# Salivary gland mucoepidermoid carcinoma is a clinically, morphologically and genetically heterogeneous entity: a clinicopathological study of 40 cases with emphasis on grading, histological variants and presence of the t(11;19) translocation

**Stephan Schwarz, Clemens Stiegler, Maximilian Müller, Tobias Ettl, Gero Brockhoff, Johannes Zenk, Abbas Agaimy**

**Angaben zur Veröffentlichung / Publication details:**

Schwarz, Stephan, Clemens Stiegler, Maximilian Müller, Tobias Ettl, Gero Brockhoff, Johannes Zenk, and Abbas Agaimy. 2011. "Salivary gland mucoepidermoid carcinoma is a clinically, morphologically and genetically heterogeneous entity: a clinicopathological study of 40 cases with emphasis on grading, histological variants and presence of the t(11;19) translocation." *Histopathology* 58 (4): 557–70. https://doi.org/10.1111/j.1365-2559.2011.03777.x.



# Salivary gland mucoepidermoid carcinoma is a clinically, morphologically and genetically heterogeneous entity: a clinicopathological study of 40 cases with emphasis on grading, histological variants and presence of the t(11;19) translocation

Stephan Schwarz,* Clemens Stiegler,[1]* Maximilian Müller, Tobias Ettl,[2] Gero Brockhoff,[3] Johannes Zenk[4] & Abbas Agaimy

*Department of Pathology, University of Erlangen, Erlangen, Germany,* [1]*Department of Pathology, University of Regensburg, Regensburg, Germany,* [2]*Department of Oral and Maxillofacial Surgery, University of Regensburg, Regensburg, Germany,* [3]*Department of Gynecology and Obstetrics, University of Regensburg, Regensburg, Germany, and* [4]*Department of Oto-Rhino-Laryngology, University of Erlangen, Erlangen, Germany*

*Aims*: To correlate World Health Organization (WHO) grade, patient's outcome and presence of t(11;19) to histological tumour variants in 40 well-characterized mucoepidermoid carcinomas (MECs).

*Methods and results*: MECs were classified as 'classical' based on the presence of equal proportions of the three cell types or the dominance (≥50%) of mucous cells together with at least one other cell type, and as 'variant' if composed of ≥80% of a single non-mucous cell type. Classical MECs were more common ($n = 23$). Variant MECs had predominant squamoid ($n = 9$), eosinophilic ($n = 5$) or clear cell ($n = 3$) morphology. Twenty-seven tumours were WHO grade 1, three grade 2 and ten grade 3. The t(11;19) was detected in 82%, 35% and 0% of classical MEC, variant MEC and non-MEC, respectively. Classical MECs were associated significantly with age ≤60 years ($P < 0.001$), grade 1 ($P < 0.001$) and t(11;19) ($P = 0.003$). Short overall survival was associated significantly with age >60 years ($P = 0.001$) and Union for International Cancer Control (UICC) stage >I ($P = 0.031$), residual tumour ($P < 0.001$), tumour grade >1 ($P = 0.001$) and squamoid variant ($P = 0.002$) in Kaplan–Meier analysis.

*Conclusions*: The results underscore the great histological diversity of MEC, the reproducibility of the WHO grading criteria and the value of histological subtypes as an additional prognostic factor.

Keywords: grading, mucoepidermoid carcinoma, salivary gland, t(11;19), variant

Abbreviations: EMC, epithelial–myoepithelial carcinoma; FISH, fluorescence *in situ* hybridization; H&E, haematoxylin and eosin; MEC, mucoepidermoid carcinoma; NOS, not otherwise specified; PAS, periodic acid-Schiff; TMA, tissue microarray; UICC, Union for International Cancer Control; WHO, World Health Organization

## Introduction

Mucoepidermoid carcinoma (MEC) is the most common primary carcinoma of major and minor salivary glands, accounting for about one-third of all salivary carcinomas.[1,2] MECs are composed of three cell types in varying proportions: mucous cells, epidermoid (squamoid) cells and undifferentiated small cells (intermediate

Address for correspondence: S Schwarz, MD, Department of Pathology, University of Erlangen, Krankenhausstrasse 12, 91054 Erlangen, Germany. e-mail: stephan.schwarz@uk-erlangen.de

*These two authors contributed equally to this study.

cells). Any of these cell types may dominate the histological picture in a given tumour, creating diagnostic difficulty.[1] Traditionally, special stains such as Meyer's mucicarmine and periodic acid-Schiff (PAS) are used to detect sparse mucous cells. Immunohistochemistry has also proved of value in recognizing foci of intermediate and epidermoid cells and, in particular, in differentiating MECs from other salivary gland carcinomas that they may mimic closely.[3,4]

Owing to the highly variable biological behaviour of MEC, a variety of prognostic factors have been evaluated in previous large series to predict aggressive tumour behaviour and hence the necessity of more aggressive treatment.[5,6] Of these, histological grading has received special attention and several grading systems have been proposed.[7–9] While the difference in behaviour of grade 1 and grade 3 MEC was confirmed in different studies, the results with regard to intermediate-grade MEC have been inconsistent, and varied from low-grade behaviour[10] to an aggressive course comparable to high-grade tumours[11] or something in between.[7] The Armed Forces Institute of Pathology (AFIP) system showed a higher reproducibility and was considered valid in the current World Health Organization (WHO) classification of head and neck tumours.[1,7,8] However, Brandwein *et al.*[12] suggested that the AFIP grading system may downgrade some MEC. They proposed considering histological features, including infiltration pattern at the invasive front, vascular invasion and bony infiltration in the assessment of tumour grade.

The translocation t(11;19) has been detected in approximately half of MECs .[13–15] However, data from different studies on the detection of this molecular alteration in Warthin tumour have been conflicting.[16–18] The presence of t(11;19) correlates with a better outcome in MEC,[13,19,20] but the explanation for this is lacking. To our knowledge, the correlation of well-defined histological variants with WHO grading, patient outcome and the presence or absence of the translocation t(11;19) has not been evaluated previously. This was the objective of this study.

## Materials and methods

### PATIENTS AND TUMOUR SAMPLES

Forty MECs were retrieved from a consecutive collection of 290 well-characterized primary salivary gland carcinomas of major and minor salivary glands with complete follow-up that were treated at the Universities of Erlangen and Regensburg, Germany, or at the City Hospital of Nuremberg, Germany, between 1990 and 2007. Clinical information was obtained from the clinical tumour registries of Erlangen-Nuremberg and Regensburg as well as from the salivary gland carcinoma registry of Erlangen. The registries and the related translational research activities are covered by ethical vota of the medical faculties of the Universities of Regensburg and Erlangen-Nuremberg.

### HISTOLOGY AND CLASSIFICATION

On the basis of haematoxylin and eosin (H&E) and PAS-stained slides from at least subtotally embedded tumours, all the 290 tumours were reviewed independently by two pathologists experienced in salivary gland tumour pathology (S.S. and A.A.) without knowledge of initial histological diagnosis, fluorescence *in situ* hybridization (FISH) results or clinical follow-up. Four cases previously considered MECs were excluded as they represented other types of salivary gland carcinomas or lacked unequivocal MEC areas. An agreed diagnosis of MEC was achieved in 40 cases which formed the study cohort.

MECs were diagnosed according to the WHO criteria,[1] and tumours categorized as either 'classical' MEC or 'variant' (non-classical) MEC. Classical MECs were tri- or biphasic neoplasms composed of clearly recognizable mucous cells (prominent single goblet cells or contiguous goblet cells forming mucinous gland-like or cystic spaces) as well as variable proportions of epidermoid (squamoid) and intermediate cells. In contrast to true squamous cells, epidermoid cells lacked intercellular bridges and intracellular keratinization. Intermediate cells were small, undifferentiated cells with round nuclei and scant basophilic cytoplasm. In difficult cases additional immunohistochemical staining for low (CK7)[21] or high molecular weight (CK5) cytokeratins was used to identify mucous, intermediate and epidermoid differentiation, respectively. CK5 antibody was purchased from Zytomed Systems Ltd (Berlin, Germany) and CK7 antibody from DCS Innovative Diagnostik-Systeme (Dr Christian Sartori Ltd, Hamburg, Germany). Immunocytochemistry was performed on 5-μm sections according to the manufacturers' instructions. Generally, all three components were easily recognised in each low-power field in classical MECs. Biphasic tumours were considered classical if a prominent mucous component (≥50%) was present beside another component (either intermediate or epidermoid cell component). The presence or absence of a prominent cystic component did not influence this classification. The presence of a focus of classical MEC in an otherwise variant MEC was judged according to the extent of both components. Tumours with ≥80% non-classical (non-mucous) histology were considered

variant MECs in this study. Applying the WHO criteria,[1] all tumours have been graded independently by the two pathologists, with complete reproducibility in all cases.

### TISSUE MICROARRAY (TMA) CONSTRUCTION

A TMA was constructed from formalin-fixed paraffin-embedded tissue blocks from these 40 patients, from an additional 250 patients with salivary gland carcinomas other than MEC, and from 60 cases of normal salivary gland tissue, as described previously.[22,23] One tissue cylinder with a diameter of 2.0 mm was punched from a morphologically representative tissue area of each donor tissue block and brought into a recipient paraffin block using a homemade semi-automated tissue arrayer. TMA sections were mounted on charged slides (SuperFrost™Plus; Menzel Ltd, Braunschweig, Germany). H&E-stained TMA sections were used for reference histology.

### FLUORESCENCE *IN SITU* HYBRIDIZATION

FISH was performed on 5-μm sections of the TMAs using commercially available, directly labelled DNA break-apart probes to detect the translocation t(11;19) (ZytoVision Ltd, Bremerhaven, Germany). The probes identified two locus specific sequences on chromosomes 11 (orange/green) spanning the break point at 11q21 and resulting in a yellow-appearing fusion signal. A translocation was evident if the fusion signal was split into an orange and a green signal. TMA sections were dewaxed for 40 min in an incubator at 72°C and for 2 × 10 min in xylene. After being rehydrated in a graded ethanol series and rinsed in distilled water, slides were placed in 0.01 M sodium citrate and steamed for 40 min in a water bath. Cell structures were digested in 0.1% pepsin (Sigma-Aldrich Chemie Ltd, Munich, Germany) and 0.01 M HCL for 10 min at 37°C. After washing in 2× standard sodium citrate (SSC) (1× SSC: 150 mM sodium chloride and 15 mM sodium citrate, pH 7) and water, slides were dehydrated in graded alcohols and air-dried. Ten millilitre of the DNA probe set were applied to the TMA area of each section. Sections were cover-slipped and the edges sealed with rubber cement. For co-denaturation of the probe and target DNA, slides were placed on a hotplate preheated to 73°C for 5 min and transferred to a warmed hybridization chamber at 37°C overnight.

After hybridization, the rubber cement was removed and the slides were immersed in 4× SSC + 0.3% Igepal (Serva Elecotrophoresis Ltd, Heidelberg, Germany), 2× SSC and 1× SSC for 10 min at 50°C, respectively. The slides were rinsed briefly in Millipore water and air-dried. Nuclei were counterstained with anti-fading DAPI (4′,6-diamidino-2-phenylindole) (Vectashield; Vector Laboratories, Burlingame, CA, USA) and analysed by epifluorescence microscopy.

Microscopy, FISH scoring and digital imaging slides were imaged with an Axio Imager Z.1 (Zeiss, Göttingen, Germany) equipped with specific filter sets for DAPI (excitation 365 ± 20 nm, emission 450 ± 25 nm; Zeiss), green (excitation 500 ± 10 nm, emission 535 ± 15 nm) and red fluorescence (excitation 545 ± 15 nm, emission 610 ± 35 nm; AHF, Tübingen, Germany). Fluorescence images were obtained with a Plan-Apochromat lens (×63, 1.4) and recorded with a charge-coupled device (CCD) camera AxioCam MRm (Zeiss). FISH scoring was performed by counting fluorescence signals in 50 malignant, non-overlapping cell nuclei for each case by two independent interpreters (C.S., M.M.). A tumour was considered positive if >50% of the cells harboured the translocation.

### STATISTICAL ANALYSES

All clinicopathological and cytogenetic data were analysed with SPSS for Windows, version 15.0 (SPSS, Inc., Erkrath, Germany). Relationships between dichotomized histological or cytogenetic markers and clinicopathological factors were examined using Fisher's exact probability test (significant association with $P < 0.05$). Overall survival (=primary outcome measure) was calculated as the time from the date of diagnosis to death from any cause or the date the patient was last known to be alive. Patients lost to follow-up were treated as censored cases based on the date they were last known to be alive. Survival curves were generated using the Kaplan–Meier method, and log-rank tests compared the distributions between groups. For multivariate analysis a Cox proportional hazards model was used in a backwards, stepwise elimination approach. At each step the least significant factor with $P > 0.10$ was eliminated with reassessment of each factor in the model at each step. A limit for the factors to be included was set at 5%.

## Results

### CLINICAL FEATURES OF MEC

The clinicopathological parameters are summarized in Tables 1 and 2. Using the current WHO criteria for grading salivary gland MEC,[1] 27 cases were low-grade (G1), three cases were intermediate-grade (G2) and 10

**Table 1.** Clinicopathological parameters related to the four subtypes of mucoepidermoid carcinoma (MEC) (significance of associations was evaluated by Fisher's exact test)

| | | Classical type ($n$ = 23) | Clear cell variant ($n$ = 3) | Eosinophilic variant ($n$ = 5) | Squamoid variant ($n$ = 9) | Fisher's exact test classical versus others | Fisher's exact test squamoid versus others |
|---|---|---|---|---|---|---|---|
| Age (years) | ≤60 ($n$ = 24) | 19 | 1 | 2 | 2 | $P < 0.001$ | $P = 0.03$ |
| | >60 ($n$ = 16) | 4 | 2 | 3 | 7 | | |
| Sex | Males ($n$ = 13) | 7 | 0 | 2 | 4 | NS | NS |
| | Females ($n$ = 27) | 16 | 3 | 3 | 5 | | |
| Site | Parotid gland ($n$ = 24) | 10 | 2 | 4 | 8 | $P = 0.125$* | $P = 0.055$* |
| | Submandibular gland ($n$ = 4) | 2 | 1 | 0 | 1 | | |
| | Oral cavity ($n$ = 12) | 11 | 0 | 1 | 0 | | |
| Stage | I ($n$ = 16) | 12 | 1 | 2 | 1 | $P = 0.107$† | $P = 0.061$† |
| | II ($n$ = 12) | 5 | 1 | 2 | 4 | | |
| | III ($n$ = 6) | 2 | 0 | 1 | 3 | | |
| | IV ($n$ = 6) | 4 | 1 | 0 | 1 | | |
| Grade | 1 ($n$ = 27) | 22 | 2 | 3 | 0 | $P < 0.001$‡ | $P < 0.001$‡ |
| | 2 ($n$ = 3) | 1 | 0 | 1 | 1 | | |
| | 3 ($n$ = 10) | 0 | 1 | 1 | 8 | | |
| Residual tumour | 0 | 22 | 3 | 4 | 8 | NS§ | NS§ |
| | 1 | 0 | 0 | 1 | 0 | | |
| | 2 | 1 | 0 | 0 | 1 | | |
| t(11;19) | Negative | 4 | 0 | 3 | 8 | $P = 0.003$ | $P = 0.001$ |
| | Positive | 19 | 3 | 2 | 1 | | |
| | Number of cases | 23 | 3 | 5 | 9 | | |

NS, Not significant.
*Major glands versus minor glands.
†Stage I versus stages II, III and IV.
‡Grade 1 versus grades 2 and 3.
§Residual tumour 0 versus residual tumours 1 and 2.

cases were high-grade (G3). Classical MEC was associated significantly with age ≤60 years ($P < 0.001$), grade 1 ($P < 0.001$) and presence of the translocation t(11;19) ($P = 0.003$).

Mean follow-up was 64.5 months (range 1.6–224 months). Ten patients (25%) died of their tumours at a mean interval of 25.2 months (range 1.6–70.9 months).

## HISTOLOGICAL FEATURES OF MEC

### *Classical MEC*

Twenty-three tumours fulfilled the above criteria of classical MEC. These revealed variable cystic areas; a prominent cystic component (>20%) was seen in seven tumours. Mucous cells were prominent in each low-power field and gland-like spaces or aggregates of

**Table 2.** Clinicopathological parameters related to presence or absence of t(11;19); significance of association was evaluated by Fisher's exact test

| | | t(11;19) negative | t(11;19) positive | Fisher's exact test |
|---|---|---|---|---|
| Age (years) | ≤60 (*n* = 24) | 6 | 18 | *P* = 0.094 |
| | >60 (*n* = 16) | 9 | 7 | |
| Sex | Male (*n* = 13) | 6 | 7 | NS |
| | Female (*n* = 27) | 9 | 18 | |
| Site | Parotid gland (*n* = 24) | 9 | 15 | NS* |
| | Submandibular gland (*n* = 4) | 2 | 2 | |
| | Oral cavity (*n* = 12) | 4 | 8 | |
| Stage | I (*n* = 16) | 5 | 11 | NS† |
| | II (*n* = 12) | 5 | 7 | |
| | III (*n* = 6) | 3 | 3 | |
| | IV (*n* = 6) | 2 | 3 | |
| Grade | 1 (*n* = 27) | 5 | 22 | *P* = 0.001‡ |
| | 2 (*n* = 3) | 3 | 0 | |
| | 3 (*n* = 10) | 7 | 3 | |
| Residual tumour | R0 (*n* = 37) | 13 | 24 | NS§ |
| | R1 (*n* = 1) | 1 | 0 | |
| | R2 (*n* = 2) | 1 | 1 | |
| | Number of cases | 15 | 25 | |

NS, Not significant.
*Major glands versus minor glands.
†Stage I versus stages II, III and IV.
‡Grade 1 versus grades 2 and 3.
§Residual tumour 0 versus residual tumours 1 and 2.

contiguous mucous cells were well discernible, even at low-power magnification (Figure 1A). Focal unusual features in classical MEC included minor components with microvesicular cytoplasm, suggesting abortive sebaceous differentiation (two cases; Figure 1B), focal syncytial overgrowth of spindled intermediate cells reminiscent of myoepithelial differentiation but lacking expression of p63 (not shown) and CK5 (expressing CK7 diffusely) (Figure 1C,D) and circumscribed tumour nests composed of peripherally located polygonal myoepithelial-like clear cells punctuated by centrally located small microcystic glands mimicking epithelial–myoepithelial carcinoma (EMC) (Figure 1E,F). However, the lumina were lined by flattened epithelial cells and lacked the well-discernible glands of EMC. Immunohistochemistry showed CK5 expression in the luminal cells and CK7 in the outer layer, thus adopting an 'inverted EMC pattern' (Figure 1E,F). A common finding in several cases was the presence of atypical pre-existing salivary ducts showing both bland ciliated epithelium and crypt-like mucous cell hyperplasia resulting in atypical budding of mucous cell-rich ductules and small solid nests of intermediate cells (Figure 1G,H). These changes were often associated with an avascular papillary epithelial tufting.

*Squamoid (epidermoid) MEC*

Squamoid tumours (*n* = 9) showed variable squamoid differentiation with horn cysts and sharp intercellular junction, but prominent intercellular bridges as seen in

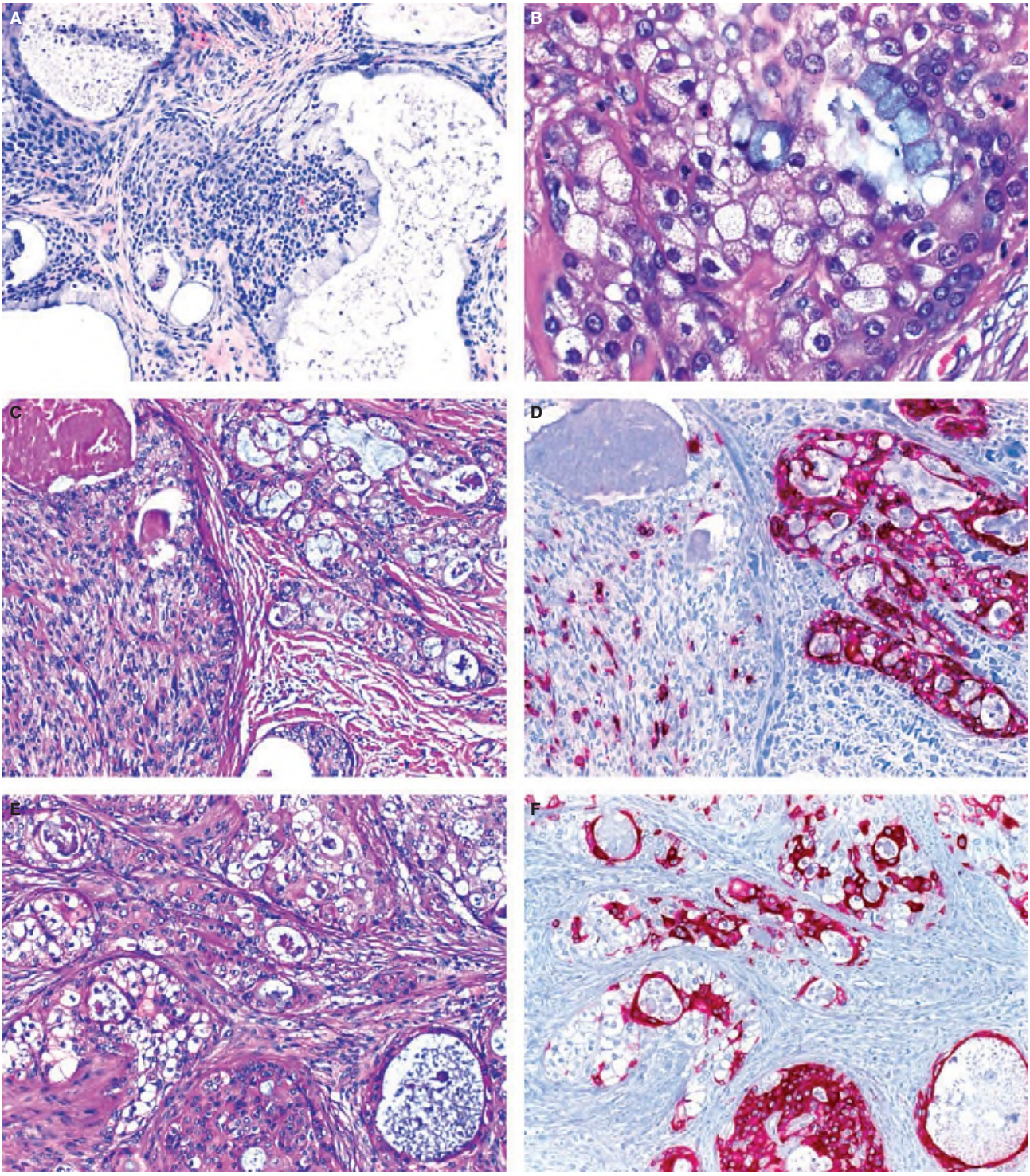


**Figure 1.** Variations of classical (low-grade) mucoepidermoid carcinoma (MEC) with unusual features. **A**, Classical and partially cystic MEC composed of mucous, epidermoid and intermediate cells. **B**, MEC with focal sebaceous-like differentiation characterized by polygonal cells with microvesicular cytoplasm. **C**, MEC exhibiting partial overgrowth of spindle-shaped intermediate cells mimicking myoepithelial differentiation but negative for myoepithelial markers (p63, not shown) and CK5 (**D**). **E**, Classical MEC (G1) with focal clear cell change of intermediate cells, negative for CK5 (**F**), but positive for CK7 (not shown), generating an inverted epithelial–myoepithelial pattern. **G**, Classical MEC entrapping a salivary duct with mucous metaplasia, possibly representing a precursor lesion of MEC. **H**, Higher magnification demonstrating a proliferation of intermediate cells within the duct lumen on the right side and a focal eosinophilic change of epidermoid tumour cells on the left side.

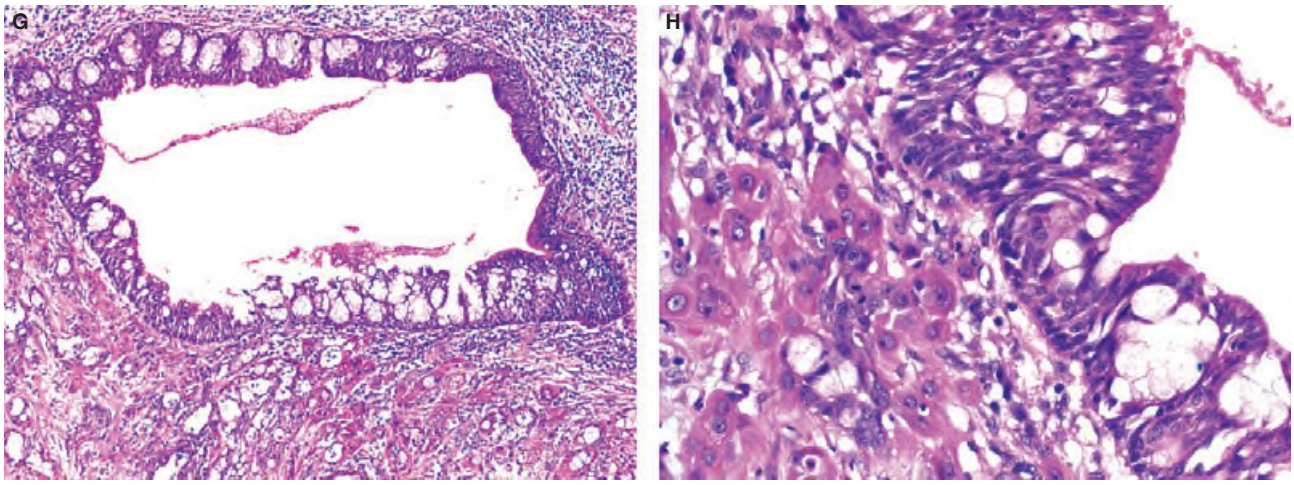


**Figure 1.** *Continued.*

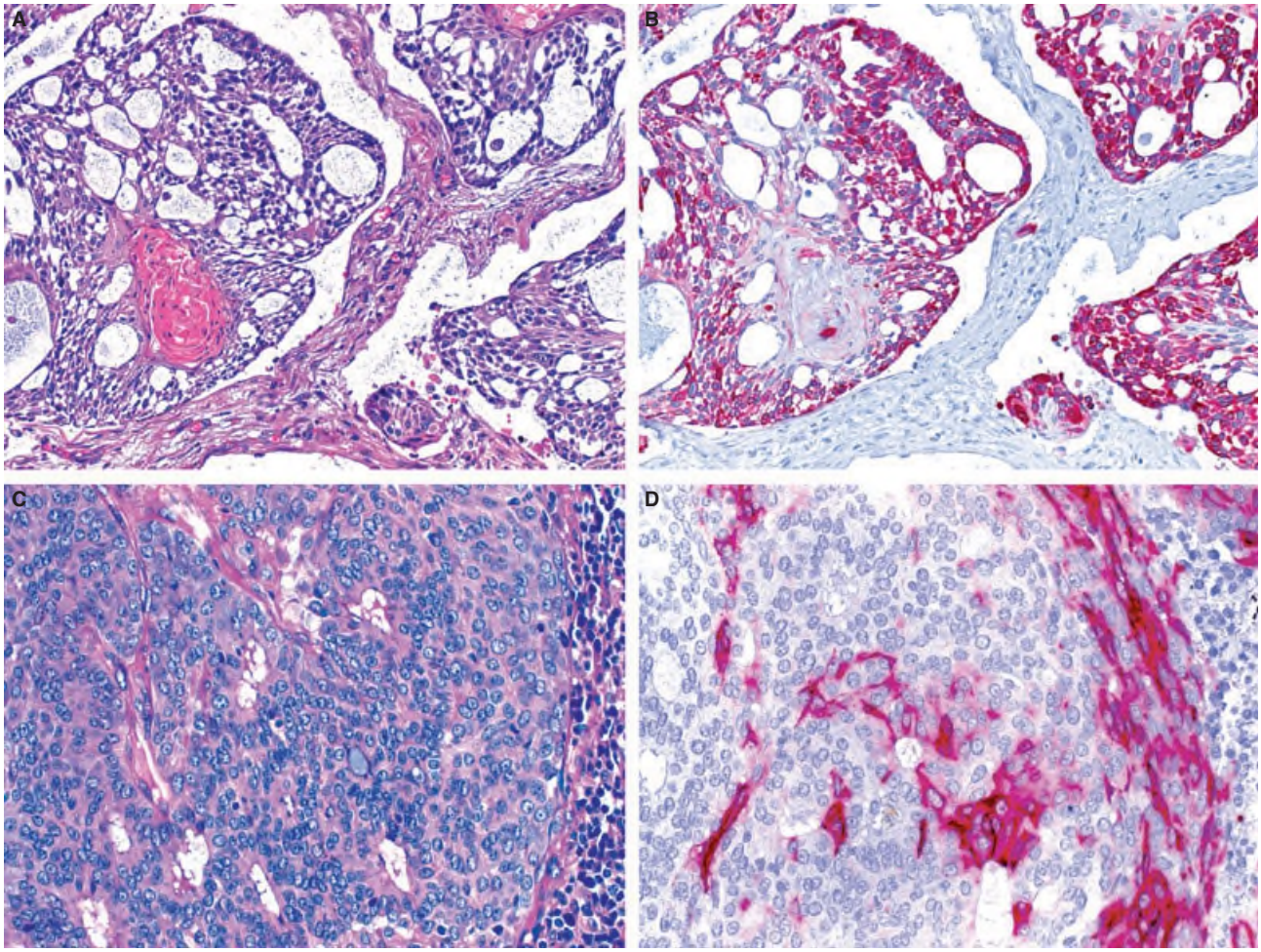


**Figure 2.** Mucoepidermoid carcinoma (MEC) of squamoid type. **A**, High-grade MEC (G3) with mucous-filled microcystic spaces and sheets of intermediate cells differentiating to mature squamoid epithelia forming horn pearls. **B**, Strong expression of CK5 in the intermediate/squamoid cells. This tumour was t(11;19)-negative. **C**, High-grade MEC (G3) composed predominantly of solid sheets of intermediate cells with interspersed single mucous cells. Partial expression of CK5 (**D**) and negativity of CK7 (not shown) indicate immature/intermediate cells. This tumour was t(11;19)-positive (not shown).

typical squamous cell carcinoma were lacking. Occasional cytoplasmic vacuoles, possibly representing abortive glandular differentiation, were seen. A few tumours revealed a focal or prominent acantholytic (pseudoglandular or pseudovascular) pattern. Another pattern seen in this group was the presence of differentiated squamoid sheets with numerous microcystic lumina filled with blue mucin and imparting a pseudocribriform pattern to the tumour (Figure 2A,B). One tumour classified into this subtype showed solid sheets of medium-sized to relatively small basophilic cells with histological and immunohistochemical features of intermediate cells with a minor component of mucous cells (Figure 2C,D). This tumour would be better categorized as 'variant MEC of intermediate cell type', but no more cases were found to warrant an additional subtype and the case was hence discussed under the subheading of squamoid MEC.

*Eosinophilic (oncocytoid) MEC*

This represented the second most common type of variant MEC ($n = 5$). Tumours in this group showed a predominance of medium-sized to large cells with deeply eosinophilic cytoplasm lacking equivocal squamoid differentiation (Figure 3A,B). Tumour cells revealed either a prominent glandular differentiation that closely resembled mature striated ducts (Figure 3C) or they were arranged into solid sheets and possessed prominent centrally located nuclei, thus strikingly mimicking an oncocytic neoplasm (Figure 3D). Occasionally, both features were seen side by side. In addition, all tumours showed either minor classical MEC areas, focal epidermoid/squamoid differentiation or scattered mucous cells, thus establishing the diagnosis of MEC (Figure 3E,F). The tumour illustrated in Figure 3A was composed of large atypical cystic glands lined by oncocytic partially ciliated cells with occasional papillary pattern reminiscent of Warthin tumour. Single striated duct-like glands seemed to bud off the cyst. However, mucous cells and other typical features were evident in small areas of the tumour. Furthermore, a lymphoid component was lacking and the stroma was hypervascular.

*Clear cell MEC*

Although in general focal clear cell areas were quite common in MEC, the clear cell variant was the least common variant in our study ($n = 3$). Clear cell variants were composed of solid sheets of medium-sized polygonal cells with cytoplasmic clearing, usually with peripheral cytoplasmic retraction with occasional lightly eosinophilic staining tumour cell groups (Figure 4A,B). The appearance of some of these tumours was reminiscent of the chromophobe renal cell carcinoma (Figure 4C). The typical nesting and hyaline bands of clear cell carcinoma, not otherwise specified (NOS), was absent. Unequivocal mucous cells or minor foci of typical MEC were seen, thus establishing the diagnosis (Figure 4B,D).

### DISTRIBUTION AND PROGNOSTIC ROLE OF THE TRANSLOCATION T(11;19) IN MEC

The t(11;19) was detected in 25 of the 40 MECs (62.5%). This corresponded to 19 of 23 classical MECs (82.6%) and 6 of 17 variant MECs (35%). Among the variant MEC types, the positivity rate was 100%, 40% and 11% in the clear cell, eosinophilic and squamoid variants respectively. All the non-MEC salivary carcinomas were translocation-negative. The close association of the translocation with the classical type of MEC was significant ($P = 0.003$), as was the negative association with the squamoid variant ($P = 0.001$). As shown in Table 2, the translocation was associated significantly with low-grade tumours ($P = 0.01$), but not with age, stage, presence of residual tumour, sex and localization. Although not significant, the occurrence of t(11;19) was associated with better overall survival ($P = 0.098$).

### ASSOCIATIONS OF THE HISTOLOGICAL VARIANTS WITH CLINICOPATHOLOGICAL PARAMETERS

The different variants of MEC have been correlated with clinical parameters (age and tumour stage) and tumour grade. The classical type predominated in younger patients, with a mean age of 42 years (range 11–80 years, $P < 0.001$), whereas the squamoid variant occurred usually in the elderly, with a mean age of 68 years (range 37–94 years, $P = 0.03$). MECs of classical type were typically low-grade (G1, $P < 0.001$), the squamoid variant was of intermediate- or high-grade ($P < 0.001$). Squamoid variant MEC tended to occur more frequently in the major glands (parotid and submandibular gland) than in the minor glands of the oral cavity ($P = 0.055$). An association of the variants with sex and stage could not be demonstrated.

### PROGNOSTIC SIGNIFICANCE OF CLINICOPATHOLOGICAL PARAMETERS IN MEC

To examine the prognostic significance of important clinicopathological parameters in MEC, Kaplan–Meier analyses were applied (Figure 5). Here, short overall survival was associated significantly with age >60 years ($P = 0.001$), high UICC stage (II, III, IV versus I; $P = 0.031$), residual tumour after primary surgery ($P < 0.001$) and higher tumour grade (G2 and

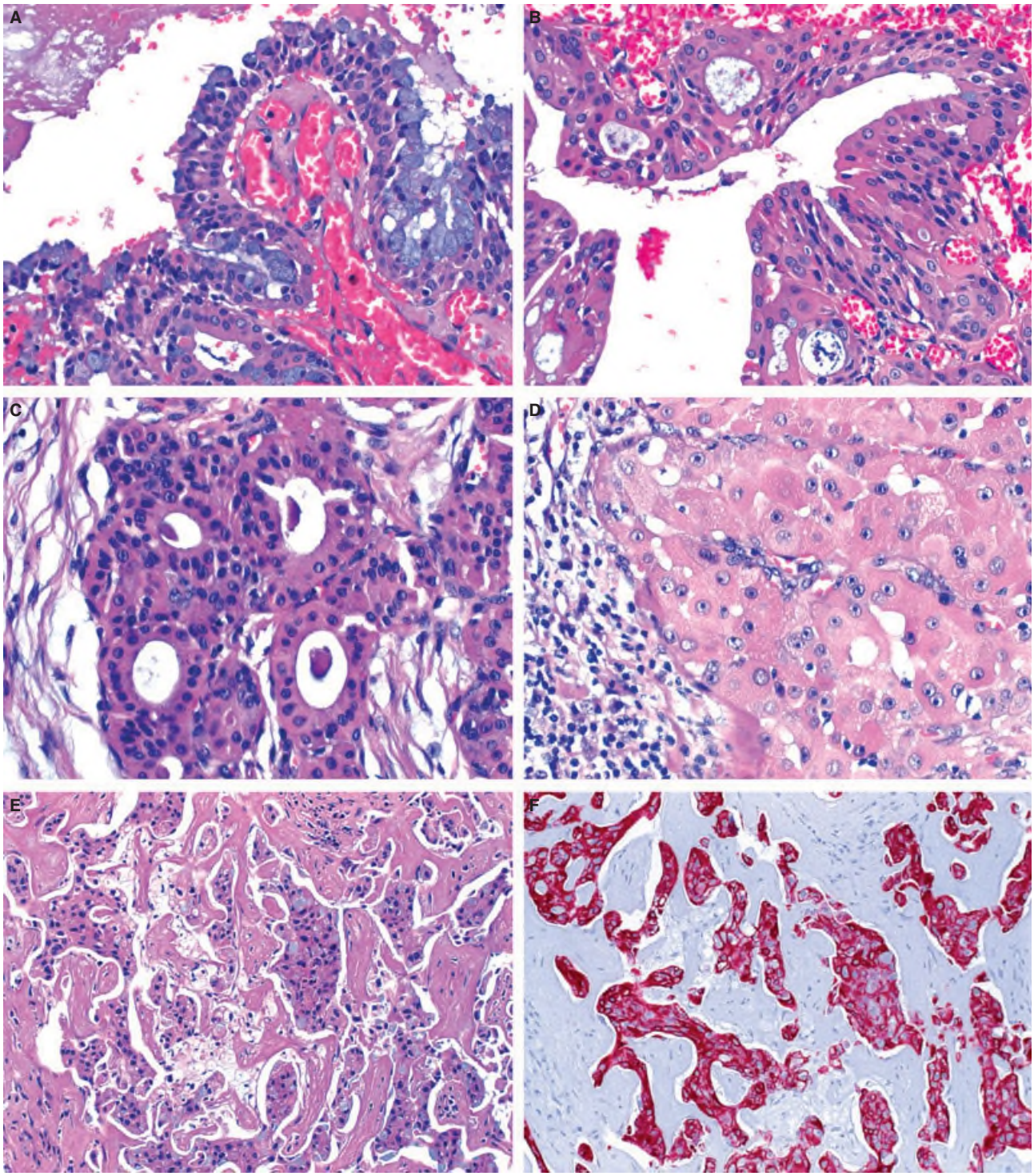


**Figure 3.** Mucoepidermoid carcinoma (MEC) of eosinophilic (oncocytoid) type/with striated duct differentiation. **A**,**B**, Cyst wall of a partially cystic MEC of eosinophilic type (G1) resembling a Warthin tumour with mucous metaplasia and without lymphoid stroma. The neoplastic tubules were strikingly similar to striated ducts of normal salivary gland. Translocation t(11;19) was negative. **C**,**D**, Another t(11;19)-positive low-grade MEC with striated duct differentiation (**C**) and solid cell sheets with characteristic oncocytic appearance (**D**). **E**, Eosinophilic MEC with predominant sclerosis (sclerosing type eosinophilic MEC, G2). The tumour was composed of solid sheets of CK7-positive (**F**) intermediate cells with interspersed mucous cells and focal positivity for CK5 (not shown). This tumour was t(11;19)-negative.

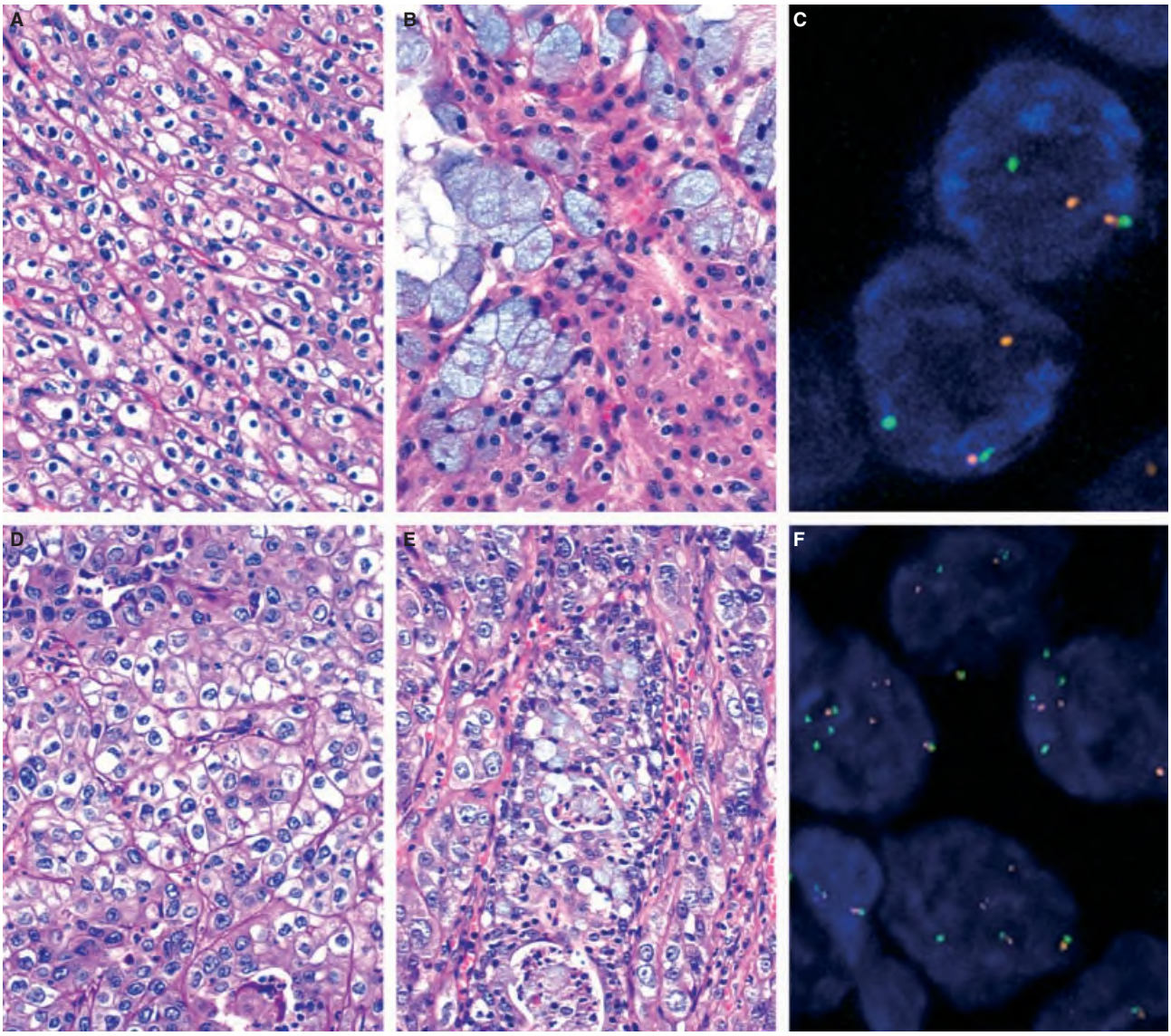


**Figure 4.** Mucoepidermoid carcinoma (MEC) of clear cell type. **A**, Low-grade MEC composed predominantly of solid sheets of clear cells with inconspicuous nuclei and 'chromophobe' appearance. **B**, A small focus with classical morphology detected on further sampling. **C**, Fluorescence *in situ* hybridization (FISH) using break-apart probes demonstrated translocation t(11;19) indicated by split orange and green signals. **D**, High-grade clear cell MEC with higher nuclear pleomorphism and focal well-differentiated areas (**E**). **F**, FISH demonstrated polysomy 11 and bi- or tri-allelic translocation t(11;19).

G3 versus G1; $P = 0.001$). Sex and localization did not influence survival significantly. Analysis of the known clinicopathological parameters was supplemented by a Kaplan–Meier analysis of the aforementioned histological variants of MEC. Here, the classical type of MEC was associated with best prognosis, whereas the non-classical MEC variants were significant negative prognosticators ($P = 0.002$).

The univariate Kaplan–Meier analysis was complemented by a multivariate Cox regression to examine the independence of the prognostically relevant clinicopathological parameters. As the tumour variant was associated strongly with tumour grade (Table 1), the Cox regression analysis could not include both parameters. Thus, two analyses were performed: one with grade (grade 1 versus others) and one with variant (squamoid versus others). The other parameters to include into the analysis were those which proved to be of prognostic significance in univariate Kaplan–Meier analysis (Figure 5): age, stage and residual tumour. The Cox regression model (Table 3) was adjusted within two reverse steps in order to exclude a

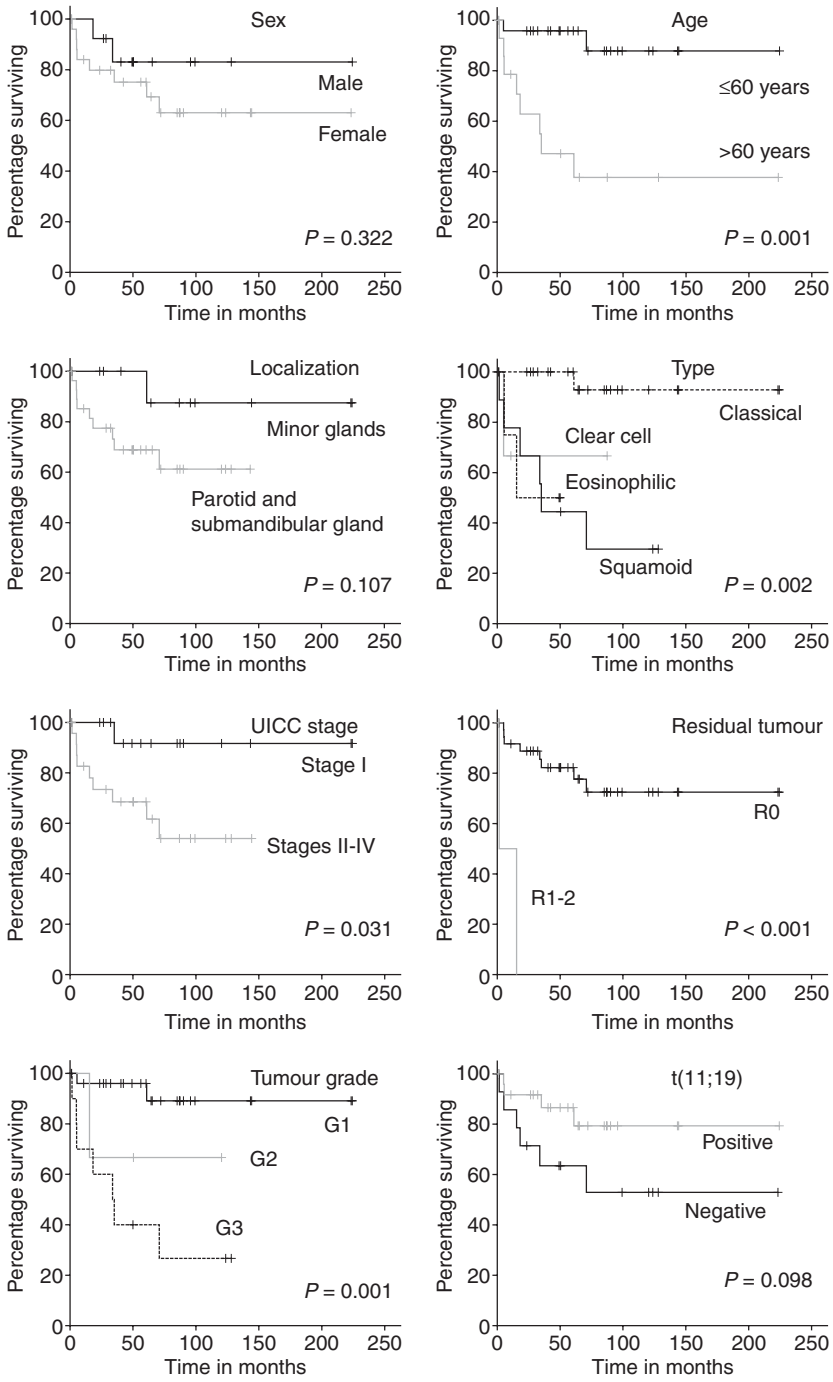


**Figure 5.** Univariate Kaplan–Meier analyses of clinicopathological parameters (sex, age, localization, type, Union for International Cancer Control (UICC) stage, residual tumour classification, grade and translocation t(11;19) regarding overall survival (in months). *P*-values according to log-rank statistics.

non-significant parameter (stage). High age, higher grade, presence of residual tumour and squamoid variant remained significant prognostic factors with hazard ratios of between 4.7 and 18.0.

## Discussion

In this study, we analysed the histological spectrum of MEC in relation to tumour site, WHO grade, stage, survival and the presence of the translocation t(11;19) in 40 well-characterized MECs of major and minor salivary glands. Our findings underscore the diverse morphology of MEC, which results from the striking variability in the proportion of the three cell types comprising the tumour. In its classical form, MEC shows a comparable proportion of three or of at least two cell types, hence the diagnosis of this classical variant is straightforward and usually poses

**Table 3.** Univariate and multivariate Cox regression with reverse stepwise selection. In the upper half age, stage, grade, and residual tumour are included; in the lower half grade is replaced by tumour type (squamoid versus others)

| Parameter | Definition | Univariate | Multivariate | | | |
|---|---|---|---|---|---|---|
| | | | Step 1 | Step 2 | Hazard ratio | 95% confidence interval |
| Age (years) | ≤60 ($n$ = 24) versus >60 ($n$ = 16) | $P$ = 0.005 | $P$ = 0.06 | $P$ = 0.05 | 5.1 | 1.0–26.9 |
| Stage | Stage I ($n$ = 16) versus stages II–IV ($n$ = 24) | $P$ = 0.064 | $P$ = 0.32 | | | |
| Grade | Grade 1 ($n$ = 27) versus grades 2–3 ($n$ = 13) | $P$ = 0.004 | $P$ = 0.11 | $P$ = 0.04 | 5.4 | 1.1–28.4 |
| Residual | R0 ($n$ = 37) versus R1–2 ($n$ = 3) | $P$ = 0.001 | $P$ = 0.13 | $P$ = 0.10 | 4.7 | 0.74–30.0 |
| Age (years) | ≤60 ($n$ = 24) versus >60 ($n$ = 16) | $P$ = 0.005 | $P$ = 0.09 | $P$ = 0.10 | 4.3 | 0.8–24.0 |
| Stage | Stage I ($n$ = 16) versus stages II–IV ($n$ = 24) | $P$ = 0.064 | $P$ = 0.31 | | | |
| Variant | Squamoid ($n$ = 9) versus others ($n$ = 31) | $P$ = 0.007 | $P$ = 0.12 | $P$ = 0.05 | 4.7 | 1.0–20.7 |
| Residual | R0 ($n$ = 37) versus R1–2 ($n$ = 3) | $P$ = 0.001 | $P$ = 0.02 | $P$ = 0.01 | 18.0 | 2.1–152.8 |

no difficulty. However, a single cell type may constitute the bulk of the tumour, thus giving rise to monophasic variants of MEC; their diagnosis and true categorization may be challenging, even for those with great experience in salivary gland pathology. We have subclassified variant MEC into squamoid, eosinophilic and clear cell subtypes based on the presence of >80% variant pattern in the tumour.

The squamoid subtype was the most common variant MEC in this study and implied commonly a high-grade tumour with a significantly adverse outcome. The diagnosis of this variant may be facilitated by detection of minor foci of classical MEC through extensive tumour sampling and demonstration of isolated mucous cells or intracellular mucin assisted by special stains (mucicarmine, PAS). Also, the presence of a population of smaller cells lacking evident squamoid differentiation (intermediate cells) represents an important clue to diagnosis. Immunohistochemistry may help to recognize such minor foci that would remain undetected upon initial H&E evaluation. Adenosquamous carcinoma, which is not included into the WHO classification of salivary gland tumours, is an important differential diagnosis of the squamoid MEC. Demonstration of unequivocal mucous cells and/or intermediate cells on H&E, mucin stains or by immunohistochemistry represents the main discriminator. In this setting, the t(11;19) may help to confirm the diagnosis of MEC in equivocal cases. Lennerz *et al.*[24] detected the t(11;19)-related rearrangements in seven of seven MECs of the uterine cervix, but in none of 14 conventional cervical adenosquamous carcinomas. However, the sensitivity of t(11;19) for squamoid MEC is low in our study (11%). Immunohistochemistry showed consistent expression of CK5 in squamoid MEC as well as in high-grade MEC.[25] One tumour in this series showed a predominance of small blue-staining CK5-negative intermediate cells, suggesting the existence of an intermediate cell variant, but the clinical significance of this remains to be studied further in larger series. We discussed this case within the spectrum of the squamoid variant.

Eosinophilic MEC (also known as oncocytoid MEC)[26,27] shows increased cytoplasmic eosinophilia irrespective of growth pattern, but some cases may appear oncocytoid as a result of prominent striated duct-like differentiation.[28] The latter pattern is exceedingly rare and may mimic MEC arising in Warthin tumour.[29] Although eosinophilic MEC may show a prominent papillary and cystic pattern, the two-layered pattern of oncocytic cells with centrally located nuclei and a prominent lymphoid stroma typical of Warthin tumour are lacking in *de novo* eosinophilic MEC. Furthermore, MEC ex Warthin tumour is usually of the classical type.[29] On the other hand, eosinophilic MEC with papillary pattern and tumour-associated lymphoid proliferation has to be distinguished from benign Warthin tumour with squamous and/or mucous metaplasia and from so-called Warthin adenocarcinoma.[30]

The clear cell variant was generally reminiscent of the clear cell carcinoma, NOS. However, on careful evaluation, a variable, but generally minor population of intermediate cells or a minute focus of classical MEC may be detected. The t(11;19) proved helpful and sensitive in the current study in detecting this variant

(three of three), but the small case number does not allow for solid conclusions. Of interest, focal clear cell change associated with a biphasic pattern very reminiscent of EMC was seen in one classical MEC. However, immunohistochemistry revealed an 'inverted EMC pattern' with the luminal CK5 positivity and CK7 expression in the outer layer. Awareness of this finding, whether in classical or in clear cell MEC, should help to avoid misinterpretation as EMC.

Our results showed high reproducibility of the current WHO grading system,[1] which is based on the AFIP criteria.[7,8] However, due to the relatively limited number of cases we could not assess whether the WHO system downgrades MEC. In line with the observation by Brandwein *et al.*,[12] the dominant histological pattern/features of tumours correlated well with the tumour grade and outcome, thus suggesting that defining histological criteria should be included in the scoring/grading of MEC. Non-classical variants tend to be of higher WHO grade than classical MEC; the latter were predominantly grade 1 tumours (96%) compared to only 29% grade 1 variant MEC. A comparable statistically significant value ($P < 0.001$) was demonstrated for WHO grade 1 and classical variant in the association analysis, indicating that recognition of the classical variant implies a grade 1 tumour.

One interesting finding in this study was the occurrence of atypical crypt-like mucous cell hyperplasia and foci of intermediate-like cells associated with residual salivary ducts in several cases of classical MEC. This finding probably represents a putative precursor lesion, but reactive changes induced by the tumour as well as secondary colonization of pre-existing ducts by tumour cells cannot be excluded reliably. However, in the experience of the authors, this finding is not common in specimens harbouring other-type salivary gland carcinomas, suggesting a precursor change associated specifically with mucoepidermoid neoplasms and arguing against a reactive ductal lesion, but this merits future studies. Williamson *et al.*[29] described similar foci of squamous and mucous cell metaplasia associated with cytological atypia within oncocytic epithelium of Warthin tumour harbouring MEC, and speculated that these might represent precursor lesions for MEC.

In summary, we analysed 40 salivary MEC, demonstrating a wide histological variation and showing that histological variants correlate well with the WHO tumour grade, biological behaviour and the presence of t(11;19). Future studies on larger series are needed to verify the value of histological variants in supplementing tumour grading and predicting treatment failure.

## Acknowledgement

The authors thank Professor Dr Peter Wünsch of the Nuremberg City Hospital for generously supplying paraffin blocks of his institution's MECs, Mrs Marietta Bock and Mr Rudolf Jung for excellent technical assistance. We also thank Dr Sabrina Petsch, Tumor Center Erlangen-Nuremberg, and Dr Monika Klinkhammer-Schalke, Tumor Center Regensburg for yielding the clinical follow-up data of the patients to the authors.

## References

1. Goode RK, El-Naggar AK. Mucoepidermoid carcinoma. In Barnes L, Eveson JW, Reichart P, Sidransky D eds. *World Health Organization classification of tumour. Pathology and genetics of head and neck tumours*. Lyon: IARC Press, 2005; 219–220.
2. Eveson JW, Cawson RA. Salivary gland tumours. A review of 2410 cases with particular reference to histological types, site, age and sex distribution. *J. Pathol.* 1985; **146;** 51–58.
3. de Araujo VC, de Sousa SO, Carvalho YR, de Araujo NS. Application of immunohistochemistry to the diagnosis of salivary gland tumors. *Appl. Immunohistochem. Mol. Morphol.* 2000; **8;** 195–202.
4. Rizkalla H, Toner M. Necrotizing sialometaplasia versus invasive carcinoma of the head and neck: the use of myoepithelial markers and keratin subtypes as an adjunct to diagnosis. *Histopathology* 2007; **51;** 184–189.
5. Ettl T, Schwarz S, Kleinsasser N, Hartmann A, Reichert TE, Driemel O. Overexpression of EGFR and absence of C-KIT expression correlate with poor prognosis in salivary gland carcinomas. *Histopathology* 2008; **53;** 567–577.
6. Subramaniam MM, Ng SB, Seah SB, Anuar D, Soong R, Lee VK. Molecular characterization of dedifferentiated mucoepidermoid carcinoma of the trachea using laser microdissection-based TP53 mutation analysis. *Histopathology* 2009; **55;** 472–475.
7. Auclair PL, Goode RK, Ellis GL. Mucoepidermoid carcinoma of intraoral salivary glands. Evaluation and application of grading criteria in 143 cases. *Cancer* 1992; **69;** 2021–2030.
8. Goode RK, Auclair PL, Ellis GL. Mucoepidermoid carcinoma of the major salivary glands: clinical and histopathologic analysis of 234 cases with evaluation of grading criteria. *Cancer* 1998; **82;** 1217–1224.
9. Luna MA. Salivary mucoepidermoid carcinoma: revisited. *Adv. Anat. Pathol.* 2006; **13;** 293–307.
10. Nance MA, Seethala RR, Wang Y *et al.* Treatment and survival outcomes based on histologic grading in patients with head and neck mucoepidermoid carcinoma. *Cancer* 2008; **113;** 2082–2089.
11. Aro K, Leivo I, Makitie AA. Management and outcome of patients with mucoepidermoid carcinoma of major salivary gland origin: a single institution's 30-year experience. *Laryngoscope* 2008; **118;** 258–262.
12. Brandwein MS, Ivanov K, Wallace DI *et al.* Mucoepidermoid carcinoma: a clinicopathologic study of 80 patients with special reference to histological grading. *Am. J. Surg. Pathol.* 2001; **25;** 835–845.
13. Nakayama T, Miyabe S, Okabe M *et al.* Clinicopathological significance of the CRTC3–MAML2 fusion transcript in mucoepidermoid carcinoma. *Mod. Pathol.* 2009; **22;** 1575–1581.

14. O'Neill ID. t(11;19) translocation and CRTC1–MAML2 fusion oncogene in mucoepidermoid carcinoma. *Oral Oncol.* 2009; **45;** 2–9.
15. Behboudi A, Enlund F, Winnes M *et al.* Molecular classification of mucoepidermoid carcinomas-prognostic significance of the MECT1–MAML2 fusion oncogene. *Genes Chromosom. Cancer* 2006; **45;** 470–481.
16. Martins C, Cavaco B, Tonon G, Kaye FJ, Soares J, Fonseca I. A study of MECT1–MAML2 in mucoepidermoid carcinoma and Warthin's tumor of salivary glands. *J. Mol. Diagn.* 2004; **6;** 205–210.
17. Bullerdiek J, Haubrich J, Meyer K, Bartnitzke S. Translocation t(11;19)(q21;p13.1) as the sole chromosome abnormality in a cystadenolymphoma (Warthin's tumor) of the parotid gland. *Cancer Genet. Cytogenet.* 1988; **35;** 129–132.
18. Tirado Y, Williams MD, Hanna EY, Kaye FJ, Batsakis JG, El-Naggar AK. CRTC1/MAML2 fusion transcript in high grade mucoepidermoid carcinomas of salivary and thyroid glands and Warthin's tumors: implications for histogenesis and biologic behavior. *Genes Chromosom. Cancer* 2007; **46;** 708–715.
19. Okabe M, Miyabe S, Nagatsuka H *et al.* MECT1–MAML2 fusion transcript defines a favorable subset of mucoepidermoid carcinoma. *Clin. Cancer Res.* 2006; **12;** 3902–3907.
20. Miyabe S, Okabe M, Nagatsuka H *et al.* Prognostic significance of p27Kip1, Ki-67, and CRTC1–MAML2 fusion transcript in mucoepidermoid carcinoma: a molecular and clinicopathologic study of 101 cases. *J. Oral Maxillofac. Surg.* 2009; **67;** 1432–1441.
21. Meer S, Altini M. CK7+/CK20− immunoexpression profile is typical of salivary gland neoplasia. *Histopathology* 2007; **51;** 26–32.
22. Schwarz S, Bier J, Driemel O *et al.* Losses of 3p14 and 9p21 as shown by fluorescence *in situ* hybridization are early events in tumorigenesis of oral squamous cell carcinoma and already occur in simple keratosis. *Cytometry A* 2008; **73;** 305–311.
23. Milanes-Yearsley M, Hammond ME, Pajak TF *et al.* Tissue microarray: a cost and time-effective method for correlative studies by regional and national cancer study groups. *Mod. Pathol.* 2002; **15;** 1366–1373.
24. Lennerz JK, Perry A, Mills JC, Huettner PC, Pfeifer JD. Mucoepidermoid carcinoma of the cervix: another tumor with the t(11;19)-associated CRTC1–MAML2 gene fusion. *Am. J. Surg. Pathol.* 2009; **33;** 835–843.
25. Lueck NE, Robinson RA. High levels of expression of cytokeratin 5 are strongly correlated with poor survival in higher grades of mucoepidermoid carcinoma. *J. Clin. Pathol.* 2008; **61;** 837–840.
26. Brannon RB, Willard CC. Oncocytic mucoepidermoid carcinoma of parotid gland origin. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 2003; **96;** 727–733.
27. Weinreb I, Seethala RR, Perez-Ordonez B, Chetty R, Hoschar AP, Hunt JL. Oncocytic mucoepidermoid carcinoma: clinicopathologic description in a series of 12 cases. *Am. J. Surg. Pathol.* 2009; **33;** 409–416.
28. Foschini MP, Marucci G, Eusebi V. Low-grade mucoepidermoid carcinoma of salivary glands: characteristic immunohistochemical profile and evidence of striated duct differentiation. *Virchows Arch.* 2002; **440;** 536–542.
29. Williamson JD, Simmons BH, el-Naggar A, Medeiros LJ. Mucoepidermoid carcinoma involving Warthin tumor. A report of five cases and review of the literature. *Am. J. Clin. Pathol.* 2000; **114;** 564–570.
30. Bell D, Luna MA. Warthin adenocarcinoma: analysis of 2 cases of a distinct salivary neoplasm. *Ann. Diagn. Pathol.* 2009; **13;** 201–207.