6. O'Brien MER, Perren T, Tan S, Wiltshaw E. Three cases of raised alpha feto protein in epithelial ovarian cancer of the clear cell subtype. *J Gynaecol Oncol*, in press.
7. Toppila M, Tyler JPP, Fay R, *et al.* Steroid receptors in human ovarian malignancy. A review of four years of tissue collection. *Br J Obstet Gynaecol* 1986, **93**, 986–992.
8. Slotman BJ, Kuhnel R, Rao BR, Dijkhuizen GH, De Graffe J, Stolk J. Importance of steroid receptors and aromatase activity in the prognosis of ovarian cancer. High tumour progesterone receptor levels correlate with longer survival. *Gynecol Oncol* 1989, **33**, 76–81.
9. Krebs HB, Goplerud DR, Kilpatrick JS, Myers MB, Hunt A. The role of CA 125 as tumor marker in ovarian carcinomas. *Obstet Gynecol* 1986, **67**, 473–477.
10. Mahlck CG, Grankvist K, Kjellgren O, Backstrom T. Relationship between CA 125 and progesterone production in women with ovarian cancer. *Cancer* 1990, **65**, 2058–2063.

Pergamon

European Journal of Cancer Vol. 30A, No. 4, pp. 445–448, 1994

0959–8049/94 $7.00 + 0.00

0959-8049(93)E0094-7

Serum Progesterone at the Time of Surgery and Survival in Women with Premenopausal Operable Breast Cancer

R.A. Badwe, D.Y. Wang, W.M. Gregory, I.S. Fentiman, M.A. Chaudary, P. Smith, M.A. Richards and R.D. Rubens

Serum progesterone and oestradiol levels have been measured in 210 premenopausal women with operable breast cancer on samples taken within 3 days of tumour excision. There was no relation between oestradiol level and time since last menstrual period, nor any effect of oestradiol value on prognosis. However, serum progesterone levels were related to the phase of the cycle as determined by time since last menstrual period. When divided on a basis of levels > 1.5 ng/ml (luteal phase) and ≤ 1.5 ng/ml, it was found that there was no difference in survival between the two groups among 117 axillary node negative cases. However, in the 93 patients with positive axillary nodes, higher progesterone levels were associated with significantly better survival. Thus, serum progesterone levels at the time of surgery may affect the prognosis of premenopausal node positive patients with operable breast cancer.
Eur J Cancer, Vol. 30A, No. 4, pp. 445–448, 1994

INTRODUCTION

FOLLOWING THE report of Hrushesky and colleagues [1] on the influence of timing of surgery within the menstrual cycle on the long-term outcome for premenopausal patients with breast cancer, a number of retrospective studies have been reported with apparently conflicting results [2–9]. The discrepancies in the findings arise in part from analysis of different time intervals within the cycle. In two studies [1,9], patients operated on around the time of ovulation (defined as 6–20 days after the onset of menstruation) were compared with those operated on at other phases of the cycle. At Guy's Hospital, we chose to examine the outcome for patients undergoing tumour excision between days 3 and 12 of the cycle (when high levels of circulating oestrogen would be expected, without opposing progesterone) with that of patients operated on at other phases of the cycle (when levels of both hormones would be expected to be either high or low together). In two series of patients managed at Guy's we have demonstrated that prognosis was significantly worse for patients operated on between days 3 and 12 of the cycle [2,3]. A study from the Memorial Sloan Kettering Cancer Centre [4] showed similar results (comparing surgery in the first and second halves of the cycle), while others using the Guy's criteria have shown no difference in outcome [5–8].

One of the possible criticisms of all of the studies reported to date is that information on phase of the menstrual cycle, gathered retrospectively from hospital case notes, could be unreliable. During the period covered by our first study (1975–1985), blood was collected around the time of primary treatment of breast cancer from a cohort of patients for subsequent prognostic factor analyses. Although blood was taken at variable times in relation to diagnostic or definitive surgery, dates of blood collection were known and the large majority were taken within 3 days of the time of excision of the primary tumour.

We report here the oestradiol and progesterone levels measured on samples from patients in our first report from whom serum had been stored. The purposes of this study were to

Correspondence to I.S. Fentiman.
The authors are at the ICRF Clinical Oncology Unit, Guy's Hospital, London SE1 9RT, U.K.
Revised and accepted 18 Oct. 1993.

EJC 30:4-B

validate the accuracy of estimation of patients being in the luteal phase from information in case notes, and to assess the prognostic significance of perioperative oestradiol and progesterone levels.

MATERIALS AND METHODS

Between 1975 and 1985, a total of 560 premenopausal patients presented to the Guy's Breast Unit with unilateral, operable, invasive breast cancer [2]. Blood had been taken and serum stored at −20°C from 271 of these patients around the time of initial diagnosis. The samples were in most cases taken 1 day before or 2 days after diagnostic excision biopsy. In a minority of cases, the samples were taken at longer intervals before or after tumour excision, either when the patient attended for preoperative investigation or when the patient was re-admitted for definitive surgery (modified radical mastectomy or a breast conserving procedure including axillary clearance). For the analysis of the influences of perioperative oestrogen and progesterone levels on outcome, only those patients who had blood taken within 3 days on either side of the date of tumour excision were included. Of the 271 patients with blood available 210 (77%) came into this category. Needle biopsy and fine needle aspiration were not undertaken in this time period. The date of surgery was taken as the day on which the breast cancer itself was excised.

Data on the time of the last menstrual cycle (LMP) and regularity of cycles was available from hospital notes for 121 of the 271 patients (45%). This compares with 249 of the 560 (44%) patients in our first report [2]. For the purpose of validation of the accuracy of LMP data from patients records on phase of the menstrual cycle, progesterone levels were compared with the estimated day of the cycle on which blood was taken. Those in whom blood was taken during the first 12 days of the cycle were expected to have low levels. 11 patients were excluded from this comparison because the estimated day of cycle at the time of blood sampling was greater than 28 days, and thus a further cycle could have intervened. This could for example have occurred if the LMP was 20 days before tumour excision and blood was taken 15 days after excision. 2 further patients were excluded because their samples were taken before their last known menstrual period. These two groups of exclusions reduced the available data for analysis of both LMP and hormone levels to 108 patients.

Blood oestradiol assay

Oestradiol was determined by double antibody radioimmunoassay using a kit from Diagnostic Products Corporation (Basingstoke, Hants, U.K., Cat No: KE2). The characteristics of the assay were satisfactory for the purposes of this study. The sensitivity was 1.4 pg/ml and the precision was less than 5%. The specificity was such that the cross-reaction with oestrone and oestriol was 1.3 and 0.2%, respectively. There was no detectable cross-reaction with progesterone. The inter- and intra-assay reaction was less than 10%.

Blood progesterone assay

Progesterone was measured in serum by solid phase radioimmunoassay using a kit (coat-a-count, Cat No: TKPG) purchased from Diagnostic Products Corporation. The minimum standard, other than zero, was equivalent to 0.1 ng/ml. The inter- and intra-assay coefficients of variation were less than 10%. The specificity was satisfactory with minimal cross-reaction to 17-hydroxyprogesterone (0.3%), 11-deoxycortisol (2.4%), 11-deoxycorticosterone (1.7%) and 5-pregnan-3, 20-dione (1.3%).

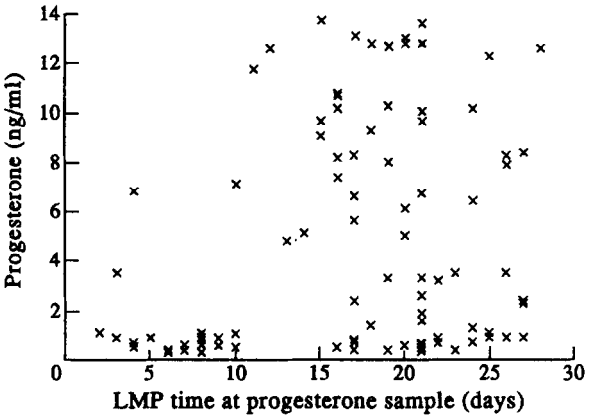

Fig. 1. Relationship between last menstrual period and serum progesterone level.

Progesterone levels of > 1.5 ng/ml were taken to show that the patient was in the luteal phase and this was confirmed by inspection of LMP intervals (see Results and Figure 2).

Statistical methods

Relapse-free survival and survival were calculated by the method of Kaplan and Meier [10] and significance was determined by the log rank test [11]. Multivariate analysis was performed with Cox's proportional hazards models [12]. Age at diagnosis and tumour size were treated as continuous variables. Lymph nodes were categorised as negative, 1–3 (metastatic nodes), 4–9 or ≥ 10. A cut-off point of 1.5 ng/ml was used for progesterone. Histology was categorised as invasive ductal in three grades, with lobular and other histologies being combined into a single group.

RESULTS

Of the 271 cases who had assays performed, 151 were node negative and only 1 received adjuvant chemotherapy. There were 119 node positive patients of whom 66 (55%) received no adjuvant therapy and 32 (27%) were given cyclophosphamide, methotrexate, fluorouracil (CMF), and 21 (18%) were given L-phenylalanine mustard (L-PAM). The median follow-up was 11 years. Figure 2 is a plot of oestradiol value against time elapsed since last menstrual period. This shows that there was no relationship between LMP interval and oestradiol level. Further analysis (data not shown) did not reveal any relationship between

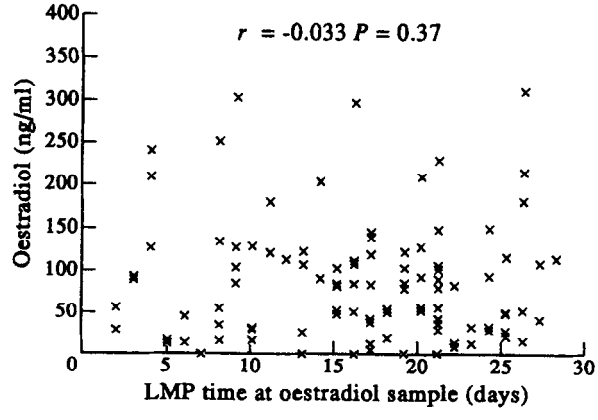

Fig. 2. Relationship between last menstrual period and serum oestradiol levels.

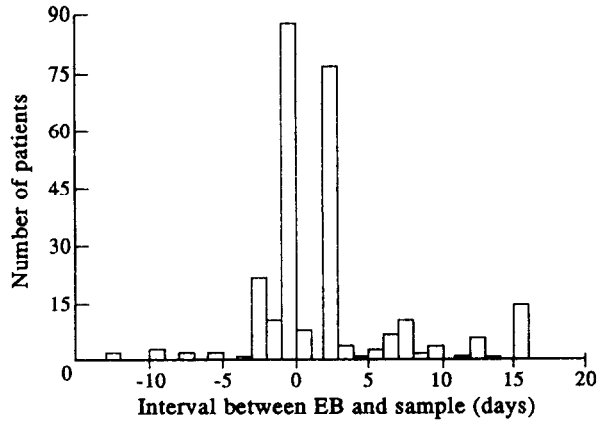

Fig. 3. Interval between excision of tumour (EB) and blood sample.

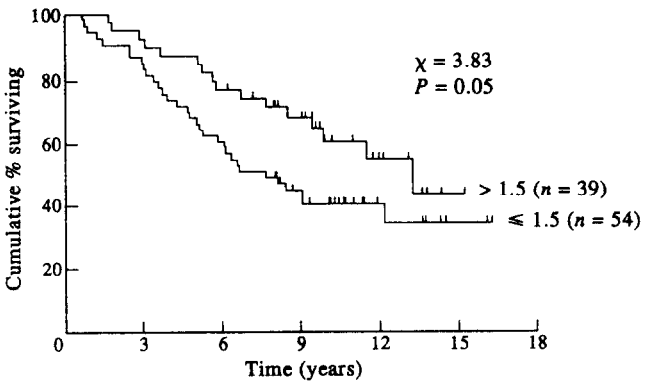

Fig. 5. Overall survival of node positive patients with serum progesterone levels ≤ 1.5 ng/ml and > 1.5 ng/ml.

oestradiol level and overall survival. A comparison between progesterone levels and calculated day of cycle for the 108 patients in whom data related to LMP could be retrieved from hospital notes is shown in Figure 1. Inspection of this figure confirms that 1.5 ng/ml is an appropriate cut-off level for separating the luteal and non-luteal phases of the menstrual cycle, since the large majority of patients sampled in the first 12 days of the cycle have values below this level. Only 5 of 32 (16%) patients in this period had positive serum progesterone levels, compared with 48 out of 76 (63%) whose blood was taken later in the cycle ($P < 0.0001$, Fisher's exact test).

Because of fluctuations in progesterone, some patients with low progesterone levels in the second half of the cycle may, of course, have had anovulatory cycles. This does provide, therefore, a validation of the general accuracy of LMP dates extracted from patient records. This group of 108 patients forms a subset of the 249 patients in our original study for whom LMP dates at time of surgery were known.

In order to assess the impact of perioperative progesterone levels on long-term survival, analysis was confined to the 210 patients from whom blood was taken within 3 days of surgery (Figure 3). 117 of these patients had no histological evidence of lymph node involvement following axillary clearance. Amongst these patients perioperative progesterone levels did not appear to influence survival (Figure 4). For the 93 patients who had positive axillary lymph nodes, high serum progesterone levels were associated with improved prognosis (Figure 5). Multivariate analysis confirmed this to be independent of other known prognostic variables including number of involved lymph nodes, tumour type and age (Table 1). Of the 210 patients with known perioperative progesterone levels, 94 also had LMP data available from the case notes. Survival for the 28 patients who had tumour excisions between days 3 and 12 of the cycle was worse than that for the 66 patients operated on at other phases of the cycle ($P < 0.0001$). Multivariate analysis showed that date of LMP provided significant prognostic information within this group ($P = 0.001$), but that when this had been taken into account, serum progesterone level gave no additional information ($P = 0.45$). The relative risk of dying for patients with progesterone levels ≤ 1.5 ng/ml was 2.1 times as great as for patients with levels > 1.5 ng/ml. Thus, those with low progesterone levels had a 2-fold increase in mortality compared with those with progesterone > 1.5 ng/ml.

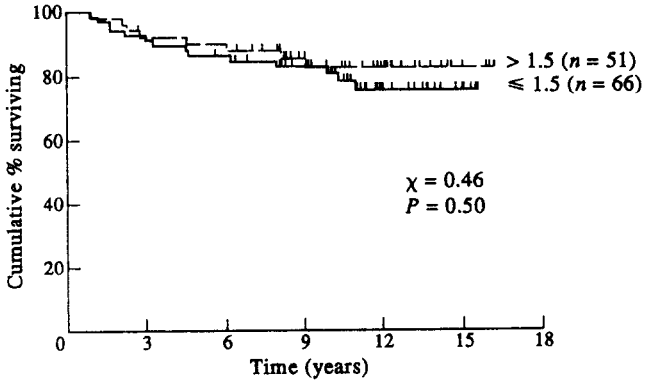

Fig. 4. Overall survival of node negative patients with serum progesterone levels ≤ 1.5 ng/ml and > 1.5 ng/ml.

DISCUSSION

This study is retrospective rather than prospective, but nevertheless provides a validation of the accuracy of estimation of the phase of the menstrual cycle from careful retrospective analysis of hospital case notes. Only a small minority (16%) of those who would have been predicted to have low progesterone levels (i.e. those in the first 12 days of the cycle) from analysis of their menstrual histories, were found to have high levels on analysis of stored serum samples. Similarly, the majority (63%) of those who were predicted to be in the luteal phase of the cycle had high progesterone levels. Others may have had anovulatory cycles, or may have been wrongly allocated on the basis of menstrual history. In addition, it is possible that the assay could

Table 1. Univariate and multivariate significance of prognostic factors for survival for node positive women

Factor†	Univariate* χ^2	Univariate* *P* value	Multivariate χ^2	Multivariate *P* value
Age	4.0	0.05	6.5	0.01
Tumour size	5.2	0.02	2.2	ns
No. of nodes involved	7.7	0.006	11.5	0.0007
Histological subtype	17.5	<0.0001	6.2	0.01
Progesterone	4.9	0.03	5.35	0.02

ns, non-significant. *From Cox model, not log-rank test. †For coding of factors, see statistical methods section.

have given false negative results. Oestradiol measurements were found to be non-contributory both in terms of phase of the cycle and prognosis. This may arise because a point estimation of oestradiol gives no indication of whether the level is rising or falling, and does not identify which phase of the menstrual cycle occurred at the time of sampling.

By both univariate and multivariate analysis, we have shown that node positive patients with high serum progesterone levels have a survival advantage over those with low levels. No significant effect was found in node negative cases. This was similar to the finding in our first study in which the 10-year survival for node negative patients undergoing surgery between days 3 and 12 was 82% as compared with 89% for those having surgery at other times of the cycle [2]. The difference in survival is not, however, as large as that observed in our previous reports related solely to the day of cycle. This can be at least partly explained by the presence in the low progesterone group of some patients who were operated on either at the end of a cycle or at the start of a cycle, when oestrogen would also be expected to be low. We have previously demonstrated that patients operated on at these times have a good prognosis. Thus, assessment of progesterone levels is probably not as reliable an indicator of prognosis as estimation of day of cycle. The small patient numbers in the node positive group ($n = 93$) combined with a correlation between number of involved nodes and tumour size, probably explain the lack of multivariate significance ($P = 0.14$) of tumour size in this group.

The mechanism by which progesterone exerts a beneficial effect is unknown. However, progesterone is known to counteract the effects of oestrogen by stimulating β-hydroxysteroid dehydrogenase, an enzyme which actively metabolises oestrogen [13]. Unopposed oestrogen stimulates secretion of proteases from breast cancer cells acting at various pH ranges in both extra and intracellular domains [14,15]. During the follicular phase of menstruation, tumour manipulation may be likely to disseminate cells which have the capacity to establish at distant sites. Since these proteases have a short half life, this activity subsides rapidly as soon as the unopposed oestrogenic environment is modified by the rising progesterone level [16]. In addition, an increased ability to secrete growth factors [17] and the low natural killer (NK) cell activity [18], under the influence of oestrogen, may create a permissive environment for micrometastases to become established. Progesterone does not appear to inhibit proliferation of breast tissue since an autopsy study showed that there were higher mitotic counts in breast epithelium during the luteal phase [19].

In conclusion, results of this study further strengthen our view that endocrine factors at the time of tumour excision are important determinants of survival. The presence of progesterone in an oestrogenic milieu appears to reduce the development of subsequent distant metastases, and thereby improves survival. Definite evidence of this will come only from prospective studies, several of which are now underway.

1. Hrushesky WJM, Bluming AZ, Gruber SA, Sothern RB. Menstrual influence on surgical cure of breast cancer. *Lancet* 1981, **ii**, 949–952.
2. Badwe RA, Gregory WM, Chaudary MA, *et al.* Timing of surgery during the menstrual cycle and survival of premenopausal women with operable breast cancer. *Lancet* 1991, **337**, 1261–1264.
3. Badwe RA, Fentiman IS, Richards MA, *et al.* Surgical procedures, menstrual cycle phase and prognosis in operable breast cancer. *Lancet* 1991, **338**, 815–816.
4. Senie RT, Rosen PP, Rhodes P, Lesser MC. Timing of breast cancer excision during the menstrual cycle influences duration of disease free survival. *Ann Intern Med* 1991, **115**, 337–342.
5. Rageth JC, Wyss P, Unger C, Hochuli E. Timing of breast cancer surgery within the menstrual cycle: influence of lymph-node involvement, receptor status, postoperative metastatic spread and local recurrence. *Ann Oncol* 1991, **2**, 269–272.
6. Powles TJ, Ashley SE, Nash AG, Tidy A, Gazet JC, Ford. Timing of surgery in breast cancer. *Lancet* 1991, **337**, 1604.
7. Goldhirsch A, Gelber R, Forbes J, *et al.* Timing breast cancer surgery. *Lancet* 1991, **338**, 691–692.
8. Low SC, Galea MH, Blamey RW. Timing breast cancer surgery. *Lancet* 1991, **338**, 691.
9. Ville Y, Briere M, Lasky S, Spyratos F, Oglobine J, Brunet M. Timing of surgery in breast cancer. *Lancet* 1991, **337**, 1604–1605.
10. Kaplan EL, Meier P. Non-parametric estimation from incomplete observations. *Am Stat Assoc J* 1958, **53**, 457–481.
11. Peto R, Pike MC, Armitage P, *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.
12. Cox DR. Regression models and life tables. *J R Stat Soc* 1972, **34**, 187–220.
13. Jarvis PM, Kuttenn F, Gompei A. Oestradiol and progesterone interaction in normal and pathological breast cells. Endocrinology of breast: basic and clinical aspects (Angeli A, Badlow HC, Dogliotti L, eds). *Ann NY Acad Sci* 1980, **464**, 152–167.
14. Rochefort H, Augereau P, Briozzo P, *et al.* Structure, function, regulation and clinical significance of the 52K pro-cathepsin D secretion by breast cancer cells. *Biochimie* 1988, **70**, 943–949.
15. Huff K, Lippman ME. Hormonal control of plasminogen activator secretion in ZR-75-1 human breast cancer cells in culture. *Endocrinology* 1984, **114**, 1702–1710.
16. Frances CW, Marden VJ. Mechanism of fibrinolysis. *Haematology* 1990, 1313–1321.
17. Lippman ME, Dickson RB, Bates S, *et al.* Autocrine and paracrine growth regulation of human breast cancer. *Breast Cancer Res Treat* 1986, **7**, 59–70.
18. Duffy MJ, Reilly D, O'Sullivan C, *et al.* Urokinase plasminogen activator, a new and independent prognostic marker in breast cancer. *Cancer Res* 1990, **50**, 6827–6829.
19. Longacre TA, Bartow SA. A correlative morphologic study of human breast and endometrium in the menstrual cycle. *Am J Surg Pathol* 1986, **10**, 382–393.

Acknowledgement—Dr D.Y. Wang is supported by the Breast Cancer Research Trust.