

Survival of Premenopausal Breast Carcinoma Patients in Relation to Menstrual Cycle Timing of Surgery and Estrogen Receptor/Progesterone Receptor Status of the Primary Tumor

Lucienne S. Cooper, M.Sc.

Cheryl E. Gillett, Ph.D.

Neera K. Patel, M.B.

Diana M. Barnes, D.Sc.

Ian S. Fentiman, M.D.

Hedley Atkins Breast Unit, Guy's Hospital, London, United Kingdom.

BACKGROUND. Premenopausal breast carcinoma patients who undergo tumor excision during the follicular phase of their menstrual cycle may have a significantly worse prognosis than those whose tumors are excised in other phases of the menstrual cycle.

METHODS. Outcome was determined in a series of 112 premenopausal women with operable breast carcinoma in relation to the timing of surgery within the menstrual cycle and the estrogen receptor (ER) and progesterone receptor (PR) status of their primary tumors as determined by immunohistochemistry.

RESULTS. Those patients with ER positive tumors who underwent surgery in the early and luteal phase of the cycle had a significantly better survival than women with ER negative tumors (chi-square test = 15.56; $P < 0.001$). This also was true for PR status (chi-square test = 18.21; $P < 0.001$). After follicular phase surgery, tumor receptor status had no effect on overall survival. Patients with the best prognosis had ER/PR positive tumors excised on Days 0–2 and 13–32 but even those women with ER or PR negative tumors removed during the luteal phase of their menstrual cycle fared better than patients whose tumors were removed during the follicular phase.

CONCLUSIONS. There was a better survival rate for patients with both ER/PR positive and negative tumors treated during the luteal phase of the menstrual cycle. This could be the result of progesterone acting on the surrounding peritumoral normal tissue, thereby exerting a straitjacket effect and improving cohesion of the primary carcinoma. Unopposed estrogen in the follicular phase of the cycle may enable more tumor emboli to escape and successfully establish micrometastases. *Cancer* 1999;86:2053–8. © 1999 American Cancer Society.

KEYWORDS: breast carcinoma, timing of surgery, menstrual cycle, estrogen receptors, progesterone receptors, prognosis.

There is mounting evidence that the timing of surgery within the menstrual cycle has a significant effect on prognosis in premenopausal women with breast carcinoma.^{1–3} Since the original suggestion by Hrushesky et al. that surgical cure of breast carcinoma was affected by the menstrual phase,⁴ there has been considerable controversy with several studies finding no effect.^{5,6} Subsequently, Badwe et al. hypothesized that unopposed estrogens might be deleterious and reported a significant worsening of prognosis in women undergoing tumor excision between Days 3 and 12 of the menstrual cycle.⁷

Although there have been several subsequent negative reports, a meta-analysis indicated that there was a significant overall effect of

Address for reprints: Ian S. Fentiman, M.D., Hedley Atkins Breast Unit, Guy's Hospital, London SE1 9RT, United Kingdom.

Received July 23, 1999; accepted August 9, 1999.

the timing of surgery.¹ Additional studies in which progesterone receptor (PR) and estrogen receptor (ER) levels were measured in blood samples taken around the time of surgery showed that among those women who had PR levels > 4 ng/mL (luteal phase) there was a significantly better prognosis.⁸ In addition, a histologic study indicated an increased likelihood of vascular invasion around tumors resected during the follicular phase of the cycle.⁹ In a subsequent study we showed that, in the follicular phase, there was an increased risk of the establishment of viable micrometastases that was most marked in tumors with higher proliferative activity.¹⁰

When the effect of timing of surgery first was demonstrated it was apparent in patients with both ER positive and negative tumors.⁷ This suggested that the mechanism in part acted via an indirect effect on the ER positive peritumoral normal tissue. To investigate this further, we immunohistochemically measured the ER and PR status in a series of 112 tumors excised from premenopausal women who underwent surgery at a known time within their menstrual cycle.

MATERIALS AND METHODS

One hundred and twelve women with operable invasive breast carcinoma treated in the Breast Unit at Guy's Hospital between 1975–1985 were studied. The cases used in this study were a subset of cases we have used previously.¹⁰ Some cases were excluded because there was insufficient tumor tissue for further analysis. All patients were premenopausal and the date of their last menstrual period prior to surgery was known. From this, the day of the menstrual cycle phase on which they underwent surgery was calculated. Surgery was comprised of either modified radical mastectomy or breast conservation (tumorectomy and axillary lymph node clearance). Subsequently, those patients treated by breast conservation therapy received an iridium implant (20 grays [Gy]) followed by whole breast irradiation (46 Gy).¹¹ Patients were seen every 3 months for 3 years, every 6 months for the next 2 years, and annually thereafter. Long term verified follow-up data were available for all cases.

The clinical size of the tumors was known and the histologic type was established using guidelines from the World Health Organization.¹² There were two main histologic types encountered: infiltrating ductal carcinoma of no special type and infiltrating lobular carcinoma. The histologic grade of all the tumors was determined by the modification of the Bloom and Richardson system as proposed by Elston and Ellis.¹³ The number of lymph nodes containing metastases and the microscopic tumor size were all determined by Dr. Rosemary Millis, a consultant pathologist on

the Clinical Oncology Unit at the time the current study was conducted.

Immunohistochemical Methods

Dewaxed and rehydrated 3- μ m, formalin fixed, paraffin embedded sections from the primary tumor underwent antigen retrieval using a pressure cooker and then were stained for ER and PR as described previously.¹⁰ Immunohistochemical staining was performed with anti-ER antibody ID5 (Dako Co., Carpinteria, CA) at a 1:70 dilution and anti-PR antibody 1A6 (Novocastra, Vector Laboratories Ltd., Peterborough UK) at a 1:40 dilution. The sections were incubated in primary antibody for 1 hour at room temperature. A standard peroxidase-conjugated streptavidin-biotin complex method was used and sites of binding were visualized with diaminobenzidine (Sigma Chemical Co., St. Louis, MO). Negative control sections in which the primary antibody was omitted and replaced with phosphate-buffered saline were included in each case. Sections of tumors previously shown to have varying levels of ER and PR positivity were used as positive controls.

Evaluation of Staining

Evaluation was undertaken by two of the authors (L.C. and N.P.) without prior knowledge of the timing of surgery using a conference microscope. Sections were evaluated by scoring the approximate proportion of cells staining and their intensity. Proportions were scored as follows: 0 (negative) 1 (1–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%). The intensity of staining was given a numeric score of 0 (no staining), 1 (weak staining), 2 (moderate staining), or 3 (strong staining). These scores were added together, giving a maximum count of 7. Scores ≤ 3 were considered to be ER and PR negative and those with scores > 3 were deemed ER and PR positive. This is in accord with our previous studies.¹⁴ Normal breast tissue, when present, was scored according to the proportion of cells staining.

Statistical Methods

Survival curves were generated using the method of Kaplan and Meier and the log rank analysis was used to compare ER and PR scores with overall survival.¹⁵ To compare ER and PR with the phase of the menstrual cycle, the chi-square test was used.

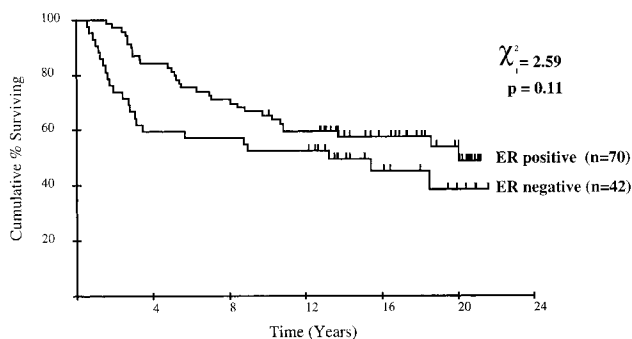
RESULTS

Of the 112 tumors, 70 (63%) were found to be ER positive and 68 (61%) were found to be PR positive. The proportion of ER positive tumors was not significantly different in those patients who underwent tumorectomy between Days 3 and 12 compared with those patients operated on at other times in the cycle

TABLE 1
Immunohistochemical Scores for ER and PR in Relation to Phase of Menstrual Cycle

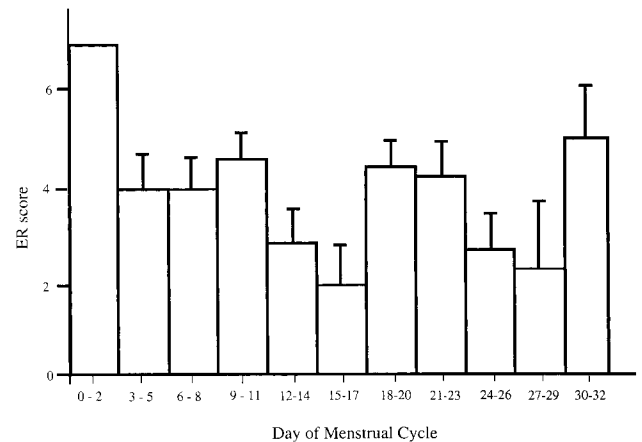
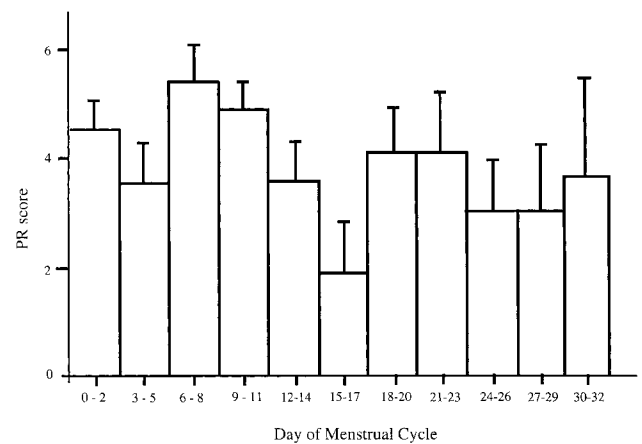
Malignant breast tissue (intensity and proportion of cells staining)		
	Score 0-3	Score 4-7
ER		
Days 3-12	19 (35%)	36 (65%)
Days 0-2, and 13-32	23 (40%)	34 (60%)
		Chi-square = 0.19
PR		
Days 3-12	19 (35%)	36 (65%)
Days 0-2 and 13-32	25 (44%)	32 (56%)
		Chi-square = 0.67
Normal breast tissue ^a (proportion of cells staining only)		
	Score 0-1	Score 2-4
ER		
Days 3-12	21 (41%)	30 (59%)
Days 0-2 and 13-32	19 (35%)	35 (65%)
		Chi-square = 0.4
PR		
Days 3-12	17 (33%)	34 (67%)
Days 0-2 and 13-32	16 (30%)	38 (70%)
		Chi-square = 0.17

ER: estrogen receptor; PR: progesterone receptor.

^a Seven cases did not have any normal tissue present.**FIGURE 1.** Overall survival by estrogen receptor (ER) status. χ^2 : chi-square test.

(65% vs. 60%; chi-square test = 0.19, degrees of freedom [df] = 1; $P = 0.66$), as shown in Table 1. Likewise, the proportion of PR positive cases was similar in both phases (65% vs. 56%; chi-square test = 0.67, df = 1; $P = 0.41$). There was no difference in the proportion of either ER positive or PR positive cells in normal tissue during different phases of the menstrual cycle (Table 1).

Overall survival by ER status, regardless of the phase of the menstrual cycle, is shown in Figure 1. It can be seen that although those women with ER positive tumors fared slightly better than their ER negative counterparts, this did not achieve statistical signifi-

**FIGURE 2.** Mean estrogen receptor (ER) values by day of menstrual cycle including standard errors.**FIGURE 3.** Mean progesterone receptor (PR) values by day of menstrual cycle including standard errors.

cance (chi-square test = 2.59, df = 1; $P = 0.11$). Similar data were obtained when the effect of PR status on overall survival was examined (chi-square test = 2.26, df = 1; $P = 0.13$) (data not shown).

The distribution of ER and PR scores according to the timing of surgery are shown in Figures 2 and 3, respectively. These histograms demonstrate that there was no significant difference in ER and PR positivity throughout the menstrual cycle.

Figure 4 shows the overall survival of those patients undergoing surgery during the two phases of the cycle (excluding five patients who died of causes other than breast carcinoma) and Figure 5 shows the precise distribution of survival according to the timing of surgery. For those who underwent surgery between Days 3 and 12, the 10-year survival rate was 45% compared with 75% for those patients undergoing tumor excision at other times during their menstrual cycle (chi-square test = 15.56, df = 1; $P < 0.01$). The effect on overall survival of the

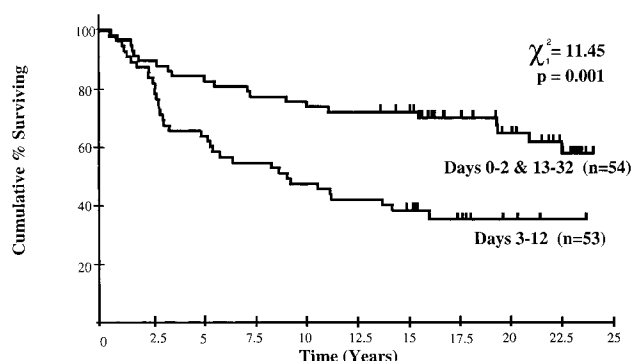


FIGURE 4. Overall survival by phase of menstrual cycle. χ^2 : chi-square test.

combination of the timing of surgery with ER status is shown in Figure 6 (chi-square test = 16.49; $P < 0.001$). This reveals a very interesting divergence by ER status in those patients undergoing surgery during the luteal phase of the menstrual cycle. The 10-year overall survival rate for the ER positive cases was 80% compared with 60% in ER negative cases. In contrast, among the women who underwent surgery during the follicular phase of their menstrual cycle, ER status appeared to have no effect on prognosis, with both groups having a 10-year survival rate of 42%.

As shown in Figure 7, a similar effect was observed in relation to the timing of surgery and PR status (chi-square test = 18.21; $P < 0.001$). The 10-year survival rate for PR positive patients who underwent surgery during the luteal phase of their menstrual cycle was 88% compared with 56% for those with PR negative tumors. In the

follicular phase, both PR positive and PR negative patients had a 10-year survival rate of 44%.

DISCUSSION

Attempts to characterize the mechanisms by which the menstrual cycle phase affects the prognosis in patients with operable breast carcinoma often raise more questions than answers.^{10,16} This study has been no exception. In the good phase of the menstrual cycle (Days 0–2 and 13–32), women with ER positive tumors have a significantly better outcome than those with ER negative tumors. The same is true for those patients with PR positive and PR negative tumors. This could be explained by the observation that ER positive and PR positive tumors are more likely to be better differentiated and hence be less aggressive.¹⁷ This is consistent with our previous findings in relation to MIB-1 staining, menstrual phase, and prognosis.¹⁰ In the luteal phase, those women with slowly proliferating tumors ($\leq 10\%$ of cells positive) had a significantly better prognosis than those with rapidly proliferating lesions (MIB-1 scores of $>10\%$). The survival of the latter group was similar to that of women with MIB-1 scores of $\leq 10\%$ who underwent surgery during the follicular phase of their menstrual cycle.

In contrast, there was no difference in the survival of patients who underwent surgery during the bad (follicular) phase of the menstrual cycle when classified according to ER and PR status. This may be a chance finding. Alternatively, it could result from an increased likelihood of the establishment of viable

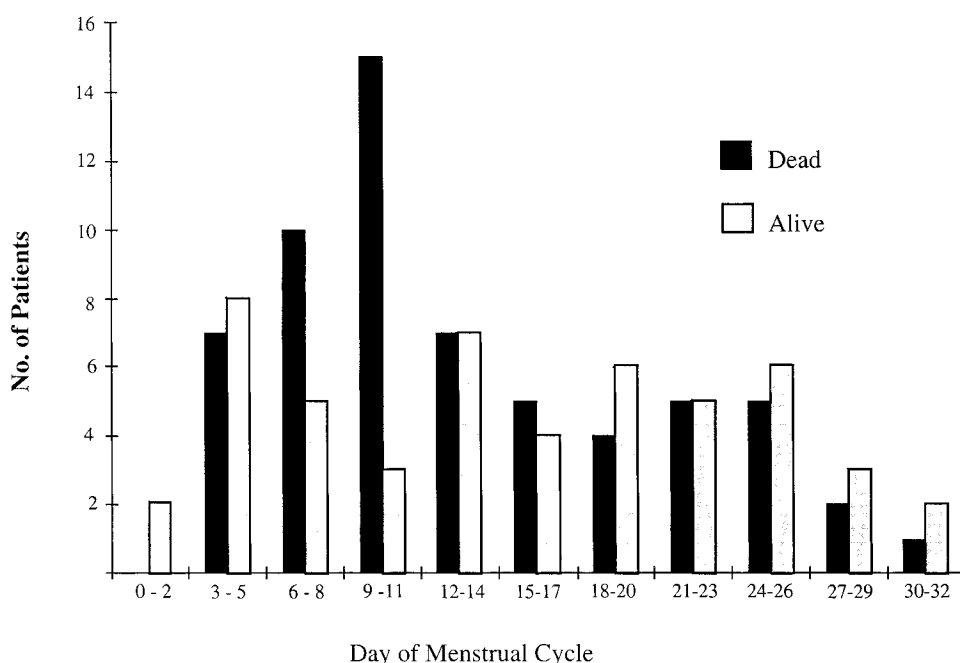


FIGURE 5. Overall survival by day of menstrual cycle.

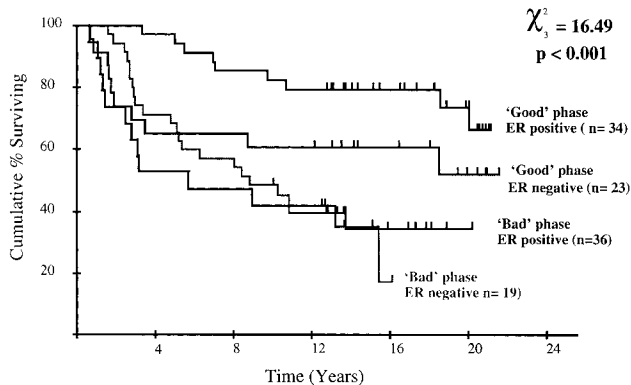


FIGURE 6. Overall survival by phase of menstrual cycle and estrogen receptor (ER) status. χ^2 : chi-square test.

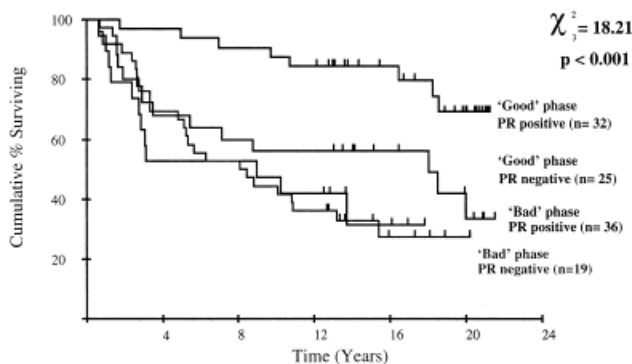


FIGURE 7. Overall survival by phase of menstrual cycle and progesterone receptor (PR) status. χ^2 : chi-square test.

metastatic cells from ER positive tumors shed during the follicular phase being matched by vascular dissemination of the less cohesive ER negative tumors. There has been considerable controversy regarding the influence of ER and PR on the prognosis of women with breast carcinoma and important effects may have been missed because of the failure to take into account the timing of surgery in premenopausal women.

The proportion of ER positive tumors (63%) was lower than usually reported in unselected series of breast carcinoma patients but is appropriate for a group of premenopausal patients because the proportion of ER positive tumors increases with age. No such effect was observed in relation to PR status, which does not change with age.¹⁸

There were no significant differences between the proportion of ER positive or ER negative tumors in the different phases of the cycle. However, this may not reflect the situation in the surrounding normal tissue. Söderqvist et al. performed fine-needle aspiration cytology in 42 healthy volunteers throughout the menstrual cycle.¹⁹ Although PR was detectable in 80% of aspirates from women in both the follicular and luteal phases, ER was found in 68% of follicular specimens

but only 32% of luteal phase aspirates. The results of the current study showed no difference in the ER and PR status of the luminal epithelial cells in normal breast tissue surrounding tumors excised during different menstrual phases.

The unopposed estrogens in the follicular phase have pleiotropic effects, including induction of cathepsin D²⁰ and increased synthesis of vascular endothelial growth factor (VEGF).²¹ The former may enhance the local invasive capacity of malignant cells and the latter is a necessary factor for angiogenesis so that tumor emboli can establish viable metastases. Recent studies have shown that there is a significant lowering of serum VEGF in premenopausal women during the luteal phase of the menstrual cycle.²²

The results of this study reinforce the importance of the timing of surgery in the prognosis of premenopausal women with operable breast carcinoma. The benefit is most pronounced in those with hormone-dependent and slowly proliferating tumors who undergo surgery during the luteal phase of the menstrual cycle. Follicular phase surgery is associated with a poor prognosis regardless of hormone status. These findings have not solved the mystery of the mechanisms involved in the timing of surgery. Nevertheless, they may influence patient management by rescheduling surgery, leading to a better prognosis for premenopausal women with operable breast carcinoma.

REFERENCES

1. Fentiman IS, Gregory WM, Richards MA. Effect of menstrual cycle phase on surgical treatment of breast cancer. *Lancet* 1994;344:402.
2. Veronesi U, Luini A, Mariani L, Del Vecchio M, Alvez D, Andreoli C, et al. Effect of menstrual phase on surgical treatment of breast cancer. *Lancet* 1994;343:1544-6.
3. Goldhirsch A, Gelber RD, Castiglione M, O'Neill A, Thürlimann B, Rudenstam C-M, et al. Menstrual cycle and timing of breast surgery in premenopausal node-positive breast cancer: results of the International Breast Cancer Study Group (IBCSG) Trial VI. *Ann Oncol* 1997;8:751-6.
4. Hrushesky WJM, Bluming AZ, Gruber SA, Southern RB. Menstrual influence on surgical cure of breast cancer. *Lancet* 1989;i:949-53.
5. Powles TJ, Jones AL, Ashley S, Tidy A. Menstrual effect on surgical cure of breast cancer. *Lancet* 1989;ii:1343-4.
6. Goldhirsch A, Gelber RD, Forbes J, Price K, Castiglione M, Rudenstam C-M, et al. Timing breast cancer surgery. *Lancet* 1991;338:692.
7. Badwe RA, Gregory WM, Chaudary MA, Richards MA, Bentley AE, Rubens RD, et al. Timing of surgery during menstrual cycle and survival of premenopausal women with operable breast cancer. *Lancet* 1991;337:1261-4.
8. Mohr PE, Wang DY, Gregory WM, Richards MA, Fentiman IS. Serum progesterone and prognosis in operable breast cancer. *Br J Cancer* 1996;73:1552-5.

9. Badwe RA, Bettelheim R, Millis RR, Gregory WM, Richards MA, Fentiman IS. Cyclical tumor variations in premenopausal women with early breast cancer. *Eur J Cancer* 1995; 31A:2181-4.
10. Cooper LS, Gillett CE, Smith P, Fentiman IS, Barnes DM. Cell proliferation measured by MIB1 and timing of surgery in breast cancer. *Br J Cancer* 1998;77:1502-7.
11. Van Dongen JA, Bartelink H, Fentiman IS, Lerut T, Mignolet F, Olthuis G, et al. Factors influencing local relapse and survival and results of salvage treatment after breast-conserving therapy in operable breast cancer: EORTC Trial 10801, breast conservation compared with mastectomy in TNM stage I and II breast cancer. *Eur J Cancer* 1992;28A: 801-5.
12. World Health Organization. International histologic diagnosis of tumors. In: Histologic typing of breast tumors. Geneva: World Health Organization, 1981:.
13. Elston CW, Ellis IO. Pathologic prognostic factors in breast cancer. *Histopathology* 1991;19:403-10.
14. Barnes DM, Harris WH, Smith P, Millis RR, Rubens RD. Immunohistochemical determination of estrogen receptor: comparison of different methods of assessment of staining and correlation with clinical outcome of breast cancer patients. *Br J Cancer* 1996;74:1445-51.
15. Peto R, Pike MC, Armitage P, Breslow NE, Cox DR, Howard SV, et al. Design and analysis of clinical trials requiring prolonged observation of each patient II: analysis and examples. *Br J Cancer* 1977;35:1-39.
16. Badwe RA, Richards MA, Fentiman IS, Gregory W, Saad Z, Chaudary MA, et al. Surgical procedures, menstrual cycle phase, and prognosis in operable breast cancer. *Lancet* 1991;338:815-6.
17. Millis RR. The relationship between pathology of breast cancer and hormone sensitivity. *Rev Endocrinol Rel Cancer* 1987;20(Suppl):13-8.
18. Skinner LG, Barnes DM, Ribeiro GG. The clinical value of multiple steroid receptor assays in breast cancer management. *Cancer* 1980;46:2939-45.
19. Söderqvist G, Von Schoultz B, Tani E, Skoog L. Estrogen and progesterone receptor content in breast epithelial cells from healthy women during the menstrual cycle. *Am J Obstet Gynecol* 1993;168:874-9.
20. Rochefort H. Cathepsin D in breast cancer: a tissue marker associated with metastasis. *Eur J Cancer* 1992;28A:1780-3.
21. Schweike D, Hin A, Neufeld G, Gitay-Goren H, Keshet E. Patterns of expression of vascular endothelial growth hormone (VEGF) and VEGF receptors in mice suggest a role in hormonally regulated angiogenesis. *J Clin Invest* 1991;91:2235-43.
22. Heer K, Kumar H, Speirs V, Greenman J, Drew PJ, Fox JN, et al. Vascular endothelial growth factor in premenopausal women - indicator of the best time for breast cancer surgery. *Br J Cancer* 1998;78:1203-7.