PCNA-LI, Ki-67 Immunostaining, p53 Activity and Histopathological Variables in Predicting the Clinical Outcome in Patients with Parathyroid Carcinoma

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Abstract. *Parathyroid carcinoma is uncommon and no reliable histological markers are available for predicting the clinical outcome of patients. The aim of this study was to assess the correlation between survival, histopatological markers, proliferating cell nuclear antigen (PCNA), Ki-67 antigen and the expression of the p53 nuclear protein in patients with confirmed parathyroid carcinoma (PC). The routine histological specimens from 15 patients (11 men, 4 women, median age 65 years) with confirmed PC who had died of the disease were reviewed. New specimens were also stained with the streptavidin-biotin-peroxidase complex standard technique. The labelling index (LI) of PCNA was quantified by counting 1000 cells from multiple areas in a random fashion, while immunostaining of both Ki-67 and p53 was evaluated as the percentage of positive cells. The PCNA-LI, Ki-67 (%) and p53 (%) values were 14.9±4.1 (median 13, range 2-70), 13.9±3.9 (median 11%, range 3-65%) and 38.5±4.6 (median 29%, range 19-65%), respectively. There was an inverse correlation between age of the patients and p53 (R=–0.73, p=0.002), but no correlation with both PCNA-LI (R=0.07, p=0.72) and Ki-67 (R=–0.07, p=0.79). A significant relationship (R=0.93, p<0.01) between PCNA-LI and Ki-67 was found, while p53 did not correlate with either PCNA-LI (R=–0.11, p=0.71) or Ki-67 (R=–0.05, p=0.86). An inverse correlation (R=–0.63, p=0.01) between survival and the presence of spindle cells and coagulation necrosis together in the standard slides was observed, but there was no correlation (p=NS) between survival and PCNA-LI (R=0.05), Ki-67 (R=0.05) or p53 (R=0.25). In conclusion, none of the tested immunohistochemical markers were useful in predicting the clinical outcome of patients with PC. However, the presence of spindle cells and coagulation necrosis together in the standard specimens should be considered as a negative prognostic factor.*

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Parathyroid carcinoma (PC) is uncommon, accounting for only 1-5% of cases of primary hyperparathyroidism (1-3). Unfortunately, the histological distinction between PCs and parathyroid adenomas is difficult and the biological behaviour of the tumour varies widely. Moreover, no reliable histological markers are usually available for predicting the clinical outcome of patients (4). The aim of this study was to assess the correlation between survival, histopathological findings, proliferating cell nuclear antigen (PCNA), Ki-67 antigen and the expression of the p53 nuclear protein in patients with confirmed PC who eventually died of the disease.

Materials and Methods

Formalin-fixed paraffin-embedded tissue sections from the surgical specimens of 15 patients (11 men, 4 women, median age 65 years, range 30-78 years) who had undergone surgery for PC were used and new standard slides were obtained and reviewed. The main clinical and biochemical parameters of the overall population, as well as the histopathological findings according to the Bondeson criteria (5), are reported in Table I. Six patients had multiple metastases (lung, bone, mediastinum), 9 had lung metastases and 14 developed local recurrences. A total of 17 re-operations were performed. All the patients died of the disease between 21 and 146 months from the first operation. The specimens were stained with the streptavidin-biotin-peroxidase complex standard technique. The monoclonal antibodies anti-PCNA (Clone PC 10, Dako Laboratories, Glostrup, Denmark), anti-Ki-67 (NCL-KI67-MM1, Novacastra Laboratories, UK) and anti-p53 (Clone DO 7, Dako

Table I. *Main clinical, biochemical and histopathological parameters of the overall patient population.*

Patient	Age	Ca	PTH	Size	TGP	MF	AF	SC	NA	CN	VI
1	30	3.43	NA	25	+	+		+	+		
2	41	4.05	518	45		+	+		+		+
3	43	3.09	312	25	+		+	+	+	+	
4	51	3.88	990	20	+	+	+		+		
5	56	4.12	313	40	+	+	+		+		
6	59	3.33	124	20	+	+	+	+			
7	65	2.65	NA	15	+		+	+	+		
8	66	3.02	107	15	+	+	+		+		
9	68	3.32	95	35		+		+	+		+
10	68	3.21	NA	25	+	+		+		+	
11	70	3.09	NA	25	+	+		+	+		
12	72	4.25	NA	30		+	+	+		+	
13	74	2.62	282	30	+		+	+		+	
14	76	2.92	291	40	+	+	+		+	+	
15	78	2.90	126	40	+	+	+	+			
Mean±SE	61.1±3.7	3.3±0.1	315.8±85.8	28.7±2.5							

Ca=basal serum calcium levels (mmol/l; normal values: 2.10-2.55), PTH=basal serum parathyroid hormone levels (ng/l; normal values: 10-65), NA=not available, Size=greatest diameter of the parathyroid tumour at pathological examination at first operation (mm), TGP=trabecular growth pattern, MF=mitotic figures, AF=areas of fibrosis, SC=spindle cells, NA=nuclear atypia, CN=coagulation necrosis, VI=blood vessels invasion, SE=standard error

Laboratories) were used as the primary antibodies (PA). The PA were localised by sequential application of biotinylated rabbit anti-mouse IgG goat immunoglobulin, streptavidin-peroxide conjugate and diaminobenzidine (6, 7). The peroxidase was visualised using a DBA tetra-acetate solution and the specimens were counterstained with hematoxylin (7). The labelling index (LI) of PCNA was quantified by counting 1,000 cells from multiple areas in a random fashion and immunostaining of both Ki-67 and p53 was evaluated as the percentage of positive cells. Only strong nuclear staining was considered as positive; cytoplasmic or weak nuclear staining was scheduled as negative. The reported data are expressed as mean ± standard error (SE). The Mann-Whitney *U*-test and the Pearson's correlation coefficient (R) calculation were used for the comparison of qualitative variables in case of a non-normal distribution and to evaluate the linear relationship between pairs of variables, respectively. The median survival time (MST) was calculated using the Kaplan-Meier method. The differences were considered significant at a p value of <0.05.

Results

A high (> 25 mitoses per high-power field [HPF]) mitotic activity (x 40 objective, x 10 ocular) was found in 5 cases, while 7 tumours showed low (5-25 mitoses per 50 HPF) mitotic activity. A weak correlation between pre-operative serum calcium levels and age was found ($R=0.52$, $p=0.048$). The survival, PCNA labelling index (PCNA-LI), Ki-67 and p53 values for each patient are reported in Table II. The PCNA-LI value ranged from 2 to 70 (median 13), the Ki-67 rate ranged from 3% to 65% (median 11%) and the p53 rate from 19% to 65% (median 29%). There was an inverse

Table II. *Survival (months), PCNA labelling index (PCNA-LI), Ki-67 (%) and p53 (%) values.*

Patient	Survival	PCNA-LI	Ki-67	p53
1	24	6	24	56
2	54	11	7	51
3	20	8	4	52
4	75	13	9	65
5	29	14	12	61
6	22	20	17	64
7	34	70	65	29
8	146	18	13	22
9	122	13	11	21
10	48	14	12	24
11	24	9	7	20
12	21	7	8	26
13	23	2	3	28
14	35	5	4	40
15	27	14	12	19
Mean±SE	46.9±10.0	14.9±4.1	13.9±3.9	38.5±4.6

SE=standard error.

Table III. *Relationship between survival, PCNA-LI, Ki-67 and p53.*

Parameter	R	r^2	t	p
PCNA-LI	0.050	0.0025	0.18	0.86
Ki-67	-0.053	0.0028	-0.19	0.85
p53	-0.255	0.065	-0.95	0.36

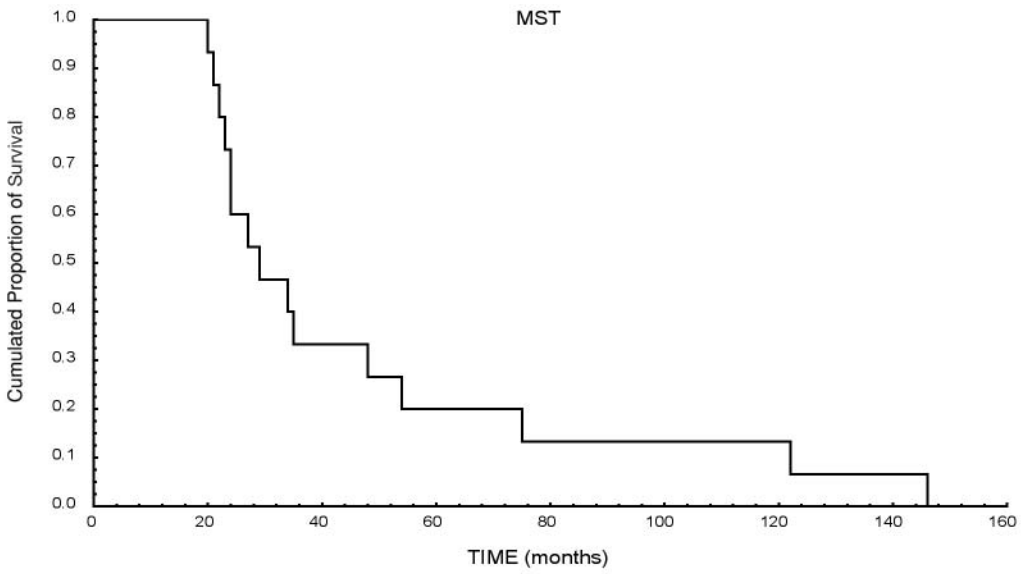

Figure 1. *Cumulated proportion of survival according to the Kaplan-Meier method in patients with parathyroid carcinomas (MST=median survival time).*

correlation between the age of the patients and p53 (R=–0.73, p=0.002), but no correlation with either PCNA-LI (R=0.07, p=0.72) or Ki-67 (R=–0.07, p=0.79). A significant relationship (R=0.93, p<0.01) between PCNA-LI and Ki-67 was found, but p53 did not correlate with either PCNA-LI (R=–0.11, p=0.71) or Ki-67 (R=–0.05, p=0.86). An inverse correlation (R=–0.63, p=0.01) between survival and the presence of spindle cells (SC) and coagulation necrosis (CN) together in the standard slides was observed, although the survival of patients with and without SC and CN were 67.8±47.3 *vs.* 36.5±1.2 (p=0.21) and 55.7±44.8 *vs.* 29.4±12.0 (p=0.10), respectively. Overall, the median survival of patients without SC and CN was 76 months, while in the presence of both SC and CN the median survival was 28 months (p<0.05). However, no correlation was found between survival, PCNA-LI, Ki-67, or p53 (Table III). As previously reported, we did not find any correlation (p=NS) between survival, age of the patients, size of the tumour, mitotic activity and pre-operative biochemical parameters (4). The median survival was 29 months and the cumulated proportions of survival according to the Kaplan-Meier method are shown in Figure 1.

Discussion

PC is a rare tumour, usually difficult to distinguish from parathyroid adenoma on frozen-section examination but easy to localise pre-operatively because of its relatively large size (8, 9). Often no reliable signs of malignancy are detectable, even on final pathology, and none of the features reported by Shantz and Castleman should be considered reliable criteria of malignancy (3, 4, 10). The National Cancer Data Base (NCDB) survey reported an overall 10-year survival rate of less than 50% for patients with PC, but it is difficult to establish parameters correlating with clinical outcome (3, 11). Tumour size appeared to have no impact on prognosis, while age and lymph node metastases have been considered relevant prognostic factors (11-13). We found no correlations between survival and either the age of the patients or size of the tumour. Several histological techniques have been investigated with the aim of predicting the biological behaviour of patients with PC, including DNA cytometry, PCNA-LI, cycle-associated antigen Ki-67 and p53. The significance of DNA ploidy in diagnosing PC is unclear and its prognostic significance is still controversial (12, 14, 15). In a previous study, no correlation was found between survival and DNA index in patients with PC (4). PCNA is a 36 kDa acidic nuclear protein involved in DNA synthesis and was found to be a reliable histological marker of cell proliferation (6). The Ki-67 antigen is a proliferation-associated nuclear antigen that is expressed in all phases of the cell cycle except in the G0-phase and is usually obtained using the MIB-1 antibody (16). p53 has been recognised as a nuclear phosphoprotein that restricts cell proliferation by inducing growth arrest and/or apoptosis (17-19). PC is usually p53- and Ki-67-positive with respect to parathyroid adenomas and normal parathyroid glands and the Ki-67 labelling index is highest in patients with PC (20, 21). Moreover, Cryns *et al.* observed that both p53 allelic loss and abnormal p53 protein expression in PC may implicate a role of p53 in the pathogenesis of a subset of the tumour (22). Finally,

Kameyama *et al.* found that patients with PC and no recurrences of their tumour showed low PCNA-LI, and a patient who died 34 years after surgery with multiple metastases had both very high PCNA-LI and Ki-67 values (23). Unfortunately, these occasional observations were not confirmed in our study. In patients with PC, the clinical and biochemical parameters usually do not correlate with histopathological findings (15). However, Bondeson *et al.,* reviewing 95 cases of PC collected from 37 hospitals, found that the triad of macronoduli, more than 5 mitoses per 50 HPF and necrosis were associated with an aggressive behaviour in terms of recurrent disease (5). We did not find a relationship between survival and mitotic activity, but a significant ($p<0.05$) inverse correlation between spindle cells (SC) and coagulation necrosis (CN) together and survival was observed. In conclusion, our data suggest that PCNA-LI, Ki-67 and p53 are not useful in predicting the clinical outcome of patients with PC. However, the presence of SC and CN together in the standard specimens should be considered to be a negative prognostic factor.

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