The many faces of acinic cell carcinomas of the salivary glands: a study of 40 cases relating histological and immunohistological subtypes to clinical parameters and prognosis

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Aims: To study the morphological heterogeneity of acinic cell carcinoma (ACC) in correlation with clinicopathological parameters.

Methods and results: Forty well-characterized ACCs were classified as solid (n = 20), microcystic (n = 15), papillary-cystic (n = 4) or follicular (n = 1), based on the dominant architectural growth pattern. Fourteen tumours exhibited eosinophilic/clear cell morphology and 18 tumours were rich in zymogen granules (so-called blue dot tumours). High-grade morphology occurred in five tumours. Based on cytokeratin (CK) 7 staining and in analogy to CK7 expression in normal salivary gland epithelia, three distinct histogenetic subtypes were recognized: acinar (CK7-negative; n = 13), ductular (diffuse CK7-positive; n = 11) and mixed ductulo-acinar (10–66% CK7-positive cells; n = 16). Most papillary-cystic tumours displayed ductular differentiation (P = 0.015), whereas blue dot tumours never did (P < 0.001). Analysis of relapse-free survival (RFS) revealed that Stage I tumours had the best prognosis without any relapse in 18 years follow-up (P = 0.06). High-grade tumours were associated with shorter RFS (P = 0.028). Concerning the histogenetic types, monophasic (pure acinar or ductular) tumours were associated with a significantly better RFS than mixed ductulo-acinar tumours (P = 0.008).

Conclusion: The results underscore the great histological diversity of ACC, and the value of histogenetic subtyping as an additional prognostic factor regarding RFS.

Keywords: acinic cell carcinoma, cytokeratin 7 immunohistochemistry, histogenetic subtypes, mixed ductulo-acinar subtype, salivary glands

Abbreviations: ACC, acinic cell carcinomas; CK, cytokeratin; DOG-1, discovered on gastrointestinal stromal tumours-1; H&E, haematoxylin and eosin; MASC, mammary analogue secretory carcinoma; OS, overall survival; RFS, relapse-free survival

Introduction

Acinic cell carcinoma (ACC) is an uncommon malignancy of salivary gland origin. It constitutes 2.5–7% of all parotid gland tumours and about 12% of carcinomas of the parotid gland.[1,2] The vast majority of ACCs involve the parotid gland (83–88%), and only a minor subset of cases (6–7%) originates in the minor salivary

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glands, predominantly of the oral cavity.[3–5] ACC was described initially as an adenoma, but since the 1950s recognition of the potential for recurrence and/or metastasis has justified the general classification of acinic cell neoplasms as carcinomas.[6,7] Bilateral development has been described infrequently, predominantly in children, and extremely rare familial occurrence of ACC is also recognised. Whether familial occurrence may constitute an inherited tumour syndrome is still under debate.[8–10]

Histologically, four architectural patterns have been described (in descending frequency): solid, microcystic, papillary-cystic and follicular.[11] Classifying ACC according to these subtypes remains difficult, as different patterns may occur in a single lesion.[12] In rare cases of recurring ACC, transformation of conventional low-grade ACC into a high-grade neoplasm has been reported as 'de-differentiation'.[13–15] The high-grade component may either keep the original solid or microcystic pattern of ACC (though in addition showing higher mitotic activity and/or other atypical features) or adopt a new 'non-acinar' line of differentiation.[15] Clinically, high-grade ACC is associated with advanced tumour stage and worse outcome and thus might represent a distinct disease entity.[13]

A histological grading system of ACC combining architecture and cytology with gross pathology of the tumour, i.e. local tumour growth, has been proposed by Batsakis *et al.*,[16] but is not generally accepted.[17] Cytologically ACCs are composed predominantly of serous acinar cells. These cells may contain large quantities of enzyme (zymogen) granules and exhibit a blue dot appearance in conventional haematoxylin and eosin (H&E) staining (hence the designation 'blue dot tumours'). However, intercalated ductal, vacuolated, clear and so-called non-specific glandular cells are recognized in many tumours and may predominate over serous cells.[18] In de-differentiated ACC the majority of cells are considered to be of intercalated ductal type.[14] Similar to the heterogeneity of their constituent tumour cells, the stromal component of ACC may show diverse appearance. ACC can be associated with a prominent lymphoid reaction; in particular, well-demarcated microcystic tumours may harbour a lymphoid stroma, constituting a lymphoid-rich subtype of ACC.[19] Concerning the expression of cytokeratins (CK), various immunohistochemical staining patterns have been reported in ACC, probably reflecting different stages of differentiation. CK8 and CK18 are expressed more consistently in ACC than CK7, CK17 and CK19.[20] A $CK7^+/CK20^-$ immunoexpression profile is a common feature of ACC, as it is of other salivary gland carcinoma subtypes.[21] Moreover, CK5 expression has been also reported in ACC, whereas CK14 is usually negative.[15] However, the CK7 expression in ACC is surprising, since normal serous (acinar) cells of the salivary glands do not express this marker.[20] Thus, ACC might be differentiating more towards intercalated ductal cells than true serous acinar cells. Among other antibodies, discovered on gastrointestinal stromal tumours-1 (DOG-1) might be a promising new marker of salivary acinar differentiation.[22]

Due to the infrequency and low aggressiveness of ACC, in the last 20 years only a few clinicopathological studies with long-term follow-up have been published in the English literature.[1,18,23–26] The largest study comprised 35 patients, with a median follow-up period of 5 years (range 3.4–210 months).[25] The 5-year overall survival (OS) was 100% for low-grade carcinomas and 69% for high-grade carcinomas ($P = 0.01$). Long-term OS (after 15–20 years) approached 80%.[26,27] Tumour relapse occurred in about 15% of patients within the first 5 years.[25] Apart from clinical parameters, prognostic impact could be demonstrated for the Ki-67 index[24] and an irregularity and a larger size of concomitant blood vessels.[23]

The aim of the present study was to review the ACC cases of the Erlangen salivary gland registry and to correlate clinical parameters, histological subtype and immunohistochemical data on the expression of CK7 with long-term follow-up of the patients.

Material and methods

PATIENTS AND TUMOUR SAMPLES

Forty ACCs were retrieved from a consecutive collection of well-characterized primary salivary gland carcinomas of both major and minor salivary glands (total $n = 290$) with complete follow-up, treated at the Universities of Erlangen and Regensburg, Germany, or at the Nuremberg Hospital, Germany, between 1990 and 2009. 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 ethics regulations of the medical faculties of the Universities of Regensburg and Erlangen-Nuremberg.

HISTOLOGY AND CLASSIFICATION

Using H&E- and periodic acid-Schiff (PAS)-stained slides from at least subtotally embedded tumours, all 290 tumours were reviewed independently by two pathol-

ogists experienced in salivary gland tumour pathology (S.S. and A.A.) without knowledge of the initial histological diagnosis or clinical follow-up. A few cases ($n = 6$) with an original diagnosis of ACC were found to represent either other types of salivary gland carcinomas, especially mammary analogue secretory carcinoma (MASC),[28] or were lacking unequivocal ACC areas, hence they were excluded. An agreement was achieved in 40 cases of ACC which represented the cohort of the current study. ACCs were diagnosed according to the well-established World Health Organization (WHO) criteria.[17] Corresponding to the major architectural pattern, the tumours were categorized as either solid, microcystic, papillary-cystic or follicular.[11]

IMMUNOHISTOCHEMISTRY

Immunohistochemical staining for low molecular weight cytokeratins (CK7; DCS Innovative Diagnostik-Systeme Dr Christian Sartori Ltd, Hamburg, Germany) was used to identify cells with terminal duct differentiation.[21] Additional markers applied were antibodies recognising Ki-67 (MIB-1; Dako Deutschland Ltd, Hamburg, Germany), amylase (Zytomed Systems Ltd, Berlin, Germany) and synaptophysin (Zytomed Systems Ltd). Staining for vimentin (Dako Deutschland Ltd), S100 (Zytomed Systems Ltd), mammaglobin (Biologo Dr Hartmut Schultheiß e.K., Kronshagen, Germany) and mucin (MUC) 4 (Santa Cruz Biotechnology Inc., Heidelberg, Germany) was performed to exclude MASC, which strongly expresses these markers.[28–30] Immunostaining was performed on 3-μm sections, according to the manufacturers' instructions. Amylase expression was considered significant/positive if at least 10% of cells stained positive. Expression of synaptophysin was considered significant/positive if at least 5% of cells stained positive. High proliferative activity was defined by a Ki-67 index >5%. Cases with high proliferative activity, nuclear pleomorphism and higher nuclear to cytoplasmic ratio were regarded as high grade. As staining for CK7 reliably detects cells with terminal duct differentiation and is absent in acinar cells of the normal salivary glands, we used the CK7 staining pattern to define three distinct immunohistochemical subtypes of ACC that probably reflect the histogenetic derivation and/or the line of differentiation in a given tumour as follows: the first group comprised cases without detectable or with no more than minimal/focal staining (<10% positive-stained cells) for CK7. As, in the vast majority, these cases exhibited a serous morphology with intracytoplasmic accumulation of zymogen granules, they were designated the 'acinar' subgroup. This group represents the prototype (classical type) of ACC that is generally well recognizable on H&E- and PAS-stained sections. The second group showed a predominant population of CK7-positive cells (>66% of tumour cells), indicating terminal/intercalated duct differentiation, and was thus designated the 'ductular' subgroup. The remainder showed variable CK7 expression (10–66% of tumour cells), corresponding to a significant admixture of acinar and terminal/intercalated duct differentiation. This group was the designated 'biphasic' or 'mixed ductulo-acinar' subgroup. For the purpose of this study, the acinar, ductular and mixed ductulo-acinar (biphasic) subtypes were referred to as types 1, 2 and 3 tumours, respectively.

STATISTICAL ANALYSES

All clinicopathological data were analysed with SPSS for Windows, version 18.0 (SPSS, Munich, Germany). Relationships between dichotomized histological or immunohistochemical markers and clinicopathological factors were examined using Fisher's exact probability test (significant association with $P < 0.05$). Relapse-free survival (RFS), considered the primary outcome measure, was calculated as the time from the date of the initial diagnosis to relapse diagnosis or the date the patient was last known to be alive and disease-free. OS 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 disease-free or alive, respectively. Survival curves were generated using the Kaplan–Meier method, and log-rank tests employed to compare the distributions between groups.

Results

CLINICAL FEATURES OF ACC

The clinical parameters are summarized in Table 1 and related to the three histological subtypes. The patients were 19 males and 21 females, with a mean age of 52.3 years (range 17–82 years). Thirty-six tumours were localized to the parotid gland (90%), two to the submandibular gland (5%) and two to the oral cavity (5%). According to the present Union for International Cancer Control (UICC) classification, 21 cases were Stage I, eight Stage II, eight Stage III, and three Stage IV, at initial presentation. Mean follow-up was 44.5 months (range 1.6–224 months). Relapse occurred in eight cases (20%), and four patients (10%) died of disease within the follow-up period.

Table 1. Clinical parameters in relation to the three predominant histological patterns of acinic cell carcinomas

Pattern	Solid*		Microcystic†		Papillary-cystic/follicular‡	
Age						
≤50 years (n = 19)	8	NS	9	NS	2	NS
>50 years (n = 21)	12		6		3	
Sex						
Males (n = 19)	12	NS	4	P = 0.034	3	NS
Females (n = 21)	8		11		2	
Site						
Parotid gland (n = 36)	18	NS	13	NS	5	NS
Submandibular gland (n = 2)	2		0		0	
Oral cavity (n = 2)	0		2		0	
Stage						
I (n = 21)	11	NS	6	NS	4	NS
II (n = 8)	4		4		0	
III (n = 8)	3		4		1	
IV (n = 3)	2		1		0	
Relapse						
Yes (n = 8)	3	NS	4	NS	1	NS
No (n = 32)	17		11		4	
Number of cases (n = 40)	20		15		5	

*Fisher's exact test: solid pattern versus others.
†Fisher's exact test: microcystic pattern versus others.
‡Fisher's exact test: papillary-cystic/follicular pattern versus others.

HISTOLOGICAL FEATURES OF ACC

Architecturally, the solid type was the most common (n = 20, Figure 1A), followed by the microcystic pattern (n = 15), whereas the papillary-cystic (n = 4) and follicular (n = 1) patterns were uncommon. However, a minor follicular pattern was present in one of four cases with papillary-cystic growth. The solid pattern (Figure 1A) was characterized by sheets of cells separated by thin fibrovascular strands, and thus often appeared trabecular. A minority of cells showed intracytoplasmic vacuoles. In contrast, the occurrence of small cystic lumina in large aggregates of cells was the hallmark of the microcystic pattern (Figure 1B). The arrangement of the cells was looser than in the cribriform pattern of adenoid cystic carcinoma. The papillary-cystic pattern occurred mainly in cystic tumours. Here, the tumour cells formed papillae with fibrous cores or pseudo-papillae (Figure 1C). In some of the pseudo-papillae microcysts were present, marking a transition to the microcystic pattern. The follicular pattern was reminiscent of thyroid parenchyma with follicle-like cystic glands containing colloid-like secretion and lined by cuboidal or flattened epithelial cells (Figure 1D).

Due to the histological overlap between them, the papillary-cystic tumours and the follicular tumours were grouped together for further analyses in this study. Clinically relevant and significant associations between clinical parameters and histological architectural types were not found (Table 1). Additional features of the tumours that were also recognized included stromal changes (presence of a 'warthinoid' lymphoid stroma commonly forming germinal centres,

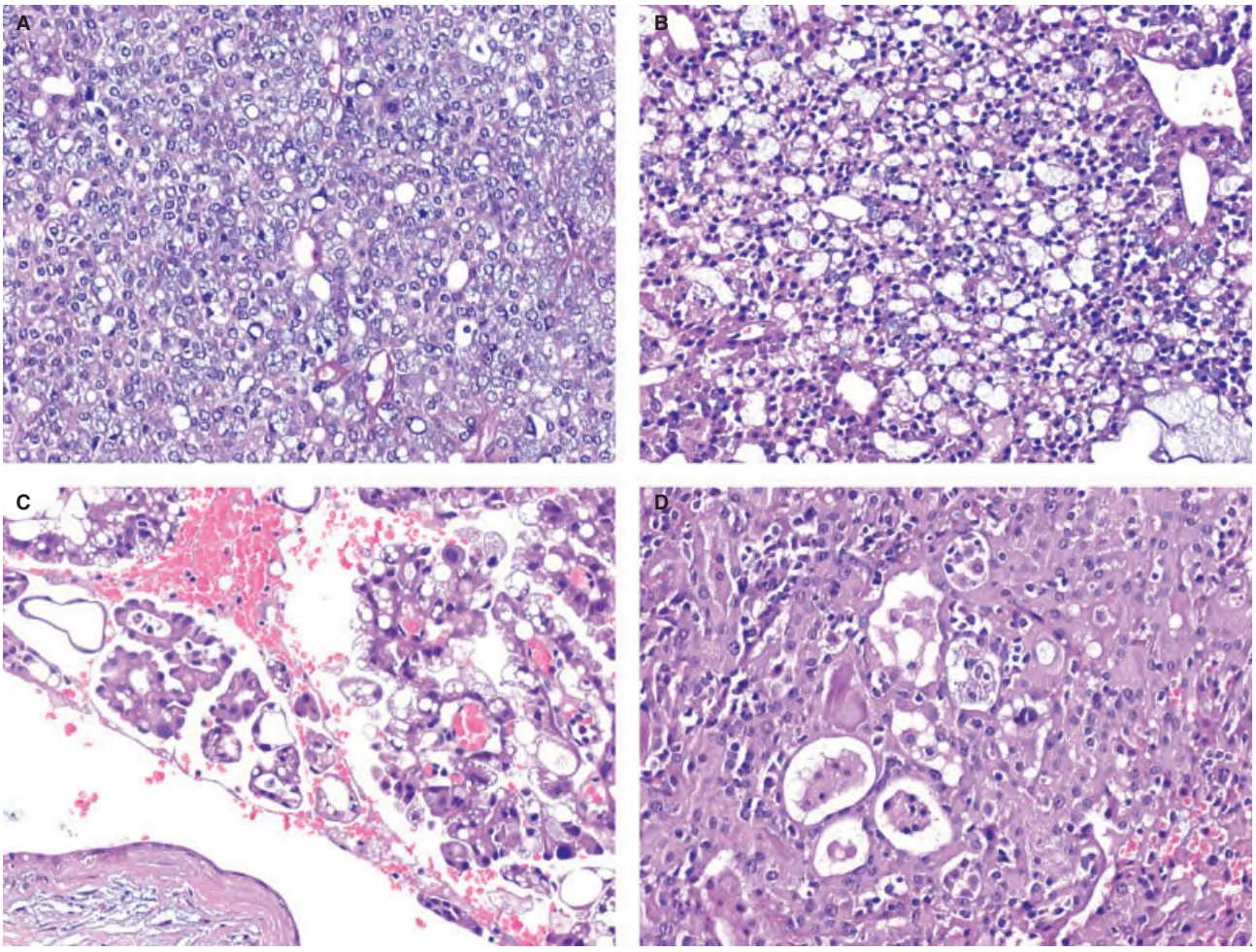

Figure 1. The four histological subtypes of acinic cell carcinoma. **A**, Solid type; **B**, microcystic type; **C**, papillary-cystic type; **D**, follicular type. Haematoxylin and eosin.

scarring and sclerosis) as well as the cytological appearance of the tumour cells (so-called blue dot tumours with high amounts of intracytoplasmic zymogen granules, and tumours with eosinophilic or clear cytoplasm). In contrast to mucoepidermoid carcinoma, where eosinophilic and clear cell variants can be differentiated,[31] transitions from eosinophilic areas to clear cell areas were often present in ACC, so these cytoplasmic variations were considered a single variant. Five high-grade tumours were characterized by higher nuclear pleomorphism, a higher nuclear to cytoplasmic ratio, and higher proliferative activity (Ki-67/MIB-1 > 5%). Three of these tumours showed considerable mitotic activity exceeding five mitoses per 10 high-power fields (1 HPF = 0.238 mm^2). Higher proliferative activity was not observed in low-grade tumours.

None of the 40 tumours in the present series exhibited a pure secretory microcystic pattern or other expanded patterns of the recently reported MASC of salivary glands, and PAS-positive zymogen granules were at least focally evident in all cases.[28,32]

Concerning the cytomorphological subtypes, the blue dot variant was seen in 18 cases (Figure 2A), whereas 14 cases were of the eosinophilic/clear cell variant (Figure 2B). Eight cases could not be attributed to a specific variant (Table 2). All solid tumours were either of eosinophilic/clear cell or of blue dot variant ($P < 0.01$).

Fifteen cases (37.5%) demonstrated a prominent accompanying lymphoid reaction (lymphoid stroma), characterized by formation of follicles with occasional germinal centres (Figure 2C). In four of these cases the cytoplasm of the tumour cells was eosinophilic, giving the tumour a 'warthinoid' appearance. In four cases sclerosis was a prominent feature (Figure 2D). One tumour was characterized by signet ring cell morphology.

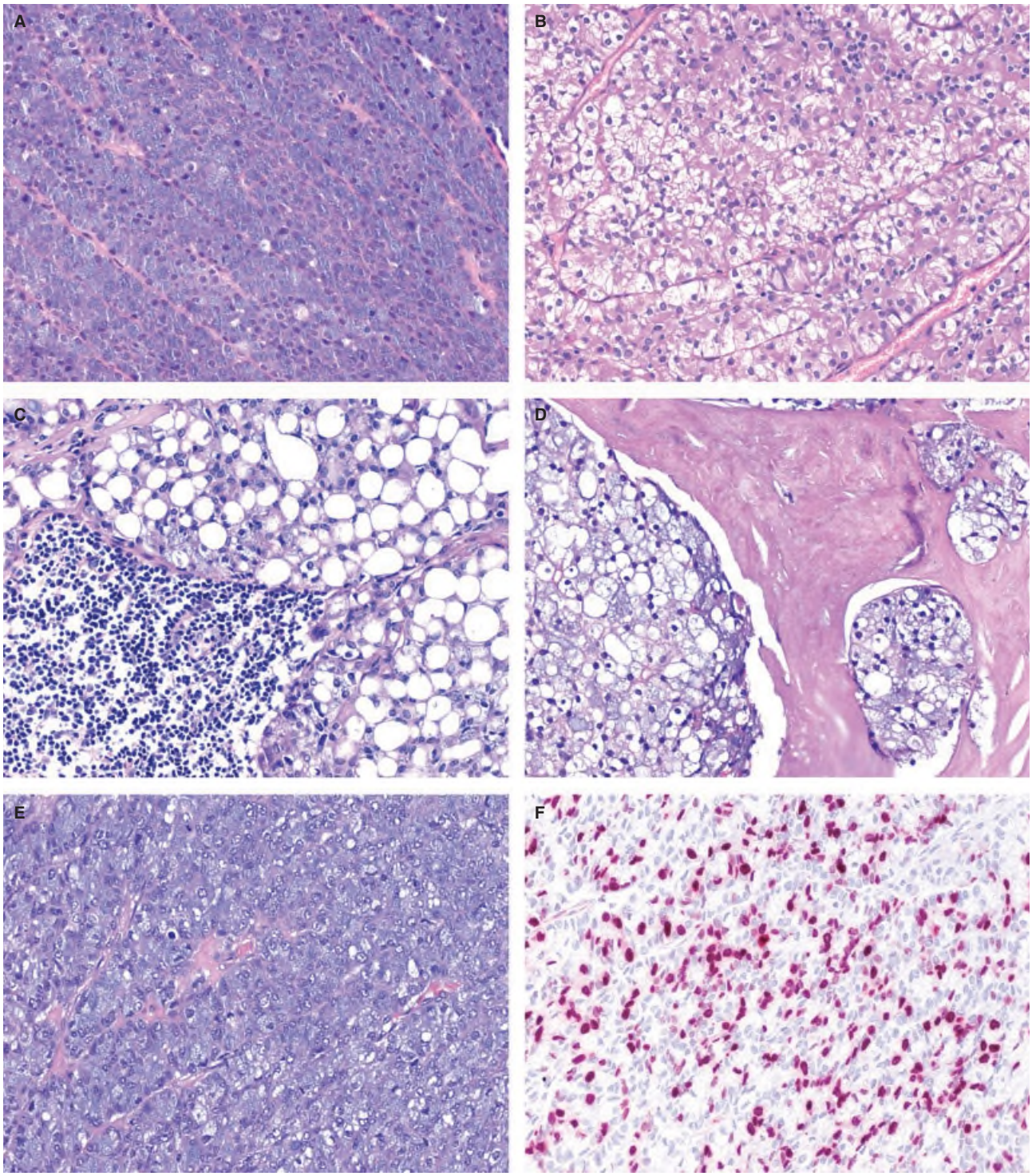

Figure 2. Morphological variants and special features of acinic cell carcinoma. **A**, Blue dot variant; **B**, eosinophilic/clear cell variant; **C**, tumour with accompanying lymphoid stroma; **D**, tumour with sclerosing areas; **E**, de-differentiated acinic cell carcinoma of solid type, with elevated proliferative activity demonstrated by Ki-67 staining (**F**).

Table 2. Histological parameters in relation to the three predominant histological patterns of acinic cell carcinomas

Subtype	Solid*		Microcystic†		Papillary-cystic/follicular‡	
Variant						
No variant (n = 8)	0	$P < 0.01$	6	NS	2	NS
Eosinophilic/clear cell (n = 14)	9	NS	3	NS	2	NS
Blue dot type (n = 18)	11	NS	6	NS	1	NS
Grade						
Low (n = 35)	16	NS	14	NS	5	NS
High (n = 5)	4		1		0	
Lymphoid reaction						
Yes (n = 15)	8	NS	5	NS	2	NS
No (n = 25)	12		10		3	
Sclerosis						
Yes (n = 3)	2	NS	1	NS	0	NS
No (n = 37)	18		14		5	
Amylase						
Significant (n = 21)	11	NS	9	NS	1	NS
Negative/weak (n = 19)	9		6		4	
Synaptophysin						
Significant (n = 13)	7	NS	4	NS	2	NS
Negative/weak (n = 27)	13		11		3	
Number of cases (n = 40)	20		15		5	

*Fisher's exact test: solid pattern versus others.
†Fisher's exact test: microcystic pattern versus others.
‡Fisher's exact test: papillary-cystic/follicular pattern versus others.

Of the five cases showing high grade morphology (de-differentiation) and a high proliferation fraction demonstrable by Ki-67 staining (Figure 2E,F), four were of solid subtype and one of microcystic subtype.

IMMUNOHISTOCHEMISTRY FOR AMYLASE AND SYNAPTOPHYSIN IN ACC

In 21 tumours significant expression of amylase (≥10% of cells) was noted, whereas 17 tumours stained very focally and two cases were completely negative (Table 2). However, one of the negative cases exhibited a characteristic microcystic architecture without secretory features but with focal PAS-positive zymogen granules, and thus could be diagnosed as ACC; the other amylase-negative tumour had a solid architecture and a blue dot appearance. Expression of amylase was not necessarily associated with the blue dot type ($P = 0.25$). High amounts of amylase were found in cases of solid, microcystic and follicular subtypes. All papillary-cystic tumours stained only focally for amylase ($P = 0.042$; papillary-cystic subtype vs. solid, microcystic and follicular subtypes).

Synaptophysin was expressed (≥5% of cells) in 13 tumours (33%). An association with a specific morphological subtype could not be demonstrated (Table 2).

CK7 AS A MARKER OF DUCTULAR DIFFERENTIATION IN ACC AND RECOGNITION OF THREE HISTOGENETIC SUBTYPES

In normal salivary glands CK7 is negative in the acini, but positive in terminal, intercalated and striated

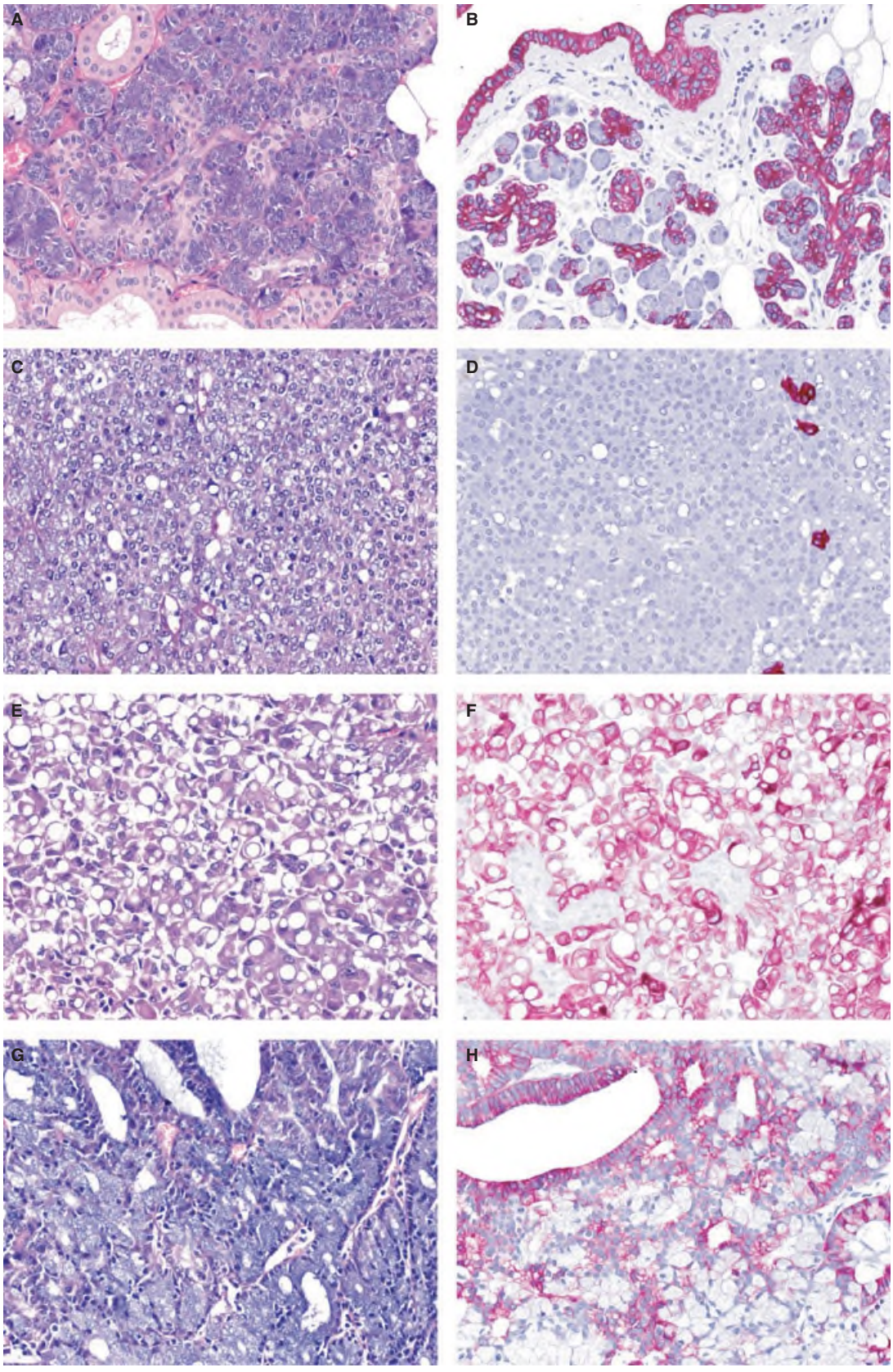
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Figure 3. Three histogenetic types of acinic cell carcinoma (ACC) demonstrated by cytokeratin (CK)7 staining. Left column: haematoxylin and eosin staining. Right column: CK7 staining. **A**, **B**, Normal salivary gland tissue with expression of CK7 in intercalating (terminal) ducts, striated ducts and excretory ducts. Acinar epithelia are CK7-negative. **C**, **D**, Type 1 tumours were commonly solid tumours with negative CK7 expression indicating acinar differentiation. **E**, **F**, Microcystic type 2 ACC with strong and diffuse expression of CK7 corresponding to terminal duct (ductular) differentiation. **G**, **H**, ACC of papillary-cystic type with CK7-positive cells forming lumina and CK7-negative cells in solid tumour areas, corresponding to biphasic (ductulo-acinar) differentiation.

ducts (Figure 3A,B). Thus, it is generally accepted that CK7-positive cells in ACC are of terminal/intercalated duct, i.e. ductular origin.[33] Thirteen of our cases lacked a significant ductular differentiation, as CK7 staining was only focally positive in <10% of cells, or completely negative (Figure 3C,D). Only these tumours featured a pure acinar cell differentiation. This subtype was named the 'acinar' subtype (or type 1 tumour). In contrast, 11 cases exhibited a strong and diffuse positive reaction for CK7 in >66% cells, forming a distinct tumour group of terminal/intercalated duct origin (Figure 3E,F). This histogenetic type was designated the ductular subtype (type 2 tumours). The third group of tumours ($n = 16$) showed an admixture of both patterns (mixed ductulo-acinar subtype or type 3 tumours); in these cases CK7 staining was seen in 20–60% of tumour cells (Figure 3G,H), giving a biphasic or mixed ductulo-acinar appearance. As shown in Table 3, the three histogenetic subtypes occurred in all ages and sexes of patients as well as at low and high UICC stages. High-grade morphology was restricted to the acinar and ductulo-acinar subtypes and was not recognized in ductular tumours ($P = 0.18$).

Associations between these histogenetic subtypes and the morphological subtypes, as well as with other histological parameters, are summarized in Table 4. The papillary-cystic and follicular tumours were associated significantly with a ductular differentiation ($P = 0.015$). A blue dot appearance was associated with ductulo-acinar type ($P = 0.016$), and did not occur in monophasic tumours with predominant ductular differentiation ($P < 0.001$). Lymphoid stroma, significant tumour sclerosis and amylase positivity were common findings in all histogenetic subtypes. Whereas approximately half of tumours with ductular differentiation expressed synaptophysin, most tumours with acinar or ductulo-acinar differentiation did not ($P = 0.075$).

PROGNOSTIC SIGNIFICANCE OF CLINICOPATHOLOGICAL PARAMETERS

To examine the prognostic significance of important clinicopathological parameters in ACC, Kaplan–Meier analyses were applied (Figure 4). Figure 4A compares OS and RFS of 40 patients with ACC. In eight patients the tumour relapsed; four patients died of their tumours. OS was 90% after 5 and 10 years, and 75% after 20 years. The longest period until relapse was 20 years. OS and RFS did not differ significantly. Due to the infrequency of tumour-related deaths in the follow-up period (10%), clinicopathological analyses regarding prognosis were restricted to RFS as the primary outcome parameter. Whereas age, sex and site did not affect RFS (data not shown), tumour stage appeared to be of prognostic impact, although statistical significance was not achieved ($P = 0.06$): UICC Stage I tumours did not recur within the first 15 years of follow-up and therefore could be considered totally cured by excision (Figure 4B). The five high-grade tumours behaved more aggressively than the majority of low-grade tumours, and were associated with shorter RFS ($P = 0.028$, Figure 4C). While architectural histomorphological subtyping had no impact on survival (data not shown), analysis according to histogenetic subtyping into acinar (type 1), ductular (type 2) and ductulo-acinar/biphasic (type 3) differentiation showed that half the relapses ($n = 4$) occurred in the patient group with type 3 tumours, three in the type 1 subgroup and one in the type 2 subgroup (Table 3). The biphasic tumours behaved more aggressively than the other (monophasic) subtypes, demonstrable by earlier relapse (Figure 4D). The first relapse had already occurred within 1 year in the biphasic group, whereas tumours with monophasic differentiation relapsed not earlier than 10 years after diagnosis. This difference in clinical behaviour was statistically significant ($P = 0.008$).

Discussion

As ACC of the salivary glands is rare,[2] few clinicopathological studies have been published in recent years.[1,18,25,26] The largest series analysed the outcome (RFS and OS) for 35 patients with respect to histopathological parameters: extracapsular extension in periparotid tissue, size >4 cm, high-grade morphology (high mitotic index, tumour necrosis and pleomorphism) were significant negative prognostic factors; and the 5-year RFS and OS were 94% and 100% for low-grade and 54% and 69% for high-grade carcinomas, respectively.[25] High-grade morphology has been described in recurrent ACC as constituting

Table 3. Clinical parameters in relation to the three histogenetic subtypes of acinic cell carcinomas

Subtype	Acinar*		Ductulo-acinar†		Ductular‡	
Age						
≤50 years (*n* = 19)	5	NS	8	NS	6	NS
>50 years (*n* = 21)	8		8		5	
Sex						
Males (*n* = 19)	4	NS	9	NS	6	NS
Females (*n* = 21)	9		7		5	
Site						
Parotid gland (*n* = 36)	12	NS	15	NS	9	NS
Submandibular gland (*n* = 2)	1		0		1	
Oral cavity (*n* = 2)	0		1		1	
Stage						
I (*n* = 21)	4	NS	10	NS	7	NS
II (*n* = 8)	4		3		1	
III (*n* = 8)	4		2		2	
IV (*n* = 3)	1		1		1	
Grade						
Low (*n* = 35)	10	NS	14	NS	11	NS
High (*n* = 5)	3		2		0	
Relapse						
Yes (*n* = 8)	3	NS	4	NS	1	NS
No (*n* = 32)	10		12		10	
Number of cases (*n* = 40)	13		16		11	

*Fisher's exact test: acinar subtype versus others.
†Fisher's exact test: ductulo-acinar/biphasic subtype versus others.
‡Fisher's exact test: ductular subtype versus others.

de-differentiation of low-grade tumours,[13–15] but might also occur *de novo*.[25] To date, few immunohistochemical markers have been analysed with respect to patient outcome,[34–36] and only the proliferation marker Ki-67/MIB-1 correlated with prognosis,[24] as high proliferative activity is associated mainly with high-grade morphology. Spiro *et al.*[37] and Batsakis *et al.*[16] introduced three-tiered grading systems, based on the growth pattern, circumscription, vascular invasion, nuclear pleomorphism and mitotic index, but the prognostic relevance of these criteria has not yet been demonstrated in larger series.

Four histopathological (architectural) subtypes of ACC have been described in previous studies: solid, microcystic, papillary-cystic and follicular.[11] Whereas Spiro *et al.*[37] found the papillary-cystic pattern to be associated with worse survival, the prognostic relevance of histopathological suptyping was not confirmed by others[38,39] or by our present study of 40 patients. Five patients in our cohort had tumours with high-grade morphology, characterized by high proliferative activity (Ki-67 > 5%), and these cases were associated with significantly worse outcome ($P = 0.028$). Comparable to the recent study by Gomez *et al.*,[25] in our series the 10-year OS was 90% and RFS was 84%.

As ACC is essentially a low-grade malignancy, long follow-up periods are needed to draw reasonable prognostic conclusions. Conversely, ACC occurs predominantly in the elderly, so that OS might not be the

Table 4. Histological parameters in relation to the three immunohistological subtypes of acinic cell carcinomas

Subtype	Acinar*		Ductulo-acinar†		Ductular‡	
Pattern						
Solid (n = 20)	6	NS	10	NS	4	NS
Microcystic (n = 15)	7	NS	5	NS	3	NS
Papillary-cystic/follicular (n = 5)	0	NS	1	NS	4	P = 0.015
Variant						
No variant (n = 8)	2	NS	2	NS	4	NS
Eosinophilic/clear cell type (n = 14)	4	NS	3	NS	7	NS
Blue dot type (n = 18)	7	NS	11	P = 0.016	0	P < 0.001
Lymphoid reaction						
Yes (n = 15)	3	NS	8	NS	4	NS
No (n = 25)	10		8		7	
Sclerosis						
Yes (n = 3)	0	NS	1	NS	2	NS
No (n = 37)	13		15		9	
Amylase						
Positive (n = 21)	7	NS	10	NS	4	NS
Negative/weak (n = 19)	6		6		7	
Synaptophysin						
Positive (n = 13)	2	NS	5	NS	6	NS
Negative/weak (n = 27)	11		11		5	
Number of cases (n = 40)	13		16		11	

*Fisher's exact test: acinar subtype versus others.
†Fisher's exact test: ductulo-acinar/biphasic subtype versus others.
‡Fisher's exact test: ductular subtype versus others.

most reliable endpoint parameter for survival analyses. Moreover, many patients are lost to further follow-up. These cases are censored in Kaplan–Meier analyses. In our study the 20-year OS was 75%, but all remaining patients had relapses. Because relapse occurred much more frequently than death, we restricted Kaplan–Meier analyses to RFS in further analyses.

In the present study we found that CK7 immunohistochemistry divides ACC into three histogenetic subtypes. It is known from previous studies that CK7 expression indicates tubuloductal differentiation (intercalated duct-like),[20] as normal acini of the salivary glands are CK7-negative. Tubuloductal differentiation was designated grade 2 by Batsakis *et al.*,[16] whereas a solid, acinar or microcystic architecture was referred to as grade 1. We divided ACC into three major histogenetic subtypes, referred to in this study as type 1 (or acinar differentiation), type 2 (or ductular differentiation) and type 3 (or mixed ductulo-acinar differentiation). We found ductular differentiation to be associated significantly with the papillary-cystic/follicular pattern (P = 0.015). Eleven tumours showed homogeneous CK7 expression (ductular type); 13 tumours were completely negative, indicating 'true' acinar differentiation. Approximately half the CK7-negative cases were so-called blue dot tumours, with prominent intracytoplasmic secretory granules. As reported by Meer *et al.*,[21] CK7 staining is frequently variable in ACC: we found a heterogeneous staining pattern in 16 tumours, with CK7-negative acinar cells intermingled with CK7-positive ductular structures, sometimes in a sheet-like arrangement. These tumours

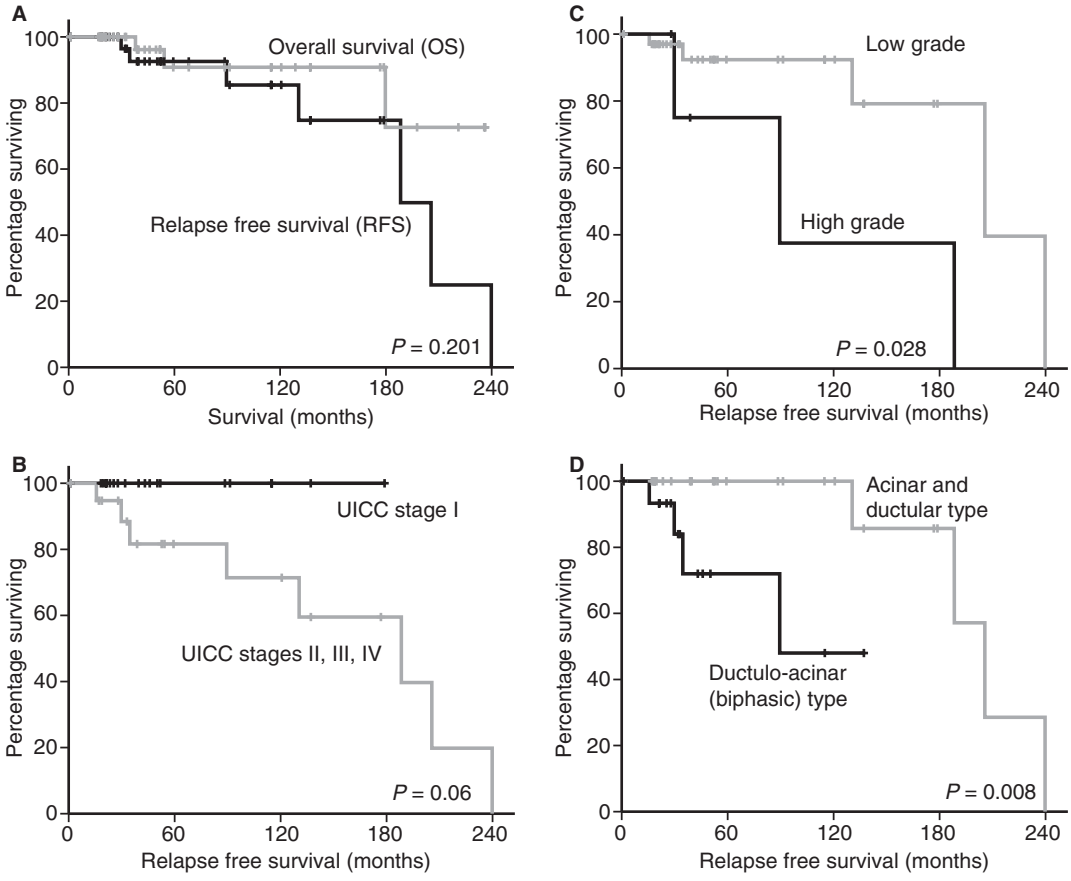

Figure 4. Univariate Kaplan–Meier analyses of clinicopathological and histogenetic parameters. **A**, Comparison of overall survival (OS) and relapse- free survival (RFS) in 40 patients with acinic cell carcinoma. **B**, RFS in relation to Union for International Cancer Control (UICC) stages (Stage I versus Stages II, III and IV). **C**, Impact of tumour grade on RFS (low-grade versus high-grade). **D**, RFS in relation to monophasic types (acinar and ductular type) and the biphasic type (ductulo-acinar type). *P*-values according to log-rank statistics.

were designated biphasic, i.e. of mixed ductulo-acinar differentiation (type 3), and we found that they behaved more aggressively than the monophasic tumours ($P = 0.008$). About 50% of patients with biphasic tumours developed relapse within 10 years, whereas no patients with monophasic tumours did.

Regarding differential diagnosis, ACC should be distinguished from the recently described MASC of salivary glands that display significant histological overlap with ACC.[28] Although MASC shows diffuse and strong CK7 staining and its architectural and cellular patterns overlaps greatly with that of ACC, serous acinar differentiation is not a feature in any of the reported cases of MASC. The authors[28] state clearly that large serous acinar cells with cytoplasmic PAS-positive zymogen-like granules typical of ACC are completely absent in their cases. Furthermore, the cytological and structural diversity characteristic of ACC seems not to be a feature of MASC. Immunohistochemical coexpression of vimentin and S100 combined with variable reactivity for mammaglobin and MUC4 in MASC may permit differentiation from ACC.[30] In equivocal cases, demonstration of the *ETV6–NTRK3* translocation by fluorescence *in-situ* hybridization (FISH) analysis is diagnostic of MASC and excludes ACC. The intercalated duct-like differentiation in these two entities (MASC and ACC) is probably responsible for their overlapping morphological features. However, it is still unclear whether patients with MASC should be treated differently from patients with ACC.

In summary, we analysed 40 salivary ACC, demonstrating wide histological variation and showing that CK7 pattern separates these tumours into three histogenetic subtypes with a significant impact on RFS. Specifically, we found that biphasic (mixed ductulo-acinar or type 3) tumours behaved more aggressively than monophasic (acinar and ductular or types 1 and 2) tumours. Follow-up of patients with type 1 and type 2 tumours should be maintained over decades, as late relapses are not unusual. In contrast, clinicopatho-

logical studies would be necessary to determine whether patients with type 3 tumours benefit from more aggressive therapy, especially from irradiation, which not is usually administered in ACC.

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