

Ki-67 staining in histological subtypes of breast carcinoma and fine needle aspiration smears

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Abstract

Thirty four cases of invasive breast carcinoma were analysed for heterogeneity of Ki-67 reactivity in a tumour, and proliferative activity in various histological subtypes was compared. The growth fractions determined in areas of central and peripheral tumour were the same. Mucinous and lobular carcinoma showed lower Ki-67 activity than ductal carcinomas. When ductal carcinomas were subdivided according to their dominant growth pattern, the carcinomas with a solid or comedo growth pattern showed the highest proliferative activity. These results largely confirm data from previous cell kinetic studies on the incorporation of radioactively labelled thymidine. A correlation between the growth fraction determined by Ki-67 in fine needle aspiration smears and cryostat sections of corresponding tumours was shown, implying that the immunostaining of cytological smears gives a reliable impression of the growth fraction of a tumour and may therefore be used in prospective studies.

Cell kinetic studies have shown that there is an association between the thymidine labelling index and the biological activity of breast carcinomas.^{1,2} Monoclonal antibody Ki-67, which defines nuclear antigen in proliferating cells, allows cells in the growth cycle to be detected without the need for time consuming methods such as external application of radioactively labelled nucleotides or mutagenic substances such as bromodeoxyuridine or iododeoxyuridine.³ This antibody is therefore used increasingly in studies on breast carcinoma to evaluate the growth fraction in relation to other known prognostic variables both in histological and cytological material.⁴⁻⁹

As assessment of the growth fraction determined by Ki-67 immunostaining may be implicated in the prognosis of a patient it is essential to follow standardised procedures. Because it is not known to what extent discrepant data may arise due to heterogeneity of the distribution of proliferating cells in a tumour, we assessed the prevalence of Ki-67 positive cells in the peripheral and central areas of tumour. Furthermore, we determined the association between different histological subtypes and the level of Ki-67 reactivity.

Because fine needle aspiration (FNA)

smears are increasingly being used in the diagnosis of breast carcinoma and consequently for immunocytochemical analysis, we assessed the reliability of Ki-67 immunostaining of FNA smears, by comparing the results found in histological sections with those found in cytological smears of the same tumour.

Methods

Thirty four cases of invasive breast carcinoma were evaluated. The tumours were classified according to the WHO classification.¹⁰ They included 21 ductal, six lobular, four mixed type (ductal/lobular) and three mucinous carcinomas. The 21 ductal carcinomas were consecutively classified according to the dominant growth pattern: two cribriform, three solid, four comedo, two mixed solid/comedo and 10 not otherwise specified (NOS). The comedo carcinoma was defined as a ductal in situ component in an otherwise invasive carcinoma.

A complete cross-section of resected tumour was snap frozen in liquid nitrogen. The areas of central and peripheral tumour were assessed separately.

Frozen tissue samples on glass slides coated with poly-L-lysine¹¹ and FNA smears on uncoated glass slides were air dried and subsequently fixed in acetone (for 10 minutes). After rinsing in phosphate buffered saline (PBS) Ki-67 (Dako, Denmark) was applied (dilution 1 in 5 in PBS with 0.02% gelatine, one hour). After rinsing with PBS a horse-radish peroxidase conjugated polyclonal rabbit anti-mouse antibody (Dako, Denmark) was used as second step reagents for half an hour. After rinsing with PBS the reaction product was visualised using diaminobenzidine as substrate.

After a final wash nuclear counterstaining was achieved by incubation in Mayer's haematoxylin for one minute. Positive and negative controls were included.

Brown speckled staining of nuclei or nucleoli was regarded as a positive reaction. This positivity was semiquantitatively assessed in tissue sections by counting at least 300 cells in the areas with highest proliferative activity, similar to the procedure described for mitotic counting.¹² In 26 cases this was done both in the central and peripheral areas of the tumour specimen.

When the invasive ductal carcinomas showed special growth patterns, these were counted separately. In FNA smears 500 cells were counted at random at a magnification of

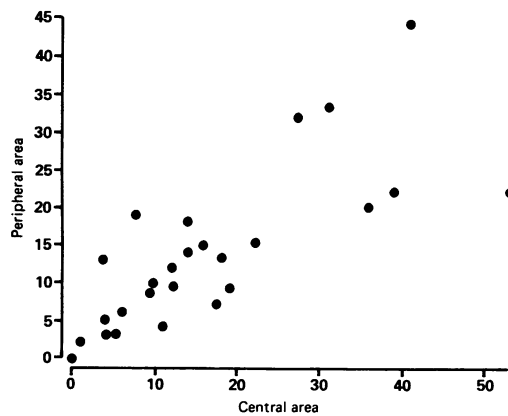
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Accepted for publication
27 September 1990

Figure 1 The correlation between the growth fraction of central and peripheral areas in frozen cross-sections of breast carcinoma determined by Ki-67.



1000 × under oil immersion and the percentage of positive nuclei was determined.⁸

The Ki-67 score of 14 FNA smears was compared with that of the frozen sections of corresponding tumours.

Results

In 25 carcinomas the Ki-67 reactivity was assessed both in the central and peripheral areas of the same tumour. Although the central areas on average reached somewhat higher values than the more peripheral ones, a strong correlation (0.78) between the two areas was seen (fig 1).

Figure 2 shows the Ki-67 reactivity of the 34 carcinomas classified according to the WHO. The mucinous carcinomas contained a low percentage of Ki-67 stained nuclei, with a mean of 10%, whereas the ductal carcinomas showed much higher values, with a mean of 23%. The lobular and mixed carcinomas showed intermediate values of 18% and 15%, respectively. Figure 2C shows the Ki-67 determined growth fraction of the ductal carcinomas according to their dominant growth pattern. Comedo and solid growth patterns showed mean values of Ki-67 staining of 33% and 31%, respectively. The NOS carcinomas showed a mean value of 19%. Of the two ductal carcinomas with a dominant cribriform growth pattern, one showed no reactivity at all, while the other one showed Ki-67 staining of 10%.

In 14 carcinomas the Ki-67 reactivity in FNA smears and frozen tissue sections of the same tumour correlated (0.71) (fig 3). In FNA smears on average lower Ki-67 values were obtained than sections of corresponding tumours. Nevertheless, a high score on FNA smears was usually associated with a high score on frozen sections (fig 4).

Figure 2 The growth fraction of invasive carcinoma determined by Ki-67 and classified according to the WHO. (A) mucinous (n = 3); (B) lobular (n = 6); (C) ductal* (type 1, solid n = 5; type 2, cribriform n = 2; type 3 comedo, n = 6; type 4 NOS, n = 10); and (D) mixed lobular ductal (n = 4). *Two showed both a solid and comedo growth pattern.

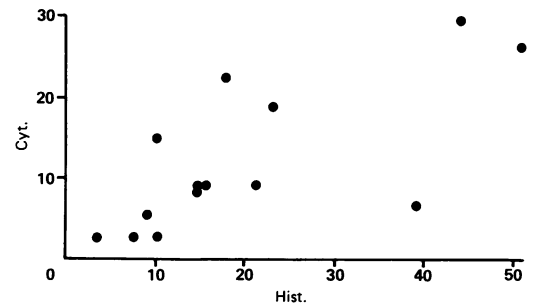
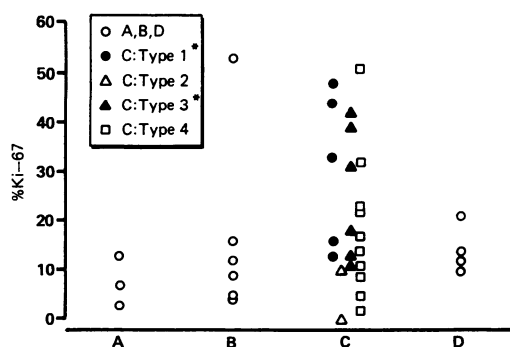


Figure 3 Correlation between the Ki-67 determined growth fraction assessed in frozen sections and in fine needle aspiration smears of the same tumour (n = 4).

Discussion

This study shows that similar levels of Ki-67 immunostaining are found both in central and peripheral areas of the tumour (fig 1). This result is at variance with the understanding that most cellular areas, and therefore the areas where active growth is most likely, are normally found at the periphery of a tumour.^{12,13} In our view this only holds true when the central area of the tumour is highly sclerotic due to poor vascularisation.

In this series the four histological types of breast carcinoma displayed different proliferative activity as defined by Ki-67, the ductal carcinomas showing the highest activity, the mucinous the lowest. This agrees with published findings,¹⁴⁻¹⁷ which state that ductal carcinomas are the most aggressive tumours compared with lobular and mucinous carcinomas, the latter two being slowly proliferating invasive carcinomas with a long survival. Similar results were obtained in the cell kinetic studies done by Meyer,¹ where both lobular and mucinous breast carcinomas showed low thymidine labelling indices. Furthermore, Lelle *et al* compared the growth fraction, as determined by Ki-67, of ductal and lobular carcinomas and found lower values in the latter.⁶

The various growth patterns of the invasive ductal carcinomas also show large differences in their proliferative activity (fig 2C). The two cribriform carcinomas showed the lowest levels of Ki-67 immunostaining. Similarly, Meyer observed low thymidine labelling indices in the intraductal carcinomas with a cribriform growth pattern.¹⁸ The same carcinomas have an excellent prognosis.^{16,19} In contrast, the in situ comedo component in otherwise invasive ductal carcinomas showed high levels of Ki-67 immunostaining comparable with the high thymidine labelling indices in intraductal comedo carcinomas.¹⁸ Surprisingly, we found similar values both in comedo and solid carcinomas; Meyer observed low labelling indices in solid intraductal carcinomas comparable with the ones found in cribriform intraductal carcinomas.

Like Lelle *et al* we found, on average, lower Ki-67 scores in FNA smears than in frozen sections.⁶ This may have been due to a different sampling and counting method. Using fine needle aspiration, cells are obtained from all parts of the tumour and counting is done

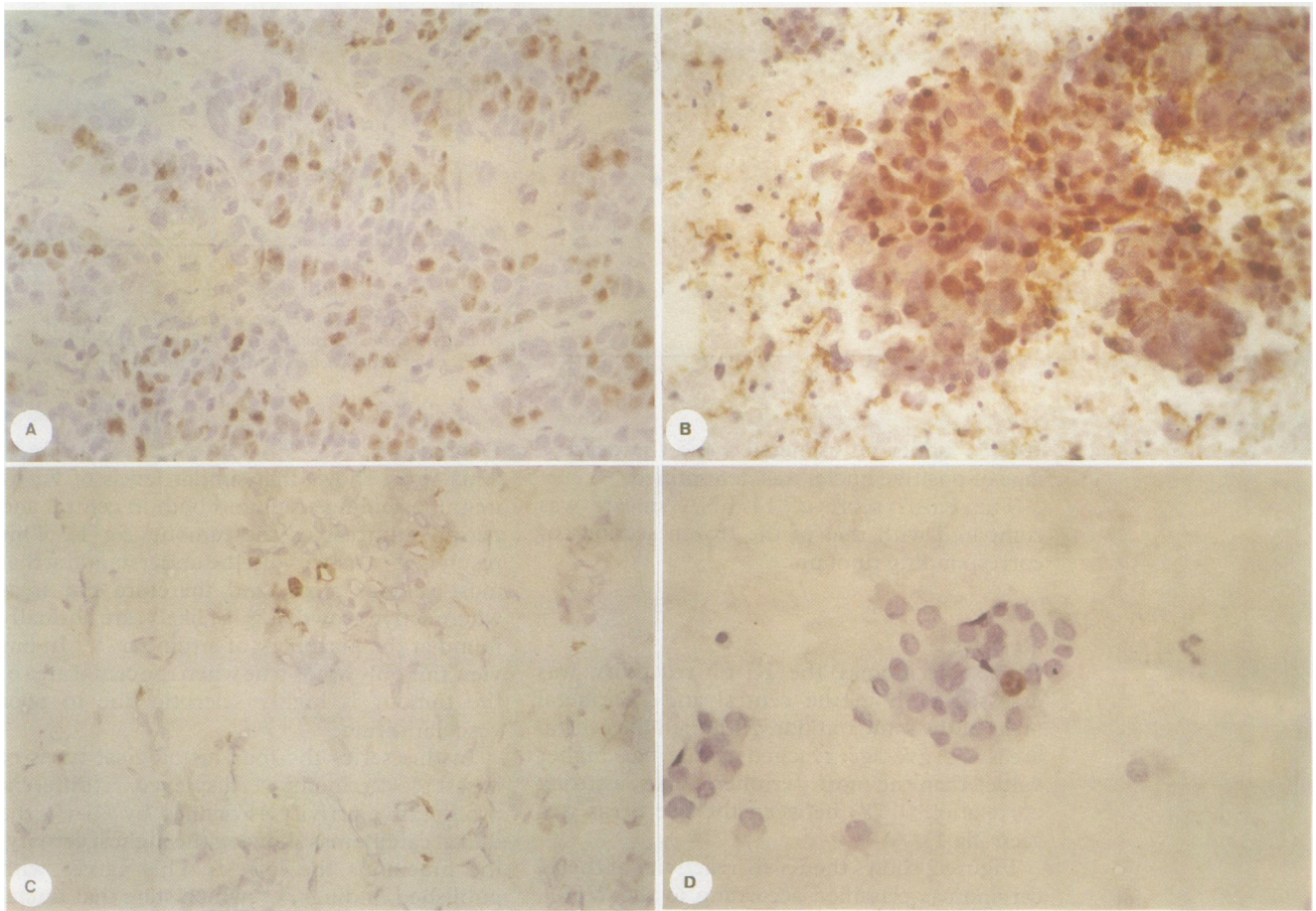


Figure 4 Comparison of Ki-67 immunostaining of frozen sections (A, C) and FNA smears (B, D) of the same tumour. A + B: ductal carcinoma, C + D: mucinous carcinoma.

randomly; frozen sections give information about only one part of the tumour and proliferative activity is determined by the areas with highest activity. Consequently, smears may give a better impression of the average proliferative activity of a tumour than histological sections.

Our results on breast cancer are in line with those found by Brown *et al.*²⁰ These authors compared cryostat sections of non-Hodgkin's lymphomas with FNA smears of corresponding tumours and found an excellent correlation.

Early detection of breast cancer as a result of screening programmes will result in smaller amounts of malignant tissue available for additional techniques. The feasibility of immunocytochemical detection of proliferative activity on FNA smears shown here allows the prognostic markers on these small sized tumours to be assessed.

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