ANTICANCER RESEARCH *25*: 611-616 (2005)

Correlation Between Serum Estradiol/Progesterone Ratio and Survival Length in Invasive Squamous Cell Cervical Cancer

DAN HELLBERG[1,2], ANNIKA K. LINDSTRÖM[3,4] and ULF STENDAHL[4]

[1]*Centre for Clinical Research, Nissers väg 3, 791 82 Falun;*
[2]*Department of Obstetrics and Gynecolology, Falun Hospital, Falun;*
[3]*The Ventrum Clinic, 79021 Bjursas;*
[4]*Department of Gynecologic Oncology, Norrlands University Hospital, 901 85 Umea, Sweden*

Abstract. *Background: There is epidemiological and laboratory evidence for an association between invasive squamous cell cervical cancer and female sex steroid hormones, such as oral contraceptives. Materials and Methods: Premenopausal (n=72) and postmenopausal (n=118) women with invasive squamous epithelial cervical cancer were included in this study. Serum estradiol and progesterone and DNA S-phase fraction as a measure of proliferative activity were analysed, in 51 pre-, and 77 postmenopausal women, among whom 13 and 43, respectively, died from disease. Results: There was a significant positive correlation between a low serum estradiol/progesterone ratio and short survival in those premenopausal women who eventually died from cancer (p=0.02). Clinical stage was similar when the estradiol/progesterone ratio was dichotomized. There was no association between estradiol/progesterone ratio and survival-months in postmenopausal women. In both pre- and postmenopausal women deceased from cervical cancer, a S-phase fraction at or above 12% was correlated with reduced survival-months (p=0.03). Conclusion: These results, if confirmed, contribute to bridging the gap between previous epidemiological and laboratory findings of an association between female sex steroid hormones and squamous cell cervical cancer.*

Cervical human papillomavirus (HPV) infection is now established as an etiological agent for cervical neoplasia (1). HPV infection is commonly referred to as a necessary but not sufficient factor for invasive cervical cancer (2), which is evident from the high proportion of women of fertile age that, at some time, will have a cervical HPV infection as compared to those who in fact develop invasive cancer (3). Immortilization of the cervical cell is necessary for progress of cervical intraepithelial neoplasia to invasive cancer. Integration of viral DNA to the host genome, which enables expression of viral oncogenes E6 and E7, seems to be a necessary step in immortilization, which probably does not occur without the presence of co-factors (2).

Among proposed risk factors for cervical neoplasia are sexual risk behavior, long-term use of oral contraceptives, smoking, dietary factors and sexually transmitted infections, mainly genital chlamydial infection (4-7). Multiparity has also been suggested as a risk factor, also when controlled for sexual risk behavior and oral contraceptive use (8). Smoking and oral contraceptive use remain as the most studied epidemiological co-factors.

Some laboratory experiments support the epidemiological findings of an association between female steroid sex hormones and HPV-related cervical cell transformation (9-13). It is not known if an increased cell transformation occurs *in vivo* when serum progesterone or gestagen levels are high, such as during pregnancy or with gestagenic contraceptives.

Increased cell proliferation is necessary for tumor growth. We previously found an increase in cell proliferation, measured as number of cells in S-phase, with high serum progesterone levels in invasive cervical cancer (14). Studies on the S-phase fraction as a prognostic factor in cervical cancer have given conflicting results (15).

There is a gap between available epidemiological and laboratory studies on the one hand, and lack of clinical studies trying to elucidate possible associations between tumor growth and survival in cervical cancer, respectively, and female sex steroid hormones, on the other. The principal aim of this study was to investigate endogenous estradiol and progesterone levels in relation to length of survival in women who died from cervical cancer.

Materials and Methods

Between 1984 and 1990, 190 women with invasive squamous cell epithelial cervical cancer stage IB to IV were admitted to the

Correspondence to: Dr Dan Hellberg, Centre for Clinical Research, Nissers väg 3, 791 82 Falun, Sweden. Tel: 0046 23 4920 00, Fax: 0046 23 490989, e-mail: dan.hellberg@ltdalarna.se

Key Words: Cervical cancer, squamous cell, estradiol/progesterone ratio.

Department of Gynecologic Oncology, Norrlands University Hospital, Umea, Sweden. At admission biopsies were taken for flow cytometry and measurement of S-phase fraction and serum was collected for estradiol and progesterone measurement.

The study was prospective, but not consecutive. This was due to a period of absence of the initiator of the project (US) in the middle of the study, when serum hormones were not analyzed and S-phase fraction was only analyzed occasionally. Thus, in total 128 women had hormone analyses and 139 women had S-phase fraction estimations. The treatment of choice was radiotherapy and surgery in accordance with contemporary routines.

In addition, the evaluation included staging, grading, treatment, age, age at menarche and menopause, last menstrual date, number of pregnancies, parity, abortions, oral contraceptive history, hormone replacement therapy, and current and previous smoking. Clinical staging was made according to FIGO, and the WHO criteria were used for histological grading. The women were followed-up for at least ten years.

Pretreatment blood samples, collected in connection with taking tumor biopsies, were centrifuged and the serum was immediately frozen in small aliquots and stored at -70˚C until analyzed. Serum progesterone and estradiol were measured in duplicate by radioimmunoassay after celite chromatography.

Biopsies for flow cytometry were taken at admission to the clinic. A suspension of cell nuclei was obtained using a combined mechanical and enzymatic technique. The DNA content was expressed in relative DNA values, where the DNA content of normal diploid G0/G1 cells was given the value 2c. Human lymphocytes with a diploid DNA content were used as an external standard. Tumors with a DNA content ranging from 1.8c to 2.2c were regarded as periploid. Tumors diverging from these values were regarded as aneuploid. S-phase fraction was calculated as the proportion of cells between the G1 and G2 peaks, corrected for the background fluorescence (16).

Unilateral significance analyses of dichotomous, categorical variables, such as smoking, were made by a Chi^2 test (likelihood ratio). *T*-test was used for continuous variables such as age. A stepwise increase of cut-off levels for serum hormone levels, estradiol/progesterone ratio and S-phase fraction was used to find the best explanatory cut-off values. S-phase fraction at 12% in the whole study population, and estradiol/progesterone ratio at 60 (which was also the median ratio) in premenopausal women were chosen as cut-off values. Curve fitting and significance testing for two continuous variables, *i.e.* serum hormone levels and survival length, were analysed by linear regression. Logistic regression was used in multifactorial analyses to check and identify for possible confounders, *i.e.* clinical stage and estradiol/progesterone ratio in relation to survival.

The study was approved by the local ethical committee.

Results

Of the 190 women with invasive squamous epithelial cancer, 118 (62%) were considered menopausal, *i.e.* not having a menstrual-like vaginal bleeding during the previous six months, and the remaining 72 (38%) women were premenopausal.

The mean number of pregnancies was 2.8 (SD 1.9) and parity was 2.5 (SD 1.7). The mean age for premenopausal women was 37.7 years, and 68.2 years for postmenopausal women. According to the FIGO classification, 85 (45%) women belonged to stage IB, 23 (12%) to stage IIA, 30 (16%) to stage IIB, 5 (2%) to stage IIIA, 38 (20%) to stage IIIB and 9 (5%) to stage IV, respectively. Twenty (11%) tumors were highly-, 105 (57%) moderately- and 52 (28%) poorly differentiated, and seven (4%) tumors were anaplastic or could not be graded.

Table I. *Correlation between risk factors and prognostic factors with duration of survival in women deceased from invasive squamous cell cervical cancer irrespective of menopausal status (n=80).*

	Study group		Comparison group		*p*-value
	No.	Mean Survival (months)	No.	Mean Survival (months)	
Stage IB-IIA *vs.* IIB-IV	28	44.6	52	22.2	0.0002
Differentiation: high/ moderate *vs.* low/anaplastic	48	31.1	27	26.9	0.52
Climacteric *vs.* not climacteric	60	32.2	20	23.6	0.22
Smoker *vs.* non-smoker	37	31.4	30	30.4	0.88
Body mass index ≥ 25 *vs.* <25	33	34.5	41	26.6	0.20
Aneuploidy *vs.* non-aneuploid	37	26.9	32	32.3	0.41
S-phase ≥12% *vs.* S-phase <12%	41	24.1	22	39.9	0.03
Serum estradiol/progesterone ratio <60 *vs.* ≥60 (only premenopausal women)	8	12.6	5	31.4	0.07

The ten-year mortality rate was 28% (20/72) in pre- and 51% (60/118) in postmenopausal women ($p=0.002$) in the total population. Advanced clinical stage and S-phase fraction at or above 12% were significantly correlated with impaired survival in those women who eventually died from their disease ($p=0.03$) (Table I). Among the 13 premenopausal women who died from disease, those who had an estradiol/progesterone ratio below 60 had a shorter survival, compared to those at or above 60, and the difference almost reached statistical significance ($p=0.07$). Mean and median survival in these 13 women was 19.8 months and 12 months, respectively. Women with a body mass index at or above 25 had slightly, but insignificantly, longer survival than those below. There was no correlation between estradiol/progesterone ratios and body mass index, either in premenopausal, or in postmenopausal women.

When serum progesterone and estradiol were analyzed as

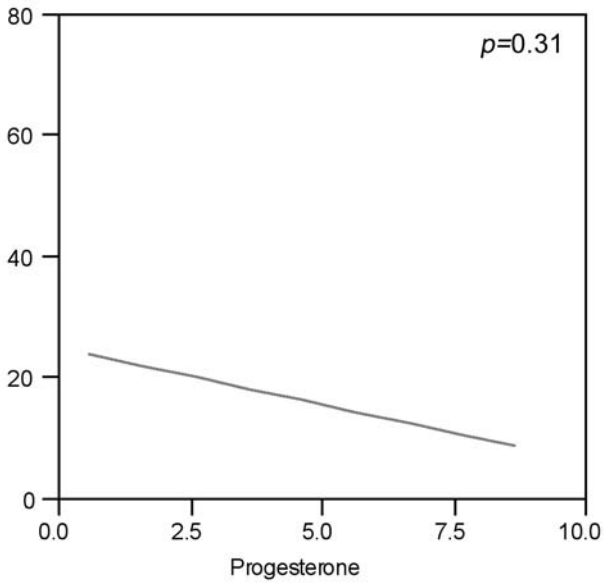

Figure 1. *Correlation between survival (y=months) and serum progesterone in 13 premenopausal women deceased from invasive cervical cancer.*

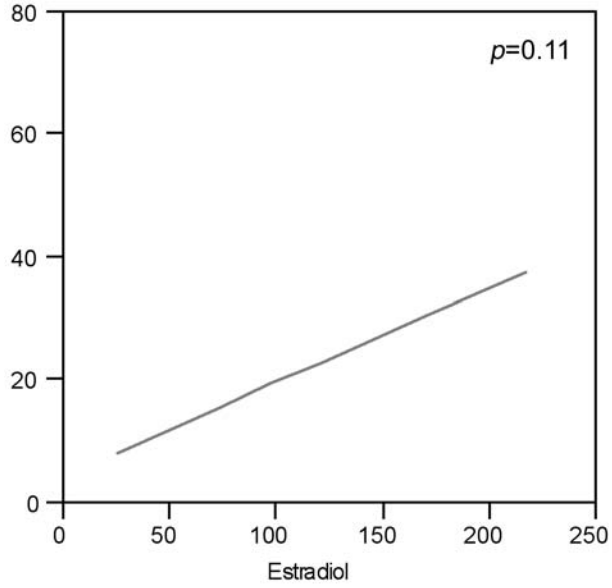

Figure 2. *Correlation between survival (y=months) and serum estradiol in 13 premenopausal women deceased from invasive cervical cancer.*

continuous variables and survival curves were estimated by linear regression in the premenopausal women, there was a slight decrease in survival with increasing serum progesterone (Figure 1) and better survival with rising serum estradiol (Figure 2). Both correlations were non-significant. However, increased estradiol/progesterone ratios were significantly ($p=0.02$) correlated with increasing survival (Figure 3).

Forty-five postmenopausal women died from disease. There was no tendency for any association between serum estradiol and progesterone and the estradiol/progesterone ratio with survival.

Serum was, on average, sampled on day 12 of the menstrual cycle in the deceased premenopusal women. There was no association between survival months and menstrual day at serum sampling. Mean and median survival time was 19.8 and 12 months, respectively. When the survival time was dichotomized to women deceased in <12 and ≥ 12 months, the former had their serum sampled on mean menstrual day 10.4 as compared to day 12.8 among the latter (NS). When cut-off level was set to 20 months, those who survived longer had their serum sampled at day 13.0 of the menstrual cycle compared to 11.3 in those with a shorter survival (NS). Among all premenopausal women, the deceased ones on average had their serum collected on day 13.3 of the menstrual cycle as compared to day 14.4 in survivors (NS).

Premenopausal deceased women were dichotomized into stage IB-IIB (n=8) *versus* IIIA-IV (n=5). The mean

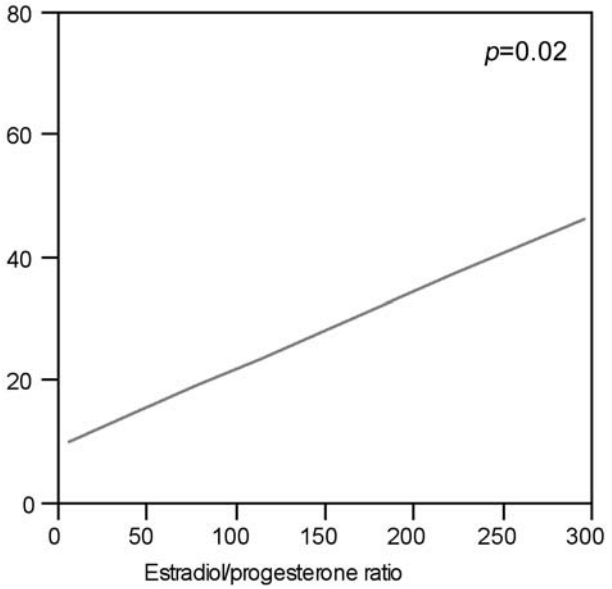

Figure 3. *Correlation between serum estradiol/progesterone ratio and survival (y=months) in 13 premenopausal women deceased from invasive cervical cancer.*

estradiol/progesterone ratio was 95.4 in the former group as compared to 60.7 in the latter (NS). Adjustment for stage did not alter the significant correlation between survival months and estradiol/progesterone ratio ($p=0.02$). Stage was dichotomized into IB-IIA (n=42) *versus* IIB-IV (n=9)

Table II. *Association between risk factors and prognostic factors for invasive squamous cell cervical cancer with S-phase fraction ≥12% in women deceased from cervical cancer irrespective of menstrual status (n=63).*

	S-phase fraction ≥12% n=41 (%)	S-phase fraction <12% n=22 (%)	*p*-value
Age	63.2	59.1	0.28
Stage IB-IIA *vs.* IIB-IV	28 (68.3)	13 (59.1)	0.47
Differentiation: high-moderate *vs.* low/anaplastic	16 (42.1)	4 (20.0)	0.08
Climacteric *vs.* non-climacteric	33 (80.5)	16 (72.7)	0.48
Smoker *vs.* non-smoker	22 (62.9)	9 (45.0)	0.20
Total radiation dosage	48.7	49.3	0.88
Aneuploidy	29 (70.7)	4 (18.2)	0.0001
Estradiol/progesterone ratio (only premenopausal)	37.6	185.1	0.02

in all premenopausal women. The mean estradiol/progesterone ratio was 118.8 in the former group, as compared to 87.1 in the latter (NS).

A high S-phase fraction was associated with fewer survival months. Table II shows associations between S-phase fraction at or above 12% with possible risk factors or prognostic factors for cervical cancer in all premenopausal women in the study population. As expected, there was an association with differentiation ($p=0.08$) and ploidy ($p=0.0001$), but also a significant correlation between a high S-phase fraction and a low estradiol/progesterone ratio ($p=0.02$).

Discussion

If confirmed by others, these results, to our knowledge for the first time, give evidence to bridge the gap between previous epidemiological associations between female sex steroid hormones (long-term use of oral contraceptives, parity) and cervical neoplasia, and laboratory findings of increased transformation and proliferation rate in cervical cell cultures in the presence of female steroid sex hormones. Despite the limited size of the population, we found a strong and significant correlation between a low estradiol/progesterone ratio and a shorter survival in premenopausal women who eventually died from their disease.

It is tempting to speculate that the apparently more aggressive behavior of the cervical tumor where a lower estradiol/progesterone ratio enhanced growth also led to detection of more advanced tumors. In real figures, this was the case in premenopausal cancer, but the association did not reach statistical significance.

The role of oral contraceptive use in cervical neoplasia is not finally established. A slight but apparent increased risk of cervical neoplasia with long-term, but not short-term, use of oral contraceptives was found in most, but not all studies (5). To our knowledge, no attempt has been made to correlate cervical cancer risk with hormonal balance in combined oral contraceptives, which could provide additional evidence to the present study. It is, however, notoriously difficult to control for all confounding factors, such as sexual, smoking, current HPV infection and reproductive history, when the role of oral contraceptives is evaluated in epidemiological studies. Additional data from experimental studies are needed.

There are three major methods to study a possible role of estrogens and gestagens in cervical neoplasia: 1) Menstrual cycle, with high serum levels of estradiol during the follicular phase and progesterone during the luteal phase. 2) Pregnancy, with high levels of progesterone in particular. 3) Oral contraceptive use with exogenous supply of hormones. Progesterone has been suggested as the major suspect because of its immunosuppressive effect and for a possible connection to HPV infection (17). In contrast, estrogen has been reported to reduce susceptibility to primary HPV infection, but this might be of no importance once the HPV infection has been established (18).

HPV seems to have a tendency to transfect cells with progesterone receptors. Both HPV 16 and HPV 18 contain progesterone and glucocortiocoid response elements that will increase expression from the HPV E6 and E7 oncogenes with gestagenic stimuli (9, 10). Increased transcription of E6 and E7 is considered crucial in cell transformation (11) and a relationship to the ras oncogene has been reported (12).

Cell transformation in HPF-transfected cervical cells has been reported to occur when progesterone and ras oncogene, or oral contraceptive gestagens, were added (13). It is not known if increased cell transformation occurs *in vivo* when serum progesterone levels are high, such as during pregnancy or with oral contraceptive use. Higher serum progesterone levels, when adjusted for menstrual phase, have been correlated to a higher prevalence of HPV infection (19).

We previously found an increase in cell proliferation, measured as number of cells in S-phase, with high serum progesterone levels in invasive cervical cancer (14). In an experimental study (20), an enhanced colony forming efficiency was found in the HPV 16 DNA integrated cervical

cancer cell line SaSki after at least three days progesterone treatment. The progesterone antagonist RU 486 was able to abrogate the enhancement of progesterone on cell growth. Progesterone and glucocorticoid hormones increase HPV mRNA and significantly stimulate viral replication (21).

There is, however, also evidence for a role of estrogens. In cervical epithelium at the transformation zone, where cervical neoplasia is initiated, 16-α-hydroxylation of estradiol occurs resulting in 16-α-hydroxyestrone (22), which is linked to malignant transformation of estrogen-sensitive cells transfected by HPV. Serum estrone was found to be elevated in patients with CIN who were HPV-positive as compared to HPV-negative women with or without CIN (23). The SiHa cervical cancer line, that is transfected by HPV 16, has shown an eight-fold increase of HPV transcripts in the presence of estrogen (24).

In two studies from the same research group (25, 26), treatment with sex hormones of transgenic mice expressing HPV 16 was evaluated. Squamous cell carcinomas developed, exclusively in the vagina and cervix (25). In a subsequent study, doses were reduced five-fold. Only carcinogenesis in the cervical transformation zone remained and neoplastic progression from metaplasia, dysplasia to squamous cancer was observed (26). Steroid sex hormone levels in women were evaluated as an aid in grading cervical neoplasia, but were not found useful (27).

It is, thus, obvious that there is both epidemiological and experimental evidence that gestagens and/or estrogens may play a role in cervical carcinogenesis. There are, however, several obstacles. Epidemiological studies may lack control for all possible confounding factors. In addition, subgroups might be overlooked, which could easily have happened in the present study. Positive laboratory results in animal studies might be species-specific. Laboratory studies on human cervical tissue, normal or cancerous, where sex hormones are added, might not be equivalent to the concentrations of hormones found *in vivo*.

When the present laboratory results were summarized, it became evident that our findings of shorter survival in women with a low estradiol/progesterone ratio, as compared to those with a high ratio, are biologically plausible.

Our second main finding, a mean shorter survival in women with a high S-phase fraction, fits with the finding of an association with a low estradiol/progesterone ratio and a high S-phase fraction. Studies in which the S-phase fraction was correlated to prognosis have given conflicting results. Horn *et al.* (28) found that patients with an S-phase fraction above 12% had a poor prognosis, while Reich *et al.* (29) found a better disease-free survival in patients with carcinomas who had an S-phase fraction below 7%, although it was not statistically significant. On the other hand, Kristensen *et al.* (30) and Graflund *et al.* (15) found that the S-phase fraction was of no prognostic importance.

There are several possible reasons for the discrepancy in these results. The S-phase fraction is difficult to measure. In some studies it could be evaluated in only 22% of aneuploid tumors (30). Experience and laboratory methods are of great importance. In addition, different definitions of prognosis have been used, such as presence of lymph node metastasis, tumor stage, recurrences and overall survival. Most patients were treated with radiotherapy and, therefore, we could not diagnose all metastases. We found no effect of S-phase fraction on overall survival, but a high S-phase fraction was associated with fewer survival months in those patients who eventually died. This seems logical as a generalized disease is more directly related to prognosis than the S-phase fraction. In patients not radically cured, the tumor proliferation rate influences survival to a limited extent. This study showed that measurement of cell proliferation could be clinically useful in selected patients, but immunochemical markers such as endothelial growth factor receptor and Ki-67/MIB-1, which are both useful tools for prognostication (15, 31), should be preferred to flow cytometry measurement in cervical cancer.

In summary, the most interesting part of our results is the finding of a clinical association between sexual steroid hormones, in this study endogenous estradiol and progesterone, and survival months in premenopausal women who died from invasive squamous cell cervical cancer, providing new evidence for a hormonal role in cervical neoplasia.

Acknowledgements

We are grateful to the Cancer Research Fund, Norrlands University Hospital, Umea, and the Centre for Clinical Research, Falun, Sweden for financial support.

References

1 Baird EM: Human papillomavirus and cervical cancer. Clin Microbiol Rev *16*: 1-17, 2003.
2 Crum CP: Contemporary theories of cervical carcinogenesis: the virus, the host and the stem cell. Mod Pathol *13*: 243-51, 2000.
3 Woodman CBJ, Collins S, Winter H, Bailey A, Ellis J, Prior P, Yates M and Rollason TP: Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. Lancet *357*: 1831-6, 2001.
4 Hellberg D, Valentin J and Nilssons: Long-term use of oral contraceptives and cervical neoplasia: an association confounded by other risk factors. Contraception *32*: 337-46, 1985.
5 Smith JS, Green J, Berrington de Gonzales A, Appleby P, Peto J, Plummer M, Franceschi S and Beral V: Cervical cancer and use of hormonal contraceptives: a systematic review. Lancet *361*: 1159-67, 2003.
6 Hellberg D, Valentin J and Nilsson S: Smoking as risk factor in cervical neoplasia. Lancet *2*: 1497, 1983.
7 Haverkos HW, Soon G, Steckley SL and Pickworth W: Cigarette smoking and cervical cancer: Part I: a meta-analysis. Biomed Pharmacother *57*: 67-77, 2003.

8 Brinton LA, Reeves WC, Brenes MM, Herrero R, deBriton RC, Gaitan E, Tenorio F, Garcia M and Rawls WE: Parity as a risk factor for cervical cancer. Am J Epidemiol *130*: 486-96, 1989.
9 Crook T, Storey A, Almond N, Osborn K and Crawford L: Human papillomavirus type 16 cooperates with activated ras and fos oncogenes in the hormone-dependent transformation of primary mouse cells. Proc Natl Acad Sci USA *85*: 8820-4, 1988.
10 Chan WK, Klock G and Bernard HU: Progesterone and glucocorticoid response elements occur in the long control regions of several human papillomaviruses involved in anogenital neoplasia. J Virol *63*: 3261-9, 1989.
11 Schiffman MH: Recent progress in defining the epidemiology of human papillomavirus infection and cervical neoplasia. J Natl Cancer Inst *84*: 394-8, 1992.
12 Matlashewski G, Schneider J, Banks L, Jones N, Murray A and Crawford L: Human papillomavirus type 16 DNA cooperates with activated ras in transforming primary cells. EMBO J *6*: 1741-6, 1987.
13 Pater A, Bayatpour M and Pater MM: Oncogenic transformation by human papillomavirus type 16 deoxyribonucleic acid in the presence of progesterone or progestins from oral contraceptives. Am J Obstet Gynecol *162*: 1099-1103, 1990.
14 Lindstrom A, Backstrom T, Hellberg D, Tribukait B, Strang P and Stendahl U: Correlations between serum progesterone and smoking, and the growth fraction of cervical squamous cell carcinoma. Anticancer Res *20*: 3637-40, 2000.
15 Graflund M, Sorbe B, Bryne M and Karlsson M: The prognostic value of a histologic grading system (IFG), DNA profile, and MIB-1 expression in early stages of cervical squamous cell carcinomas. Int J Gynecol Cancer *12*: 149-57, 2002.
16 Heiden T, Strang P, Stendahl U and Tribukait B: The reproducibility of flow cytometric analyses in human tumors. Methodological aspects. Anticancer Res *10*: 49-54, 1990.
17 Stendahl U and Rogo K: Cervical cancer: role for progesterone during pregnancy and contraception? Am J Obstet Gynecol *163*: 685-6, 1990.
18 Brabin L: Interactions of the female hormonal environment, susceptibility to viral infections and disease progression. AIDS Patient Care *16*: 211-21, 2002.
19 Kedzia W, Gozdzicka-Josefiak A, Kwasniewska A, Schmidt M, Miturski R and Spaczynski M: Relationship between HPV infection of the cervix and blood serum levels of steroid hormones among pre- and postmenopausal women. Eur J Gynaecol Oncol *21*: 177-9, 2000.
20 Yuan F, Auborn K and James C: Altered growth and viral gene expression in human papillomavirus type 16-containing cancer cell lines treated with progesterone. Cancer Invest *17*: 19-29, 1999.
21 De Villiers E-M: Relationship between steroid hormone contraceptives and HPV, cervical intraepithelial neoplasia and cervical carcinoma. Int J Cancer *103*: 705-8, 2003.
22 Auborn KJ, Woodworth C, DiPaolo JA and Bradlow HL: The interaction between HPV infection and estrogen metabolism in cervical carcinogenesis. Int J Cancer *49*: 867-9, 1991.
23 Salazar EL, Mercado E, Sojo I and Salcedo M: Relationship between estradiol 16 alpha-hydroxylation and human papillomavirus infection in cervical cell transformation. Gynecol Endocrinol *15*: 335-40, 2001.
24 Mitrani-Rosenbaum S, Tsvieli R and Tur-Kaspa R: Oestrogen stimulates differential transcription of human papillomavirus type 16 in SiHa cervical carcinoma cells J Gen Virol *70*: 2227-32, 1989.
25 Arbeit JM, Howley PM and Hanahan D: Chronic estrogen-induced cervical and vaginal squamous carcinogenesis in human papillomavirus type 16 transgenic mice. Proc Natl Acad Sci USA *93*: 2930-5, 1996.
26 Elson DA, Riley RR, Lacey A, Thordarson G, Talamantes FJ and Arbeit JM: Sensitivity of the cervical transformation zone to estrogen-induced squamous carcinogenesis. Cancer Res *60*: 1267-75, 2000.
27 Shields TS, Falk RT, Herrero R, Schiffman M, Weiss NS, Bratti C, Rodriguez AC, Sherman ME, Burk RD and Hildesheim A: A case-control study of endogenous hormones and cervical cancer. Br J Cancer *90*: 146-52, 2004.
28 Horn LC, Raptis G and Nenning H: DNA cytometric analysis of surgically treated squamous cell cancer of the uterine cervix, stage pT1b1-pT2b. Analyt Quant Cytol Histol *24*: 23-9, 2002.
29 Reich O, Purstner P, Haas J, Lahousen M, Tamussino K and Winter R: Prognostic significance of preoperative DNA flow cytometry in surgically-treated cervical cancer. Eur J Gynaecol Oncol *24*: 13-7, 2003.
30 Kristensen G, Kaern J, Abler V, Hagmar B, Trope C and Pettersen E: No prognostic impact of flow-cytometric measured DNA ploidy and S-phase fraction in cancer of the uterine cervix: a prospective study of 465 patients. Gynecol Oncol *57*: 79-85, 1995.
31 Gafney DK, Haslam D, Tsodikov A, Hammond E, Seaman J, Holden J, Lee RF, Zempolich K and Dodson M: Epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) negatively affect overall survival in carcinoma of the cervix treated with radiotherapy. Int J Radiat Oncol *56*: 922-8, 2003.

Received June 21, 2004
Revised November 16, 2004
Accepted December 20, 2004