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Acknowledgements—We thank Mrs Janice Davidson for help in the statistical analysis and Miss Carolyn Victor-Smith for typing the manuscript.

Eur J Cancer, Vol. 27, No. 12, pp. 1555–1559, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00
Pergamon Press plc

Prognostic Factors in Axillary Lymph Node-negative (pN−) Breast Carcinomas

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Axillary lymph node-negative (pN−) breast carcinomas (n = 281) were analysed histoquantitatively for two mitotic indexes (MAI, mitotic activity index; M/V, volume corrected mitotic index) and nine nuclear factors with special emphasis on disclosing prognostic factors during a follow-up of 12 years. The M/V index (P = 0.0018), tumour size (P = 0.0052), MAI (P = 0.0115) and histological grade (P = 0.0565) predicted the recurrence-free survival. MAI (P = 0.0007), M/V index (P = 0.0046), tumour size (P = 0.0133), histological grade (P = 0.0528) and S.D. of the nuclear perimetry (P = 0.07) predicted the disease-related survival. In Cox's analysis, MAI (P = 0.004), adjuvant therapy (P = 0.03) and tumour size (P = 0.09) predicted survival independently. Recurrence-free survival was related independently to nuclear perimetry (P < 0.001), SD of nuclear area (P = 0.01) and MAI (P = 0.019) in Cox's analysis. In small (diameter ≤20 mm) tumours, S.D. of nuclear perimetry predicted recurrence-free survival (P = 0.03) in Cox's analysis. The results advocate the use of mitotic indexes and nuclear factors in place or in combination with conventional histological grading in predicting the survival and tumour recurrence in axillary lymph node-negative breast carcinomas.
Eur J Cancer, Vol. 27, No. 12, pp. 1555–1559, 1991.

INTRODUCTION

QUANTITATIVE VARIABLES have significant predictive value in several epithelial tumours [1–20]. S-phase fraction [5, 12, 13], DNA ploidy [3, 5, 11, 12, 14], mitotic activity [1, 2, 12, 16, 17] and some nuclear factors [1, 2, 16, 18, 19] have proved to be significant prognostic factors in breast cancer as well.

Recently, the role of flow cytometry [3, 5] in prognostication of breast cancer has been studied more intensely than that of quantitative morphometry [1, 2]. For axillary lymph node-negative breast carcinomas, the majority of the prognostic data is derived from the studies using flow cytometry [11, 13, 14, 16]. However, in other epithelial tumours, multivariate analyses have shown that flow cytometric variables are inferior or at best equal to the mitotic indexes and morphometrically measured nuclear factors in predicting the long-term survival [8]. Thus, there is ample evidence to indicate that relatively simple morphometric methods are accurate in prediction of even the localised (T1) breast carcinomas [4, 8, 10]. It seems to be established that, compared with, e.g. flow cytometry, there are major advantages confined to morphometric methods, including a high reproducibility [6, 20] and relative simplicity.

From the clinical point of view, an accurate prognostic prediction of axillary lymph node-negative breast carcinomas is one of the key issues in breast oncology [22–24]. The currently used histological grading systems are subject to considerable variation [25], and consequently, the estimates of prognosis in individual cases are inaccurate [25].

On the basis of the above, the present study was prompted, in which the predictive value of the morphometric factors was assessed in 281 patients with axillary lymph node-negative (pN−) breast carcinomas followed-up for over 12 years at Kuopio University Hospital during 1968–1990.

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Revised 12 Sep. 1991; accepted 19 Sep. 1991.

PATIENTS AND METHODS

Patients

A total of 281 consecutive women with axillary lymph node-negative breast carcinomas were treated and followed up at Kuopio University Hospital of Kuopio during 1968–1990. From these patients, complete follow-up histories and archival paraffin

Table 1. Patients' characteristics

No. of patients	281
Age at diagnosis, mean (S.D.)	62.2 (13.5)
Follow-up time, mean(S.D.)(S.E.)(years)	12.2(4.7)(0.3)
Diameter of tumour, mean(S.D.)(S.E.)(cm)	3.1(1.9)(0.02)
0–2.0	111
2.1–5.0	141
>5.0 cm	21
Not known	8
Type of primary treatment	
Modified mastectomy	233
Mastectomy and adjuvant therapy	45
No mastectomy	1
Number of recurrent cases	99(35.2%)
Causes of death during the follow-up	
Breast cancer	56
Other	38

embedded peroperative biopsy specimens of their primary tumours were available for analysis. Tumour size was recorded as the maximum tumour diameter as measured in fresh mastectomy specimens by the operating surgeon which was a standard practice in our hospital during the study period. The decision to perform a mastectomy was based on a frozen section study or on a preoperative open biopsy study. The follow-up was every 3 months during the first year, every 6 months during the next 2 years and annually thereafter. Determination of the deaths due to breast cancer was made by critical review of both the death certificates and the clinical data. The pertinent clinical data of patients are summarised in Table 1. In total 19/281 (7%) of tumours were inflammatory (T4) carcinomas.

Histological assessment

The peroperative biopsy specimens (not specimens for frozen section) fixed in buffered formalin immediately after removal (pH 7.0) and embedded in paraffin were available at the files of the Department of Pathology. From each paraffin embedded biopsy specimen, 5 μm thick sections were cut in 1990 and stained with haematoxylin and eosin for light microscopy. The histological grading was done according to Bloom and Richardson [26] and histological typing was completed according to WHO [27]. The grading and typing was performed simultaneously by two board certified pathologists (VMK, SM) using a consultation microscope in a blinded manner, i.e. the examiners were unaware of the clinical data. In cases where intraductal growth was exclusively present, no grading was done [28]. In histological typing, 236 tumours proved to be ductal carcinomas, 30 were lobular carcinomas and the remaining 15 were other special histological types [27].

Axillary lymph node status was assessed by histological examination in all cases. Usually 2–4 lymph nodes were analysed and all macroscopically suspicious lymph nodes were screened for metastasis. Usually one slice was studied (stained with haematoxylin and eosin) and the nodes were sectioned so that the hilar region of nodes could be screened for cancer cells.

Mitotic indexes

The mitotic figures were counted with a consultation microscope by two observers (P.L. and S.A.) using an objective magnification of 40× (field diameter 490 μm). The mitotic figures were identified as described earlier [6] from the most cellular areas of the tumour (usually the tumour margins) and avoiding the frequently necrotic central areas. The mitotic activity index (MAI) was the number of mitotic figures in 10 consecutive fields. The volume corrected mitotic index (M/V index) was estimated using the method described by Haapasalo *et al.* [6]. In final analyses, both these mitotic indexes were corrected to correspond the mitotic activity in 1 mm^2 of a section.

Morphometric measurements

In morphometric measurements, the semiautomatic IBAS 1&2 image analyser was used. The images of the most atypical (adequately preserved) microscopic fields were selected and projected on a video screen through a video camera attached to the microscope (magnification ×40). In each tumour, a mean of 75 nuclei were traced using a digitiser tablet and a mouse connected to the computer. The computer automatically calculated the following nuclear factors: the mean nuclear area (NA), standard deviation of the nuclear area (SDNA), nuclear perimetry (PE), standard deviation of the nuclear perimetry (SDPE), largest nuclear diameter (D_{max}), shortest nuclear diameter (D_{min}) and the mean area of the 10 largest nuclei (NA10). Form factor of the nuclear perimeter, FFPE = $(4 \times \pi \times NA)/PE$, and form factor of nuclear area, FFNA = $NA (\pi \times D_{max} \times D_{min})$, were calculated on the basis of the above values.

Statistical analyses

The basic statistical analysis was done by the SPSS/PC+ program package in a Toshiba T3200 computer. Recurrence-free survival was defined as the time elapsed between the primary therapy and the first confirmed metastasis or recurrent growth. Survival was calculated by counting as breast cancer deaths only those women who died with known metastases of their disease. Univariate survival analysis was based on the life-table method with the Lee–Desu statistics. The group limits for the mitotic indexes were adopted from the previous reports [1, 2]. Several group limits were tested for the nuclear factors. Finally pathological variables and tumour size were included in a multivariate analysis together with menopausal status (under/over 50 years) and adjuvant therapy to control possible confounding factors. Multivariate survival analysis was performed (P.L.) with the BMDP computer program (2L) (BMDP Statistical Software, University of California, Los Angeles).

RESULTS

The values of the morphometric measurements as related to different histological grades are summarised in Table 2. The differences in the mean values of morphometric variables among the histological grades were significant ($P < 0.0001$).

The morphometric variables were not related significantly to tumour size. The mean (S.E.) value of M/V index in recurring tumours was 20.2 (2.0) as compared with 15.5 (1.3) in the non-recurring tumours (Wilcoxon rank sum test $P = 0.008$). The MAI values were 13.1 (1.5) and 10.5 (1.1) in the recurring and non-recurring tumours, respectively (Wilcoxon rank sum test $P = 0.018$).

Tumour size (Fig. 1), histological grade ($P = 0.0565$), M/V index (Fig. 2) and MAI ($P = 0.0115$) accurately predicted the recurrence-free survival. Tumour size (Fig. 3), histological grade (Fig. 4), MAI (Fig. 5) and M/V index ($P = 0.0036$) predicted disease-related survival. Of the nuclear factors, SDPE showed some predictive value in survival analysis (Fig. 6) while others had no significant predictive value ($P > 0.1$). The two best nuclear factors predicting the disease-related survival in

Table 2. The morphometric variables related to histological grades

Variable	Range	Grade 1 (n = 20)	Grade 2 (n = 151)	Grade 3 (n = 74)
NA(μm^2)	24.3–203.5	59.0(4.8)	72.2(1.8)	105.5(3.5)
NA10(μm^2)	31.7–458.8	83.0(7.4)	104.1(2.9)	164.5(6.8)
SDNA(μm^2)	2.3–99.9	14.4(1.5)	19.3(0.7)	33.6(1.6)
PE(μm)	9.6–55.7	31.0(5.6)	33.6(0.5)	40.6(0.7)
SDPE(μm)	1.8–30.0	3.7(0.3)	4.6(0.2)	6.4(0.2)
Dmax(μm)	5.0–19.6	10.8(0.4)	11.8(0.2)	14.4(0.2)
Dmin(μm)	1.2–7.0	6.5(0.4)	7.9(0.4)	9.0(0.2)
FFPE	14.9–71.4	23.3(1.0)	26.5(0.5)	31.9(0.5)
FFNA*	0.03–5.2	0.3(0.1)	0.3(0.03)	0.2(0.0)
M/V index	0–138	4.0(1.3)	11.4(1.1)	36.5(2.5)
MAI	0–125	1.4(0.4)	5.7(0.5)	28.9(2.2)

NA = nuclear area, SDNA = S.D. of nuclear area, PE = nuclear perimetry, SDPE = S.D. of nuclear perimeter, D_{max} = largest nuclear axis, D_{min} = shortest nuclear axis, FFPE = form factor of perimetry, FFNA = form factor of nuclear area, M/V index = volume corrected mitotic index, MAI = mitotic activity index.
*No significant difference among the grades.
Mean (S.E.).

small (diameter ≤20 mm) tumours were SDPE (P = 0.07) and SDNA (P = 0.1) whereas recurrence-free survival could not be predicted significantly by any of the variables ($P > 0.1$).

The adjuvant therapy had no significant impact on recurrence-free survival ($P > 0.1$). Tumour treated with adjuvant methods had a more unfavourable prognosis in univariate analysis than tumours without adjuvant therapy (P = 0.001).

The independent predictors of recurrence-free survival and survival according to Cox's analysis are shown in Table 3. In small tumours (diameter ≤20 mm) none of the variables could predict survival in multivariate analysis whereas nuclear perimetry [P = 0.06; coefficient (S.E.) = 0.45 (0.16)] and S.D. of nuclear perimetry [P = 0.03; coefficient (S.E.) = −0.37(0.17)] were related to recurrence-free survival.

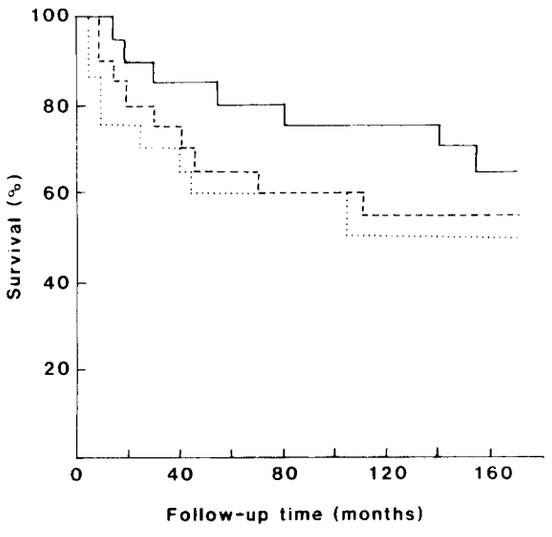

Fig. 1. The recurrence-free survival of patients subdivided according to tumour size. The difference among the curves is significant (χ^2 = 10.5, P = 0.0052). ———: diameter ≤20 mm, n = 111; ----: diameter 21–50 mm, n = 141; ····: diameter > 50 mm, n = 21.

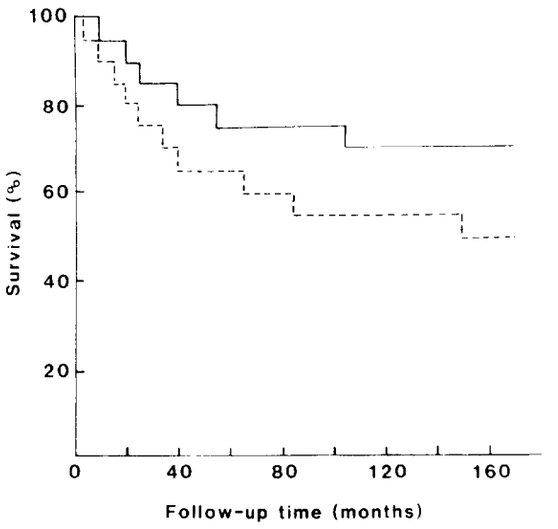

Fig. 2. The recurrence-free survival time of patients subdivided according to M/V index. The difference between the curves is significant (χ^2 = 9.7, P = 0.0018). ———: M/V index ≤10, n = 141; ----: M/V index > 10, n = 140.

DISCUSSION

According to conventional practice, all breast carcinomas have been treated by mastectomy and axillary lymph node evacuation. Recent data suggests that small axillary lymph node-negative breast carcinomas can be safely treated by segmental resection [21–23]. Among these women, a certain group exists who will develop a tumour recurrence [21–24]. At current understanding, we are not able to accurately identify this subgroup of women who would benefit from an aggressive adjuvant therapy.

According to previous studies, DNA aneuploidy [11, 14] and SPF [13] have had a potential to predict recurrences in axillary lymph node-negative breast carcinomas. However, because of the many negative results, the predictive value of DNA aneuploidy in these tumours has been questioned at present [13, 15]. The results from flow cytometry, however, suggest that the

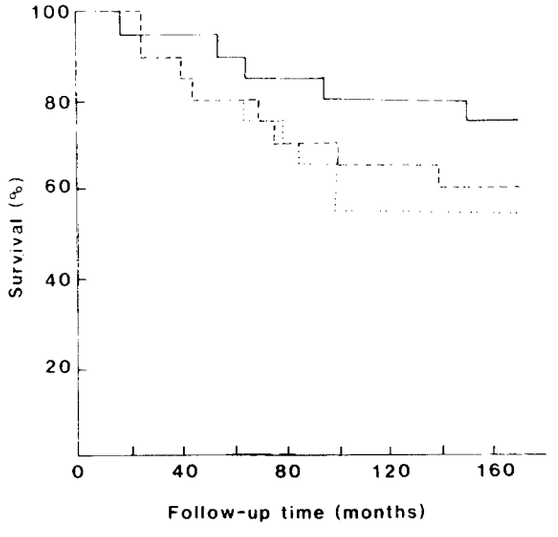

Fig. 3. The corrected breast cancer-related survival of patients subdivided according to tumour size. The difference among the curves is significant (χ^2 = 8.6, P = 0.0133). The group limits and number of cases are identical to those in Fig. 1.

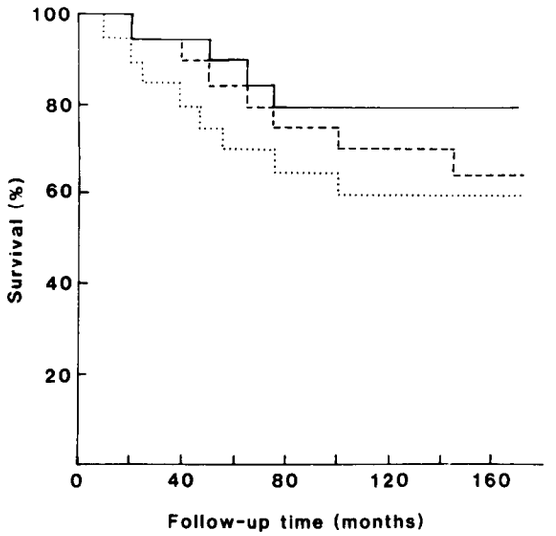

Fig. 4. The corrected breast cancer related survival of patients subdivided according to histological grade. The difference among the curves is significant ($\chi^2 = 5.9$, $P = 0.528$). ———: grade I, $n = 20$; ----: grade II, $n = 165$; ·····: grade III, $n = 74$.

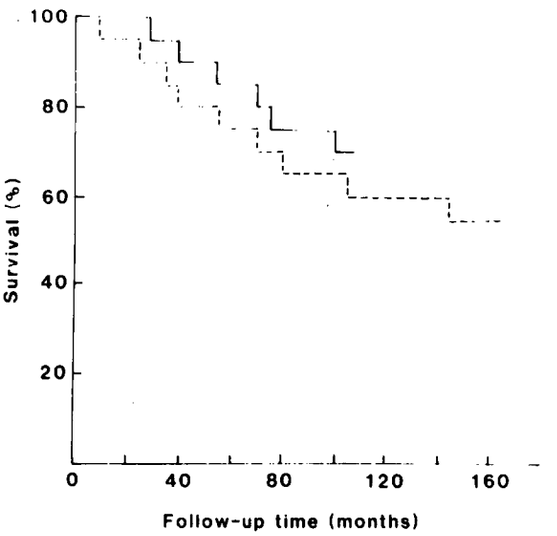

Fig. 6. Corrected breast cancer-related survival of patients subdivided according to S.D. of nuclear perimeter. The difference between the curves is almost significant ($\chi^2 = 3.1$, $P = 0.0762$). ———: SDPE ≤ 6 μm, $n = 205$; ----: SDPE > 6 μm, $n = 76$.

mitotic activity and nuclear factors might have predictive value like in unselected series of breast carcinomas [1, 2, 12, 19]. Their role as prognostic variables in axillary lymph node-negative tumours has been incompletely studied, whatsoever [18].

Tumour size and histological grade were significant predictors in univariate analysis. Both these observations are in alignment with the previous reports [11, 13]. The low reproducibility of both these factors due to their subjective assessment reduces their predictive value, however [25]. The accurate measurement of tumour size is skewed by the invasive growth pattern of the tumours. The critical role of the measurement of tumour size has been clearly shown by a number of clinical trials in which the predictive value of the resection margins has been tested [24]. Consequently, tumour size has limited prognostic value as shown by the present multivariate analysis. Histological grading, although variable, is suitable for routine use since grade can be simply assessed in routine paraffin embedded sections although it had no independent predictive value in Cox's analysis.

None of the nuclear factors had significant predictive value in survival analysis, SDPE showing only a suggestive predictive value. However, nuclear perimetry and S.D. of nuclear area were independently related to recurrence-free survival after the confounding effect of adjuvant therapy and menopausal status were controlled by multivariate analysis. According to experimental studies these shape factors may be related to intrinsic malignancy of cancer cells [29]. Only mitotic frequency predicted the recurrence-free survival and survival significantly both in univariate and multivariate analysis which confirms previous observations [1, 2, 12, 16, 17]. Although several histological types were included in the present series, the histological type had no independent prognostic value.

The M/V index [6] proved to be a significant prognostic predictor although it disregards the cellularity in the microscopic

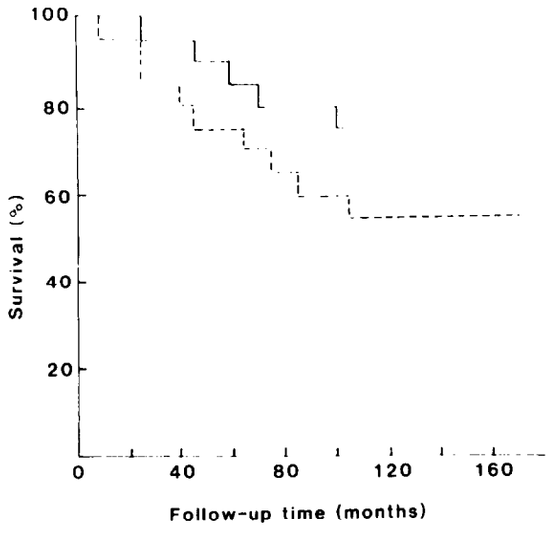

Fig. 5. The corrected breast cancer related survival of patients subdivided according to MAI. The difference between the curves is significant ($\chi^2 = 11.5$, $P = 0.0007$). ———: ≤ 10, $n = 191$; ----: MAI > 10, $n = 90$.

Table 3. Independent predictors of recurrence-free survival and survival in Cox's multivariate analysis

Factor	β(S.E.)	β/S.E.	*P*	Hazard rate
Relapse-free survival				
PE	0.58(0.13)	4.62	<0.001	1.78(1.38–2.31)
SDNA	−0.44(0.14)	−3.23	0.010	0.64(0.48–0.85)
MAI	0.36(0.15)	2.36	0.019	1.43(1.06–1.93)
Survival				
MAI				
Adjuvant	0.57(0.24)	2.32	0.004	1.78(1.10–2.85)
therapy	0.55(0.28)	1.96	0.030	1.73(0.99–3.03)
Tumour size	0.32(0.18)	1.72	0.087	1.38(1.21–1.97)

The β coefficient indicates how each factor contributes to the hazard. β/S.E. describes their significance (*z*-value). The hazard rates with 95% confidence intervals is given for each factor.

fields [6]. On the other hand, it is well established that the proportion of cancer cells in the microscopic image has significant predictive value in breast cancer [30] which probably favoured MAI in the present multivariate analysis. The major advantage of the M/V index, however, is its high reproducibility [6, 20]. In the previous studies, the morphometrically determined nuclear factors have had a limited independent predictive value in breast carcinomas [2, 17, 19]. Also in tumours of the bladder, pancreas, and ovary, mitotic indexes have been shown to predict tumour progression, recurrence and survival [4, 6–10] more efficiently than nuclear factors or histological grading systems in current use. More importantly, the mitotic indexes are potent predictors in localised T1 tumours [4, 8–10].

In the small (diameter ≤20 mm) breast carcinomas, none of the variables assessed could accurately predict survival whereas nuclear factors predicted recurrence free survival in Cox's analysis. The nuclear factors that showed predictive value are related to intrinsic malignancy of cancer cells [29]. So, it seems difficult to elaborate significant prognostic predictors by analysing the factors confined to cancer cell structure only, without taking into account the cell-mediated and humoral immune responses [16, 31].

The assessment proliferative activity alone in small tumours is an insufficient means to predict the prognosis. This is in contrast to the large tumours, in which the proliferative activity as measured by the mitotic indexes [1, 2, 12, 17] or SPF [5, 13] is a highly significant predictor. In small breast carcinomas, factors related to invasion, and cell differentiation (e.g. lymphatic invasion, tumour margin circumscription and intraductal growth, nuclear shape factors) seem to have significant predictive value [11]. This was also true in the present series, where tumours with predominantly an intraductal growth had a more favourable prognosis.

In conclusion, the present results demonstrate that the mitotic activity in axillary lymph node-negative breast tumours is a significant predictive variable. The prognostic value established by the light microscopic quantitative measurements reached similar statistical level than that obtained by flow cytometry in the previous reports. The present results might have clinical implications while making decisions on adjuvant therapy for axillary lymph node-negative patients. Moreover, quantitative measurements could be used as an adjuct to histological grading to improve its prognostic accuracy. Further randomised prospective follow-up studies employing large number of patients are necessary, however, to fully elucidate this issue.

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Acknowledgements—The study was supported in part by research grants from the Savo Cancer Fund and Paavo Koistinen Foundation.