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Original Paper

Cyclical Tumour Variations in Premenopausal Women with Early Breast Cancer

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The hormonal milieu at the time of tumour excision may have a significant impact on survival in premenopausal patients with breast cancer, with those undergoing surgery between days 3 and 12 of the menstrual cycle having a worse prognosis. To investigate possible mechanisms which might explain this finding, histological features of tumours from 363 patients included in two studies from Guy's Hospital have been reviewed. Axillary nodal involvement occurred in 71/115 (62%) of patients whose primary tumour was excised between days 3 and 12 of the cycle, compared with 116/248 (47%) of patients undergoing surgery at other phases of the cycle (χ^2 = 7.04, $P < 0.01$). Vascular invasion was observed in 54/115 (47%) of primary tumours removed between days 3 and 12 and 82/248 (33%) of tumours removed at other times (χ^2 = 6.47, $P < 0.02$). Multivariate analysis of factors influencing survival indicated that both axillary nodal status and phase of the cycle were highly significant independent predictors of prognosis.

Key words: breast cancer, menstrual phase, prognosis, histology
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INTRODUCTION

FOR PREMENOPAUSAL women with early breast cancer, there is some evidence that the phase of the menstrual cycle at the time of surgery affects survival [1–3]. In our first report [1] involving patients diagnosed between 1975 and 1985, we compared survival for patients who underwent excision of the primary tumour during a phase of predicted unopposed oestrogen secretion (3–12 days after onset of menstruation), with that of patients operated on at other phases of the cycle. After day 12 of the cycle, both oestrogen and progesterone are normally secreted, whereas levels of both hormones are low between 0 and 2 days after the onset of menstruation. In the first study, patients operated on between days 3 and 12 had a 54% 10 year actuarial survival rate, compared with 84% for those operated on at other times ($P < 0.001$). In a second cohort of patients (treated since 1985), similar results were reported [4]. Although some other groups have been unable to confirm this finding [5–9], a recent meta-analysis of all published studies has shown that overall there is a significant effect of timing of surgery [10]. The odds reduction on survival for surgery in the luteal phase was 16%.

In this study, data from both cohorts of patients have been combined and a detailed review of histological parameters undertaken. In neither of the previous reports were any significant differences observed in the distribution of conventional prognostic factors, such as tumour size or type, between those undergoing surgery between days 3 and 12 after the last menstrual period (LMP) and those operated on at other times. However, in both reports, a non-significant excess of patients with positive axillary lymph nodes was observed amongst those undergoing tumorectomy between days 3 and 12.

One hypothesis that might explain the findings related to timing of surgery is that tumour handling may lead to spread of malignant cells and the likelihood of such tumour dissemination occurring may be influenced directly or indirectly by the endocrine environment. Evidence for the link between hormones and prognosis was provided by a recent study which found that node-positive patients with peri-operative progesterone levels >1.5 ng/ml (luteal phase) had a significantly better survival than those with progesterone levels ≤1.5 ng/ml [11]. Dissemination can occur via lymphatics or blood vessels [12], and so we were interested in investigating the relationship between phase of the menstrual cycle and vascular invasion by tumour cells.

PATIENTS AND METHODS

Clinicopathological data of 823 premenopausal patients with invasive operable breast cancer treated at Guy's between 1975

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and 1990 were retrieved from a computerised database. Hospital case notes were scrutinised for information on the last menstrual period before excision of tumour, together with duration and regularity of the cycle, as previously described [1]. Data on the date of last menstrual period (LMP) were not available for 199 patients. A further 132 patients were recorded as having irregular periods, making assessment of the phase of the cycle at the time of surgery unreliable. 51 patients had had a hysterectomy and 72 were excluded due to factors affecting hormonal milieu (oral contraceptives, hormone replacement therapy, pregnancy and lactation). As a result, 369 evaluable patients were available for the analysis.

Most patients with single tumours less than 4 cm were treated with breast conservation treatment (excision biopsy, axillary dissection and radiotherapy), and those with cancers greater than 4 cm were treated with modified radical mastectomy. 10 patients with tumours greater than 4 cm had wide excision followed by radiotherapy to breast and gland fields, and their histological lymph node status was unknown. The pathological specimens were dissected when fresh.

Cancers were typed according to WHO criteria and infiltrating ductal carcinomas were graded using the Bloom and Richardson system [13]. Invasion of lymphatics and/or blood vessels was determined both within and around the primary tumour, assessment being made on standard haematoxylin and eosin stained sections. Lymphatic and/or blood vessel invasion was defined as the presence of tumour cells within endothelial-lined channels with or without a smooth muscle wall.

Statistical methods

Survival (S) and relapse-free survival (RFS) were calculated by the method of Kaplan and Meier [14], with significance being determined using the log-rank test [15], and multivariate analysis being undertaken with Cox's proportional hazards model [16]. Survival data were analysed using the SUREAL package. Two by two tables were compared using Fisher's Exact Test, or the chi-square with Yates correction for larger groups. Proportions across more than two groups were compared using chi-square statistics. The Mann–Whitney test was used to test for differences in the number of involved nodes between the two groups.

RESULTS

A total of 369 patients who were diagnosed between 1975 and 1990 had regular menstrual cycles and had known the date of the LMP before excision of the primary tumour. Histological material was available for review in 363 (98%) of these cases. 115/363 (32%) underwent surgery between days 3 and 12 of the cycle (Group 1), 248 (68%) undergoing surgery either earlier or later in the cycle (Group 2). The actuarial 10 year survival rate for patients in Group 1 was 52%, compared with 80% for those in Group 2 ($P < 0.001$, Figure 1).

The tumour characteristics for the two groups of patients are shown in Table 1. As in our previous reports, no significant imbalances were observed between the groups in relation to tumour size ($P = 0.93$) or histological grade ($P = 0.86$). In this combined data set, however, a significant excess of patients with positive axillary lymph nodes was observed among those in Group 1 ($P = 0.01$). A detailed breakdown of lymph node involvement according to phase of the cycle is shown in Table 2 and Figure 2. A similar excess of cases with positive lymph nodes was observed both amongst patients who underwent axillary clearance at the time of tumorectomy (61% node positive for Group 1 compared with 44% node positive for Group 2) and amongst those whose axillary surgery was undertaken at a second operation, usually 5 days later (60% node positive for Group 1 compared with 47% node positive for Group 2).

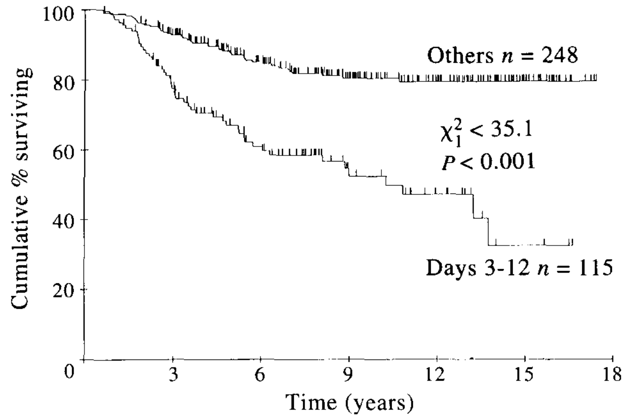

Figure 1. Overall survival of patients undergoing tumour excision between days 3 and 12 and at other times.

Table 1. Distribution of tumour characteristics between LMP subgroups

	Group 1	Group 2
	(Days 3–12)	(Other)
n	115	248
T size		
0–2	45 (40%)	100 (40%)
2–5	65 (56%)	139 (56%)
>5	5 (4%)	9 (4%)
Lymph node status		
Negative	44 (38%)	132 (53%)
Positive	71 (62%)	116 (47%)
Grade		
I	10 (9%)	27 (11%)
II	43 (37%)	97 (39%)
III	39 (34%)	75 (30%)
Other	23 (20%)	49 (20%)
Vascular invasion		
Present	54 (47%)	82 (33%)
Absent	61 (53%)	166 (67%)
ER		
Positive	70 (61%)	155 (63%)
Negative	29 (25%)	58 (23%)
Unknown	16 (14%)	35 (14%)
PR		
Positive	64 (56%)	119 (48%)
Negative	32 (28%)	84 (34%)
Unknown	19 (17%)	45 (18%)

ER, oestrogen receptor; PR, progesterone receptor.

Vascular invasion was observed in 136/363 (37%) of primary tumours. This feature was more common in node positive patients than in node negative patients (93/187 (50%) versus 43/176 (24%), $P < 0.0001$). Vascular invasion was observed significantly more frequently in tumours excised between days 3 and 12 ($P = 0.02$, Table 1). Amongst the 176 patients who had negative axillary lymph nodes, there was little difference in the

Table 2. Pattern of lymph node involvement according to phase of cycle at time of tumour excision

	Group 1 (Days 3–12) $n = 115$	Group 2 (Other) $n = 248$
Lymph nodes		
0	44 (38%)	132 (53%)
1	25 (22%)	37 (15%)
2	12 (10%)	23 (9%)
3	4 (4%)	12 (5%)
4–6	8 (7%)	18 (7%)
7–9	8 (7%)	10 (4%)
≥10	14 (12%)	16 (7%)
Mean no. of nodes	4.2	2.6
Median no. of nodes	1	0

$P = 0.02$ for difference in number of nodes between groups 1 and 2 (Mann–Whitney U-test).

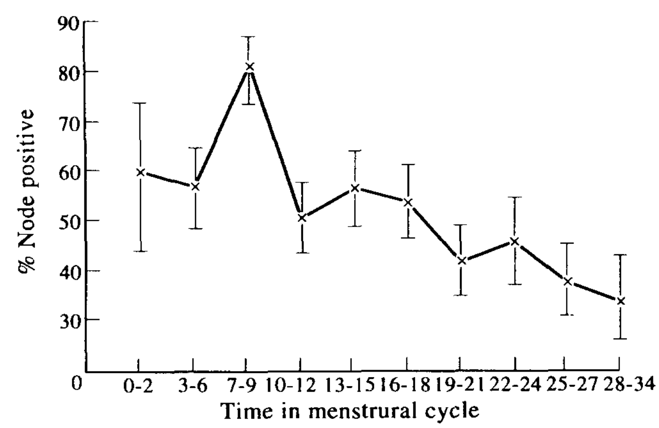

Figure 2. Percentage of patients with positive axillary lymph nodes by time in the menstrual cycle.

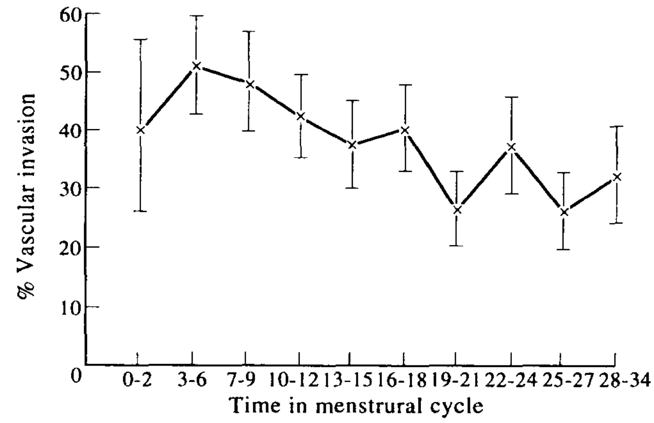

Figure 3. Percentage of patients with vascular invasion in relation to time in the menstrual cycle.

Table 3. Significance of prognostic factors for survival

Factor	Univariate* χ^2	Univariate* P	Multivariate χ^2	Multivariate P
No. of nodes†	47.9	<0.0001	40.7	<0.0001
LMP day	30.8	<0.0001	27.5	<0.0001
Tumour type/grade‡	35.4	<0.0001	36.8	<0.0001
Tumour size§	10.4	0.0013	3.9	0.05
Age	4.9	0.03	2.0	0.16
Vascular invasion	4.2	0.04	2.0	0.15

LMP, last menstrual period.
* See statistical methods; † 0 versus 1–3 versus 4–9 versus ≥10; ‡ Ductal grade (1–3) with non-ductal histologies coded as 2; § ≤2 cm versus >2 cm.

proportion of patients with vascular invasion in the two groups (30% for Group 1 versus 23% for Group 2, $P = 0.48$). This effect was mainly confined to patients with positive axillary lymph nodes (58% versus 45%, $P = 0.1$). The proportion of patients found to have vascular invasion is shown in relation to the phase of the cycle when tumorectomy was undertaken in Figure 3.

Because of the significant inter-relationships between phase of cycle, nodal involvement and vascular invasion, each of which influenced survival in a univariate analysis, a multivariate analysis of the effects of these parameters on survival was undertaken (Table 3). Both lymph node involvement and phase of cycle were highly significant independent predictors of survival.

DISCUSSION

An inter-relationship between phase of the menstrual cycle at the time of surgery and histological parameters has not been reported previously. In this study, an increased incidence of lymph node involvement ($P = 0.01$) and vascular invasion ($P = 0.02$) was observed in women undergoing tumorectomy between days 3 and 12 of the cycle. In addition, as has been observed in many other studies [12, 17, 18], vascular invasion was related to lymph node involvement ($P < 0.0001$).

It is important to examine the magnitude of the difference in the number of involved nodes between the follicular and luteal phases as a prelude to explaining this effect. It can be seen from Table 2, by examining both the mean and median number of nodes, that there is an increase of approximately one node only between the follicular and luteal phases. The distribution of patients with different numbers of nodes is also consistent with this "1 node shift", as can be seen in Table 2.

Two immediate questions arise from these findings. Firstly, have these associations between menstrual phase and vascular invasion and lymph node involvement arisen by chance? Secondly, if this was the case, does this explain the observed relationship between phase of the cycle at the time of surgery and long term survival? Although the possibility that the association arose by chance cannot be dismissed, the strength of the relationship makes this unlikely. However, the results of the multivariate analysis show that the effect on prognosis cannot be explained by the imbalance in either the number of involved nodes, or the presence of vascular invasion between the follicular and luteal phases of the cycle.

If the associations are not due to chance, what other possible hypotheses are there? Is it possible that surgery itself disseminates tumour in a different way in different phases of the menstrual cycle, and does the endocrine environment at the time of surgery significantly influence the likelihood of tumour invading lymphatics and spreading to axillary nodes? Even if this were so, it is difficult to conceive how the hormonal milieu at the time of surgery could affect the presence of established nodal involvement. The presence of lymph node metastases has always been regarded as a likely indicator of disease duration. In many cases axillary nodal metastases form substantial tumour masses which, by virtue of their incorporation into the node, have been present for a considerable time.

There is, however, a range of size of nodal metastases and some are very small indeed. Such micrometastases might possibly arise peri-operatively from disseminated tumour emboli. A similar process has been suggested for tumour emboli which have been detected in the bone marrow of patients undergoing surgery for breast cancer [19]. Furthermore, Nissen-Meyer has shown that a short course of immediate postoperative chemotherapy can reduce the risk of relapse, suggesting that surgery may sometimes disseminate viable tumour cells [20].

It is possible that the establishment of growth within the parenchyma of the node by a small tumour embolus within the peripheral sinus is influenced by host factors, including the hormonal milieu. However, the majority of such sinusoidal metastases are detected only by serial sectioning of blocks of lymph nodes, with or without the use of immunohistochemical markers for epithelial cells. The nodal metastases in this study were all detected by examination of haematoxylin and eosin stained sections taken from just two levels of the block of each node, and are, therefore, not of this type. Thus, there are no conclusive explanations for the observed relationship between the number of involved nodes and the timing of surgery in relation to the stage of the menstrual cycle.

There are hypotheses which could explain the apparent relationship between vascular invasion and phase of the menstrual cycle. Proteolytic enzymes which may facilitate transendothelial invasion of malignant cells have been shown to be modulated by oestrogens [21] as have intravascular enzymes such as plasminogen activator [22], which can lyse clots that can form a barrier to tumour embolisation.

However, this all remains speculative. From the two series of patients treated between 1975 and 1989, the timing of surgery during the menstrual cycle had a highly significant effect on the prognosis of premenopausal women in breast cancer. This report demonstrates that this effect on prognosis is not explained by an imbalance in the number of involved nodes or by the differences in vascular invasion between the follicular and luteal phases of the cycle.

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