Pergamon

European Journal of Cancer Vol. 33, No. 1, pp. 39–44, 1997

Printed in Great Britain
0959-8049/97 $17.00+0.00

PII: S0959-8049(96)00368-1

Original Paper

p53 Protein Expression, Cell Proliferation and Steroid Hormone Receptors in Ductal and Lobular *In Situ* Carcinomas of the Breast

M. Rudas,[1] R. Neumayer,[1] M.F.X. Gnant,[2] M. Mittelböck,[3] R. Jakesz[2] and A. Reiner[1]

[1]Institute for Clinical Pathology, University of Vienna, Allgemeines Krankenhaus, Währinger Gütel 18-20, A1090 Vienna; [2]Surgical University Clinic, University of Vienna, Allgemeines Krankenhaus, Währinger Gütel 18-20, A1090 Vienna; and [3]Institute for Medical Computer Science, University of Vienna, Allgemeines Krankenhaus, Währinger Gütel 18-20, A1090 Vienna, Austria

p53 and c-erbB-2 expression, and their correlation with cell proliferation and steroid hormone receptors, were investigated in 121 carcinomas, 23 lobular *in situ* carcinomas (LCIS), 74 intraductal carcinomas (DCIS) and 24 minimal invasive carcinomas. DCIS were classified according to the EORTC classification. All markers were measured immunohistochemically on paraffin sections. None of the LCIS, 9 DCIS and 9 minimal invasive cancers showed nuclear positivity for p53. A strong association between histological type and p53 expression was found. Proliferation rates correlated with p53 expression. c-erbB-2 positivity was found in 1 LCIS, 27 DCIS and 12 minimal invasive cancers. There was a significant correlation between p53 expression and c-erbB-2. Both parameters were associated with high proliferation rate and negativity for steroid hormone receptor status. Nuclear pleomorphism could become a comparable prognostic marker in DCIS as it is for infiltrating carcinomas.

Key words: DCIS, CLIS, minimal invasive cancer, p53, c-erbB-2, growth fraction, steroid hormone receptors

Eur J Cancer, Vol. 33, No. 1, pp. 39–44, 1997

INTRODUCTION

MAMMOGRAPHIC SCREENING programmes have been intensified over the last decades, and radiographic techniques have been improved. Both factors led to earlier detection of breast cancer. Consequently, the proportion of early, non-invasive stages of breast cancer is increasing and will represent a greater diagnostic problem in the future [1–4].

Prognostic parameters in invasive breast cancer are well documented and thoroughly investigated. Due to the previous rarity of intraductal breast carcinomas (ductal carcinoma *in situ*, DCIS), there exist only few reports on prognostic parameters in DCIS. Therefore, the aim of our study was to evaluate morphological factors which are known to be of prognostic importance in invasive breast cancers and describe them in LCIS (lobular carcinoma *in situ*) and DCIS.

Correspondence to M. Rudas.
Received 2 Aug. 1995; revised 2 Aug. 1995; accepted 2 Aug. 1995.

MATERIALS AND METHODS

We investigated a series of 121 breast cancers consisting of 23 LCIS and 74 DCIS from patients operated on between 1978 and 1995. DCIS were classified according to the recommendations of the Breast Cancer Study Group of the EORTC [5]. Additionally, we investigated 24 predominantly intraductal carcinomas with minimal stromal invasion, which meant that they showed only focally periductal tumour spread according to the description given by Tavassoli [6]. However, for some tumours, there was insufficient material to examine all parameters (see Results).

Tumour size could be determined only in 29 (39%) DCIS and ranged from 0.3 to 6 cm. In 45 DCIS (61%) tumour size could not be determined grossly because only few ducts were involved. In some cases, DCIS showed a diffuse growth pattern within the specimen and, therefore,

could not be measured. 17 DCIS (23%) were classified as well differentiated, 30 (41%) met the criteria of intermediately differentiated DCIS, and 27 (36%) were assigned to poorly differentiated DCIS. An intraductal component in minimal invasive cancers was measurable in 17 (71%) cases and ranged between 0.5 and 5 cm. The intraductal component of those tumours was well differentiated in 2 (8%) cases, intermediately differentiated in 10 (42%) cases and poorly differentiated in 13 (54%) cases.

Furthermore, we wanted to compare different DCIS classifications with regard to whether differences in subtyping would affect the significance and correlations of the parameters investigated. We first subdivided DCIS into comedo/non-comedo applying the widely used classification scheme by the presence/absence of necrosis, then stratified DCIS according to their nuclear grade into polymorphous/non-polymorphous and compared both with the EORTC scheme we used for our study.

Histological sections of tumour tissue were prepared according to the routine paraffin histological technique and stained with haematoxylin–eosin.

Oestrogen (ER) and progesterone receptors (PgR) were determined immunohistochemically on paraffin sections using the ER-ICA and PgR-ICA kit (Abbott Laboratories, N. Chicago, Illinois, U.S.A.). For detecting ER, slides were pretreated with a 0.1% solution of protease type XIV (Sigma) at 37°C for 10 min. For PgR detection, slides were not enzyme pretreated. Slides were incubated overnight with the primary antibody at 4°C. Prediluted ER was approximately 5-fold reconcentrated by ultracentrifugation using Ultracent 30 tubes (BioiRad, California, U.S.A.). PgR was used as provided with the kit. The further steps of the staining procedure were performed using the immunohistochemical ABC method. Tumours showing at least 10% receptor-positive nuclei were scored positive.

For the determination of proliferating cell fractions, we used MIB1 (Clone MIB1, Dianova GmbH, diluted 1:50), a monoclonal antibody directed against the proliferation associated Ki-67 antigen, which can be used on formalin-fixed, paraffin-embedded tissues [7]. Paraffin sections were incubated in citrate buffer (pH 6) in the microwave oven first at 120 W for 20 min, then twice for 5 min at 450 W. Citrate buffer had to be refilled several times in order to keep sides moist. The slides were cooled for 15–20 min at room temperature and, after incubation with the primary antibody for 1 h, were further processed using the immunohistochemical ABC method.

Immunohistochemical staining for the p53 protein was carried out using a mouse monoclonal antibody (PaB 1801, Cambridge Research Biochemicals, diluted 1:4000) directed against mutant p53 protein [8]. For p53 staining, paraffin sections were incubated with Target Unmasking Fluid (TUF, Kreatech Biotechnology B.V., The Netherlands, dilution 1:3) at 90°C for 10 min and then left to cool for 15 min. After rinsing several times with distilled water and tris-Tween, slides were incubated with the primary antibody for one hour. Further immunohistochemical staining was performed with a streptavidin-complex according to the ABC method.

c-erbB-2 overexpression was detected using CB 11 (Medac, dilution 1:40), a mouse monoclonal antibody recognising the internal domain of the c-erbB-2 oncogene protein on formalin-fixed, paraffin-embedded tissue [9]. For c-erbB-2 staining, paraffin sections were incubated with the primary antibody for one hour at room temperature, further staining was done using the ABC method.

For all ABC immunohistochemical procedures, products from Vector Laboratories (Burlingame, California, U.S.A.) were used. Briefly, after incubation with the primary antibody and incubation with a biotinylated antimouse-antibody, incubation with the ABC complex for one hour followed. The reaction product was developed with diaminobenzidine tetrahydrochloride. Finally slides were counterstained with Harris' haematoxylin. All steps of incubation were performed at room temperature.

The MIB1 labelling index (MIB1/LI) was obtained by counting a minimum of a 1000 tumour cells over a 1 cm^2 ocular grid under 400× magnification and calculating the percentage of positive cell nuclei. Nuclei showing even brown staining were regarded as positive. Areas which showed high labelling were chosen for counting.

p53 expression was estimated semiquantitatively. The cut-off point was set at 10% positive tumour cells. c-erbB-2 overexpression was assessed as present or absent regarding positive membrane staining, cytoplasmic staining was ignored.

Statistics

Data were stored in an IBM main frame computer of the Vienna Medical School using an SAS software (SAS Institute, Cary, North Carolina, U.S.A.) for data entry, management and statistical analysis.

Continuous factors were described with the median, and differences between groups were tested in a nonparametric manner by the Wilcoxon test.

All prognostic factors considered in the analyses were categorised by 2 or 3 groups using suitable cut-offs to simplify the presentation of results. Differences between these groups were tested by means of the standard chi-squared trend test and Fisher's exact test.

RESULTS

All 23 cases of LCIS were p53 negative, although 6 LCIS showed scattered and faint nuclear staining, the percentage of positive cells being far less than 10%. c-erbB-2 overexpression was absent in 22 LCIS (96%) and 1 LCIS (4%) was c-erbB-2 positive. Cell proliferation rates in LCIS were low. The median MIB1-LI was 1.3%. Individual MIB1-LI ranged from 0.2% to 9.9%. The maximum MIB1-LI was achieved by the LCIS showing c-erbB-2 overexpression. The majority of LCIS were ER-ICA and PgR-ICA positive (80% and 90%, respectively).

9 DCIS (13%) showed p53 positivity, and 62 (87%) did not express p53 at all or showed only eventual and weak nuclear staining less than 10%. p53 expression was completely absent in tumours with small, monomorphous nuclei and, therefore, low-grade nuclear anaplasia. We found p53 expression only in tumours with large, pleomorphic nuclei and, therefore, high nuclear grade which is reflected by the classification score we used as poorly differentiated DCIS (Figure 1).

Figure 1 shows the distribution of c-erbB-2 overexpression in DCIS. In 27 (38%), c-erbB-2 positivity could be demonstrated. c-erbB-2 staining was never seen in DCIS

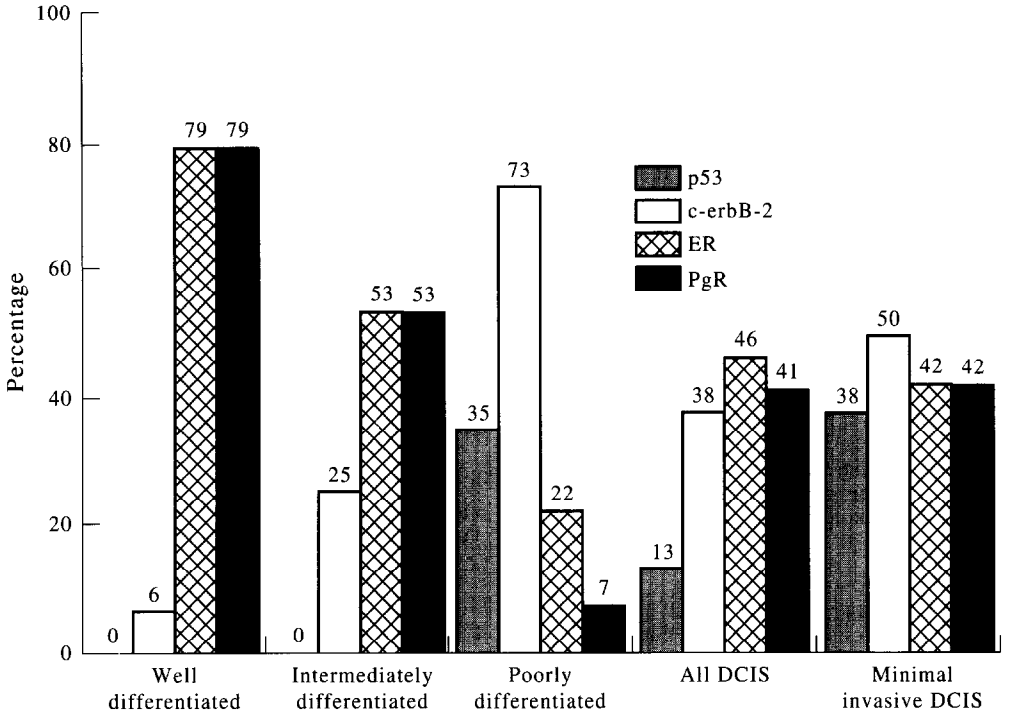

Figure 1. p53 in DCIS (n = 71; pos = 9; neg = 62; P = 0.0000516) and minimal invasive carcinomas (n = 24; pos = 9; neg = 15); c-cerbB-2 in DCIS (n = 71; pos = 27; neg = 44; P = 0.00001) and minimal invasive carcinomas (n = 24; pos = 12; neg = 12); ER-ICA in DCIS (n = 71; pos = 33; neg = 38; P = 0.00156) and minimal invasive carcinomas (n = 24; pos = 10; neg = 14); and PgR-ICA in DCIS (n = 71; pos = 29; neg = 42; P = 0.00001) and minimal invasive carcinomas (n = 24; pos = 10; neg = 14).

with small, monomorphous nuclei, but occurred mainly in DCIS with large, pleomorphic nuclei which belonged to the poorly differentiated DCIS subgroup.

The median MIB1-LI for DCIS was 13.5%. When analysing the proliferation rates of the three subgroups, we found a consistent increase from well-differentiated to poorly differentiated DCIS. The median MIB1-LI of tumours composed of large, pleomorphic cells exceeded those of tumours composed of small cell DCIS by more than 6-fold (Figure 2).

ER (ER-ICA) and PgR (PgR-ICA) were determined in 71 cases. A total of 33 (46%) DCIS were ER-ICA positive and 29 (41%) were PgR-ICA positive. The distribution of steroid hormone receptor positivity within the subgroups is shown in Figure 1. A statistically significant correlation between ER and PgR expression and nuclear anaplasia could be demonstrated. The majority (79%) of well-differentiated DCIS were both ER-ICA and PgR-ICA positive. In contrast, only 22% of poorly differentiated DCIS were ER-ICA positive and even fewer (7%) were PgR-ICA positive (Figure 1).

In the group of intraductal carcinomas with minimal stromal invasion, the distribution of cell proliferation was similar to intermediately differentiated DCIS. Differences that occurred between DCIS and minimal invasive cancers with respect to this parameter did not reach statistical significance. p53 positivity was similar to poorly differentiated DCIS, and c-erbB-2 and steroid hormone receptor status was in between these two groups. Minimal invasive carcinomas had a higher proportion (38%) of p53 positive tumours than other DCIS and, in contrast to these, p53 positivity was also seen in tumours with intermediately differentiated intraductal component. This increase was statistically significant. The results obtained for p53, c-erbB-2 overexpression, MIB1 and steroid hormone receptor status are shown in Figures 1 and 2.

The association between p53 and c-erbB-2 expression, hormone receptor status and cell proliferation were evaluated. p53 positivity was significantly associated with an absence of steroid receptors, high cell proliferation (as indicated by higher MIB1-LI) and c-erbB-2 overexpression. Whereas p53 negative DCIS were evenly distributed with respect to ER and PgR status, there was only 1/9 (11%) p53 positive DCIS also positive for ER and 0/9 positive for PgR (n = 69; ER, P = 0.031; PgR, P = 0.008030). In p53 positive DCIS, cell proliferation rates exceeded the median MIB1-LI (13.5%) in 8/9 (89%) cases, while 38/62 (61%) p53 negative tumours had an MIB1-LI below the median (n = 71, P = 0.00873). 7/9 DCIS positive for p53 also overexpressed c-erbB-2, and 41/61 (67%) of p53 negative tumours were also negative for c-erbB-2 (n = 70, P = 0.023).

c-erbB-2 overexpression was also correlated with cell proliferation, with 22/27 (81%) DCIS with c-erbB-2 positivity having an MIB1-LI over the median (13.5%), and 30/43 (70%) c-erbB-2 negative DCIS having an MIB1-LI below the median (n = 70, P = 0.0000578). c-erbB-2 positivity was correlated with an absence of steroid receptors, with 24/27 (89%) DCIS positive for c-erbB-2 negative for ER, and 25/27 (93%) negative for PgR receptors. 28/41 (68%) and 26/41 (63%) of c-erbB-2 negative DCIS were positive for ER and PgR, respectively (n = 68; ER, P = 0.00000385; PgR, P = 0.00000304). The correlation between MIB1-LI and steroid receptors was also significant, with 24/35 (69%)

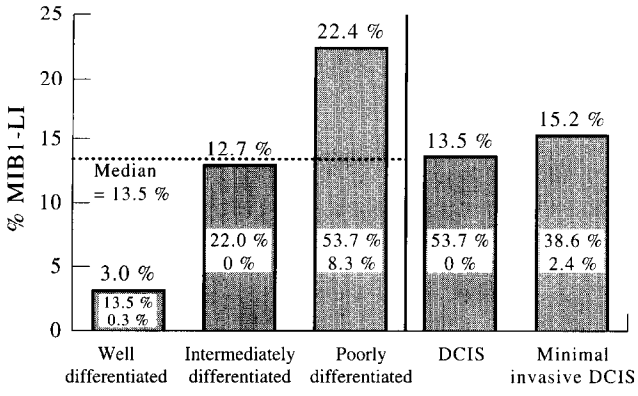

Figure 2. MIB 1 in DCIS ($n = 71$; $P < 0.00001$) and in minimal invasive carcinomas ($n = 24$). Percentages within bars give minimum and maximum labelling index within the group. Percentage on top of the bar gives median labelling index of the group.

DCIS with an MIB1-LI above the median (13.5%) also negative for ER, and 28/35 (80%) negative for PgR. 21/34 (62%) and 22/34 (65%) DCIS with an MIB1-LI below the median were positive for ER and PgR, respectively ($n = 69$; ER, $P = 0.012$; PgR, $P = 0.00023$).

The results of the comparison between different DCIS classifications are displayed in Table 1. Neither p53, c-erbB-2 expression nor steroid hormone receptor status were significantly associated with the amount of necrosis reflected by the comedo/non-comedo subtype. As mentioned before, p53 and c-erbB-2 overexpression were significantly correlated to nuclear polymorphism as was steroid hormone receptor status. The correlation between DCIS subtype and proliferative activity, however, remained statistically significant when subtyping was done by the presence/absence of necrosis. EORTC classification and nuclear grade showed nearly identical results.

DISCUSSION

Previous studies have thoroughly described prognostic factors and their relationship with each other in invasive breast cancer. Our study was undertaken in order to describe some of these well-known prognostic factors in noninvasive and minimal invasive breast cancer. In this study, no LCIS and 13% of the DCIS showed p53 positivity. We could find no previously published data describing p53 overexpression in a larger series of LCIS.

Our results for DCIS are in accordance with the literature. Several investigators have reported a proportion of p53 positive DCIS ranging between 13% and 25% [10–12]. The differences in percentages of p53 positive DCIS may be attributed to different cut-off levels as well as to different antibodies used, and histomorphological subtypes of the tumour sample may vary between different reports. In our series of DCIS with minimal stromal invasion, 38% of tumours were p53 positive. In the literature, the proportion of tumours overexpressing p53 increases in invasive breast cancers to 23–25% [10, 11, 13, 14], and p53 overexpression is associated with certain histomorphological tumour features, particularly nuclear anaplasia in both intraductal and infiltrating ductal carcinomas [11, 15, 16].

c-erbB-2 staining in LCIS was negative in 96%. Most previous reports have not found c-erbB-2 positivity in LCIS [17–19]. One study reports only one of 9 LCIS staining positive for c-erbB-2 [20].

In our series 38% DCIS were c-erbB-2 positive, which is lower than that reported in previous publications [12, 17, 19, 21]. This could be explained by a higher percentage of DCIS with higher nuclear grade in these series compared to ours, since all published data accordingly confirm the strong statistical correlation between histological tumour differentiation and c-erbB-2 overexpression. Several investigators have focused on c-erbB-2 overexpression in breast cancer progression. In contrast to p53 overexpression, which is maintained during tumour progression, there exists widespread agreement that c-erbB-2 overexpression is lower in infiltrating ductal carcinomas compared to DCIS. However, the strong association with high nuclear grade can be found in infiltrating ductal carcinomas as well [18, 19]. In our study, 50% of minimal invasive cancers were c-erbB-2 positive which did not differ significantly from pure DCIS. This could indicate that c-erbB-2 overexpression is maintained in early stages of stromal invasion.

Cell proliferation in LCIS was low compared with DCIS. In DCIS, the proliferative rate correlated with histomorphological subtype, especially nuclear anaplasia. MIB1-LI of tumours composed of large, pleomorphic cells exceeded those of tumours with small cells by more than 6-fold. Until recently, only a few reports have commented on the proliferative activity of non-invasive breast cancer [22]. Although in that study TLI was used for assessing growth fractions, the results are comparable with the results we obtained using MIB1.

Several study groups report that the strong association of histological tumour grade and cell proliferation can also be seen in infiltrating ductal carcinomas, even though they used different methods for measuring cell proliferation [13, 15, 16, 23]. As shown in a previous publication, the results obtained by TLI and Ki-67 are statistically significantly correlated [23].

Table 1. P *values of histoprognostic parameters in different DCIS schemes*

	EORTC	Polymorphous/non-polymorphous	Comedo/non-comedo
	P	*P*	*P*
ER	0.00156	0.00153	0.464
PgR	0.00000326	0.00000372	0.180
p53	0.00000516	0.00000927	0.138
MIB 1	0.00000124	0.00000207	0.025
c-erbB-2	0.00000921	0.000000621	0.092

ER and PgR status in LCIS was positive in the majority of cases (80% and 90%, respectively).

In DCIS a positive ER and PgR status was observed in less than 50%. Thus, the incidence of ER and PgR positivity in DCIS we demonstrated was slightly lower than in infiltrating ductal carcinomas, where 55–70% of the tumours are ER positive and 30–70% of the tumours are PgR positive [6].

The reports on steroid hormone receptors in both LCIS and DCIS yield controversial results. Furthermore, most published data on steroid hormone receptors in noninvasive breast cancer concentrate on ER alone. In LCIS, the rate of ER positive cases varies between 60% and 100% [24–26]. Data on PgR in LCIS are rare: one investigator reported 3 out of 5 LCIS to be PgR rich [24]. In the literature the proportion of ER positive DCIS varies between 33.5% and 75% [12, 24–27]. Until recently, only few investigators have evaluated PgR in DCIS. In a previous report, 30% of DCIS showed PgR positivity [24]. The wide range of steroid hormone receptor deviation can partly be attributed to different cut-off levels in immunohistochemical studies (5–25%), and partly to the application of other than immunohistochemical methods [24]. In addition, varying proportions of ER and PgR expression may be due to varying proportions of poorly differentiated, large cell DCIS included in the study.

A strong correlation between ER and PgR and nuclear anaplasia could be demonstrated. A similar inverse correlation has already been demonstrated in infiltrating ductal carcinomas. Immunohistochemically assessed ER and PgR expression decreased with increasing degree of nuclear anaplasia, indicating increasing cellular dedifferentiation [11, 13, 23, 28].

Our observation of the inverse relationship between p53 and c-erbB-2 expression, cell proliferation and both ER and PgR status and the direct relationship between p53 and c-erbB-2 expression are in keeping with data published on both DCIS and infiltrating ductal carcinomas [13–16, 20, 29]. Although several authors have also demonstrated a coincidental expression of p53 and c-erbB-2 [13, 16, 29], some studies have failed to find a significant correlation between these prognostic factors [12].

Finally, we compared different DCIS classifications with respect to the influence of subtyping on the significance and correlations between the various parameters. Nuclear grade was the best predictor for the presence or absence of the prognostic factors we used in our study. Similar observations and conclusions stressing the importance of nuclear grade as an indicator for cellular dedifferentiation and as a predictor for expression of several prognostic factors and biological behaviour can be found in the literature [1, 5, 25].

The histological tumour grading according to Bloom and Richardson [30] has been used as a prognostic factor in infiltrating ductal carcinomas for a long time and is still one of the most important predictive factors for tumour behaviour. Among the grading factors, nuclear grade reflecting nuclear anaplasia and, therefore, cell dedifferentiation, is the most important single factor [31, 32].

As can be demonstrated from our data for intraductal carcinomas, correlations with various prognostic parameters correlate most strongly to DCIS subclassification schemes which take into consideration nuclear pleomorphism. Therefore, we conclude that nuclear pleomorphism in DCIS could become a comparably important prognosticator in DCIS as it already is for infiltrating ductal breast cancer.

1. Patchefsky AS, Schwartz GF, Finkelstein SD, *et al.* Heterogeneity of intraductal carcinoma of the breast. *Cancer* 1989, **63**, 731–741.
2. Posner MC, Wolmark N. Non-invasive breast carcinoma. *Breast Cancer Res Treat* 1992, **21**, 155–164.
3. Lagios MD. Heterogeneity of ductal carcinoma *in situ* of the breast. *J Cell Biochem Suppl* 1993, **17G**, 49–52.
4. Recht A, van Dongen JA, Fentiman IS, *et al.* Third meeting of the DCIS working party of the EORTC (Fondazione Cini, Isola S. Giorgio, Venezia, 28 February 1994)—Conference report. *Eur J Cancer* 1994, **30A**, 1895–1901.
5. Holland R, Peterse JL, Millis RR, *et al.* Ductal carcinoma *in situ*: a proposal for a new classification. *Semin Diagnostic Pathol* 1994, **11**, 167–180.
6. Tavassoli FA. *Pathology of the Breast.* Norwalk, Connecticut, Appleton & Lange, 1992, 298–300.
7. Key G, Becker MHG, Bron B, *et al.* New Ki-67 equivalent murine monoclonal antibodies (MIB 1-3) generated against bacterially expressed parts of the Ki-67 cDNA containing three 62 base pair repetitive elements encoding for the KI-67 epitope. *Lab Invest* 1993, **68**, 629–636.
8. Banks L, Matlashewski G, Crawford L. Isolation of human p53 specific antibodies and their use in the study of human p53 expression. *Eur J Biochem* 1986, **159**, 529–534.
9. Corbett IP, Henry JA, Angus B, *et al.* NCL-CB11, a new monoclonal antibody recognizing the internal domain of the c-erbB-2 oncogene protein effective for use on formalin-fixed, paraffin-embedded tissue. *J Pathol* 1990, **161**, 15–25.
10. Davidoff AM, Kerns B-JM, Iglehart JD, *et al.* Maintenance of p53 alterations throughout breast cancer progression. *Cancer Res* 1991, **51**, 2605–2610.
11. Thor AD, Moore DH, Edgerton SM, *et al.* Accumulation of p53 tumor suppressor gene protein: an independent marker of prognosis of breast cancers. *J Natl Cancer Inst* 1992, **84**, 845–855.
12. Poller DN, Roberts EC, Bell JA, *et al.* p53 expression in mammary ductal carcinoma *in situ*: relationship to immunohistochemical expression of estrogen receptor and c-erbB-2 protein. *Hum Pathol* 1993, **24**, 463–468.
13. Barbareschi M, Leonardi E, Mauri FA, *et al.* p53 and c-erbB-2 expression in breast carcinomas: an immunohistochemical study including correlations with receptor status, proliferation markers, and clinical stage in human breast cancer. *Am J Clin Pathol* 1992, **98**, 408–418.
14. Davidoff AM, Herndon JE, Glover NS, *et al.* Relation between p53 overexpression and established prognostic factors in breast cancer. *Surgery* 1991, **110**, 259–264.
15. Cattoretti G, Rilke F, Andreola S, *et al.* p53 expression in breast cancer. *Int J Cancer* 1988, **41**, 178–183.
16. Martinazzi M, Crivelli F, Zampatti C, *et al.* Relationship between p53 expression and other prognostic factors in human breast carcinoma: an immunohistochemical study. *Am J Clin Pathol* 1993, **100**, 213–217.
17. Ramachandra S, Machin L, Ashley S, *et al.* Immunohistochemical distribution of c-erbB-2 in *in situ* breast carcinoma—a detailed morphological analysis. *J Pathol* 1990, **161**, 7–14.
18. Somerville JE, Clarke LA, Biggart JD. c-erbB-2 overexpression and histological type of *in situ* and invasive breast carcinoma. *J Clin Pathol* 1992, **45**, 16–20.
19. Bartkova J, Barnes DM, Millis RR, *et al.* Immunohistochemical demonstration of c-erbB-2 protein in mammary ductal carcinoma *in situ*. *Hum Pathol* 1990, **21**, 1164–1167.
20. Barnes DM, Meyer JS, Gonzalez JG, *et al.* Relationship between c-erbB-2 immunoreactivity and thymidine labelling index in breast carcinoma *in situ*. *Breast Cancer Res Treat* 1991, **18**, 11–17.
21. Allred DC, Clark GM, Molina R, *et al.* Overexpression of HER-2/neu and is relationship with other prognostic factors change during the progression of *in situ* to invasive breast cancer. *Hum Pathol* 1992, **23**, 974–979.

22. Meyer JS. Cell kinetics of histologic variants of *in situ* breast carcinoma. *Breast Cancer Res Treat* 1986, 7, 171–180.
23. Rudas M, Gnant MFX, Mittlböck M, *et al.* Thymidine labeling index and Ki-67 growth fraction in breast cancer: comparison and correlation with prognosis. *Breast Cancer Res Treat* 1994, **32**, 165–175.
24. Pallis L, Wilking N, Cedermark B, *et al.* Receptors for estrogen and progesterone in breast carcinoma *in situ*. *Anticancer Res* 1992, **12**, 2113–2116.
25. Bur ME, Zimarovsky MJ, Schnitt SJ, *et al.* Estrogen receptor immunohistochemistry in carcinoma *in situ* of the breast. *Cancer* 1992, **69**, 1174–1181.
26. Giri DD, Dundas SAC, Nottingham JF, *et al.* Oestrogen receptors in benign epithelial lesions and intraductal carcinomas of the breast: an immunohistochemical study. *Histopathology* 1989, **15**, 575–584.
27. Poller DN, Snead DRJ, Roberts EC, *et al.* Oestrogen receptor expression in ductal carcinoma *in situ* of the breast: relationship to flow cytometric analysis of DNA and expression of the c-erbB-2 oncoprotein. *Br J Cancer* 1993, **68**, 156–161.
28. Reiner A, Reiner G, Spona J, *et al.* Histopathologic characterization of human breast cancer in correlation with estrogen receptor status: a comparison of immunocytochemical and biochemical analysis. *Cancer* 1988, **61**, 1149–1154.
29. Ji H, Lipponen P, Aaltomaa S, *et al.* c-erbB-2 oncogene related to p53 expression, cell proliferation and prognosis in breast cancer. *Anticancer Res* 1993, **13**, 1147–1152.
30. Bloom HJ, Richardson WW. Histological grading and prognosis in breast cancer. *Br J Cancer* 1957, **11**, 359–377.
31. le Doussal V, Tubiana-Hulin M, Friedman S, *et al.* Prognostic value of histologic grade nuclear components of Scarff–Bloom–Richardson (SBR): an improved score modification based on a multivariate analysis of 1262 invasive ductal breast cancers. *Cancer* 1989, **64**, 1914–1921.
32. Schumacher M, Schmoor C, Sauerbrei W, *et al.* The prognostic effect of histological tumor grade in node-negative breast cancer patients. *Breast Cancer Res Treat* 1993, **25**, 235–245.