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Proliferating cell nuclear antigen in breast carcinomas

An immunohistochemical study with correlation to histopathological features and prognostic factors

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Abstract Proliferating cell nuclear antigen (PCNA), was examined by immunohistochemistry in 509 breast carcinomas. The immunoreactivity was found to be independent of the length of fixation when the tissue sections were microwaved before incubation with the primary antibody. The PCNA immunoreactivity was assessed by two semi-quantitative methods, which were correlated but not exchangeable. The comedo type of intraductal carcinomas and invasive ductal carcinomas had a higher PCNA score than other types. Lymph node metastases had a significantly higher PCNA score than primary carcinomas. High PCNA immunoreactivity was correlated with the presence of lymph node metastases, absence of tubule formation, numerous mitoses, severe nuclear pleomorphism, high histological grade and absence of progesterone receptors (PgR). PCNA in lymph node positive tumours was correlated with tumour type, especially with ductal carcinomas, absence of tubule formation, high histological grade and absence of PgR, whereas PCNA in lymph node negative tumours was correlated with large tumour size, numerous mitoses, severe nuclear pleomorphism and high histological grade. Number of mitoses and nuclear pleomorphism were the two most important factors in predicting the PCNA score; the absence of PgR and nuclear pleomorphism were important in lymph node negative and positive tumours, respectively. In a univariate analysis high PCNA score was found to be correlated with shorter relapse-free period and poorer over-all survival.

Key words Proliferating cell nuclear antigen
Immunohistochemistry · Breast carcinomas

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Introduction

The proliferative capacity of a malignant neoplasm is an important adjunct to histological grading and may be an indicator of treatment response and relapse in many tumours (Meyer et al. 1984; McGuire 1987; Tubiana and Courdi 1989; Tubiana et al. 1989). Good correlation has been found between high cell proliferation rates measured by flow-cytometry and poor short-time survival in primary breast cancers (Kallioniemi O-P 1988; Kallioniemi O-P et al. 1991). Cell kinetics have been determined by thymidine-incorporation, flow-cytometry or counting mitotic figures; the two first techniques are time consuming, costly and difficult to use in a routine diagnostic procedure. Mitotic figures, which can be identified by light microscopy, appear only in a short fraction of time in the proliferative phase of the cell cycle, in the M-phase and mitotic counting is subject to significant variability because of such factors as delay in fixation and differences in counting procedures (Graem and Helweg-Larsen 1979; Donhuijsen et al. 1990; Clayton 1991). These factors ensure that the number of mitotic figures does not represent the total number of cells in proliferative phase. Immunohistochemistry, in contrast, is a relatively simple technique which has the advantage that the morphology of the tissue is maintained. Antigens in proliferative cells must be resistant to formalin-fixation to allow assessment of the proliferative potential in paraffin-embedded archival tissue from malignant neoplasms.

PCNA is an 36 kDa auxiliary protein for DNA polymerase-δ (Bravo et al. 1987; Prelich et al. 1987), and is mainly present in cells where DNA-replication or DNA-synthesis occur, which is in late S-phase and G2-phase of the replicative cycle of the cells (Mathews et al. 1984; Celis et al. 1984). However, PCNA synthesis is not only dependent on DNA synthesis, since its synthesis can be induced by various growth factors (Bravo and Macdonald-Bravo 1984; Macdonald-Bravo and Bravo 1985; Bravo 1986). Cells held in hydrourea continue to

produce PCNA (Bravo and Macdonald-Bravo 1985), and pharmacological inhibition of DNA synthesis does not always depress the synthesis of PCNA (Macdonald-Bravo and Bravo 1985). In quiescent cells the amount of PCNA is low, whereas the production increases prior to DNA replication (Kurki et al. 1986). In breast tissue or carcinoma PCNA immunoreactivity has been shown to correlate with thymidine labelling index (Battersby and Anderson 1990), DNA flow-cytometry (Garcia et al. 1989; Siitonen et al. 1993a) and Ki-67 immunoreactivity (Dawson et al. 1990).

In this study we have examined the immunoreactivity of PCNA in primary breast cancers from 497 patients, and have correlated the staining results with various histopathological features and prognostic factors.

Materials and methods

Four hundred and ninety seven patients with primary breast carcinoma, diagnosed at the Department of Pathology, Gentofte University Hospital during 1980–85 were included. Twelve patients had bilateral tumours of which 7 were synchronous and 5 asynchronous. Thus, 509 tumours were available for examination. One hundred and seventy eight patients had concomittant lymph node metastases. Information about treatment, relapse-free period and time of over-all survival was obtained from the secretary of the Danish Breast Cancer Cooperative Group (DBCG) (Andersen et al. 1981). The primary treatment was in all cases either wide local excision or mastectomy. One hundred and thirty five patients received adjutant chemotherapy and/or local radiotherapy as part of the secondary treatment. The mean follow-up time was over 10 years. Menopausal status (premenopausal versus postmenopausal) was known for all patients. Operation specimens were examined according to the guide-lines of DBCG. Tumour size was measured on the operation specimen and was recorded as either more or less than 30 mm in the largest diameter. Lymph node status was recorded as either negative or positive regardless of the number of positive lymph nodes. Tissue for microscopic examination was formalin-fixed and paraffin-embedded following routine procedures. Histological typing and grading was performed on haematoxylin and eosin (HE) stained sections. Intraductal carcinomas were classified according to the classification proposed by Page et al. (1987). If more than one type was present the predominant type was recorded. The invasive carcinomas were classified according to the World Health Organization (WHO) typing of breast tumours (1968). Histological grading of the invasive ductal carcinomas not otherwise specified (NOS) was done according to a modification of the method described by Bloom and Richardson (1957) (Table 1). The score from each item was added. Tumours with a total score of 3–5 points were classified as grade I, 6–7 as grade II, and 8–9 as grade III.

Table 1 Mitosis were counted in ten high-power fields (HPF) in the area with most mitoses using an Olympus BH-2 microscope, with a HPF of 490 nm in diameter

Score	Grading of breast carcinomas		
	Tubule formation	Nuclear pleomorphism	Mitoses
1	Pronounced	Minimal	0–1
2	Moderate	Moderate	2–3
3	Little or none	Severe	4 or more

Oestrogen receptor (ER) and progesterone receptor (PgR) status of the primary carcinomas was determined by the dextran-coated charcoal (DCC) technique, if enough fresh tissue was available. The biochemical measurements of ER and PgR were expressed as fmol/mg protein. Values below 10 fmol/mg protein were considered negative.

For immunohistochemistry 5 μm sections from both primary and secondary tumours were cut, dewaxed and pretreated in a microwave-oven (Voss Micro 361-1, Model 58907) at 650 W twice for 5 min in distilled water. The sections were thereafter incubated overnight at 4° C with the primary antibody, PC10 a monoclonal mouse antibody (DAKO, Copenhagen, Denmark), used in a 1:600 dilution in TRIS buffered saline (TBS) pH 7.6 with azide/bovine albumin. The second layer was biotinylated rabbit-anti-mouse immunoglobulin (DAKO) used in a 1:400 dilution. The sections were then incubated with avidin-biotin peroxidase ABC-complex (DAKO) for 30 min. Between incubations the sections were washed three times in TBS pH 7.6 for 5 min. The colour was developed using 3-amino-9-ethylcarbazole (AEC). Sections were counterstained with Mayer's haematoxylin. Sections from a tonsil with hyperplasia were assayed as a positive control in each run. Negative controls in which the primary antibody was replaced by PBS or mouse IgG_2-immunoglobulin were also included.

Tissue blocks from five primary breast carcinomas, a tonsil and a seminoma of the testis were fixed in 4% buffered formaldehyde for various lengths of time (6, 24, 48, 72 h and 1 week respectively) before processing through to paraffin embedding followed by the the above described method.

Tumour cells with nuclear staining were recorded as positive. The PCNA immunostaining was registrered semi-quantitatively in two ways. Method I: tumours were scored as 0, if none or less than 1% of the tumour cells were positive; 1, 1–10%; 2, 11–25%; 3, 26–50%, and 4, if more than 50% of the tumour cells were positive. Method II: the tumours were scored 0, if none or less than 1% of the tumour cells were positive; +, if few of the tumour cells were positive; ++, if a moderate number, and +++, if most of the tumours cells were positive.

Chi-squared test or Fischer's exact probability test were used when PCNA immunoreactivity was compared with clinical and histopathological parameters. Multiple logistic regression was performed by JMP 2,0 program, SAS Institute on a Macintosh computer to assess which parameters were of most importance for predicting PCNA immunoreactivity. The PCNA score, when assessed by method I was registered as either more or less than 25% positive tumour cells when this parameter was entered in the logistic regression model.

Relapse-free period and over-all survival were estimated by the method of Kaplan-Meier (1958) and compared by the log-rank test (Cox and Oakes 1984).

When the two methods of scoring PCNA-immunoreactivity were compared Cicchetti's Weighted Kappa was used. The different entries in the contingency table were weighted depending on how important the evaluation by the two methods was. The weights were equal to 1 in the diagonal, when the two methods agreed, and 0.75, when the score was one category higher or lower than the diagonal.

When the two methods for assessing PCNA immunoreactivity were compared group 1 and 2 in method I were combined into one group.

When the PCNA immunoreactivity in the intraductal carcinoma was compared with the invasive carcinomas and the invasive carcinomas with lymph node metastases McNemar's test was used (Armitage and Berry 1987).

Results

The percentage of cells positive for PCNA and the intensity of the immunostaining in five invasive ductal carcinomas, a tonsil with hyperplasia, and a seminoma

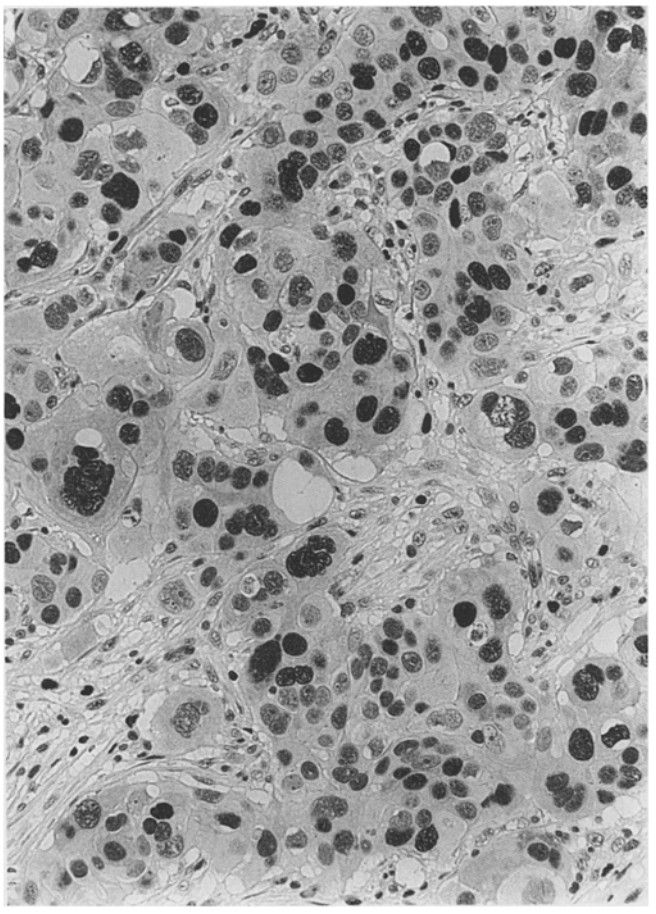

Fig. 1 A low grade invasive ductal carcinoma with severe nuclear pleomorphism showing characteristic immunoreactivity for PCNA (PC10 in a 1:600 dilution), ABC-technique, ×200

Table 2 Distribution of clinical, histological and prognostic features in 509 primary breast carcinomas. Tubule formation: 1 = pronounced, 2 = moderate, 3 = little or none; nuclear pleomorphism: 1 = minimal, 2 = moderate, 3 = severe; mitoses: 1 = 0–1, 2 = 2–3, 3 = more than 4 per high power field. Histological grade: 1 = grade I, 2 = grade II, 3 = grade III

		Number
Patients with one primary tumour		497
Patients with bilateral tumours		12
Menopausal status	Premenopausal	142
	Postmenopausal	367
Axillary lymph nodes	With metastases	178
	Without metastases	331
Tumours size	Less than 30 mm	305
	More than 30 mm	204
Tumour type	Invasive ductal	397
	Invasive lobular	49
	Miscellaneous	63
Tubule formation	1	69
	2	140
	3	188
Mitoses	1	179
	2	174
	3	44
Nuclear pleomorphism	1	75
	2	267
	3	55
Histological grade	1	149
	2	189
	3	59
Oestrogen receptor	Positive	93
	Negative	280
Progesterone receptor	Positive	90
	Negative	195

of the testis was the same whether the tissues had been fixed from 6 h up to 1 week before microwave processing and staining. The immunoreactivity of PCNA was, thus, found to be independent of the length of fixation when this immunohistochemical method was used. Figure 1 shows a characteristic nuclear immunoreactivity for PCNA in an invasive poorly differentiated invasive ductal carcinoma where more than 50% of the tumour cells are positive.

The two semi-quantitative methods for assessing PCNA-score agreed in 95% of intraductal carcinomas (Kappa value 0.87), in 87% of the primary invasive carcinomas (Kappa value 0.76), and in 92% of the lymph node metastases (Kappa value 0.79).

PCNA-scores in the intraductal carcinomas showed that the comedo type had a statistically significant higher PCNA-score compared with the other types. Over 80% of the intraductal carcinomas of the comedo type had a high PCNA score (34 or 45 in method I and + + + in method II), compared with only 35% in the other types.

The PCNA-scores in intraductal and invasive ductal carcinomas, were identical in 52% of the cases. In the remaining cases, no correlation between the immunoreactivity in the intraductal and invasive components could be demonstrated. Lymph node metastases had a statistically significant higher PCNA-score than the corresponding invasive carcinomas ($p < 0.05$), especially when the PCNA score in the primary tumours was above score 1 assessed by method I and above + assessed by method II.

Number of tumours, menopausal status, number of patients with and without lymph node metastases, tumour size and histopathological features such as tumour type, tubule formation, number of mitoses, nuclear pleomorphism, histological grade, ER and PgR are shown in Table 2. Three hundred and ninety seven tumours were invasive ductal carcinomas, 49 were invasive lobular and 63 were classified as miscellaneous carcinomas, which included 35 invasive ductal carcinomas with a predominant intraductal component, 16 mucinous, 5 medullary, 2 papillary and 4 tubular carcinomas, and 1 carcinoma with squamous metaplasia. In 125 specimens, an intraductal carcinoma was also present, 50 of these were of the comedo type, 54 of the cribriform, 10 of the papillary and 11 of the solid type.

Table 3 Correlation between the immunoreactivity for PCNA in all the invasive breast carcinomas and the clinical, histological and prognostic features, whether the immunoreactivity was recorded according to method I or II

	Method I	Method II
Menopausal status	NS	NS
Axillary lymph nodes	$p<0.032$	$p<0.034$
Tumours size	$p<0.01$	NS
Tumour type	NS	$p<0.0004$
Tubule formation	$p<0.0001$	$p<0.0002$
Mitoses	$p<0.0001$	$p<0.0006$
Nuclear pleomorphism	$p<0.0001$	$p<0.0027$
Histological grade	$p<0.0000$	$p<0.0002$
Oestrogen receptor	$p<0.045$	NS
Progesterone receptor	$p<0.0114$	$p<0.0103$

High PCNA score, in all the primary tumours, was associated with the presence of lymph node metastases, with absence of tubule formation, numerous mitoses, severe nuclear pleomorphism, high histological grade (poorly differentiated), and absence of PgR. This applied whether the score was assessed by method I or II (Table 3). When PCNA score was assessed by method I, additional positive correlations were found between high PCNA score and tumour size above 30 mm and absence of ER. When method II was used, a positive correlation was observed between high PCNA score and tumour type, showing that invasive ductal carcinomas had a higher PCNA score than any of the other types of carcinomas.

High PCNA-score in lymph node positive tumours was correlated with tumour type, including those with invasive ductal carcinomas, absence of tubule formation, high histological grade (poorly differentiated) and absence of PgR. High PCNA-score in lymph node negative tumours was correlated with large tumour size, numerous mitoses, severe nuclear pleomorphism in addition to high histological grade (poorly differentiated), but not with tumour type or PgR status. The same correlations were observed whether method I or II was used (Table 4).

Lymph node positive tumours were larger, more frequently of invasive lobular type, often ER negative and had a higher PCNA score than the lymph node negative tumours. No differences in menopausal status, tubule formation or PgR status could be observed between the two groups.

In a multiple regression model including all tumours, mitotic index and nuclear pleomorphism were the two most important factors for predicting a high PCNA-score with odds ratios of 1.52 and 2.56, respectively. For lymph node positive tumours the absence of PgR was

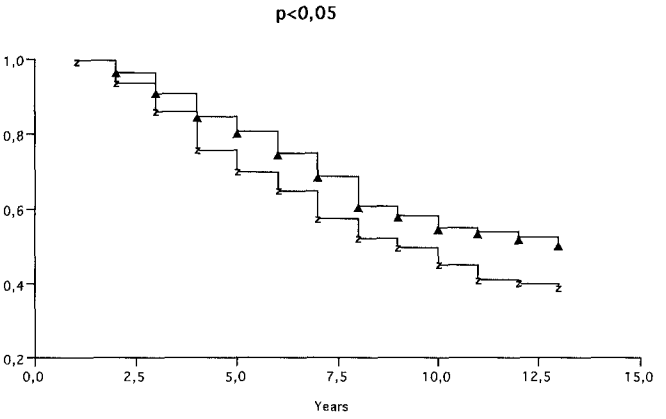

Fig. 2 Over-all survival for groups with high (>25%) and low (<25%) PCNA score. (Z – high PCNA (>25%); ▲ – low PCNA (<25%))

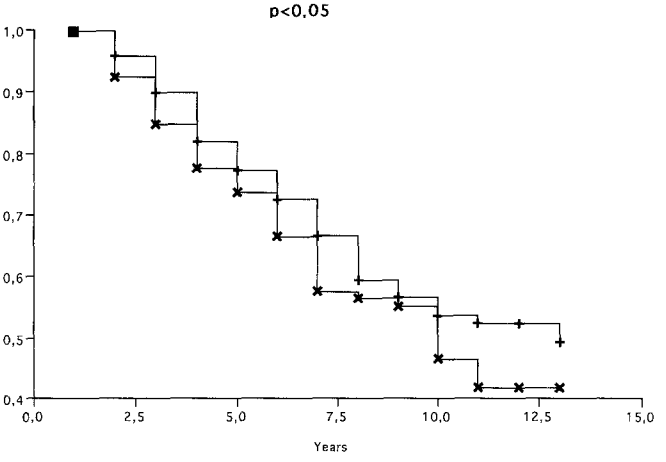

Fig. 3 Over-all survival for groups with high (+ + +) and low (+,+ +) PCNA score. (+ – low PCNA (+,+ +); X – high PCNA (+ + +))

Table 4 Correlation between the immunoreactivity for PCNA in tumours from lymph node positive and negative patients, and clinical, histological and prognostic factors, whether the immunoreactivity was recorded by method I or II. *NS*, Not statistically significant; ER, oestrogen receptor; PgR, progesterone receptor

Feature	Lymph node positive tumours		Lymph node negative tumours	
	Method I	Method II	Method I	Method II
Menopausal status	NS	NS	NS	NS
Tumour type	$p<0.249$	$p<0.0258$	NS	NS
Tumour size	NS	NS	$p<0.01$	$p<0.003$
Tubule formation	$p<0.0117$	$p<0.0117$	NS	NS
Mitoses	NS	NS	$p<0.0001$	$p<0.0008$
Pleomorphism	NS	NS	$p<0.0001$	$p<0.0008$
Histological grade	$p<0.0227$	$p<0.0044$	$p<0.0001$	$p<0.0205$
ER	NS	NS	NS	NS
PgR	$p<0.0373$	$p<0.0388$	NS	NS

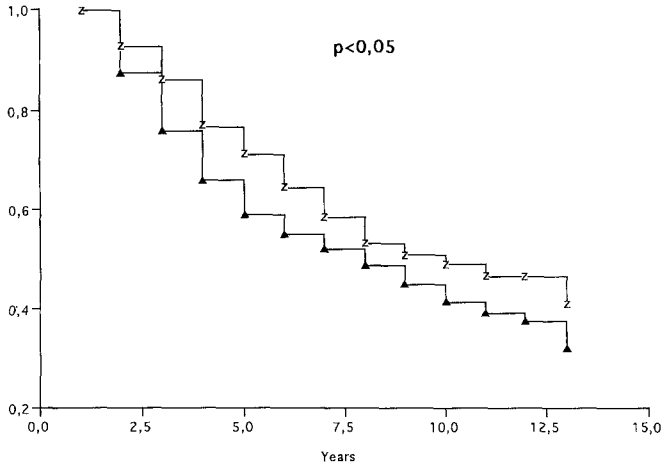

Fig. 4 Relapse-free period for groups with high (>25%) and low (<25%)
PCNA score. (Z – low PCNA (<25%); ▲ – high PCNA (>25%))

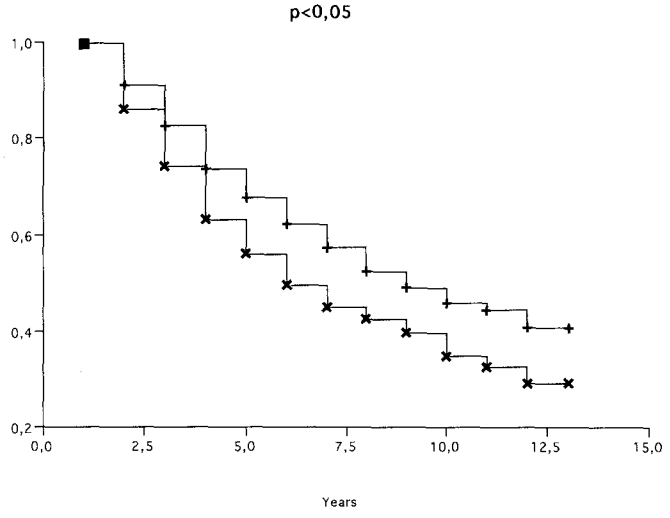

Fig. 5 Relapse-free period for groups with high (+ + +) and low (+,+ +) PCNA score. (+ – low PCNA (+,+ +); X – high PCNA (+ + +))

the only important variable (odds ratio 4.8) whereas in lymph node negative tumours nuclear pleomorphism was the only variable of importance (odds ratio 2.58).

Based upon the PCNA score in the primary tumours, the patients were separated into two groups. Patients with low PCNA score assessed by method I were those with less than 25% positive tumour cells (score 1 and 2), and those with high PCNA score were the cases with more than 25% positive tumour cells (score 3 or 4). The group with low PCNA score, assessed by method II, were those scored + or + +, and the group with high PCNA score was scored + + +. It was only possible to yield statistically significant differences in relapse-free period and over-all survival between the group with high and low PCNA score when the above limits were used. Kaplan-Meier plots of the over-all survival and relapse-free period are shown in Figs. 2–5 for patients with high and low PCNA scores identified by method I and II, respectively.

The group with high PCNA scores, regardless of the method used for scoring, had a statistically significantly poorer overall survival and a shorter relapse-free period compared with the group with low PCNA score. The major differences in relapse-free period and over-all survival between the groups were mainly manifested 5 years after diagnosis. The difference in over-all survival between groups with high or low PCNA scores was almost 10% and was maintained through the follow-up period.

Discussion

Cell kinetic data are among the most important factors of treatment response and relapse in many tumours (Meyer et al. 1984; McGuire 1987; Tubiana et al. 1989a, b). Various methods of estimating tumour proliferation rate has been developed; most of these are time consuming, costly and difficult to use in a routine diagnostic procedure. Immunohistochemistry has the advantages that the morphology of the tissue is maintained, is easy to apply, and is inexpensive. However, immunoreactivity to proliferating cell antigens such as PCNA is dependent on the fixative used and the length of fixation, which make and assessment of the proliferative capacity in formalin-fixed neoplasms unreliable (Hall and Levison 1990; Suzuki et al. 1992; Leong et al. 1993). Siitonen et al. (1993b) showed that satisfying immunoreactivity was achieved in formalin-fixed and paraffin-embedded sections after microwave treatment in saturated lead thiocyanate. The use of a lead solution is, however, not attractive, because of its poisonous potential. The relationship between the immunoreactivity for PCNA and the length of fixation was not examined by Siitonen et al. (1993b). In the present study we found that the immunoreactivity of PCNA was independent of the length of fixation if the sections were microwaved in distilled water before incubation with the primary antibody. We did, therefore, not considered the length of fixation when evaluating the PCNA score in this study.

We have assessed the immunoreactivity of PCNA using semiquantitative methods rather than actual cell counting, since in a minor study we have shown poor inter- and intraobserver correlations (Kappa = 0.59) whether the immunoreactivity was assessed by actual counting or by one of the two semi-quantitative methods (unpublished). The actual cell counting technique is time consuming and not useful as a routine diagnostic procedure, since thousands of tumour cells must be counted to get a reliable assessment of the PCNA immunoreactivity. We found that the semi-quantitative methods are as reliable and reproducible as the actual cell counting technique. Method I has 5 intervals, whereas method II has only 4. Method II is based upon a traditional pathological semi-quantitative scale, whereas method III has more detailed intervals in the

lower end of the scale. We found that the two methods were correlated but not exchangeable.

The comedo type of intraductal carcinomas was found to have a higher PCNA score compared with the other types, which may reflect an increased malignant potential, since the comedo type is known to have an increased potential for local recurrence and increased metastatic potential (Page et al. 1987; Bellamy et al. 1993). The PCNA scores in intraductal and invasive carcinoma did not differ. However, invasive ductal carcinomas had higher PCNA score than the other types. Like, Tahan et al. (1993), we found that the PCNA score in axillary lumph node metastases was significantly higher than in the primary tumours. The higher PCNA score in the lymph node metastases may reflect an increased proliferative potential of the metastatic tumour cells, an increased DNA damage or an altered/increased growth factor profile. To determine this it should be examined whether PCNA scores in the lymph node metastases are associated with the mitotic index.

Our findings are in agreement with those of most other studies (Siitonen et al. 1993a, b; Tahan et al. 1993) which also have compared the immunoreactivity of PCNA with different clinical and histopathological variables, using an univariate analysis. Some studies have found a correlation between high PCNA score and menopausal status, tumour size, hormone receptor status and nodal status, while others have not. These differences may occur because of variation of the composition of the examined population, of the immunohistochemical method used and of the method use to assess the PCNA immunoreactivity. The latter was demonstrated in our study since different correlations were found whether the PCNA immunoreactivity was assessed by method I or II. Thus, in studying correlations between PCNA immunoreactivity and histopathological variables it is important to evaluate the method used to assess the immunoreactivity as well as the staining method used.

To determine which of the histopathological variables was of most importance in predicting PCNA score we used a multiple logistic regression, which showed that high PCNA score in all tumours was correlated with both the number of mitoses and the degree of nuclear pleomorphism. The correlation between the PCNA immunoreactivity and the number of mitoses implies that PCNA is a reliable marker of cell proliferation. However, the strong correlation between high PCNA score and nuclear pleomorphism suggests that PCNA may also be associated with either the differentiated state of the tumour, or with other yet unknown factors such as increased DNA damage and the presence of growth factors which can stimulate the PCNA synthesis (Bravo and Macdonald-Bravo 1984; Macdonald-Bravo and Bravo 1985; Bravo 1986).

Some studies have found that PCNA is related to shorter relapse-free period and poorer over-all survival; others have not (Gasparini et al. 1992; Siitonen et al. 1993a, b; Tahan et al. 1993).

Regardless of the method used to assess the PCNA immunoreactivity, high PCNA score in this study was correlated with a shorter relapse-free period and a poorer over-all survival. Gasparini et al. (1992), who also used a semi-quantitative scoring system of PCNA in primary breast carcinomas, found no correlation between high PCNA score and relapse-free period or over-all survival. However, the follow-up time in that study was only 4 years. Our results show that it was only after 5 years follow-up that statistically significant differences occurred between patients with low or high PCNA score. This emphasises the importance of a long follow-up period before the value of prognostic factors can be determined. Tahan et al. (1993) examined the PCNA score in 86 patients with primary breast carcinomas with a follow-up period up to 220 months and high PCNA score (over 25% positive tumour cells) was found to be correlated with both shorter relapse-free period and poorer over-all survival using an univariate analysis. A 20% difference was found between the over-all survival in patients with tumours with low and high PCNA score. We found 12% and 8% differences in the overall survival between patients whose tumours had low and high PCNA assessed by method I and II, respectively. The smaller difference found in our study may reflect that the composition of the population examined was different, that another immunohistochemical method was used and that the PCNA immunoreactivity was assessed by semi-quantitative methods. Although 135 patients received additional therapy after the primary treatment, this could not explain the smaller difference found in overall survival found in our study, as no difference in the proportion of patients receiving additional therapy was found between those with high or low PCNA score. In this study, the differences in relapse-free period and overall survival between the groups with low and high PCNA score, although small, were significantly different.

Immunoreactivity for PCNA has been shown to be correlated with Ki-67 in malignancies (Dervan et al. 1992; Leong et al. 1993; Mango et al. 1992; Sabatti et al. 1993; Yu et al. 1992), although PCNA score is found to be higher than the Ki-67 score. The reason among others may be that PCNA has a long half life (Yu et al. 1992). Furthermore, antibodies against PCNA and Ki-67 recognize different nuclear antigens. It is currently claimed that Ki-67 is a better marker of the proliferative activity than PCNA, as Ki-67 reacts with a well known epitope, which is only expressed during the proliferative phase of the cell cycle (Gerdes et al. 1984), while the antibody PC10 has been shown to react with two forms of PCNA (Bravo and Mcdonald Bravo 1987), one which is present during DNA synthesis and associated with the entry of the cell into S-phase and one which is present although the cell is no longer actively proliferating. Thus, the presence of PCNA does not always reflect proliferation but can also identify cells in which DNA synthesis occurs for other reasons. In our study high PCNA expression was not only correlated with high

mitotic number, but also with marked nuclear pleomorphism. Whether PCNA is a true reflection of cell proliferation or not, several studies have found good correlations between PCNA indexes and poor clinical prognosis (Mango et al. 1992; Tahan et al. 1993). Further evaluation of the prognostic significance of PCNA must be carried out, including multivariate analysis in prospective studies. Multivariate analysis has not been performed in this study, since the aim was mainly to examined PCNA immunoreactivity in breast carcinomas in relation to various histopathological and prognostic factors. Whether PCNA can be used as an independent prognostic factor or not is to be determined by multivariate analysis in an on-going study.

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