A Prospective Prognostic Study of the Hormonal Milieu at the Time of Surgery in Premenopausal Breast Carcinoma

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BACKGROUND. Despite numerous studies, the influence of timing at surgery in relation to the menstrual cycle on the prognosis of breast carcinoma is still controversial. Most studies are retrospective, and the reliability of the menstrual history data is limited by the lack of hormonal assessment at the time of surgery. The authors prospectively studied the influence of the menstrual cycle phase as determined by circulating hormones at the time of surgery on the outcome of breast carcinoma.

METHODS. A population of 360 premenopausal women with nonmetastatic breast carcinoma operated on from 1992 to 1995 was analyzed. Serum estradiol, progesterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) levels were assayed the day of surgery to define the menstrual cycle phase (follicular, n = 186; ovulatory, n = 24; luteal, n = 150). The mean follow-up was 48 months.

RESULTS. There were no relations between the menstrual phase at surgery and tumor size, cathepsin D level, Scarff–Bloom–Richardson grade, Pg receptor (PgR), and the number of positive lymph nodes. The mean estrogen receptor level was higher during the follicular phase than in the ovulatory and luteal phases ($P < 0.02$). Univariate analysis of recurrence free survival (RFS) and overall survival (OS) showed no relations with the menstrual phase or the level of estradiol and progesterone at the time of surgery. High LH or FSH levels (above the medians) were associated with shorter RFS ($P = 0.02$ and $P = 0.04$, respectively) or OS ($P \leq 0.01$ and $P = 0.01$, respectively). In multivariate analysis, lymph node status, PgR status and LH level were the most significant parameters for predicting OS. There appeared to be no survival differences between menstrual cycle groups after stratification by lymph node status.

CONCLUSIONS. This prospective study showed a lack of prognostic value of timing at surgery in relation to the menstrual period or to estrogen and progesterone levels in premenopausal breast carcinoma. Conversely, high gonadotropin levels could predict OS independently of other prognostic factors. ***Cancer* 2001;91: 1854–61.** © *2001 American Cancer Society.*

KEYWORDS: breast carcinoma, menstrual cycle, estrogen, progesterone, gonadotropin, prognosis.

Experimental and clinical data have revealed that estrogens play a crucial role in the growth of breast carcinoma cells. Over the last decade, the importance of the hormonal milieu in patients with breast carcinoma has raised the question of whether the menstrual cycle phase at the time of surgery could affect the long term prognosis of premenopausal patients.

Initial studies of mice and humans suggest that unopposed es-

trogens at surgery may unfavorably affect the outcome of breast carcinoma.[1,2] The deleterious effects of unopposed estrogen could be conceptually supported further by the finding that high circulating estradiol level was found associated with an increased risk of breast carcinoma.[3] Although two previous studies found that a high serum progesterone level at the time of tumor surgery was associated with a better prognosis,[4,5] there are no published studies to our knowledge documenting an effect of estradiol level at surgery on the outcome of breast carcinoma.

Many clinical studies[5–13] and two meta-analyses[14,15] have shown a decreased overall survival rate in women with breast carcinoma who had undergone surgery in the follicular phase. However, some other studies failed to show such a significant effect.[16–22] These discrepancies might be explained by the limited reliability of the menstrual history data, the absence of hormonal measurement at time of surgery, and the fact that these studies were retrospective.

We prospectively analyzed the outcome of premenopausal women with breast carcinoma according to the menstrual status and estradiol, progesterone, and gonadotropin levels at the time of surgery.

PATIENTS AND METHODS

Study Design

A total of 487 consecutive premenopausal patients with primary breast carcinoma entered a monocentric prospective study from 1992 to 1995 (Montpellier Cancer Center, France). Menopausal women were clinically defined as patients with the last regular menses occuring more than 6 months earlier. Exclusion criteria were preoperative radiotherapy or chemotherapy (n = 35), metastasis (n = 17), use of oral contraceptives less than 1 month before surgery (n = 14), perimenopausal women (patients with the last regular menses occuring $\leq$ 6 months earlier and with the hormone profile defined below, n = 60), and pregnancy (n = 1). The remaining 360 patients qualified for the study. Informed consent was obtained from all patients entering the study.

The hormonal phase of the menstrual cycle was determined according to levels of circulating hormones at the day of surgery (day of tumor resection or mastectomy). Hormone level assessments, including estradiol (E2), progesterone (Pg), follicle-stimulating hormone (FSH), and luteinizing hormone (LH), were routinely performed in our laboratory on fresh sera daily. Patients with high FSH (> 15 International Unit [IU/L) and low estradiol level (< 40 pg/mL) were considered perimenopausal and thus excluded from the study (n = 60). Women with progesterone values exceeding 2.5 ng/mL were considered to be in the luteal phase of the cycle, based on manufacturer's threshold recommendation of the progesterone kit (CIS, Gif sur Yvette, France). The ovulatory period was defined on the basis of both high LH (> 10 IU/L) and E2 (> 100 pg/mL) values. The remaining premenopausal women were classified in the follicular phase.

Patients and Follow-Up

Tumor classifications were evaluated according to the International Union Against Cancer classification for breast carcinoma. Patients underwent mastectomy (n = 190) or sector resection followed by radiotherapy (n = 170). At the time of the study, our clinical standard procedure consisted of operating on patients immediately after confirmation of diagnosis by surgical biopsy (thus excluding two-stage surgery procedure). Patients with lymph node positive and estrogen receptor (ER) negative status were recommended for six courses of cyclophoshamide, methotrexate, 5-fluorouracil (CMF). Patients with ER positive and lymph node positive status received a combination of adjuvant chemotherapy with endocrine therapy. Patients with ER positive and no lymph node involvement received endocrine therapy, generally consisting of a combination of ovariectomy and tamoxifen for 5 years. Patients with lymph node negative and ER negative status were recommended for adjuvant chemotherapy (six courses of CMF) when poor pronostic indicators were present (such as Grade 3 and/or tumor size > 3 cm). There were no statistically significant differences in the treatment distribution between phases of the menstrual cycle (data not shown).

Recurrence free survival (RFS) and overall survival (OS) were defined as survival until recurrence or death from breast carcinoma, or censorship at the last checkup before the study closing date (October 1995). Patients were followed up at regular intervals, i.e., every 6 months for the first 2 years, then yearly. Deaths unrelated to breast carcinoma (n = 2) were censored at death. A letter to the general practician was sent when patients did not show up for the last yearly visit at the study closing date. Two patients were lost to follow-up. During the mean follow-up period of 48 months, 66 patients experienced recurrences and 29 died.

Cytosolic Assays

Estrogen receptor and Pg receptor (PgR) were assayed using the dextran-coated charcoal method with ^{3}H-estradiol and ^{3}H-progesterone (specific activity, 87 Ci/mmol; NEN, Paris, France). Quality control included both internal controls and European Organization for

Research and Treatment of Cancer standards. Estrogen receptor or PgR levels of 10 fmol/mg protein or more were considered positive. Total cathepsin D levels were measured with a one-step double determinant solid-phase immunoradiometric assay (ELSA-cathD kit, CIS).

Circulating Hormones

Estradiol and Pg were measured using two commercially available radioimmunoassay kits, ESTR-US-CT and PROG-CTRIA (CIS). Luteinizing hormone and FSH were assessed using LH Coatria and FSH Coatria kits (Biomérieux, Crapone, France).

Statistical Analysis

For sample size calculation, given an expecting difference in OS of 16% between follicular and luteal phases based on the meta-analysis by Fentiman et al.,[14] the number of examinable patients (354) was estimated to have a power of 90% (β= 0.1) with a two-sided significance level (α) of 0.05 or less.

Recurrence free survival and OS curves obtained with the Kaplan–Meier method were compared using the log rank test. For circulating hormones (including E2, Pg, FSH, and LH) the cutoff level was defined as the median value in the entire cycle or within each phase of the menstrual cycle. After checking whether the variables were proportional hazards, the most significant prognostic factors were identified by the Cox method (BMDP Statistical Software, Berkeley, CA). The Kruskall–Wallis test was used to analyze nonnormal quantitative parameters. Chi-square or Fisher tests were used to compare qualitative parameters. The significance level (*P* value) was set at 0.05.

RESULTS

Characteristics of the Population

The study population included 360 patients in cycle (186 patients in the follicular phase, 24 patients in the ovulatory period, and 150 patients in the luteal phase). The mean and median levels of circulating hormones according to the menstrual status are shown in Table 1. Two hundred twenty-two patients were ER positive (62%), and 264 were PgR positive (73%, Table 2). Two hundred twenty patients were lymph node negative (61%).

Relation between Prognostic Factors and the Menstrual Cycle Phase

Tumor size, lymph node status, PgR, cathepsin D, and Scarff–Bloom–Richardson grade were not correlated with the menstrual cycle phase. There was a higher mean ER level in the follicular phase (44 fmol/mg) than in the ovulatory period (27 fmol/mg) and the luteal phase (28 fmol/mg, $P = 0.02$; Fig. 1, left). There was also a trend toward a higher proportion of ER positive tumors in the follicular phase (65%) than in the ovulatory (62%) and luteal phases (57%), but the difference did not reach statistical significance ($P = 0.14$; Fig. 1, right).

Survival Analysis According to Menstrual Status

In the overall population, there were no statistically significant differences in RFS or OS according to the phase of the menstrual cycle, as defined by hormonal

TABLE 1
Hormonal Characteristics of the Study Population

Hormonal characteristic	Follicular (n = 186) (mean ± SD, median)	Ovulatory (n = 24) (mean ± SD, median)	Luteal (n = 150) (mean ± SD, median)
E2 (pg/mL)	51 ± 53 (31)	190 ± 114 (162)	66 ± 48 (54)
Pg (ng/mL)	0.46 ± 0.1 (0.4)	0.77 ± 0.4 (0.65)	5.1 ± 0.5 (3.3)
FSH (IU/L)	6.3 ± 13 (8)	13.0 ± 8 (10)	9.2 ± 2.8 (4)
LH (IU/L)	7.8 ± 9 (4.5)	47.1 ± 30 (40.5)	8.1 ± 33 (2.8)

SD: standard deviation; E2: serum estradiol; Pg: progesterone; FSH: follicle-stimulating hormone; LH: luteinizing hormone.

TABLE 2
Characteristics of Patients According to Menstrual Status

Characteristic	Follicular (n = 186) (%)	Ovulatory (n = 24) (%)	Luteal (n = 150) (%)	*P* value[a]
ER				
Positive	121 (65)	15 (62.5)	86 (57)	0.14
Negative	65 (35)	9 (37.5)	64 (43)	
PgR				
Positive	136 (76)	19 (79)	109 (73)	0.94
Negative	50 (24)	5 (21)	41 (27)	
Cathepsin D (mean, pmol/mg protein)	32.2	34.3	36.3	0.41
Tumor classification				
T1	77 (42)	5 (21)	60 (41)	0.18
T2	104 (57)	19 (79)	82 (56)	
T3–T4	3 (2)	0 (0)	5 (3)	
No. of lymph nodes				
0	112 (61)	19 (79)	89 (60)	0.41
1–2	39 (21)	3 (12.5)	29 (19)	
> 2	33 (18)	2 (8.5)	31 (21)	
SBR grade				
1	19 (13)	1 (5)	12 (10)	0.48
2	77 (54)	8 (42)	62 (54)	
3	47 (33)	10 (53)	42 (36)	

ER: estrogen receptor; PgR: progesterone receptor; SBR: Scarff–Bloom–Richardson.
[a] Subgroup comparisons were performed using chi-square test.

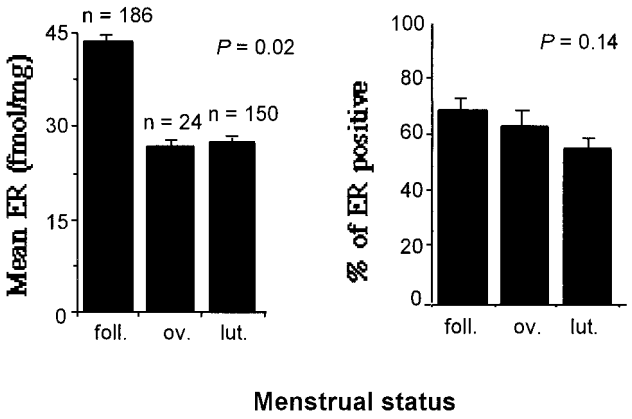

FIGURE 1. Changes in estrogen receptor (ER) according to menstrual status. (left) Mean ER level ; (right) percentage of ER positive. foll.: follicular phase; ov.: ovulatory period; lut.: luteal phase.

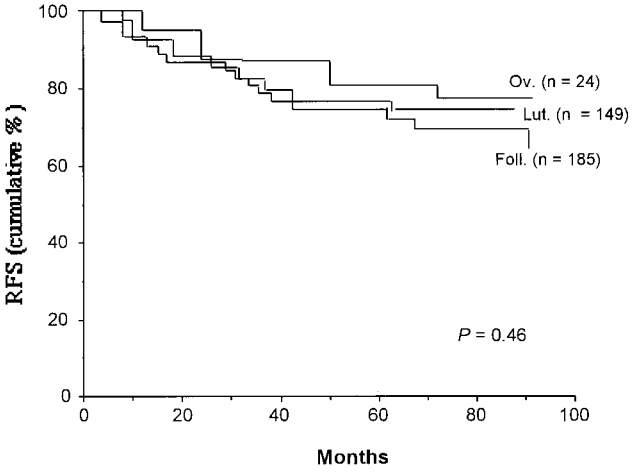

FIGURE 2. Survival analysis according to menstrual status. foll.: follicular phase; lut.: luteal phase; ov.: ovulatory period; RFS: recurrence free survival.

measurements (Fig. 2; Table 3A) or by the date of the last menstrual period (data not shown). The univariate analysis showed that lymph node status (N− vs. N+), tumor classification (T1 vs. T2 vs. T3–T4), ER (ER+ vs. ER−), PgR status (PgR+ vs. PgR−), and histologic grade (1 vs. 2–3) were significant factors for predicting a longer RFS (Table 3B). A lower cathepsin D level than the median (29 pmol/mg protein) also tended to predict a better RFS ($P = 0.07$). Lymph node status, tumor size, PgR, and cathepsin D status were significantly related to OS (Table 3). Stratification by lymph node status also revealed no difference in survival relative to the menstrual cycle phase. In the lymph node negative subgroup, the differences in RFS and OS between the luteal and the follicular phases were not statistically significant ($P = 0.10$ and $P = 0.60$, respectively). Also,

TABLE 3A
Univariate Analysis of RFS and OS: Overall Population

Parameter	Lymph node status	Tumor size	SBR	Cath D	ER	PgR
RFS	< 0.001	< 0.001	0.02	0.07	0.002	< 0.001
OS	< 0.001	0.02	0.12	0.02	0.08	0.001

Parameter	Menstrual phase	E2	Pg	FSH	LH	E2/Pg[a]
RFS	0.46	0.90	0.66	0.04	0.02	0.75
OS	0.76	0.37	0.72	0.01	0.002	0.97

TABLE 3B
Univariate Analysis of RFS and OS: ER Positive and PgR Positive Patients

Parameter	Menstrual phase	E2	Pg	FSH	LH	E2/Pg
RFS	0.95	0.67	0.44	0.07	0.30	0.33
OS	0.94	0.86	0.59	0.07	0.002	0.17

RFS: recurrence free survival; OS: overall survival; SBR: Scarff–Bloom–Richardson; ER: estrogen receptor; PgR: progesterone receptor; E2: estradiol; FSH: follicle-stimulating hormone; LH: luteinizing hormone; Cath D: cathepsin D.

[a] E2/Pg: ratio of circulating estradiol and progesterone levels.

in lymph node positive patients, there were no relations between the different phases of the cycle and RFS or OS ($P = 0.55$ and $P = 0.94$, respectively).

Survival Analysis According to Circulating Hormone Levels

Because the circulating hormone levels were not normally distributed, estradiol, progesterone, and gonadotropins levels were analyzed as dichotomous variables by using the median as the cutoff. There were no significant relations between the estradiol and progesterone levels at time of surgery and RFS or OS (Table 3). In addition, the ratio estradiol:progesterone, which theoretically could reflect unopposed estrogens, was not associated with RFS or OS. Because the effects of estradiol and progesterone might be restricted to ER positive and PgR positive tumors, the population was stratified according to the steroid receptor status. In the subpopulation of patients with both ER positive and PgR positive tumors, no effects of steroid hormones on RFS and OS were observed (Table 4).

Conversely, a high LH level (above the median) was significantly associated with a poorer RFS (Fig. 3A) and OS ($P = 0.02$ and $P = 0.002$, respectively). A higher FSH than the median value also indicated a shorter

TABLE 4
Multivariate Cox Analysis of RFS and OS

	RFS				OS			
Parameters	***P* value**	**β coeff.**	**RR**	**95% CI**	***P* value**	**β coeff.**	**RR**	**95% CI**
Lymph node	< 0.01	1.06	2.9	1.8–6	< 0.01	0.96	2.6	1.6–4.2
PgR	< 0.01	−0.93	0.39	0.2–0.6	0.06	−0.73	0.4	0.2–1.0
T classification	0.07	0.53	1.7	1.0–2.8	NS	—	—	—
LH	NS	—	—	—	< 0.01	1.28	3.6	1.4–9.0
SBR	NS	—	—	—	NS	—	—	—
ER	NS	—	—	—	NS	—	—	—
FSH	NS	—	—	—	NS	—	—	—

RFS: recurrence free survival; OS: overall survival; coeff.: coefficient; RR: relative risk; CI: confidence interval; PgR: progesterone receptor; NS: not significant; LH: luteinizing hormone; SBR: Scarff–Bloom–Richardson; ER: estrogen receptor; FSH: follicle-stimulating hormone.

RFS (Fig. 3B) and OS ($P = 0.04$ and $P = 0.01$, respectively).

Because estradiol, progesterone, and gonadotropins underwent marked cyclic variations throughout the menstrual phases, we also defined an adjusted threshold by using the corresponding median values within each menstrual cycle phase. When these adjusted cutoffs were used, estradiol and progesterone levels were not related to RFS or OS. In contrast, relations between high FSH or LH and a worse RFS and OS were observed.

Multivariate Analysis

In the multivariate analysis (which included lymph node involvement, tumor size, histologic grade, ER, PgR, FSH, and LH), the absence of lymph node involvement, a PgR positive status, and a low LH value predicted a longer OS independently of other factors (Table 4). Lymph node involvement and PgR status could predict RFS, whereas tumor size showed only a trend toward statistical significance.

DISCUSSION

This prospective study showed no effects of timing at surgery according to the hormonal menstrual cycle phase on the prognosis of breast carcinoma. In addition, our results did not support the hypothesis that circulating estrogen or progesterone at the time of surgery could be independent prognostic factors of breast carcinoma.

There has been considerable controversy in the literature over the past decade concerning the effect of time at surgery on the outcome of premenopausal breast carcinoma. Between-study comparisons by analyzing the effect of the menstrual cycle phase have been complicated by variability in the studied populations, the limited availability of accurate menstrual history data, a lack of hormonal measurements, and the involvement of various therapeutic protocols. The most important confounding factor is likely related to the definition of the menstrual cycle phase itself for at least two reasons: the reliability of the menstrual phase according to the menstrual history data and the use of different cutoff points when analyzing cyclic covariates.

First, the menstrual anamnesis generally is poorly recorded on retrospective study charts, and patients' menstrual histories often are not accurate. In our study, the potential misclassification of menstrual cycle status because of misreported data from the last menstrual phase was minimized by determinating circulating hormone levels. According to the observed discrepancies between hormonal measurements and dates of the last menstrual period, 16% of the population would have been misclassified in the follicular phase and 36% in the luteal phase. These differences might be explained, for instance, by the higher incidence of anovulatory cycle in aging premenopausal women. Recently, Mohr et al. used serum collected at the time of surgery to more accurately define the hormonal milieu and thus reduce the likelihood of misclassification of the menstrual cycle phase according to the menstrual history data.[5] This latter study revealed that a high Pg level (> 4 ng/mL) had a prognostic value for predicting a longer RFS, but no effect of estrogen on survival was observed. Using the same cutoff levels, we failed to note any differences in survival in our population (data not shown). However, this discrepancy may be due to differences in the population studied and/or the treatment protocol.

Second, a review of the literature revealed marked variations in the cutoff points for menstrual periods in

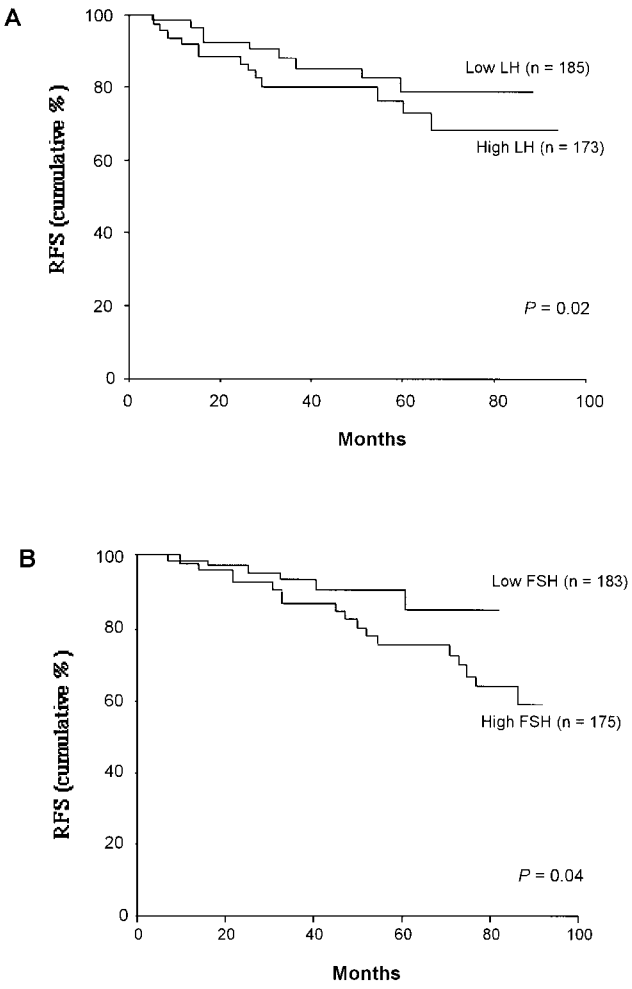

FIGURE 3. Recurrence free survival according to gonadotropin levels. (A) RFS according to LH level. (B) RFS according to FSH level. Cutoff levels for FSH and LH correspond to median values (4 IU/mL and 6 IU/mL, respectively). FSH: follicle-stimulating hormone; LH: luteinizing hormone; RFS: recurrence free survival.

the different studies. The effects of statistical data evaluation strategies have been discussed with respect to analyzing cyclic covariates,[22,23] and using multiple cutoff points may increase the risk of obtaining false-positive test results (type 1 error). The use of hormonal measurements to define menstrual cycle phase might circumvent this problem and may be helpful in the future for comparison between studies. Because the distribution of circulating hormone levels was not normal in our study population, even after the appropriate transformation, prognostic analysis of hormones as a continuous variable was not performed. Because we assumed that there was no biologic rationale for using a specific cutoff for hormones values, we used median values as thresholds for all hormone levels for survival analysis, as previously proposed for biologic prognostic markers.[24] Because of their physiologic variations throughout the cycle, the cutoffs also were set separately at the median value for each phase of the menstrual cycle, and similar results were obtained.

Another possible explanation for the effect of timing of surgery as a confounding factor could be that biologic changes related to different phases of menstrual cycle unfavorably affect the quality of diagnosis and/or treatment indications. For instance, we found that ER decreased in the luteal phase, as we previously described.[25] Because we used a competitive binding assay, the determination of ER content may have been influenced by levels of circulating estradiol during the menstrual cycle phases. However, cyclic variations in ER were not related to circulating E2 levels. Moreover, other biologic markers such as pS2, PgR, epidermal growth factor receptor, vascular endothelial growth factor, cathepsin L, or tumor size might be affected by the hormonal cycle.[25–29] Because it cannot be excluded that ER changes could reflect intratissular estrogen variations throughout the cycle, immunoenzymatic or immunohistochemestry studies would be of interest to address this question. It is also noteworthy that the effect of the menstrual phase on steroid receptors potentially could modify the prediction of hormone sensitivity and thus bias the choice of adjuvant hormone therapy and finally the outcome analysis.

Several clinical studies[5–13] and two previously published meta-analyses[14,15] showed a better survival in women operated on during the follicular phase of the cycle. However, it could be speculated that some negative studies remain unpublished, and therefore that the meta-analyses may favor the hypothesis of an effect. Moreover, the marked heterogeneity within these meta-analyses also could be argued. Unidentified confounding factors such as therapeutic bias related to cyclic variations in pronostic factors also could influence the outcome.

A noteworthy finding of this study was that high gonadotropin levels, particularly LH, could predict a poorer prognosis. To our knowledge, this is the first report analyzing the effects of gonadotropins on the outcome of breast carcinoma. Luteinizing hormone-releasing hormone (LHRH) analogs are used in premenopausal women with advanced breast carcinoma, and several large randomized trials are now in progress to assess their potential role as adjuvant therapy for early breast carcinoma.[31] It is established that LHRH agonists or antagonists inhibit the gonadotropin secretion.[31,32] Because breast carcinoma cell lines express LH receptors,[33] the LH decrease in patients

treated with LHRH analogs thus may represent a potential mechanism of antitumor action, in addition to the decreased circulating steroid levels and the possible direct growth inhibitory effect of LHRH analogs on breast carcinoma cells.[32] Because gonadotropins could modulate growth factor synthesis within ovarian follicles,[34] including insulin-like growth factor-1, transforming growth factor-α, or epidermal growth factor, a stimulation of mitogenic growth factor synthesis by gonadotropins also may be hypothesized for breast carcinoma cells. Finally, it cannot be ruled out that high FSH and LH values may reflect anovulatory cycles with unopposed estrogen, although the lack of pronostic value of the E2:Pg ratio noted in the current study does not favor this hypothesis.

The results of this prospective study with hormonal measurement do not support the hypothesis that premenopausal women with breast carcinoma would benefit from scheduling surgery at a particular time of the menstrual cycle. Further randomized studies are needed to report on the effects of the menstrual cycle phase together with steroid hormone and gonadotopin levels on the prognosis of breast carcinoma.

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