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Timing of Surgery During the Menstrual Cycle for Breast Cancer: Possible Role of Growth Factors

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Premenopausal patients undergoing surgery for breast cancer were prospectively studied. Data regarding menstrual history, pathological parameters and hormone receptor status were collected. Serum oestradiol, prolactin and progesterone levels, tumour epidermal growth factor receptor (EGFR) levels, tumour epidermal growth factor (EGF) levels and flow cytometry were measured. Patients were allocated to the follicular or luteal phase of their cycle both by history and progesterone level. No significant differences were seen in hormone receptor levels, pathological parameters or EGF levels between the two groups. EGFR levels were significantly higher in women undergoing surgery during the follicular phase of their cycle, when classified by menstrual history. Patients operated on during this phase have previously been found to have a poorer prognosis, and these results may provide a basis for this finding. This may have implications for prognosis and timing of surgery, and further investigation is warranted.

Key words: breast, menstrual cycle, surgery, receptors, epidermal growth factor, urogastrone
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INTRODUCTION

STUDIES IN mice have shown a lower rate of pulmonary metastases when mammary tumours were excised during the oestrous cycle [1]. Hrushesky and associates [2] reported on a series of 41 women, where disease-free and overall survival were better if they had their surgery midcycle, compared to perimenstrual. Four subsequent reports [3–6], with larger numbers, failed to find any statistically significant difference in survival or recurrence using the same criteria. More recently, two articles have appeared showing a survival benefit after 10 years if surgery was carried out during the luteal phase, compared to the follicular phase of the menstrual cycle [7, 8]. The proffered hypothesis is that unopposed oestrogen during the follicular phase affects either the spread of malignant cells or the survival of cells that have already metastasised.

Growth factors have been implicated in this hypothesis, both in the serum [9] as well as in the region of the tumour [10]. Growth factors such as epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) are mitogenic to breast cancer cells, and oestradiol stimulates the production of these growth factors *in vitro* [10], raising the possibility of autocrine growth stimulation *in vivo*. In addition to the possible influence of oestradiol on breast cancer, some previous work has shown a direct relationship between prolactin levels and tumour size and grade [11]. We have investigated the relationship between growth factors and serum sex hormones in patients with breast cancer, and report our findings in the subset of premenopausal women.

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PATIENTS AND METHODS

All women undergoing surgery for primary, palpable carcinoma of the breast at the Sir Charles Gairdner Hospital were approached to enter the study. Informed consent was obtained in all cases, and approval for the study was obtained from the ethics committee. Information regarding date of last menstrual period as well as length of cycle was obtained from all patients.

A pre-operative, fasting, early morning blood sample was taken and the serum separated and stored at −70°C until assayed in batches. Oestradiol, prolactin and progesterone levels were measured in all cases.

After surgical excision of the breast lump, a portion was removed, trimmed of fat and necrotic tissue, and stored in liquid nitrogen until assayed.

Tumour preparation

Fresh frozen tumour was thawed, then minced in phosphate buffer at 4°C in an UltraTurrax homogeniser. The sample was centrifuged at 40 000 ***g*** for 1 h, and the supernatant collected (as cytosol fraction) for EGF assay. The pellet was resuspended in phosphate-buffered saline and centrifuged at 1500 ***g*** for 5 min. The supernatant was collected to be used as the membrane fraction. Protein levels were measured using Coomassie Brilliant Blue.

EGF assay

Cytosolic EGF was measured using a radioimmunoassay (RIA) kit supplied by Amersham (#IM1961). Specificity was claimed by the manufacturers as 0% for rat TGF-α and 88% for mouse EGF. Results were expressed as ng EGF/mg protein.

EGFR assay

Membrane fractions were incubated with ^{125}I-labelled EGF in the presence and absence of an excess of unlabelled tracer. Non-specific binding was subtracted from total binding to obtain specific binding, with the result being expressed as fmol EGFR/mg protein.

Flow cytometry

A 2-mm cube of fresh tissue was minced with a scalpel blade in culture medium containing propidium iodide and ribonuclease, then strained through 45-μm nylon mesh. The samples were analysed on a Becton Dickinson FACS analyser using chicken red blood cells as standards. The histograms were then analysed on an IBM compatible computer using Modfit V.5 (Verity Software).

Hormone assays

Total oestradiol, progesterone, sex hormone binding globulin and prolactin levels were measured using commercially available RIA kits.

Statistical methods

The results were separately analysed according to the reported menstrual history (follicular = days 3–12, luteal = days 0–2, 13–28), and progesterone levels (follicular <5, luteal >5).

Continuous data were expressed as mean ± S.E.M. and compared using the two-sample *t*-test. The EGF receptor (EGFR) and EGF levels were not normally distributed, and were compared using the Mann–Whitney U-test. Categorical data were analysed by χ^2 with Yates correction. Statistical significance were defined as $P < 0.05$.

Oestrogen receptor (ER) positive was defined as >4 fmol/mg, progesterone receptor (PR) positive as >10 fmol/mg and EGFR positive as >10 fmol/mg.

RESULTS

A total of 37 premenopausal women were studied between March 1990 and November 1992, from which the menstrual history was available in 33 cases, and hormone levels were available in 36 cases. The mean age was 41 years, with a range of 33–51. There were 18 women in follicular phase and 15 in luteal phase according to the menstrual history, and 23 women in follicular phase and 13 in luteal phase according to progesterone levels.

EGFR levels ranged from 0 to 98 fmol/mg, with a median of 4 fmol/mg. EGF levels ranged from 0 to 6.5 ng/mg, with a median of 0.36.

Hormone levels are shown in Table 1. There was no significant difference in either oestradiol or prolactin using the menstrual classification. Both oestradiol and prolactin levels were significantly higher in the luteal group when classified by progesterone levels.

Pathological parameters and hormone receptor levels are shown in Table 2. The only significant difference detected was a higher S-phase fraction in the luteal group when classified by progesterone levels.

The growth factor results are shown in Table 3. Patients operated on in the follicular phase had a higher mean EGFR level, and patients having their operation during the luteal phase tended to have EGFR-negative tumours. This was true for both methods of classification, but reached significance only in the menstrual history group.

Levels of tumour EGF were not different in either phase of the cycle, whether classified by menstrual history or progesterone levels.

DISCUSSION

After a report that the development of pulmonary metastases was dependent upon the timing of excision of the primary tumour during the oestrous cycle in the mouse [1], Hrushesky *et al.* [2] published a report of 41 women, where those operated on perimenstrually had better disease-free and overall survival. Following this, four further reports, containing 81 [4], 245 [5], 165 [3] and 224 [6] patients, respectively, failed to show any benefit from surgery during different times of the menstrual cycle.

Two later reports, using subgroupings according to the putative time of ovulation, then appeared. Senie *et al.* [8] initially used the same groupings as Hrushesky *et al.* [2] and found a trend in the opposite direction. Re-analysing their data by defining those operated on within the first 14 days as being in the follicular phase and those operated on from day 15 onwards as being in the luteal phase, they found better disease-free survival in women operated on during the luteal phase, particularly in the node-positive subset.

The other report [7] used slightly different criteria for their analysis. Women operated on during days 3–12 were defined as being in the follicular phase, and those in days 0–2 and 13–32 as being in the luteal phase. They found that women having surgery in the luteal phase had a better disease-free and overall survival at 10 years. Guy's Hospital changed their policy after this report, so that all breast cancer patients had their operation at least 12 days after their last menstrual period. This caused much debate in the literature, particularly in view of the publicity in the lay press. Several other centres subsequently reported their results [12–15], some showing no difference in survival between the two subgroups while one from the Yorkshire Breast Cancer Group showed completely the opposite result, i.e. the group having surgery during the follicular phase of the menstrual cycle had a better survival [16]. All of these reports are retrospective and uncontrolled. They have differing subgroupings within the menstrual cycle, and the groups had differing adjuvant therapy. This makes direct comparisons between studies difficult. Further prospective trials with accurate menstrual histories backed up by hormone measurements need to be carried out to better investigate this problem. One such study co-ordinated by the Yorkshire Breast Cancer Group is currently underway.

The finding that operating during the follicular phase of the menstrual cycle may be detrimental to long-term survival could have a hormonal basis. This phase of the cycle is characterised by relatively high levels of oestradiol and low levels of progesterone. Animal studies have shown a reduction in activity of natural killer lymphocytes in the presence of oestradiol [17], and a cycling of the T4/T8 lymphocyte ratio has been demonstrated in menstruating women [18].

Locally produced growth factors, like EGF and TGF-α, are important regulators for breast cancer growth. They are both ligands for EGFR, a transmembrane receptor. When EGF or TGF-α bind to the EGFR, cell replication is stimulated. Adding oestradiol to breast cancer cells *in vitro* causes the release of

Table 1. Hormone levels

	Menstrual			Hormonal		
	Follicular	Luteal	*P*	Follicular	Luteal	*P*
Oestradiol (pmol/l) $n = 36$	327 ± 72	336 ± 60	ns	317 ± 64	414 ± 49	0.03
Prolactin (mU/l) $n = 36$	1143 ± 376	1404 ± 379	ns	1057 ± 283	2189 ± 521	0.04
Progesterone (nmol/l) $n = 36$	5 ± 3	15 + 4	ns	1.5 ± 0.16	26 ± 4.8	0.0001

ns, non-significant.

Table 2. Pathological parameters

	Menstrual			Hormonal		
	Follicular	Luteal	*P*	Follicular	Luteal	*P*
Size (mm)	23 ± 3	27 ± 4	ns	23 ± 3	29 ± 3	ns
Node-positive	9	8		12	8	
Node-negative	9	7	ns	11	5	ns
ER-positive	16	13		22	10	
ER-negative	2	2	ns	1	3	ns
Aneuploid	4	4		4	5	
Diploid	8	7	ns	12	5	ns
S phase (%)	9.5 ± 2	6.8 ± 2	ns	6.4 ± 0.9	11.4 ± 3	0.0003
Grade 1	3	0		3	0	
Grade 2	6	3		8	1	
Grade 3	3	6	ns	5	4	ns

ns, non-significant.

Table 3. Growth factors

	Classification					
	Menstrual			Hormonal		
	Follicular	Luteal	*P*	Follicular	Luteal	*P*
EGFR (fmol/mg protein) $n = 35$	22 ± 6	4 ± 1	0.004	17 ± 5	6 ± 2	ns
EGF (ng/mg protein) $n = 35$	1.0 ± 0.4	1.3 ± 0.4	ns	1.2 ± 0.4	0.8 ± 0.2	ns
EGFR +	9	3		9	5	
EGFR−	7	12	0.04	12	8	ns

EGFR, epidermal growth factor receptor; ns, non-significant.

TGF-α [10], which may be acting in an autocrine fashion on the breast cancer cells. Similar growth factors have been detected in the serum after removal of breast tumours in mice [9].

The EGFR has been shown to be an independent prognostic indicator for relapse-free and overall survival in clinical breast cancer studies [19]. Patients with higher EGFR levels tend to have a worse disease-free and overall survival. It has also been shown to be a better indicator of response to hormonal therapy than ER status [20].

The use of progesterone to differentiate between follicular and luteal phases of the menstrual cycle provides a much more valid grouping than menstrual history, particularly since recollection of the date of the last menstrual period can be unreliable. 4 of the patients in this series could not reliably remember the date of their last menstrual period, and so have not been included in analyses classified by menstrual history. 7 patients in this series that were allocated to the luteal phase on menstrual history were reclassified as follicular on the basis of progesterone levels, and

similarly 2 patients in the follicular group were subsequently reclassified as being in the luteal group. This probably accounts for the failure to achieve statistical significance when comparing progesterone levels between the two groups according to menstrual history.

Oestradiol levels were higher in the luteal group, particularly when using progesterone to classify the groups, which is what is expected from known hormone changes during the menstrual cycle. Prolactin levels were much higher in the luteal group. Our previous studies showed this to be associated with larger, more poorly differentiated tumours, but mainly in postmenopausal women [11]. This would tend to give a poorer prognosis to patients in the luteal group, which is at variance with the two previously cited series [7, 8].

No significant difference in DNA content was seen in either classification. However, using the progesterone classification, patients in the luteal group had tumours with significantly higher S-phase fractions. Progesterone has many effects on breast cancer cells *in vitro*, including increasing the expression of EGF [21], which allows actively cycling cells to progress through the G1 phase of replication. This may explain the increased S-phase in patients operated on during the luteal phase. Despite the effects of progesterone on growth factors, the overall effect is to modulate or decrease cell growth.

Our study showed higher tumour EGFR levels in the follicular group when using the menstrual history to allocate the phase of the menstrual cycle. The difference in EGFR levels was independent of any histological parameters and was unrelated to ER status. The patients with higher EGFR levels tended to be in the follicular phase of their menstrual cycle, which was the group with the poorer outlook in the studies by Badwe *et al.* [7] and Senie *et al.* [8], as well as being the group with unopposed oestradiol levels. It is possible that differences in growth factors or their receptors is one explanation for the observed differences in outcome in these studies, but EGFR levels were not reported in either study.

We have demonstrated differences in hormone levels and some prognostic factors in premenopausal patients undergoing surgery during different times of their menstrual cycle. Some of the differences favoured the follicular phase, and some the luteal phase. Obviously many factors can influence survival, and some of these may have more effect at different times during the menstrual cycle. This may explain why the reported studies have shown such conflicting results.

It must be stated that the numbers in our study are small, and no follow-up is available at this stage. However, the differences found are highly significant and warrant further investigation in the setting of a prospective trial, with a larger number of patients and with long-term follow-up of patient outcome.

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