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Prognostic Factors in Mucoepidermoid Carcinomas of Major Salivary Glands: a Clinicopathologic and Flow Cytometric Study

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Mucoepidermoid carcinomas (MEC) of the major salivary glands from 48 patients who received their treatment at a single institution were studied for prognostic indicators. Uni- and multivariate statistical analyses were performed on several clinicopathologic factors and also on flow cytometric (FCM) DNA content data of the carcinomas. Clinical prognostic factors associated with decreased survival included age >60 years ($P=0.01$), male gender ($P=0.002$), symptoms at diagnosis ($P=0.03$), stage of disease ($P\leqslant 0.0001$), type of surgery ($P=0.0006$), and recurrence ($P=0.0001$). Histopathological prognostic factors associated with decreased survival included MEC tumour grade ($P=0.0001$), tumour size >3.0 cm ($P=0.02$), lymph node involvement ($P=0.0004$) and positive surgical margins ($P=0.007$). DNA FCM factors associated with decreased survival included aneuploid tumours ($P=0.08$) and proliferative activity ($S+G_2M>5\%$, $P=0.07$). Multivariate analysis indicated that histological grade, proliferative activity, symptoms at diagnosis, clinical stage of disease and type of surgery were significant ($P\leqslant 0.05$) prognostic/survival factors in the biological assessment of this neoplasm.

Keywords: mucoepidermoid carcinoma, major salivary glands, prognostic factors, DNA flow cytometry

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INTRODUCTION

FLOW CYTOMETRICALLY-DERIVED DNA content (ploidy and proliferative S-phase fractions) as a prognostic factor in salivary gland neoplasms has not had wide application. This is especially true for mucoepidermoid carcinomas in which the studies have been preponderantly cytoflurometric and univariate analyses of a relatively small number of carcinomas [1–3].

We report here our findings of a uni- and multivariate analysis of the DNA content of mucoepidermoid carcinomas, assessed by flow cytometry, compared with conventional clinicopathologic prognostic factors. The carcinomas were of the major salivary glands (parotid, submandibular) only and were treated at a single institution (University of Texas M. D. Anderson Cancer Center, U.S.A.).

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MATERIALS AND METHODS

Mucoepidermoid carcinoma of major salivary glands from patients, who received their primary treatment at the University of Texas M. D. Anderson Cancer Center from 1956 to 1986, formed the material for this study. For inclusion in the study, the following criteria had to be met: (1) fulfilment of the histocytological criteria for the diagnosis of mucoepidermoid carcinoma; (2) availability of histological sections and pretreatment tissue blocks for microscopic examination and flow cytometric studies; (3) availability of pathology reports for data regarding gross appearance; (4) availability of medical charts, with adequate documentation of previous medical history, initial presentation, physical examination and documentation of extent of disease at presentation (i.e. local and distant metastatic to allow for clinical staging); and (5) documentation of follow-up for at least 5 years after initial diagnosis, unless the patient died of disease or other causes before reaching the end of the 5-year period.

Histological examination

Hematoxylin–eosin- and mucicarmine-stained sections (range two to six per case) of all tumours were reviewed. The tumours were evaluated for the following histopathological

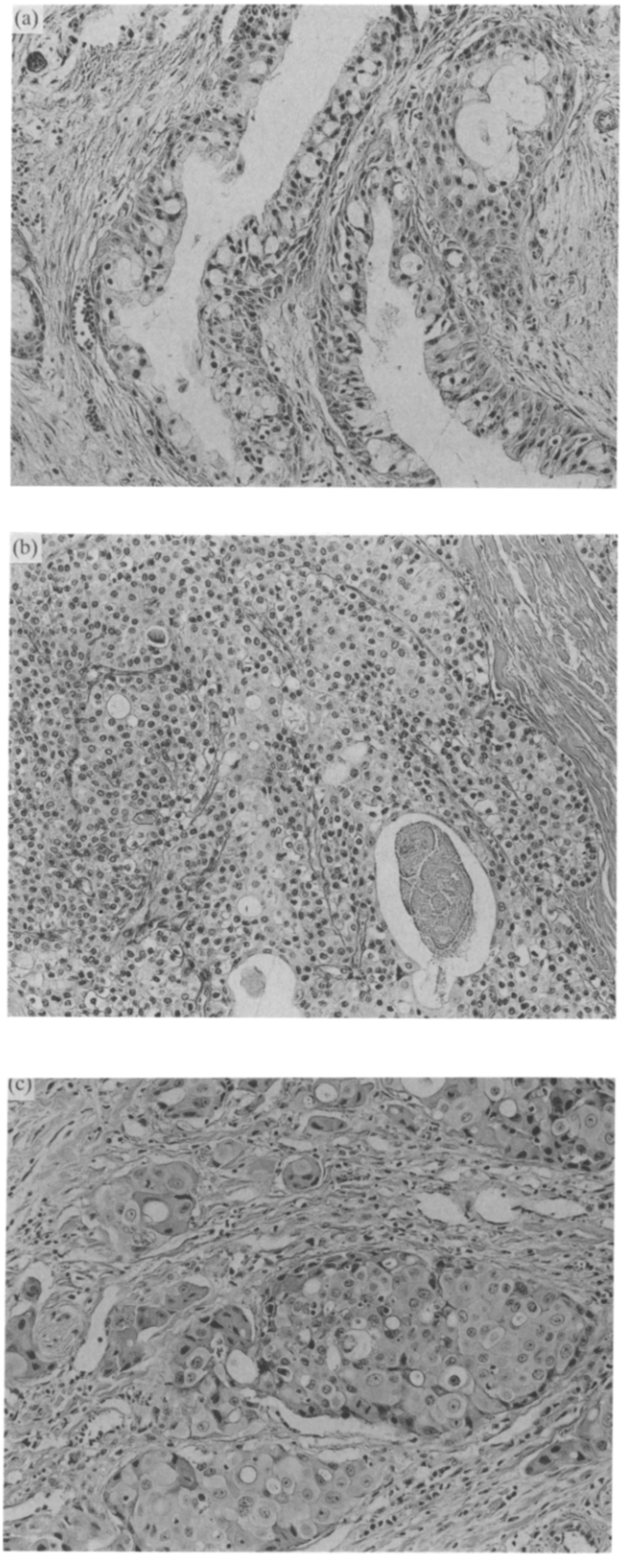

Fig. 1. Histocytological grades of mucoepidermoid carcinoma (MEC). (a) Grade 1 (low-grade). Cystic carcinoma with differentiation manifested primarily towards mucous cells (haematoxylin and eosin ×200); (b) grade 2 (intermediate grade). Microcysts only with solid growth pattern composed of intermediate cells and scattered vacuolated cells (haematoxylin and eosin ×140); (c) grade 3 (high grade). Solid growth with perineural invasion, nuclear and cellular pleomorphism and mitoses.

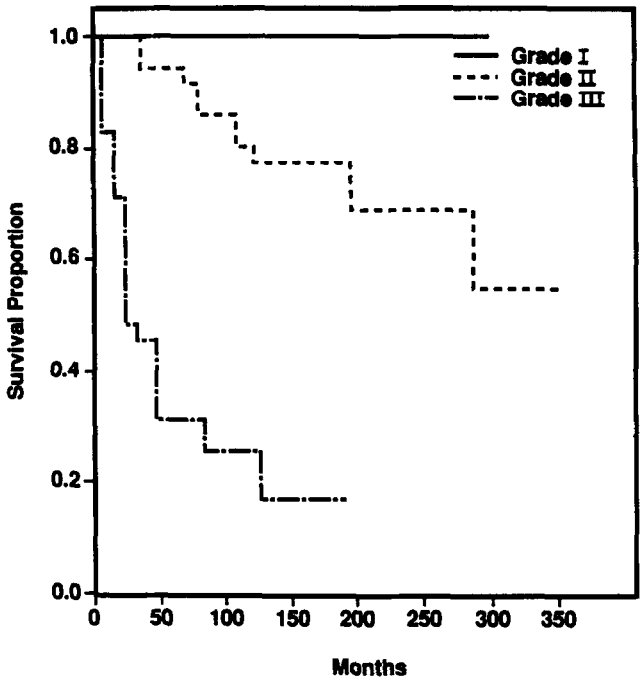

Fig. 2. Survival comparison among individuals with grade 1 (low grade), grade 2 (intermediate grade) and grade 3 (high grade) mucoepidermoid carcinoma (Kaplan–Meier method for survival curves and log-rank test).

features: histocytological grade, margin of resection involvement, facial nerve involvement, lymph node metastases and local soft tissue invasion. The histocytological grading system used in this study was a three-tiered grading system modified from Healy *et al.* [4] by Batsakis and Luna [5]. Representative photomicrographs of the three histocytologic grades are presented in Fig. 1a–c.

DNA flow cytometric analysis

Nuclear suspensions from selected paraffin-embedded blocks of neoplastic tissue were prepared using a technique based upon the methods of Hedley *et al.* [6] and McLemore *et al.* [7]. Once the samples were determined to be adequate, they were analysed on a EPICS Profile I Flow Cytometer (EPICS Division, Coulter Electronics, Miami, Florida, U.S.A.) equipped with a 5-W argon laser operated at 488 nm, with a 610-nm long-pass filter and 488-nm band pass. With each sample, at least 10 000 nuclei were measured. The DNA histograms were analysed using the "boxogram" gating method of Johnston *et al.* [8]. Neoplastic cells were considered to be aneuploid when a distinct, second G_0/G_1 peak (or any number of such separate peaks), accounting for at least 10% of the total cells analysed, was present. The proliferative activity was determined as the proportion of cells in $S+G_2M$ phases of the cell cycle, according to the technique of Baisch *et al.* [9]. High proliferative activity was considered when the $S+G_2M$ was >5% of the total tumour cell population. The coefficient of variation for all cases was less than 3%.

Statistical analyses

The continuous and categorical variables were dichotomised at points which represent patients with different clinical outcomes (survived vs. died of disease). In the univariate

Table 1. Mucoepidermoid carcinoma of major salivary glands: clinical factors and survival

Variable	Died of disease (No. of deaths/cases)	Median survival (years)	Log-rank *P* value
All patients	44% (21/48)	24.1	—
Age			
<60 years	32% (10/31)	*	0.01
⩾60 years	58% (10/17)	3.7	
Gender			
Male	65% (13/21)	8.8	0.002
Female	30% (8/27)	*	0.002
Race			
Caucasian	49% (18/37)	16.0	0.08
Black/Hispanic	27% (3/11)	*	
Symptoms			
Symptoms present	47% (17/36)	10.4	0.03
Symptoms absent	17% (2/12)	*	
Location			
Parotid gland	39% (17/43)	24.1	0.004
Submandibular gland	80% (4/5)	6.7	
Stage of disease			0.0001
Stage I	12% (1/8)	*	
Stage II	9% (1/11)	*	
Stage III	63% (10/16)	8.9	
Stage IV	69% (9/13)	2.6	
Type of surgery			0.0006
Total parotidectomy	23% (3/13)	*	
Total parotidectomy with neck dissection	67% (12/18)	3.7	
Superficial parotidectomy	25% (2/8)	*	
Superficial parotidectomy with neck dissection	0% (0/4)	*	
Submandibular resection	80% (4/5)	6.7	
Radiotherapy			
Radiotherapy	55% (11/20)	16.0	0.17
No radiotherapy	32% (9/28)	*	
Recurrence			
Overall recurrence	85% (17/20)	6.0	0.0001
No recurrence	14% (4/28)	*	
Local recurrence	82% (9/11)	6.8	0.0018
No local recurrence	27% (10/37)	*	
Metastatic recurrence	89% (17/19)	6.0	0.0001
No metatatic recurrence	7% (2/29)	*	

*Median survival has not been reached yet.

analyses, the Kaplan–Meier method [10] was used to plot survival curves for prognostic factors. The prognostic effect of a variable was tested using the log-rank test. The stepwise, multivariate proportional hazards model of Cox [11] was employed to examine the independence of prognostic factors found to be associated with survival in the univariate analyses. Finally, the association between categorised variables was analysed using the Wilcoxon, Kruskal–Wallis [12] and χ^2 tests, as appropriate. Relative risk ratios were calculated based on regression coefficients in the proportional hazards model. This ratio indicates increased (unfavourable) or decreased (favourable) risk of death at any time point for patients with a given characteristic, and compensates for all other patient characteristics.

RESULTS

Forty-eight mucoepidermoid carcinomas (43 in parotid glands and five in submandibular glands) were studied. The mean age of the subjects was 50.4 (range 10–77) years at the time of diagnosis with a peak incidence in the fifth to the seventh decades. 31 patients (65%) were <60 years of age; 17 (35%) were >60 years. There was a female:male ratio of 1:3. Racial distribution of patients was 77% ($n=37$) Caucasian, 15% ($n=7$) black and 8% ($n=4$) Hispanic. 36 (75%) patients presented with one or more signs or symptoms, including facial nerve paralysis or paresthesia, facial muscle dysfunction and facial pain.

The clinical stage of the carcinomas at first presentation was 17% stage I, 23% stage II, 33% stage III and 27% stage IV.

All patients were treated with surgical excision of their neoplasms. This consisted of superficial parotidectomy in 8, superficial parotidectomy with neck dissection in 18 and submandibular gland resection in 5 patients. 20 patients also received postoperative radiation therapy. Only 1 patient received chemotherapy after surgery. Follow-up periods ranged from 0.3 to 29.3 years (median 15).

Table 1 presents the univariate analysis of clinical factors and survival. Significant differences ($P<0.005$) between survival and patient age, gender, signs and symptoms at first clinical

Table 2. Mucoepidermoid carcinoma of major salivary glands: gross, histopathological and flow cytometric factors and survival

Variable	Died of disease (No. of deaths/cases)	Median survival (years)	Log-rank *P* value
All patients	44% (21/48)	24.1	—
Size of tumor mass			
<3.0 cm	31% (9/29)	*	0.02
⩾3.0 cm	63% (12/19)	6.7	
Grade			0.0001
MEC grade 1	0% (0/7)	*	
MEC grade 2	30% (7/23)	*	
MEC grade 3	78% (14/18)	2.2	
Lymph node involvement			
Lymph nodes involved	72% (13/18)	6.0	0.0004
No lymph nodes involved	27% (8/30)	*	
Margins of resection			0.0007
Positive margins	71% (15/21)	8.9	
Negative margins	23% (4/17)	*	
Local tissue invasion			
Local tissue invasion	69% (11/16)	8.8	0.10
No local tissue invasion	34% (10/29)	*	
Facial nerve involvement			
Facial nerve involved	33% (5/15)	*	0.46
Facial nerve not involved	45% (15/33)	24.1	
DNA ploidy			
Diploid	38% (15/39)	*	0.08
Aneuploid	67% (6/9)	6.8	
Proliferative activity			
⩽5%	20% (2/10)	*	0.07
>5%	50% (19/38)	10.4	

*Median survival has not been reached yet.

Table 3. Analysis of association between prognostic factors and survival

Variable	Multivariate (*P* value)	Relative risk ratio
Proliferative activity	0.003	1.3
Histocytological grade	0.0002	12.6
Overall recurrence	0.003	9.9
Symptoms (at presentation)	0.006	7.2
Type of surgery	0.006	3.6
Stage of disease	0.0001	2.9

presentation, location of neoplasm, clinical stage, type of surgery and recurrences were found.

Postoperative radiotherapy or chemotherapy had no apparent impact on survival of patients.

The univariate analysis of gross histopathological factors and DNA content is presented in Table 2. Significant differences ($P<0.05$) in survival were found with tumour size, histocytological grade, lymph node involvement and margins of resection.

Table 3 presents the results of multivariate analyses of clinicopathological data and relative risk ratio associated with mortality from disease. Significant independent factors in predicting survival included recurrence, symptoms at presentation, type of surgical procedure and stage of disease. The relative increased risk of dying from disease associated with clinical factors ranged from 9.9 times for recurrence of disease to 2.9 times for stage of disease.

Multivariate analyses of the gross and histopathological data (Table 3) indicated that only tumour grade represented an independent factor in predicting survival. An increased relative risk ratio (12.6) for dying of disease was associated with increased histocytological tumour grade.

DNA flow cytometric factors

Table 2 includes the results of univariate analyses of DNA flow cytometric factors. Although significant differences ($P=0.08$) were not found between aneuploid and diploid tumours, trends regarding survival were noted (Fig. 3). Two-thirds of patients with aneuploid tumours died of disease. This compares with only 38% of patients with diploid tumours. Proliferative activity ($S+G_2M$) had a mean value of 9%, ranging from 3 to 26% (median 8%). Although no significant difference ($P=0.07$) was found between tumours with low (<5%) vs. high (>5%) proliferative activity values, 50% of patients with high proliferative activity tumours died of disease (Fig. 4). This compares with 20% for patients with low proliferative activity tumours. When the proliferative activity was >10%, 87% of patients died of disease. In contrast, when the proliferative activity was <10%, only 24% of patients died of disease.

With multivariate analyses of the DNA flow cytometric data (Table 3), proliferative activity was found to be an independent factor in predicting survival. Longer survival would be expected for neoplasms with proliferative activities of <5% and the relative risk ratio for dying of disease was increased by 1.3 times when the proliferative activity was greater.

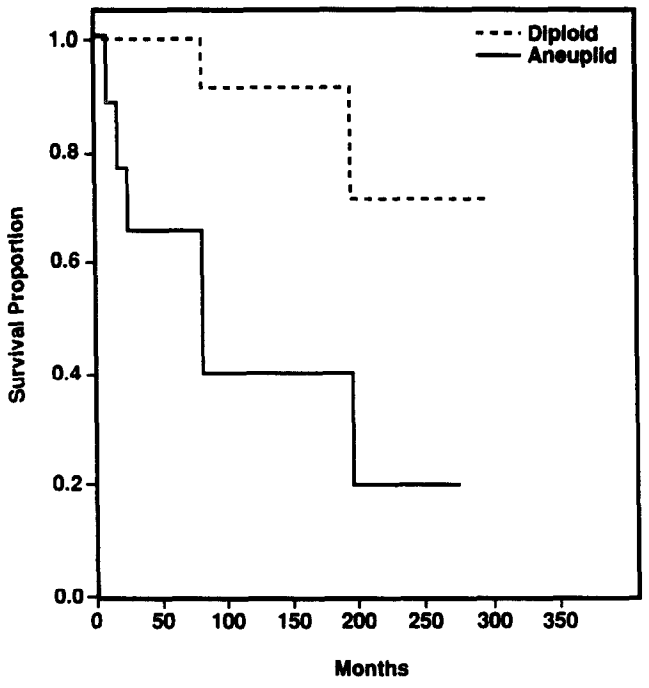

Fig. 3. Survival comparison between individuals with aneuploid mucoepidermoid carcinoma and diploid mucoepidermoid carcinoma (Kaplan–Meier method for survival curves and log-rank test).

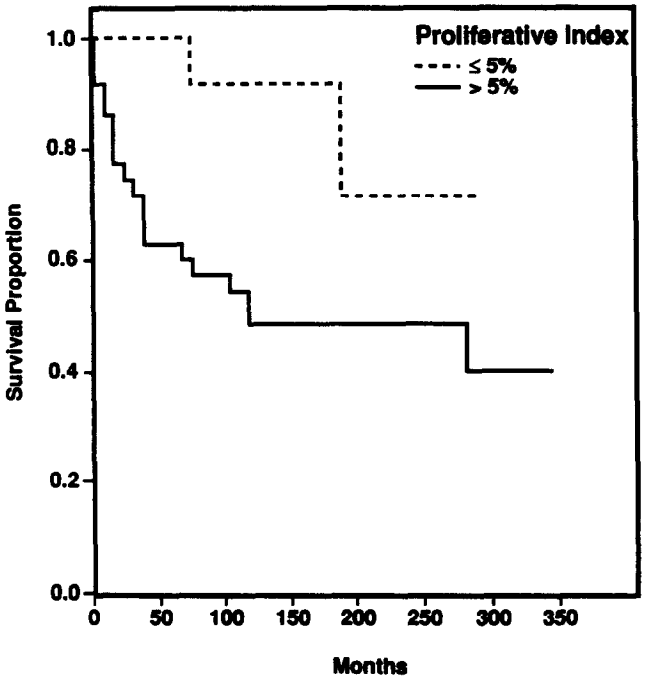

Fig. 4. Survival comparison between individuals with low (<5%) proliferative activity tumours and high (>5%) proliferative activity tumours (Kaplan–Meier method for survival curves and log-rang tests).

DISCUSSION

In the present study, a number of clinical, pathological and DNA flow cytometric indicators of mucoepidermoid carcinomas were linked to survival using univariate and multivariate statistical analyses.

Both age and gender are known to affect survival in individuals with mucoepidermoid carcinoma [13–17]. Shorter survival has been associated with male gender [17–20]. This was confirmed in our study in which the mortality rate for males was slightly more than two times that for females. Despite the level of significance in the univariate analysis, neither age nor gender was identified as a significant independent prognostic factor in the multivariate analysis.

Symptoms associated with facial nerve involvement have been reported [17–20] as important factors in survival and within our study were also found to significantly affect outcome in the univariate analysis. Multivariate analysis showed that this was a significant, independent predictor of survival.

A malignant salivary gland neoplasm, including mucoepidermoid carcinoma, within the submandibular gland has been shown to correlate with a particularly poor outcome [20, 21]. In the present study, four of five individuals with submandibular mucoepidermoid carcinoma (MEC) tumours died of their disease and had a shortened median survival (6.7 years) when compared with that for patients with parotid MEC tumours (24.1 years). The limited number of submandibular MEC cases may have resulted in failure to detect a significant difference between parotid and submandibular locations in the multivariate analyses.

Stage of disease and type of surgery proved to be significant prognostic factors in survival following both univariate and multivariate analyses. These findings are not surprising and have been reported previously by others [14, 18–20]. No doubt the extent of surgery is also intimately related to the stage of disease. The need for radiotherapy is probably related to both stage of disease and extent of surgery. Although no significant difference in survival was found with or without radiotherapy, there was a definite trend toward increased mortality in those individuals receiving radiotherapy and would tend to support the previous suppositions.

The association between recurrence and an adverse outcome has previously been established [4, 21, 22]. Within our study, over 80% of individuals with recurrences, either local or metastatic, died of disease. This finding was also found to be significant both with univariate and multivariate analyses. This prognostic factor is also linked with stage of disease and type of surgery. It has been shown that when the tumour has been completely resected the recurrence rate is only 3%; whereas when resection is incomplete, the recurrence rate is almost 70% [4].

Tumour size and survival were found to be significantly related in the univariate analysis. Tumours >3 cm in size were associated with a mortality rate that was twice that for smaller tumours. A direct correlation between tumour size and histological grade in mucoepidermoid carcinomas has been identified by a number of investigators [4, 18, 19].

Both univariate and multivariate statistical analyses showed the histocytological grade of the tumour to be an important independent prognostic factor for survival. Mortality rates increased dramatically with rising histocytological tumour grade. These rates compare favourably with other reports using different three-tiered grading systems [4, 22, 15, 17–19]. Survival rates have ranged from 95 to 100% for grade 1 tumours, from 70 to 92% for grade 2 tumours, and from 25 to 43% for grade 3 tumours.

Histocytological grade in our study was the only histopathological parameter shown to be an independent prognostic factor in survival by multivariate analyses. This factor also proved to

have the highest relative risk ratio (× 12.6) for dying of disease. This statistical finding is supported by the fact that the mortality rate was almost 80% with a median survival of only 2.2 years in grade 3 MEC tumours.

Relatively few studies have assessed the nuclear DNA content of mucoepidermoid carcinomas [1–3]. These studies have used cytophotometric image analyses rather than flow cytometric methods and have assessed only ploidy status of the tumours and not proliferative activity. Employing cytophotometric imaging techniques, diploid tumours have tended to have a "favourable" course; while tumours with an atypical (aneuploid) DNA pattern tended to have an "unfavourable" course [2, 3]. Within the present study, both DNA content and proliferative activity were determined using flow cytometry. There was a substantial difference between the mortality rate with diploid tumours (38%) when compared with aneuploid tumours (67%). The median survival was not reached during this study for diploid tumours, but was only 6.8 years for aneuploid tumours. Although this difference was not statistically significant ($P = 0.08$), the ploidy status of MECs showed a trend toward decreased survival in those patients with aneuploid tumours. Perhaps statistical significance may not have been reached due to the limited number of aneuploid tumours. Similar trends were found when survival was compared with proliferative activity ($S + G_2M$). When the proliferative activity was $>5\%$, the mortality rate was increased by 2.5 times over that for tumours with proliferative activities of $<5\%$. Proliferative activity was found to be a significant independent prognostic factor.

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