limits and invade the maxilla, difficulting its interpretation as intra-osseous or antral lesions[16]. In our review of 173 cases of mucoepidermoid carcinomas, we could not find any case originating in the maxilla, but we found 7 cases from the maxillary sinus (data not

shown). It is important to stress these differences, because antral MEC can reach considerable size before any complaint, and are usually diagnosed in late stages as high-grade tumors, carrying a poor prognosis. In contrast, although maxillary MEC have a worst prognosis than mandibular cases, they have a better prognosis than antral cases, reinforcing the importance of their differentiation.

Besides clinical and radiographic parameters, biopsy is necessary for the final diagnosis and treatment planning in all cases of central MEC. Literature reports consider the origin of central MEC from odontogenic cysts and tumors. Most of the cases are associated to dentigerous cysts, but there are evidences of their association to periodontal apical cysts, residual cysts, glandular odontogenic cysts, and odontogenic tumors[3,6,7,9,11,16].

Establishment of final histological diagnosis of central MECs can be somewhat easy in cases showing the classic histological features of these tumors[11]. However, as most of the cases are cystic low-grade tumors, differentiation from glandular odontogenic cyst is important[11]. These cysts can be uni or multilocular, showing several cystic spaces lined by epidermoid and mucoid cells, as in MEC[16]. However, the presence of intermediate cells and the solid proliferation in MEC are not seen in glandular odontogenic cysts[9,11]. Sometimes, MEC can show extensive clear cell component and these cases should be differentiated from clear cell odontogenic tumors, and other malignant and metastatic clear cell tumors[11]. Most of the MEC cases show at least one typical diagnostic area, or a mucicarmine or alcian blue-positive area, but some cases composed exclusively by clear cells can bring diagnostic difficulties[11]. Nevertheless, none of our cases showed difficulties to be diagnosed as MEC.

Most of the reported central MEC are histologically low-grade tumors and usually carry a favorable prognosis[4,7,11,14,16]. However, maxillary cases have a worse prognosis due to the possibility of local extension to important vital structures[14]. As a rule, even being low-grade tumors, MEC should be managed by wide local resection, en bloc resection or hemimandibulectomy[3,7,14]. Conservative approach is undesirable and can favor recurrence[3,10,16]. Neck dissection is

indicated when clinically involved regional lymph nodes are present and radiotherapy seems to be an adjunctive useful measure in cases with close surgical margins and high-grade tumors[10,11]. Our case 3 was submitted to neck dissection and postoperative radiotherapy due to the size of the tumor, rupture or the cortical plates, close surgical margins and proximity of the tumor to neck lymph nodes, and the patient is alive without signs of disease for 76 months.

Prognosis of MEC from the salivary glands is usually related to clinical stage of the patients and histological grade of the tumors[5]. However, this seems to be not the rule for central MEC and Brookstone & Huvos[3] alternatively suggested a three-graded classification for intraosseous MEC: grade I (without expansion or rupture of the cortical plates), grade II (with expansion but without rupture of the cortical plates) and grade III (with rupture of the cortical plates or presence of regional metastasis). Two of our cases were classified as grade II tumors and two as grade III tumors, according to this classification, and one case of each group showed local recurrence. This grading system seems to be useful, adjunctive to clinical stage, localization and histological grade of the tumors, in establishing proper management and prognosis of central MEC[11]. However, one of the difficulties in evaluating the usefulness of this system is the absence of large comparative series of central MEC in the literature.

Immunohistochemical findings of our central MEC cases showed that the intermediate-grade tumor presented higher expression of proliferative markers (PCNA and ki-67) than low-grade tumors and, inversely, low-grade tumors showed higher expression of CEA than intermediate-grade tumors. P53, c-erbB-2 and bcl-2 proteins also showed higher expression in the intermediate-grade tumor. Although there were some relationship between histological grading and immunohistochemical findings, the small number of cases does not allow any solid conclusion. Case 1, for example, a low-grade tumor, showed local recurrence and, irrespectively to histological and immunohistochemical profile of the tumor, its size and the rupture of one cortical plate seemed to be the important factors regarding local recurrence. This same patient (Case 1) also developed two other metachronic

different tumors (breast ductal carcinoma and uterine squamous cell carcinoma), and died from distant metastasis from the breast carcinoma. It is well-reported in the literature the possibility of second primary tumors in patients that developed a salivary gland tumor, the most common being other salivary or thyroid cancers[13]. The association of central MEC of the mandible, breast ductal carcinoma and uterine squamous cell carcinoma was not previously reported in the literature, but we could not find a convincingly explanation for such association.

MEC patients must be followed-up for long periods, due to the possibility of late local recurrence or regional metastasis[5]. Patient of case 1 showed local recurrence 17 years after initial presentation that was well-controlled with a second surgical approach. Case 2 showed a local recurrence after 240 months that was treated with wide local resection, but another local recurrence occurred in November 2001, after 384 months of follow-up. Few series of central MEC have shown long follow-up interval, bringing difficulties in analyzing prognosis and survival of these tumors[4,10]. Our results showed a recurrence rate of 50%, higher than the 10 to 25% rate reported in the literature. Surely, these values depend on the biological behavior of these tumors, and surgical approach. MEC regional metastasis are reported in 10 to 15% of the cases, and these tumors rarely show distant metastasis. Only about 10% of the central MEC patients can evolve to death and, in general, these tumors usually show a good overall prognosis[3,4,10,16].

In conclusion, central MECs are uncommon salivary gland tumors that usually affect the posterior mandible as uni or multilocular radiolucent irregularly-bordered lesions. They are commonly low-grade tumors and treatment includes essentially wide local excision. Long-term follow-up is necessary, as some cases can develop late local recurrences and regional metastasis, even after decades.

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## TABLES

Table 1. Clinical, treatment and follow-up data of 4 cases of central mucoepidermoid carcinomas of the mandible.

| Case | Age | Complaint (months) | Radiograph | Size (cm) | Treatment | Recurrence | Last information (months) |
|---|---|---|---|---|---|---|---|
| 1 | 59 | 18 | Multilocular | 8.0 | Surgery | Local | DOC[1] (324) |
| 2 | 24 | 5 | Unilocular | 3.0 | Surgery | Local | AWD[2] (384) |
| 3 | 31 | 60 | Multilocular | 6.0 | Surgery + ND + RXT[3] | No | NED[4] (76) |
| 4 | 55 | 18 | Multilocular | 3.0 | Surgery | No | NED[4] (80) |

1 Dead by other cause; 2 Alive with disease; 3 Surgery + Supra-omohyoid neck disection + radiotherapy; 4 No evidence of disease.

Table 2. Histological grade and expression of immunohistochemical markers in three cases (Cases 1, 2 and 4) of central mucoepidermoid carcinomas of the mandible.[1]

| Case | Histological grade | PCNA | Ki-67 | P53 | c-erbB-2 | CEA | Bcl-2 |
|---|---|---|---|---|---|---|---|
| 1 | Low | Negative | Negative | Negative | Weak | Weak | Weak |
| 2 | Low | Weak | Negative | Negative | Weak | Weak | Weak |
| 4 | Intermediate | Moderate | Weak | Weak | Moderate | Negative | Moderate |

1 Paraffin blocks were not available for case 3.

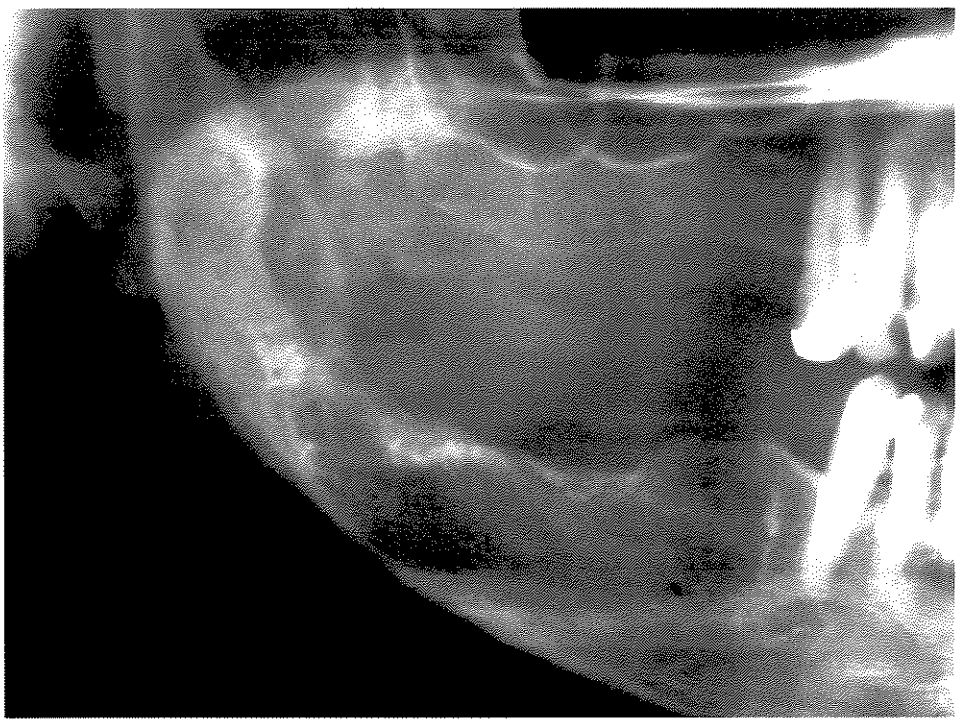

Figure 1. Panoramic radiograph of case 2 showing an irregular radiolucent area in the right mandibular body and ramus, that proved to be a recurrent central low-grade mucoepidermoid carcinoma.

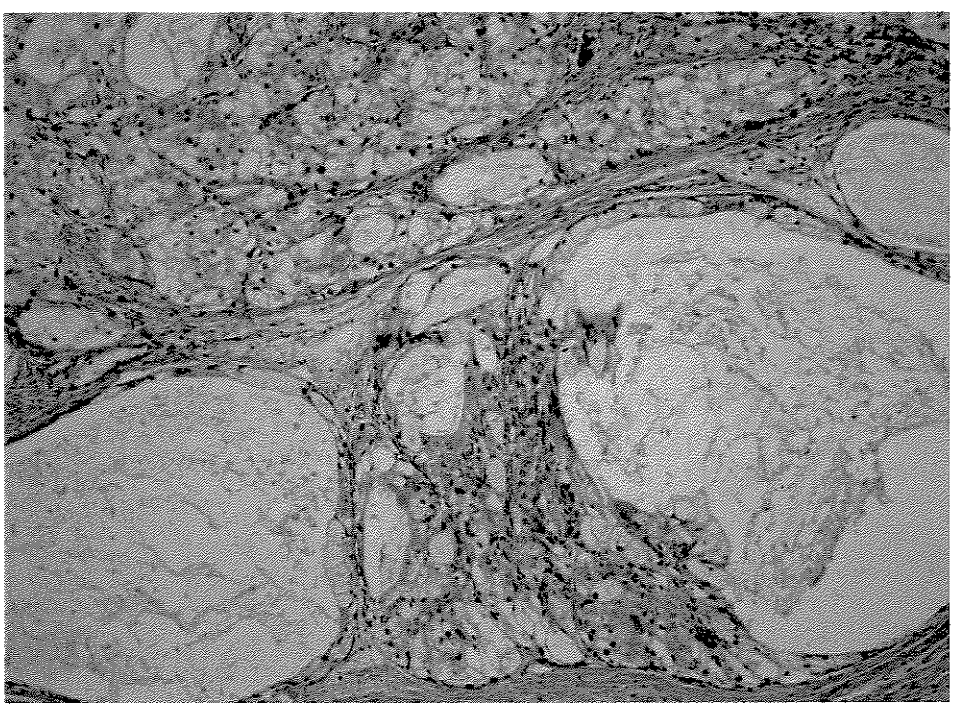

Figure 2. Low-grade central mucoepidermoid carcinoma of case 1, showing cystic structures lined by mucous and epidermoid cells (H&E, 80x).

## CAPÍTULO 5

# MEL-CAM (CD146) EXPRESSION IN PAROTID MUCOEPIDERMOID CARCINOMA

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## ABSTRACT

**Background:** Mel-CAM (CD146) is a cell-cell adhesion protein found in normal and tumoral tissues. **Objective:** To analyse Mel-CAM expression in mucoepidermoid carcinoma (MEC), and assess its importance in prognosis and its utility in differentiating high-grade MEC from squamous cell carcinoma (SCC).

**Material and Methods:** Immunohistochemical expression of Mel-CAM in 41 parotid MEC was correlated with clinical parameters. Ten cases of oral cavity SCC were included for comparison. **Results and Conclusions:** Mel-CAM expression was found in 92.7% of the MEC but was not expressed by none of the SCC. Mel-CAM expression was greater in intermediate/high grade tumors, was weaker in patients that presented local recurrence, regional and distant metastasis, but no correlation between Mel-CAM and clinical stage and survival of the patients was found. Decreased Mel-CAM expression can impair cellular contact properties, facilitating growth, cell spreading and metastasis in MEC. Mel-CAM can also be useful in differentiating high grade MEC from SCC.

**KEY-WORDS:** Mucoepidermoid carcinoma; salivary glands; parotid; Mel-CAM; CD146; squamous cell carcinoma.

## INTRODUCTION

Mucoepidermoid carcinoma (MEC) is the most common malignant salivary gland tumor, affecting mainly parotid and palatal minor salivary glands [1]. Prognosis of MEC has been usually related to clinical stage of the patients and histological grading of the tumors [2]. Additionally, expression of some immunohistochemical markers, such as ki-67, has been associated with a worst prognosis [1,3].

Mel-CAM (CD146 or MUC18) is a 113kD heterophilic cell-cell adhesion glycoprotein belonging to the immunoglobulin supergene family, whose gene is mapped to the short arm of the chromosome 11 [4]. This transmembrane molecule has been included in the group of the new endothelial antigens, and its expression is found in many normal tissues including endothelium, smooth muscle, Schwann cells and ductal and myoepithelial cells of salivary glands [5]. This protein is related to focal adhesion, cytoskeletal organization, intercellular interactions, maintenance of the cell shape, and cellular migration and proliferation control [4]. Mel-CAM is

also expressed in tumor tissues, being related to tumor size and progression, metastatic potential and agressiveness [5].

Most available anti-Mel-CAM antibodies are only useful in frozen sections, limiting its use in paraffin specimens. Recently, a monoclonal antibody directed against Mel-CAM protein was developed and tested by Shih et al. [5] in paraffin-embedded specimens. The authors described Mel-CAM expression in several normal and tumoral tissues, including five cases of salivary gland tumors. To our knowledge, this is the only report of Mel-CAM expression in salivary gland tumors.

The aim of this paper is to assess the expression of Mel-CAM in parotid MEC, its correlation to clinical stage, histological grade and prognosis of these tumors, and its utility in differentiating high-grade MEC from squamous cell carcinoma (SCC).

## MATERIAL AND METHODS

Forty-one cases of parotid MEC were retrieved from the files of the Department of Head and Neck Surgery and Otorhinolaryngology, AC Camargo Cancer Hospital, São Paulo, Brazil. Clinical, treatment and follow-up data were obtained from the patients records. Histological slides were stained with haematoxilin & eosin, Schiff periodic acid and mucicarmine techniques, reviewed and graded according to Ellis and Auclair [1] in low, intermediate and high-grade tumors. Ten cases of oral cavity SCC were retrieved from the files of the Oral Pathology Laboratory, School of Dentistry of Piracicaba, State University of Campinas (UNICAMP), Piracicaba, Brazil, reviewed and graded in well-differentiated, moderately differentiated and poorly differentiated, according to the criteria described by Anneroth et al. [6].

Immunohistochemical reactions were performed using a primary polyclonal antibody against Mel-CAM (CD146). The 3μm histological sections were deparaffinized, hydrated and washed in 10% hydrogeneous peroxide for 30 minutes. Antigen retrieval was not necessary [5]. The histological slides were incubated overnight with a primary antibody against Mel-CAM in a dilution of

1:2000. Secondary antibodies associated to a streptavidin-biotin-peroxidase method were used (StreptABComplex Duet, Mouse/Rabbit, Dako A/S, Denmark), complemented by diaminobenzidine as the chromogen. The slides were counterstained with Carazzi hematoxylin and mounted.

All slides were analysed by one of the authors (FRP) and the percentage of cells expressing Mel-CAM was classified as 0-5% (negative), 6-25% (weak), 26-50% (moderate) and 51-100% (strong), according to the median index of positive cells obtained from 10 high-power fields. Mel-CAM expression was also classified in diffuse cytoplasmic and/or membranous for each case.

Mel-CAM expression was correlated to the clinical stages of the MEC patients, histological grade, local recurrence, regional and distant metastasis and prognosis. Data were statistically analysed by the SPSS program and Person's test with a significance level of 5%.

## RESULTS

### Parotid MEC

The mean age of the forty-one patients was 40 years (range 6-78 years), with 22 males (53.7%) and 19 females (46.3%), and 75.6% were Caucasians. The mean complaint time reported by the patients was 38.8 months (range 1 to 240 months) and the most common complaints were the presence of a tumor/nodule in the area (97.6% of the cases), pain (36.6%) and facial paralysis (12.2%). The mean size of the tumors was 4.6 cm (ranging from 2 to 10 cm), and 51.3% were fixed in the adjacent tissues. Invasion of the adjacent structures was seen in 26.8% of the tumors, including skin, facial nerve and bone. TNM distribution and clinical stage of the tumors is shown in Table 1.

Twenty-one cases (51.2%) were classified as low-grade, 6 (14.6%) as intermediate-grade and 14 (34.2%) as high-grade tumors. Surgical treatment was employed in all cases, radiotherapy in 58.5% and chemotherapy in 4.9%. Neck dissection was performed in 46.3% of the cases and 19.5% of the cases showed regional metastasis after histological analysis.

Local recurrence was found in 8 patients (19.5%), and regional and distant metastasis in 10 (24.4%) and 7 (17.1%) cases, respectively. The mean follow-up of the patients was 105.7 months (ranging from 1 to 376 months). Twenty patients (48.9%) were alive without disease, 1 (2.4%) patient was alive with disease, 10 (24.4%) were dead due to the disease, 1 (2.4%) died during treatment, 1 (2.4%) died by other causes and 8 (19.5%) were lost to follow-up.

Mel-CAM expression was found in 38 cases (92.7%) and considered weak in 18 (43.9% of the cases), moderate in 9 (22.0%) and strong in 11 (26.8%) (Fig. 1). Cytoplasmic and membranous staining were observed in 32 (84.2%) and 23 (60.5%) cases, respectively, and both patterns concurrently were found in 15 (39.5%) cases (Fig. 2). Mel-CAM was expressed in mucous, intermediate and epidermoid cells, and two cases containing clear cells were also positive for Mel-CAM (Fig. 3). Also, two cases showed stronger Mel-CAM immunoreactivity in the tumoral front (Fig. 4), and cells were negative for Mel-CAM expression in areas of perineural invasion.

No correlation was found regarding the association between Mel-CAM expression and clinical stage and survival of the patients ($p>0.10$) (Table 2). Intermediate and high-grade tumors showed moderate/strong Mel-CAM expression more frequently than low-grade tumors, but the differences were not statistically significant (Table 3). Mel-CAM expression was weaker in patients who presented local reccurrence, regional and distant metastasis, although the differences were also not statistically significant (Table 4).

**Oral cavity SCC**

The mean age of the 10 patients was 56 years (ranging from 41 to 68 years), nine cases affected males and the site distribution included the lateral borders of the tongue (5 cases), floor of the mouth (3 cases) and soft palate (2 cases). Histological classification included 3 well-differentiated, 3 moderately differentiated and 4 poorly differentiated tumors. Mel-CAM immunohistochemical

expression was absent in all cases. As the positive controls, the blood vessels in these tissues were immunoreactive to the antibody.

## DISCUSSION

Immunohistochemical expression of several tumor and proliferation markers have been described in MEC but few have been associated to prognosis in these tumors [3]. Mel-CAM is a cell-cell adhesion molecule expressed in several normal tissues and also in certain tumoral tissues. It has been correlated to agressiveness, development of metastasis and a worst prognosis in some cancers [5]. Tumoral invasion and metastasis have been correlated to Mel-CAM expression in melanoma cells, however in other tumors, as ductal breast carcinoma, Mel-CAM expression seems to be inversely associated to a more aggressive behavior [4]. Mel-CAM has been also associated to malignant transformation and metastatic potential in prostate carcinoma [7].

Mel-CAM immunohistochemical expression in salivary gland tumors was only once reported in the literature [5]. The authors studied 5 salivary gland tumors and demonstrated that 3 cases of MEC expressed Mel-CAM, in contrast to 2 negative cases of adenoid cystic carcinomas. Our results showed that Mel-CAM is frequently expressed in low, intermediate and high grade MEC, and in the various cell types present in these tumors: mucous, intermediate, epidermoid and clear cells. Two of our cases showed stronger Mel-CAM immunoreactivity in the tumoral front, as already shown in primary SCC of the lung [5]. This could be related to an increased ability of invasion of these tumors. However, although these two patients had histologically intermediate and high-grade tumors, none had local recurrences and both were alive without disease after 80 and 360 months of follow-up, respectively. We did not find areas of vascular invasion histologically, so we could not associate cellular and endothelial Mel-CAM expression and the consequent vascular invasion and metastatic potential of the tumors, as already described in the literature [4].

As reported by Shih et al. [5] antigen retrieval was not necessary for Mel-CAM immunohistochemical reactions. We tested 20% of our cases both with and without antigen retrieval and the pattern of expression was similar. Mel-CAM usually shows both membranous and cytoplasmic staining and this combinated pattern seems to be the prevalent one [8]. This pattern was found in 39.5% of our cases, and most isolated cells showed both staining types, confirming these previous findings. Wu et al. [7] demonstrated that Mel-CAM expression in normal and benign hyperplastic prostatic tissue showed a predominantly membrane staining, while in carcinomas the pattern changed to a mostly cytoplasmic one. As a membrane protein, it could be suggested that cytoplasmic expression could be associated to mutant Mel-CAM protein. As most of our cases showed both patterns in the same tumor, it was not possible to correlate this pattern of expression with the histological grade of the tumors.

Mel-CAM expression has been correlated to local invasion and metastasis in melanomas [9], but our results indicate that in MEC a decreased expression is related to local recurrences and neck and distant metastasis. These data suggest that Mel-CAM expression in MEC is more similar to what happens in breast ductal carcinoma than in melanomas. Decreased Mel-CAM expression in these tumors could be related to alterations in cellular contact properties, increasing the capacity of cell spreading and metastatic potential [10].

It has been suggested that Mel-CAM expression is useful in differentiating histogenesis of spindle cell tumors, and even discriminating benign and malignant variants [8]. In addition, Mel-CAM is rarely found in normal and benign prostate tissue, but it has been consistently found in carcinomas, suggesting that it should have a role in malignant progression in this organ [7]. Although 92.7% of our cases showed Mel-CAM expression, we could not correlate the immunohistochemical findings to clinical stage, prognosis and survival of the patients. However, in intermediate and high-grade tumors, expression of Mel-CAM was stronger than in low-grade ones, although the results were not statistically significant.

Mel-CAM is rarely found in carcinomas, except for choriocarcinoma, some primary SCC of the lung and ductal carcinoma of the breast [5], and there is no reports demonstrating the Mel-CAM expression pattern in SCC of the oral cavity or major salivary glands. Therefore, we tested Mel-CAM expression in 10 cases of oral SCC and found that none of the cases was positive for Mel-CAM. It was also negative for SCC of the skin [5].

Primary SCC of glandular origin is an extremely rare and aggressive tumor, representing less than 2% of all salivary gland tumors [11,12]. They usually present as invasive moderate or well-differentiated tumors, with frequent individual intracellular keratinization and keratin pearl formation [1,12-14]. Its diagnosis requires the exclusion of a primary SCC of the skin invading the gland, a metastasis from a SCC, usually from the skin of the head and neck or from the oral and nasopharyngeal mucosa, and a high grade MEC [12-14]. The last is usually characterized for at least residual glandular pattern, presence of basaloid intermediate cells and presence of intracellular mucin, but these characteristics are frequently absent in high grade tumors [1,12]. In some instances, it is extremely difficult to distinguish clinically and histologically high grade MEC from SCC, specially in ulcerated lesions and superficial biopsies [12,14].

Chu et al. [15] have suggested that expression of cytokeratin 14 is a useful tool in differentiating SCC from other epithelial tumors. He had included nine parotid tumors in his cases, but did not specified their histological diagnosis. Other cytokeratins have also been suggested as adjunctive useful markers to differentiating MEC from other malignant salivary gland tumors and epidermoid carcinomas [16,17]. Based on our results, we suggest that Mel-CAM can be a useful marker for differentiation of high grade MEC from SCC, but other studies regarding this subject should be encouraged.

In summary, Mel-CAM is frequently expressed in MEC as either a cytoplasmic and/or membranous protein. Decreased Mel-CAM expression by MEC tumor cells can impair cellular contact properties, facilitating growth, cell spreading and metastasis of MEC. In adittion, Mel-CAM was expressed in 92.7% of the

MECs, but in none of our SCC, and seems to be a useful tool in differentiating these two entities.

**ACKNOWLEDGEMENTS**

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## TABLES

Table 1. Distribution of TNM criteria and clinical stage of 41 cases of parotid mucoepidermoid carcinomas.

| Clinical parameter | Number of cases (%) |
|---|---|
| **T (Size of the tumor)** | |
| T1 | 4 (9.7%) |
| T2 | 17 (41.5%) |
| T3 | 9 (22.0%) |
| T4 | 6 (14.6%) |
| Tx | 5 (12.2%) |
| **N (Regional metastasis)** | |
| N0 | 36 (87.8%) |
| N1 | 2 (4.9%) |
| N2a | 2 (4.9%) |
| N2b | 1 (2.4%) |
| N2c | 0 |
| N3 | 0 |
| **M (Distant metastasis)** | |
| M0 | 40 (97.6%) |
| M1 | 1 (2.4%) |
| **Clinical stage** | |
| I | 19 (46.3%) |
| II | 7 (17.1%) |
| III | 2 (4.9%) |
| IV | 8 (19.5%) |
| Without staging | 5 (12.2%) |

Table 2. Distribution of Mel-CAM expression according to clinical stage in 41 cases of parotid mucoepidermoid carcinoma.

| Clinical stage | Mel-CAM Expression | | | Total |
|---|---|---|---|---|
| | Negative | Weak | Moderate/Strong | |
| I | 0 (0%) | 8 (42.1%) | 11 (57.9%) | 19 (46.3%) |
| II | 0 (0%) | 4 (57.1%) | 3 (42.9%) | 7 (17.1%) |
| III | 0 (0%) | 1 (50.0%) | 1 (50.0%) | 2 (4.9%) |
| IV | 2 (25.0%) | 3 (37.5%) | 3 (37.5%) | 8 (19.5%) |
| Without grading | 1 (20.0%) | 2 (40.0%) | 2 (40.0%) | 5 (12.2%) |
| **Total** | **3 (7.3%)** | **18 (43.9%)** | **20 (48.8%)** | **41 (100%)** |

Table 3. Distribution of Mel-CAM expression according to histological grade in 41 cases of parotid mucoepidermoid carcinoma.

| Histological grade | Mel-CAM Expression | | | Total |
|---|---|---|---|---|
| | Negative | Weak | Moderate/Strong | |
| Low grade | 1 (4.8%) | 10 (47.6%) | 10 (47.6%) | 21 (51.2%) |
| Intermediate grade | 1 (16.7%) | 2 (33.3%) | 3 (50.0%) | 6 (14.6%) |
| High grade | 1 (7.1%) | 6 (42.9%) | 7 (50.0%) | 14 (34.1%) |
| **Total** | **3 (7.3%)** | **18 (43.9%)** | **20 (48.8%)** | **41 (100%)** |

Table 4. Distribution of Mel-CAM expression by local recurrence, regional metastasis and distant metastasis of 41 cases of parotid mucoepidermoid carcinomas.

| Parameter | Mel-CAM Expression | | | Total |
|---|---|---|---|---|
| | Negative | Weak | Moderate/Strong | |
| **Local Recurrence** | | | | |
| No | 3 (9.1%) | 12 (36.4%) | 18 (54.6%) | 33 (80.5%) |
| Yes | - | 6 (75.0%) | 2 (25.0%) | 8 (19.5%) |
| Total | 3 (7.3%) | 18 (43.9%) | 20 (48.8%) | 41 (100%) |
| **Neck Metastasis** | | | | |
| No | 2 (6.5%) | 13 (41.9%) | 16 (51.6%) | 31 (75.6%) |
| Yes | 1 (10.0%) | 5 (50.0%) | 4 (40.0%) | 10 (24.4%) |
| Total | 3 (7.3%) | 18 (43.9%) | 20 (48.8%) | 41 (100%) |
| **Distant Metastasis** | | | | |
| No | 3 (8.8%) | 13 (38.2%) | 18 (53.0%) | 34 (82.9%) |
| Yes | - | 5 (71.4%) | 2 (28.6%) | 7 (17.1%) |
| Total | 3 (7.3%) | 18 (43.9%) | 20 (48.8%) | 41 (100%) |

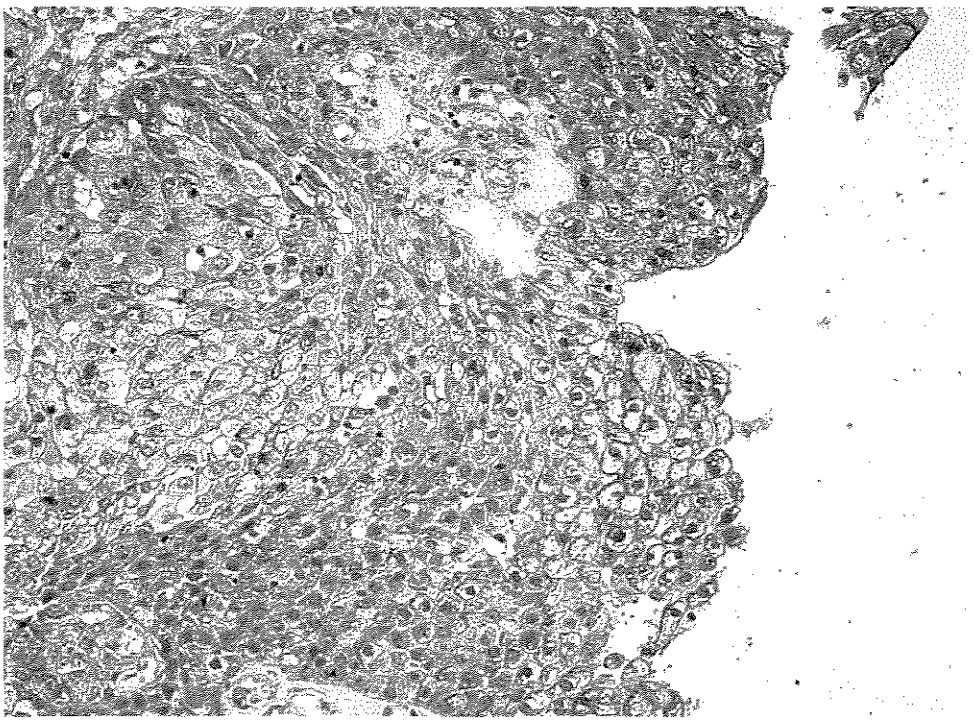

Figure 1. Low-grade MEC showing immunohistochemical expression of Mel-CAM in intermediate and mucous cells (Immunoperoxidase, 100x).

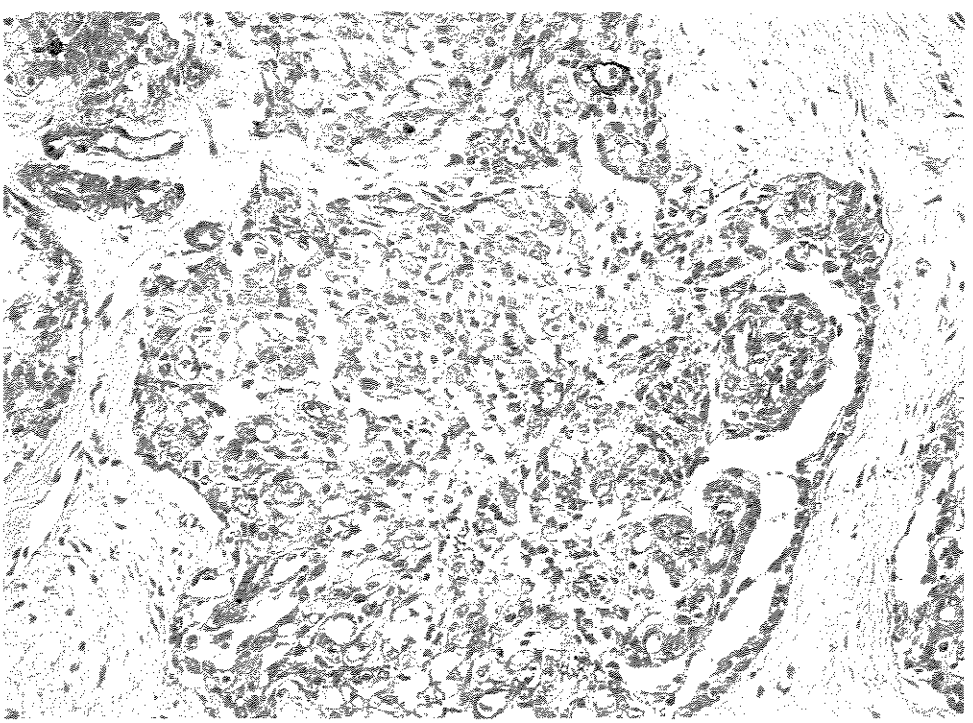

Figure 2. Intermediate-grade MEC showing both membranous and cytoplasmic expression of Mel-CAM (Immunoperoxidase, 100x).

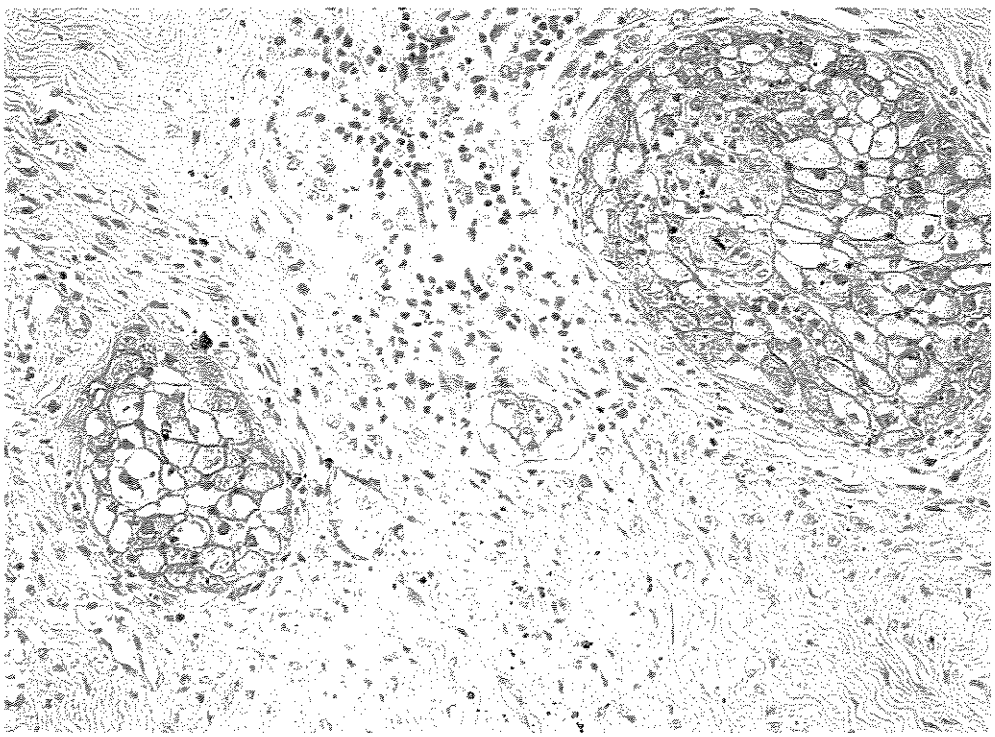

Figure 3. Mel-CAM expression in clear cells of an intermediate-grade MEC (Immunoperoxidase, 160x).

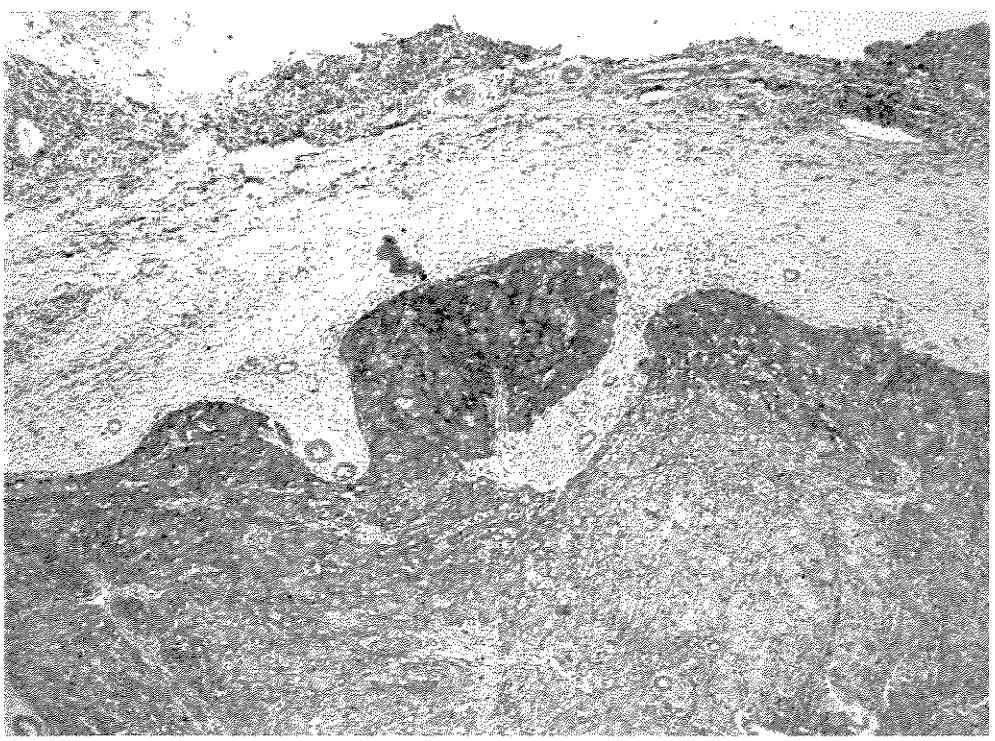

Figure 4. Strong Mel-CAM expression in the tumoral front of an Intermediate-grade MEC (Immunoperoxidase, 40x).

## CONCLUSÕES

1. Carcinomas mucoepidermóides afetaram principalmente a parótida e glândulas salivares menores intra-orais de pacientes entre a 4ª e 6ª décadas de vida, sem predileção por sexo.

2. Metade dos casos encontravam-se em estádios clínicos I e II, e 63,7% dos tumores eram de baixo grau ou grau intermediário de malignidade, determinando sobrevida global em 5 e 10 anos de 70% e 60%, respectivamente, e prognóstico relativamente favorável.

3. Parâmetros de relevância prognóstica na análise univariada de sobrevida foram: idade dos pacientes (> 40 anos), gênero masculino, fixação dos tumores, tumores na submandibular, invasão de estruturas adjacentes, T, N, estadiamento clínico e gradação histológica dos tumores.

4. A expressão imunohistoquímica de PCNA, ki-67 e p53 mostrou correlação com pior prognóstico e, de forma contrária, a expressão de CEA e bcl-2 foi relacionada a melhor prognóstico.

5. A expressão de Mel-CAM foi encontrada em 92.7% dos CME e demonstrou tendência a relação inversa com a recorrência local e com metástases regionais e a distância.

6. A análise multivariada de sobrevida revelou que a idade dos pacientes (> 40 anos), fixação dos tumores, T, N e o grau histológico mostraram-se fatores prognósticos independentes significantes após análise multivariada.

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CENTRO DE TRATAMENTO E PESQUISA

**HOSPITAL DO CÂNCER**

A. C. CAMARGO

São Paulo, 01 de setembro de 1999.

**Dr. Fábio Ramôa Pires**
**At. Dr. Luiz Paulo Kowalski**
**Departamento de Cirurgia de Cabeça e Pescoço/Otorrinolaringologia**

**Assunto: Projeto de Pesquisa nº 198/99**

Prezado Doutor,

Seu projeto de pesquisa intitulado **"Estudo imunohistoquímico e análise multivariada de fatores prognósticos de carcinomas mucoepidermóides de glândulas salivares"** foi apreciado na última reunião do Comitê de Ética em Pesquisa do Hospital do Câncer de São Paulo, realizada em 31 de agosto 1999. Este Comitê declara que seu projeto foi **aprovado**

Atenciosamente,

**Dr. Arnaldo Anelli**
**Coordenador da Comissão de Ética em Pesquisa do Hospital do Câncer**

## Ficha Clínica padronizada para o estudo

## Hospital do Câncer AC Camargo - Fundação Antônio Prudente
## Departamento de Cabeça e Pescoço
## Carcinomas mucoepidermóides de glândulas salivares

1. Número no estudo: ..... |__|__|__|
2. Registro Hospitalar: ..... |__|__|__|__|__|__|__|__|
3. Idade: _____ (anos) ..... |__|__|
4. Sexo: | 1 | masculino | 2 | feminino ..... |__|
5. Grupo étnico: | 1 | branco | 2 | amarelo | 3 | negro | 4 | outro | 9 | ignorado.... |__|
6. Tempo de queixa: _____ meses |999| ignorado ..... |__|__|__|
7. Queixas:
   a) Dor: | 0 | não | 1 | sim | 9 | ignorado..... |__|
   b) Tumor/ferida: | 0 | não | 1 | sim | 9 | ignorado..... |__|
   c) Paralisia nervo facial/mandibular: | 0 | não | 1 | sim | 9 | ignorado.. |__|
   d) Alteração salivar: | 0 | não | 1 | sim | 9 | ignorado..... |__|
   e) Outras: | 0 | não | 1 | sim | 9 | ignorado_____ ..... |__|
8. Localização Maiores: | 0 | não se aplica | 1 | parótida | 2 | submandibular | 3 | sublinguais ..... |__|
9. Localização Menores: | 0 | não se aplica | 1 | palato | 2 | rebordo alveolar | 3 | assoalho de boca | 4 | língua | 5 | mucosa jugal | 6 | pilar amigdaliano | 7 | lábio | 8 | área retromolar ..... |__|
10. Outras localizações: | 0 | não se aplica | 1 | antro maxilar | 2 | mandíbula | 3 | valécula | 4 | supraglote | 5 | rinofaringe | 6 | cordas vocais | 7 | fossa nasal ..... |__|
11. Lateralidade: | 1 | direita | 2 | esquerda | 3 | mediana | 4 | bilateral | 9 | ignorado ..... |__|
12. Topografia - CID.0: _____ (_____) ..... |__|__|__|__|
13. Maior diâmetro do tumor: _____ (cm) | 99 | ignorado..... |__|__|
14. Mobilidade: | 1 | móvel | 2 | semi-fixo | 3 | fixo | 9 | ignorado..... |__|
15. Invasão de estruturas adjacentes: | 0 | não | 1 | pele | 2 | osso | 3 | nervo | 4 | outras | 9 | ignorado ..... |__|
16. Estádio clínico (TNM):
Critério T: | 0 | não se aplica | 1 | T1 | 2 | T2 | 3 | T3 | 4 | T4 | 5 | Tx ..... |__|
Critério N: | 0 | não se aplica | 1 | N0 | 2 | N1 | 3 | N2a | 4 | N2b | 5 | N2c | 6 | N3 | 7 | Nx ..... |__|
Critério M: | 0 | não se aplica | 1 | M0 | 2 | M1 | 3 | Mx ..... |__|
Local: | 4 | pulmão | 5 | osso | 6 | fígado | 7 | cérebro | 8 | outros | 9 | não se aplica _____ ..... |__|
Estádio: | 0 | Tx | 1 | I | 2 | II | 3 | III | 4 | IV ..... |__|
17. Biópsia prévia: | 0 | não | 1 | sim ..... |__|

18. Tipo: | 0 | Não se aplica | 1 | BAAF | 2 | incisional ..... |__|__|
AP da biópsia: | 0 | Não se aplica ..... |__|__|__|__|__|__|__|__|
Resultado: | 0 | não se aplica | 1 | CME | 2 | AdPleom | 3 | CAC
| 4 | CCA | 5 | AdenoCA | 6 | Warthin | 7 | Outros _____ ..... |__|__|
19. Congelação: | 0 | não | 1 | sim ..... |__|
20. Resultado: | 0 | Não se aplica | 1 | CME | 2 | AdPleom | 3 | CAC | 4 | CCA
| 5 | AdenoCA | 6 | Warthin | 7 | Outros _____ ..... |__|__|
21. Número do AP final: | 0 | Não se aplica ..... |__|__|__|__|__|__|__|__|
| 0 | Não se aplica | 1 | CME bg | 2 | CME gi | 3 | CME ag | 4 | Só CME
| 5 | Sem lesão..... |__|
22. PAS: | 0 | Não realizado | 1 | Negativo | 2 | Positivo ..... |__|
23. Mucicarmim: | 0 | Não realizado | 1 | Negativo | 2 | Positivo ..... |__|
24. Critérios de Gradação:
CIC <20%: | 0 | Não realizado | 1 | Negativo | 2 | Positivo ..... |__|
Invasão neural: | 0 | Não realizado | 1 | Negativo | 2 | Positivo ..... |__|
Presença de Necrose: | 0 | Não realizado | 1 | Negativo | 2 | Positivo ..... |__|
Mitoses: | 0 | Não realizado | 1 | Negativo | 2 | Positivo ..... |__|
Presença de Anaplasia: | 0 | Não realizado | 1 | Negativo | 2 | Positivo ... |__|
Gradação: | 0 | Não realizada | 1 | Baixo grau | 2 | Grau intermediário
| 3 | Alto grau ..... |__|
25. AP - Linfonodos comprometidos: ___/___ | 99 | Não se aplica ..... |__|__|
26. Local linfonodos +: | 0 | 0 | 1 | I | 2 | II | 3 | III | 4 | IV | 5 | V
| 9 | não se aplica ..... |__|
27. Linfonodos contralaterais +: _____ | 99 | Ignorado ..... |__|__|
28. Tratamento oncológico prévio: | 0 | não | 1 | sim ..... |__|
29. Início do tratamento: ..... |__|__| / |__|__| / |__|__|
Seqüência: | 0 | não | 1 | Cirurgia | 2 | RXT | 3 | QT ..... |__|__|__|__|
30. Cirurgia: Data ___/___/_____ ..... |__|__| / |__|__| / |__|__|
Tipo: | 0 | sem cirurgia | 1 | local com margem | 2 | parotidectomia
| 3 | submandibulectomia | 4 | ressecção nasal | 5 | ressecção maxilar
| 6 | laringectomia | 7 | outras _____ ..... |__|
31. Esvaziamento cervical: | 0 | não realizado | 1 | SHOuni | 2 | SHObi
| 3 | RadicalUni | 4 | RadicalBi | 5 | Outros ..... |__|
32. Ressecção de estruturas: | 0 | não | 1 | pele | 2 | mandíbula | 3 | maxila
| 4 | nervo | 5 | músculo | 6 | outras ..... |__|
33. Reconstrução: | 0 | não | 1 | RRLocal | 2 | RRmiocutâneo | 3 | TL
| 4 | outros ..... |__|
34. Tumor residual pós-cirúrgico: | 0 | não | 1 | macroscópico
| 2 | microscópico ..... |__|
35. Complicações: | 0 | não | 1 | hematoma | 2 | seroma | 3 | infecção
| 4 | necrose retalho | 5 | paralisia | 6 | Frei | 7 | óbito pós-cirúrgico
| 8 | outras _____ ..... |__|

36. RXT: | 0 | não | 1 | primário | 2 | primário + pescoço ..... |__|
Data início ..... |__|__| / |__|__| / |__|__|
Dose local (cGy) ..... | 0 | Não se aplica | 9 | Ignorada ..... |__|__|__|__|
Dose cervical (cGy) ..... | 0 | Não se aplica | 9 | Ignorada ..... |__|__|__|__|
| 0 | Não se aplica | 1 | ortovoltagem | 2 | cobalto | 3 | acelerador linear
| 4 | Outras ..... |__|
37. QT: | 0 | não | 1 | sim _____ ..... |__|
Data início ..... |__|__| / |__|__| / |__|__|
38. Recidiva Local: | 0 | não | 1 | sim .... Data 1ª recidiva: ... |__|__| / |__|__| / |__|__| .... |__|__|
39. Recidiva regional: | 0 | não | 1 | sim ..... Data 1ª recidiva: . |__|__| / |__|__| / |__|__| . |__|__|
Pescoço: | 0 | Não se aplica | 1 | homolateral | 2 | contra-lateral ..... |__|
40. Recidiva distância: | 0 | não | 1 | sim .. Data 1ª metástase: . |__|__| / |__|__| / |__|__| ... |__|__|
Local: | 0 | Não se aplica | 1 | pulmão | 2 | osso | 3 | fígado | 4 | cérebro
| 5 | outros ..... |__|
41. Tratamento da recidiva: | 0 | s/ recidiva | 1 | ressecção local
| 2 | ressecção rec. Cervical | 3 | EC | 4 | Ressecção meta pulmonar
| 5 | RXT | 6 | QT | 7 | RHD ..... |__|
42. Data diagnóstico 2º tumor: ..... |__|__| / |__|__| / |__|__|
43. Data de admissão ..... |__|__| / |__|__| / |__|__|
44. Data da última informação: ..... |__|__| / |__|__| / |__|__|
45. Tempo de acompanhamento (meses): ..... |__|__|__|
46. Situação na última informação: | 1 | vivo sem doença | 2 | vivo com doença
| 3 | morte pela doença | 4 | morte durante/pelo tratamento
| 5 | morte por outras causas | 6 | perdido de seguimento
_____ ..... |__|
47. PCNA: | 0 | Não realizado | 1 | Negativo | 2 | Fraco/focal (<10% céls)
| 3 | Moderado (10-50% céls) | 4 | Forte (>50% céls) ..... |__|
48. Ki-67: | 0 | Não realizado | 1 | Negativo | 2 | Fraco/focal (<5% céls)
| 3 | Moderado (5-20% céls) | 4 | Forte (>20% céls) ..... |__|
49. P53: | 0 | Não realizado | 1 | Negativo | 2 | Fraco/focal (<5% céls)
| 3 | Moderado (5-20% céls) | 4 | Forte (>20% céls) ..... |__|
50. CerbB2: | 0 | Não realizado | 1 | Negativo | 2 | Fraco/focal (<10% céls)
| 3 | Moderado (10-50% céls) | 4 | Forte (>50% céls) ..... |__|
51. CEA: | 0 | Não realizado | 1 | Negativo | 2 | Fraco/focal (<10% céls)
| 3 | Moderado (10-30% céls) | 4 | Forte (>30% céls) ..... |__|
52. Bcl-2: | 0 | Não realizado | 1 | Negativo | 2 | Fraco/focal (<10% céls)
| 3 | Moderado (10-50% céls) | 4 | Forte (>50% céls) ..... |__|
53. Estrógeno: | 0 | Não realizado | 1 | Negativo | 2 | Fraco/focal (<10% céls)
| 3 | Moderado (10-50% céls) | 4 | Forte (>50% céls) ..... |__|
54. MelCAM: | 0 | Não realizado | 1 | Negativo | 2 | Fraco/focal (<25% céls)
| 3 | Moderado (26-50% céls) | 4 | Forte (51-75% céls)
| 5 | Intenso (>75% céls) ..... |__|