Pergamon

European Journal of Cancer, Vol. 34, No. 4, pp. 548–553, 1998

Printed in Great Britain
0959-8049/98 $19.00+0.00

PII: S0959-8049(97)10072-7

Original Paper

Tumour Grade Does Not Change Between Primary and Recurrent Mammary Carcinoma

R.R. Millis, D.M. Barnes, O.T. Lampejo,* M.K. Egan and P. Smith

Imperial Cancer Research Fund, Clinical Oncology Unit, Guy's Hospital, St Thomas' Street, London SE1 9RT, U.K.

The primary tumour grade in 115 patients with infiltrating ductal carcinoma of the breast was compared with the type of the ductal carcinoma *in situ* (DCIS) component and with the grade of 169 locally recurrent and metastatic lesions. 102 patients had axillary lymph node metastases at the time of primary surgery, 49 had subsequent recurrences and 36 had both. There was concordance of grade between the primary tumour and axillary lymph node metastases and with subsequent locally recurrent and metastatic lesions. The type of the DCIS component was also significantly associated with the grade of the infiltrating component. No evidence of progression of tumour grade between these phases of mammary carcinoma was found. © **1998 Elsevier Science Ltd. All rights reserved.**

Key words: histological grade, malignancy progression, mammary carcinoma
Eur J Cancer, Vol. 34, No. 4, pp. 548–553, 1998

INTRODUCTION

FACTORS INVOLVED in the progression of breast cancer from the *in situ* phase to invasion and from invasion to the development of metastases are of obvious importance. It is not known whether such factors occur within the malignant cells, within the surrounding host tissue or both. Most biological markers which have been examined within the *in situ* and invasive components of a carcinoma, such as hormone receptor expression, oncoprotein expression and DNA ploidy, appear to be similar in any one tumour [1–4]. There is also usually good agreement between the expression of such markers within primary tumours and their metastases [5–8]. These findings suggest that events such as overexpression of oncogenes occur early in the development of malignancy. Other features of malignancy, however, may progress with time.

It has been suggested that one of the reasons for the success of breast cancer screening programmes in reducing mortality from this disease is that many tumours are detected whilst they are still of a relatively low malignant potential and before they develop more aggressive characteristics which lead to metastases and death [9–12]. This could explain the high proportion of grade I tumours seen in breast cancer screening programmes as compared with tumours presenting symptomatically.

Histological tumour grade is one of the major prognostic indicators of invasive mammary carcinoma. Prediction of the behaviour of mammary carcinoma *in situ* has been less well evaluated and is currently of considerable interest as this latter condition is now being diagnosed more frequently due to the widespread use of mammography. The traditional classification based on architectural growth pattern has proved unsatisfactory for several reasons. There is not only marked interobserver variation but also little correlation with the subsequent development of recurrence or progression to invasion in patients treated by breast conservation therapy. Recently, several new classifications have been proposed [13], one of which is based on nuclear morphology and architectural differentiation or cellular polarisation [14]. These features are similar to those used in the grading of infiltrating carcinomas. It divides ductal carcinoma *in situ* (DCIS) into three groups: poorly, intermediately and well differentiated.

In a previous study of the relationship between the type of DCIS component and the grade of the infiltrating component in tumours in which both were present, we found a significant correlation between DCIS type and tumour grade [15]. This suggests that there is no change in grade of malignancy as tumours progress from an *in situ* to an invasive phase. This study has now been extended to include locally recurrent and metastatic tumours. These have been graded using the same histological criteria as used for grading invasive primary

Correspondence to D.M. Barnes.
Received 20 Jun. 1997; revised 30 Aug. 1997; accepted 3 Sep. 1997.
*Present address: Division of Anatomic Pathology, University of Alabama at Birmingham, Birmingham, Alabama, U.S.A.

carcinoma. The grade of the metastasis has then been compared with the grade of the infiltrating component of the primary tumour.

MATERIALS AND METHODS

All the patients in this study had been included in a previous report correlating the DCIS type with the grade of infiltrating carcinoma [15]. They all had infiltrating primary mammary carcinoma, Manchester stage I and II, diagnosed between 1980 and 1982 in the Imperial Cancer Research Fund, Breast Unit at Guy's Hospital. All had been treated by either a total mastectomy with axillary lymph node clearance or breast conservation consisting of excision of the tumour, complete axillary lymph node clearance and radiotherapy.

Only patients with an infiltrating ductal carcinoma of no specific type (NST) in which there was also an *in situ* component were included in this and the previous study. Infiltrating ductal carcinoma NST comprises approximately 75% of all infiltrating carcinomas seen in the Unit. An *in situ* component is present in approximately 80% of such tumours. In the current study, the patients were further selected to include only those with recurrent or metastatic tumour available for histological review. These consisted of a metastasis within axillary lymph nodes removed at the time of primary treatment and subsequent locally recurrent or metastatic lesions at various sites of the body. Recurrent carcinoma within the breast and subsequent contralateral mammary carcinomas were not included, as some of these represent new primary tumours.

One hundred and seventy-seven locally recurrent or metastatic tumours were examined. In 8 cases the lesion was too small or too distorted to allow accurate grading, thus leaving 169 recurrences which occurred in 115 patients. 102 patients had axillary lymph node metastases at the time of diagnosis, 49 had one or more subsequent locally recurrent or metastatic lesions at other sites and in 36 there were both. The subsequent lesions occurred in the skin over the chest wall (22), in supraclavicular lymph nodes (41) and at other sites (4). All the tissue received in the laboratory was handled in a standard manner. The specimens were collected immediately after removal from the patient and were dissected and fixed straight away.

Haematoxylin and eosin (H&E) stained histological sections of the locally recurrent and metastatic tumours were reviewed for the study. In the previous study, the DCIS type and the grade of the infiltrating component of the primary tumour were assessed in the same section, so to avoid bias, the grade was taken from the original histological report. The tumours had been divided into three grades (I, II and III) using the modified criteria of Bloom and Richardson [16, 17]. All of the cases had been reported by one of the authors (RRM). The DCIS component had been classified into three groups (well, intermediately and poorly differentiated) according to criteria described by Holland and associates [14]. This was carried out by two of the authors (RRM and OTL) and where there was disagreement this was settled by reviewing the cases together using a double-headed microscope. This classification is based primarily on nuclear morphology and to a lesser extent on architectural differentiation (polarisation of cells). In a few cases where more than one DCIS type was present, the lesion was classified according to the most poorly differentiated. For the present study, the grade of the metastatic tumour was assessed using the same criteria as for the primary tumour by one of the authors (RRM) without knowledge of the DCIS type or the grade of the primary tumour.

Statistics

Spearman's ranked correlation was used to determine the relationship between the grade of the infiltrating component of the primary tumour and the DCIS type. The kappa statistic was used to determine the measure of agreement between the grade of the primary tumour and the metastases. Table 1 shows the strength of agreement as determined by the kappa statistic [18]. For example, a kappa value equal to or less than 0.20 indicates a poor level of agreement, whereas a value equal to or greater than 0.81 indicates a very good strength of agreement.

RESULTS

The relationship between the DCIS type and the grade of the associated infiltrating carcinoma is shown in Table 2. Well differentiated DCIS was associated with grade I infiltrating carcinomas and, in the majority of patients, intermediately differentiated DCIS was associated with grade II infiltrating carcinoma. The infiltrating carcinoma associated with poorly differentiated DCIS was almost equally divided between grade II and grade III. In 2 cases, however, the associated carcinoma was grade I (Spearman rank correlation $r = 0.57$, $P < 0.0001$).

The relationship between the grade of the primary tumour and that of the lymph node metastases diagnosed at the time of initial surgery is shown in Table 3, and with subsequent recurrences in Table 4. There were few patients with grade I primaries who had nodal metastases, but in 6 of the 8 cases where this occurred the metastases were also grade I. Only 1 patient with a grade I primary carcinoma had a subsequent recurrence. In this case there was a synchronous chest wall recurrence and supraclavicular nodal metastasis and both were also grade I (Figure 1). The majority of patients with grade II primaries had grade II nodal secondaries and recurrences and most of those with grade III primaries had grade III nodal metastases and recurrences (Figure 2).

Table 1. *Kappa statistic values*

Value of kappa	Strength of agreement
< 0.20	Poor
0.21–0.40	Fair
0.41–0.60	Moderate
0.61–0.80	Good
≥ 0.81	Very good

Table 2. *Relationship between DCIS type and grade of infiltrating component*

	Grade of infiltrating component		
	I	II	III
DCIS type			
Well differentiated	3	0	0
Intermediately differentiated	4	15	1
Poorly differentiated	2	41	49

DCIS, ductal carcinoma *in situ*. Spearman rank correlation $r = 0.57$, $P < 0.0001$.

Table 3. *Relationship between grade of primary tumour and grade of nodal metastases*

	Grade of nodal metastases		
	I	II	III
Grade of primary tumour			
I	6	2	0
II	2	41	8
III	0	11	32

Kappa = 0.60, standard error = 0.073, 95% confidence interval, 0.46–0.74, proportion of agreement expected by chance = 0.44.

Table 4. *Relationship between grade of primary tumour and grade of subsequent recurrences*

	Grade of subsequent recurrences		
	I	II	III
Grade of primary tumour			
I	1	0	0
II	1	19	2
III	0	8	18

Kappa = 0.58, standard error = 0.11, 95% confidence interval, 0.36–0.80, proportion of agreement expected by chance = 0.46.

In the 49 patients with both an axillary node metastasis and a subsequent recurrent lesion available for histological evaluation (although the number was small), there was still moderate agreement between the grade of the nodal metastases and the subsequent recurrence (Table 5).

There were too few patients with more than one recurrence to make statistical analysis meaningful. However, of the 7 patients with grade II primaries, 5 had subsequent asynchronous recurrences which were all grade II; 1 had two subsequent asynchronous grade I recurrences and the other one grade II and a later grade III recurrence. Of the 6 patients with grade III primaries there were 3 with grade III and 3 with grade II first and subsequent recurrences.

DISCUSSION

A number of groups [19–21] including ourselves [15] have found a strong correlation between the grade or type of DCIS and the grade of infiltrating mammary carcinoma in tumours in which both components are present. Well differentiated, or low grade, DCIS is associated with well differentiated infiltrating carcinoma and poorly differentiated, or high grade, DCIS with either intermediately or poorly differentiated infiltrating carcinoma.

This homogeneity of grade between DCIS and invasive components of the same tumour suggests that there is no progression of the morphological features of malignancy with the development of invasion. The findings in the present study, that in the majority of patients the concordant lymph node metastases and subsequent local recurrences or distant metastases are of similar grade to the primary carcinoma and to each other, suggest that progression of grade does not occur at this stage either.

Although several grading systems have been described for invasive mammary carcinoma, the one most widely used is based on that of Bloom and Richardson [16] as modified by Elston [17, 22]. In this system, tumours are divided into three grades on the basis of evaluation of three features: growth pattern (tubule formation), nuclear morphometry (pleomorphism) and the number of mitotic figures.

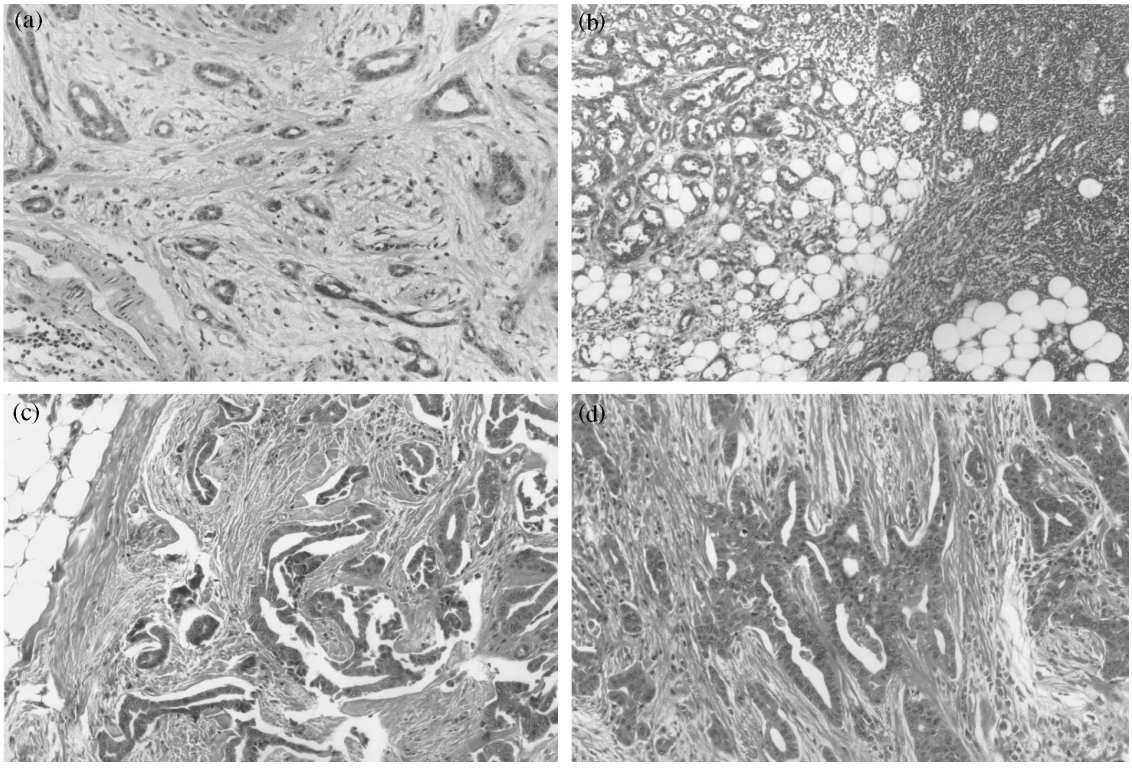

Figure 1. Infiltrating ductal carcinoma grade I: (a) primary, (b) axillary lymph node metastasis, (c) subsequent mastectomy scar recurrence and (d) subsequent supraclavicular lymph node metastasis (H & E × 200).

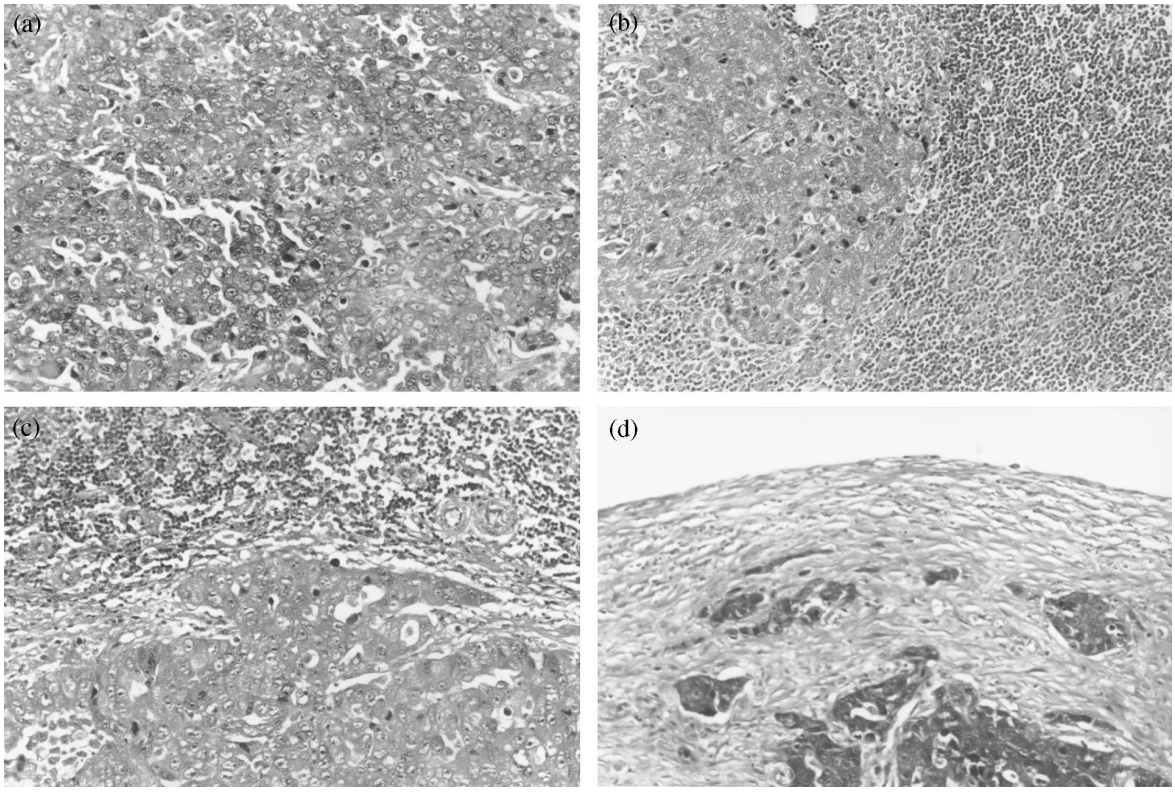

Figure 2. Infiltrating ductal carcinoma grade III: (a) primary, (b) axillary lymph node metastasis, (c) subsequent contralateral axillary lymph node metastasis and (d) subsequent pleural metastasis (H & E × 200).

Pathologists are familiar with the fact that mitoses are usually most marked at the growing edge of the tumour and tubule formation is usually most marked in the centre, both of which suggest that tumours are more malignant at the periphery. The sparsity of mitoses in the centre could, however, be explained by anoxia. It is also possible that during the formation of tubules the malignant cells initially grow in trabeculae and clumps, which subsequently develop into recognisable tubules. Although when grading a carcinoma the appearance of the whole tumour is taken into account in order to evaluate tubule formation, mitotic figures are counted in the area of highest mitotic activity and nuclear pleomorphism is assessed on the basis of the most pleomorphic area of the tumour. Therefore, tumours which have mixed features are more likely to be graded higher than lower. In general, however, although some tumours do show variation in the morphological features of differentiation from one area to another, most are fairly uniform throughout.

A few mammary carcinomas are composed of a mixture of histological types, such as mixed mucinous carcinomas, and in this case there is more likely to be a difference in the grade of the two components [23]. The mucinous area is quite often better differentiated than the non-mucinous area and metastases from such tumours usually resemble the non-mucinous rather than the mucinous component. Interestingly, during the course of this study, we encountered 1 patient with a mixed mucinous and ductal NST primary carcinoma who had an axillary nodal metastasis at the time of surgery which was malignancy grade III, as was the ductal NST component of the primary carcinoma. A subsequent local recurrence in the mastectomy scar, however, consisted of well differentiated mucinous carcinoma similar to the mucinous component of the primary tumour (Figure 3).

Table 5. *Relationship between grade of nodal metastases and grade of subsequent recurrences*

	Grade of subsequent recurrences		
	I	II	III
Grade of nodal metastases			
I	1	0	0
II	0	16	2
III	0	6	11

Kappa = 0.58, standard error = 0.13, 95% confidence interval, 0.32–0.88, proportion of agreement expected by chance = 0.48.

Metastases from any carcinoma usually closely resemble their primary tumour. Indeed it is standard practice to compare the appearance of the metastasis with that of the primary tumour from which it is thought to have arisen, in order to ensure that its appearance is compatible with its derivative there from. There are few studies, however, in which the grade of metastases has been formally studied. Bloom and Richardson [16] and Haagensen [24] found agreement between the grade of primary tumour and metastatic tumour in 82% and 71% of cases, respectively. Fisher [25] reported almost complete agreement. In a comparative study of various factors in primary and metastatic breast cancer, Hitchcock and colleagues [26] found that grade remained constant in 20 of 36 cases (56%). In the cases with a change, there was no consistent trend in the direction of change.

On the basis of the reduction of mortality seen in breast cancer screening programmes, and of histological evaluation of breast cancers detected by mammographical screening, Duffy and Tabàr suggest that there is a progression of tumour

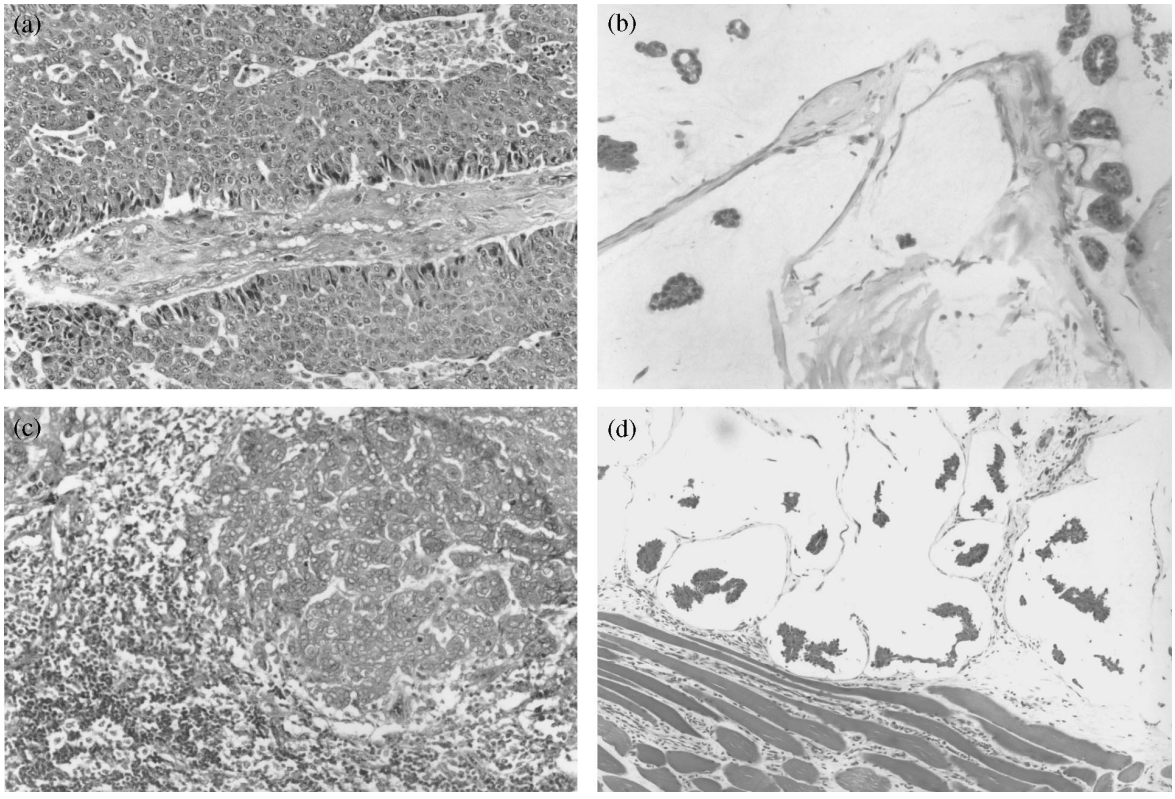

Figure 3. Mixed infiltrating ductal carcinoma grade III and mucinous carcinoma: (a) primary non-mucinous component, (b) primary mucinous component, (c) axillary lymph node metastasis and (d) subsequent mastectomy scar recurrence (H & E × 200).

grade with time [9–11]. Screen-detected carcinomas are smaller and of lower grade when compared with those in non-screened women. Duffy and Tabàr believe that as tumours grow larger they become less well differentiated, possibly because they are heterogeneous and poorly differentiated components grow at the expense of well differentiated ones. They point out that others have demonstrated such heterogeneity within tumours in terms of thymidine index, steroid hormone receptors and DNA index [27]. In a study to test their hypothesis, they found that the significant increase in grade III tumours noted in non-screened women was lost if the study was controlled for tumour size, indicating that the grade III tumours were all larger tumours. Their view on tumour progression is supported by Tubuana and Koscienly [28] who observed a similar relationship between tumour grade and size.

The phenomenon of progression of malignancy, or phenotypic drift of tumours, is disputed by Blamey [29], who, on re-analysis of the Swedish data, found no increase in grade III tumours diagnosed in the non-screened population at the end of the study (observed/expected ratio: grade I 1:9; grade II 1:4; grade III 1:0). In addition, Fisher and associates [30] have noted fewer poorly differentiated carcinomas among patients with the longest duration of symptoms even though such tumours are larger or associated with other characteristics which might suggest a later stage in the development of their disease.

In a recent paper, Tabàr and colleagues [12] proposed that some tumours are capable of progression but others are not. Using a modelling system, they estimated the sojourn time according to age and histological type of tumour and assessed the evidence for a change of malignancy grade and also the proportion of tumours which are capable of progression. They estimated that 91% of tumours are capable of progression in women aged 40–54 years, but only 38% are so capable in women aged 55–69 years.

The question of progression of malignancy is vital to the diagnosis and treatment of breast cancer and whether or not systemic therapy should be given to all patients, even those with small screen-detected carcinomas. In the past decade, Fisher [31] has suggested that breast cancer is systemic at the time of diagnosis and that tumours which are going to metastasise do so early in their evolution and so there is little point in trying to diagnose them earlier by screening. The theory of Duffy and Tabàr and their colleagues upholds the value of screening which they consider detects tumours when they are smaller and better differentiated and less malignant with less metastatic potential. They believe that many cancers become systemic with viable metastases developing between the time that they can be diagnosed by mammography and the time at which clinical diagnosis usually occurs [11].

Whether or not breast cancer becomes less differentiated with time remains unanswered, but the findings in the present study suggest that there is little change in the morphological features of malignancy of mammary carcinoma between the *in situ*, invasive, locally recurrent and metastatic phases and certainly there is no trend for a progression of grade.

1. Barnes R, Masood S. Potential value of hormone receptor analysis in carcinoma *in situ* of breast. *Am J Clin Pathol* 1990, **94**, 533–537.

2. Iglehart JD, Kerns B-J, Huper G, Marks JR. Maintenance of DNA content and erbB_2 alterations in intraductal and invasive phases of mammary cancer. *Breast Cancer Res Treat* 1995, **34**, 253–263.
3. Maguire HC, Hellman ME, Greene MI, Yeh I. Expression of c-erbB-2 in *in situ* and in adjacent invasive ductal adenocarcinomas of the female breast. *Pathobiology* 1992, **60**, 117–121.
4. Umekita Y, Takasaki T, Yoshida H. Expression of p53 protein in benign epithelial hyperplasia, atypical ductal hyperplasia, non-invasive and invasive mammary carcinoma: an immunohistochemical study. *Virchows Archiv* 1994, **424**, 491–494.
5. Iglehart JD, Kraus MH, Langton BC, Huper G, Kerns B-J, Marks JR. Increased *erb*B_2 gene copies and expression in multiple stages of breast cancer. *Cancer Res* 1990, **50**, 6701–6707.
6. Robertson JFR. Oestrogen receptor: a stable phenotype in breast cancer. *Br J Cancer* 1996, **73**, 5–12.
7. Bartkova J, Bartek J, Vojtesek B, *et al.* Immunochemical analysis of the p53 oncoprotein in matched primary and metastatic human tumours. *Eur J Cancer* 1993, **29A**, 881–886.
8. Agthoven T van, Timmermans M, Dorssers LCJ, Henzen-Logmans SC. Expression of estrogen, progesterone and epidermal growth factor receptors in primary and metastatic breast cancer. *Int J Cancer* 1995, **63**, 790–793.
9. Duffy SW, Tabàr L, Fagerberg G, *et al.* Breast screening, prognostic factors and survival—results from the Swedish two county study. *Br J Cancer* 1991, **64**, 1133–1138.
10. Tabàr L, Fagerberg G, Duffy SW, Day NE, Gad A, Gröntoft O. Update of the Swedish two county program of mamographic screening for breast cancer. *Radiol Clin North Am* 1992, **30**, 187–210.
11. Tabàr L, Fagerberg G, Day NE, Duffy SW, Kitchin RM. Breast cancer treatment and natural history: new insights from results of screening. *Lancet* 1992, **339**, 412–414.
12. Tabàr L, Fagerberg G, Chen HH, Duffy SW, Gad A. Tumour development, histology and grade of breast cancers: prognosis and progression. *Int J Cancer* 1996, **66**, 413–419.
13. Millis RR. Classification of ductal carcinoma *in situ* of the breast. *Adv Anat Pathol* 1996, **3**, 114–129.
14. Holland R, Peterse JL, Millis RR, *et al.* Ductal carcinoma *in situ*: a proposal for a new classification. *Semin Diagn Pathol* 1994, **11**, 167–180.
15. Lampejo OT, Barnes DM, Smith P, Millis RR. Evaluation of infiltrating carcinomas with a DCIS component: correlation of the histologic type of the *in situ* component with grade of the infiltrating component. *Semin Diagn Pathol* 1994, **11**, 215–222.
16. Bloom HJG, Richardson WW. Histological grading and prognosis in breast cancer. *Br J Cancer* 1957, **11**, 359–377.
17. Elston CW, Gresham GA, Rao GS, *et al.* The Cancer Research Campaign (King's/Cambridge) trial for early breast cancer: clinico-pathological aspects. *Br J Cancer* 1982, **45**, 655–669.
18. Altman D. *Practical Statistics for Medical Research*. London, Chapman & Hall, 1991.
19. Moriya T, Silverberg SG. Intraductal carcinoma (ductal carcinoma *in situ*) of the breast. *Cancer* 1994, **74**, 2972–2978.
20. Douglas-Jones AG, Gupta SK, Attanoos RL, Morgan JM, Mansel RE. A critical appraisal of six modern classifications of ductal carcinoma *in situ* of the breast (DCIS): correlation with grade of associated invasive carcinoma. *Histopathology* 1996, **29**, 397–409.
21. Goldstein NS, Murphy T. Intraductal carcinoma associated with invasive carcinoma of the breast. *Am J Clin Pathol* 1996, **106**, 312–318.
22. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow up. *Histopathology* 1991, **19**, 403–410.
23. Clayton F. Pure mucinous carcinomas of breast. *Human Pathol* 1986, **17**, 34–38.
24. Haagensen CD. The bases for the histological grading of carcinoma of the breast. *Am J Cancer* 1933, **19**, 285–327.
25. Fisher ER. Prognostic value of histopathology in breast and prostatic cancer. *Rev Endocrine-related Cancer* 1982, **11**, 11–14.
26. Hitchcock A, Ellis IO, Robertson JFR, *et al.* An observation of DNA ploidy, histological grade and immunoreactivity for tumour-related antigens in primary and metastatic breast carcinoma. *J Pathology* 1989, **159**, 129–134.
27. Meyer JS, Wittliff JL. Regional heterogeneity in breast carcinoma: thymidine labelling index, steroid hormone receptors, DNA ploidy. *Int J Cancer* 1991, **47**, 213–220.
28. Tubiana M, Koscielny S. Natural history of human breast cancer: recent data and clinical implications. *Breast Cancer Res Treat* 1991, **18**, 125–140.
29. Blamey RW. Phenotypic drift does not happen but lots of interesting things do. *The Breast* 1993, **2**, 190.
30. Fisher ER, Redmond C, Fisher B. A perspective concerning the relation of duration of symptoms to treatment failure in patients with breast cancer. *Cancer* 1977, **40**, 3160–3167.
31. Fisher B. Laboratory and clinical research in breast cancer—a personal adventure. *Cancer Res* 1980, **40**, 3863–3874.