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Steroid Hormone Profile in Postmenopausal Women with Ovarian Cancer

M.E.R. O'Brien, M. Dowsett, I. Fryatt and E. Wiltshaw

Raised levels of steroid hormones are not expected in postmenopausal women. Therefore, if detected in postmenopausal women with ovarian cancer, they must be assumed to be related to the presence of the tumour and, therefore, may be of use as tumour markers. Serum levels of CA125, progesterone, 17-hydroxyprogesterone, sex hormone binding globulin and oestradiol were measured in 44 postmenopausal women with ovarian cancer, postsurgery and prior to chemotherapy. The relationship between the four hormone levels, CA125, patient age, stage, residual disease after surgery and differentiation were tested using the Spearman and Kendall rank coefficients. A significant inverse association was found between CA125 and progesterone levels, and CA125 and 17-hydroxyprogesterone. A positive association between 17-hydroxyprogesterone and progesterone was also found, and positive correlations between stage and CA125, and residual disease and CA125 were confirmed.

Key words: steroid hormones, CA125, progesterone, oestradiol, ovarian cancer
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INTRODUCTION

THE IDEA that ovarian cancer may be a hormone-sensitive tumour is attractive, and if true would mean the availability of a large number of therapeutic options in the treatment of this disease. Raised levels of steroid hormones are not expected in any postmenopausal woman, although normal postmenopausal ovaries do continue to secrete steroids other than oestrogen [1]. However, if raised levels were detected in postmenopausal women with ovarian cancer one must assume that they are in some way related to the presence of tumour. Ovarian cancer has

been reported to produce small amounts of steroid hormones [2–4]. In addition, elevated levels of human choriogonadotrophin [5] and α feto-protein [6] have been demonstrated in the plasma of some patients. Studies have reported low levels of oestrogen receptors (ER) and progesterone receptors (PR) in ovarian cancer tissue [7], the incidence of detectable ER and PR depending on the cutoff point of the assay. However, there is only one study which reports a positive correlation between PR and survival [8]. Serum levels of CA125 have been shown to be helpful in predicting disease response to chemotherapy and detecting early relapse for ovarian cancer; it is a good indicator of bulk of disease, and remains the best available tumour marker for ovarian cancer [9].

Mahlck and colleagues have looked at several endocrine parameters in a group of 48 women with ovarian cancer, and reported a highly significant positive correlation between CA125 and serum progesterone ($P < 0.00032$), sex hormone binding globulin (SHBG) ($P < 0.00008$) and progesterone/albumin ratio ($P < 0.000015$) [10]. This report motivated us to look retrospectively at hormone levels on stored serum samples in 44 consecutive women with documented pretreatment CA125 levels in order to address three issues. The first was to attempt to confirm the results of a positive relationship between CA125 with progesterone and SHBG. The second was to look for any significant relationships between the five assay values of CA125, SHBG, 17-hydroxyprogesterone (17-OHP), progesterone and oestradiol (E2). The third and final issue was to look for the expected relationship between stage and residual disease with CA125.

PATIENTS AND METHODS

Forty-four women with ovarian cancer were selected in chronological order by hospital number. These patients were all postmenopausal at presentation or by virtue of their surgery and had stored serum and a CA125 level measured before treatment with chemotherapy. No patient had any other malignancy.

The median age was 55 years (range 31–82). Using new FIGO staging, the number of patients in stage I was 9, stage II 9, stage III 16, stage IV 8 and unknown stage in 2 cases. After initial surgery, 16 patients had no residual disease, 8 had <2 cm (minimal residual disease), 5 had 2–5 cm, and 15 had >5 cm. The pathological subtypes were non-adeno 6 cases, adeno 9, serous 16, mucinous 5, clear 3 and endometrioid 5. There were 2 borderline cases, 4 well differentiated, 8 moderately, 19 poorly, and 11 with unreported differentiation. Serum for CA125 and hormone profile were taken on arrival at the above hospital, i.e. 3–4 weeks after initial surgery. Patients had no further surgery prior to chemotherapy.

Biochemical analysis

CA125 was analysed using the CIS CA125 radioimmunoassay kit. The CA125 system is a solid phase sandwich radioimmunoassay with a mouse monoclonal anti-CA125 antibody being both the solid phase antibody and the ^{125}I-labelled antibody. The sensitivity of the assay is 5.0 U/ml. The intra- and interassay coefficients of variation are 7 and 9%, respectively. A CA125 level of greater than 35 U/ml was taken as elevated, as determined in previous reports [10–12].

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Table 1. Spearman rank correlation coefficients

	CA125	Progesterone	SHBG	17-OHP	E2
CA125	1.00				
Progesterone	−0.29	1.00			
SHBG	0.29	−0.06	1.00		
17OHP	−0.38*	0.56†	−0.06	1.00	
E2	−0.19	−0.03	−0.09	0.2	1.00

* $P < 0.02$, † $P < 0.001$.

The assays for 17-OHP, E2 and SHBG were conducted as described previously [13–15]. The progesterone and 17-OHP assays had sensitivities of <0.34 nmol/l and <0.30 nmol/l, respectively.

Statistical analysis

The levels of the four hormones and their association with CA125 were analysed using the Spearman and Kendall rank correlation coefficient and matrices constructed. Both CA125 and progesterone had skewed distributions which, although theoretically transformable to normal, remained skewed when converted to logarithms because of the large number of values in each measure that fell outside the range of the respective assays. A large number of tied values were observed in the data (patients with the same value for the hormone levels)—28 patients (64%) had a progesterone level <0.34 nmol/l and 16 had progesterone levels recorded as >0.34 nmol/l, 3 of whom were recorded as having a value of 1.2 nmol/l. In order to take into account these tied values, a more pragmatic approach to the statistical analysis was adopted: a three by two, two-way contingency table was calculated for the data, and the χ^2 statistic calculated and then partitioned by the method of least squares, into the proportion of the statistic due to trend and due to the residual variation.

RESULTS

The Spearman and Kendall rank correlation coefficient matrices for the five hormone levels showed two significant values: the pair CA125 and 17-OHP (Spearman −0.38, Kendall −0.25, $P = 0.02$) and 17-OHP and progesterone (Spearman 0.56, Kendall 0.46, $P = 0.001$) (Tables 1 and 2). There was also a negative correlation between CA125 and progesterone, although this was less significant than for 17-OHP (Spearman −0.29, Kendall −0.24, $P = 0.05$).

A positive correlation between progesterone and CA125 has been reported as highly significant [9], and thus this was examined further in our data. 14 patients (32%) had a CA125 level reported as <20 U/ml and one (2%) as >500 U/ml, and there were 28 patients (64%) who had a progesterone level

Table 2. Kendall rank correlation coefficients

	CA125	Progesterone	SHBG	17-OHP	E2
CA125	1.00				
Progesterone	−0.24	1.00			
SHBG	0.20	−0.05	1.00		
17-OHP	−0.25*	0.46†	−0.05	1.00	
E2	−0.12	−0.02	−0.05	0.16	1.00

* $P < 0.02$, † $P < 0.001$.

<0.34 nmol/l. The Spearman rank correlation coefficient for the whole data set was −0.37 ($P = 0.01$) and the Kendall rank correlation coefficient was −0.30 ($P = 0.02$). Patients were categorised into three groups (Table 3) on the basis of their CA125 values, these being <20 U/ml (below the level of sensitivity of the assay), 20–35 U/ml (assessible but still normal) and >35 U/ml (abnormal). They were further divided on the basis of their progesterone level: <0.34 nmol/l (below level of sensitivity of the assay) and >0.34 nmol/l. The calculated χ^2 value for this contingency table was 6.92 ($P = 0.03$ with two degrees of freedom) where 6.38 was due to trend ($P = 0.01$ with 1 degree of freedom) and 0.54 was due to residual variation.

All of the values for E2 and 17-OHP were either low or within the normal range. Only five out of 44 values for SHBG were above the upper limit of normal. A positive correlation was found between stage and CA125 (Spearman rank 0.54, $P = 0.01$), and residual disease and CA125 (Spearman rank 0.46, $P = 0.02$), but no other correlations were found between hormone levels and age, stage, residual disease after surgery and differentiation of tumours.

DISCUSSION

This study describes a random population of 44 postmenopausal women with epithelial ovarian cancer, 36% (16/44) of whom had a detectable level of serum progesterone postsurgery and prior to chemotherapy. We found a negative association between the CA125 levels with progesterone and SHBG, using the standard method (the Spearman correlation). Postulating that the nature of these data, with a large number of tied values, might make analysis of the data by this form misleading, we also analysed the data by a contingency table. We found a trend between CA125 and progesterone ($P = 0.01$).

The second question of a relationship between the five assay values of CA125, SHBG, 17-OHP, progesterone and E2 was answered by the Spearman rank correlation matrix; two pairs had significant probabilities — CA125 and 17-OHP (Spearman −0.38) and 17-OHP and progesterone (Spearman 0.56), but not CA125 and progesterone. The interpretation of these results needs to take into consideration the fact that five different assays with nine separate comparisons were made (10 including CA125 and progesterone already done); one result in 20 could have been expected by chance alone. With this in mind, a significance level of 0.02 from the negative association between CA125 and 17-OHP is only of marginal significance, and if a Bonferoni correction was incorporated because of the large number of tests carried out, i.e. 10, then the association between CA125 and 17-OHP would not be significant. The relationship between 17-OHP and progesterone remains significant and is expected as these hormones are closely associated in the steroid pathway. We failed to confirm any association between CA125 and the other sex hormones, E2 and SHBG.

As for the third question of a possible association between other prognostic factors and CA125, this was not found between the other four hormone assays or between CA125 and age, pathology or grade of tumour. The association known to exist between CA125 and stage, and between CA125 and residual disease was demonstrated in our patient group.

Mahlck and colleagues showed a strongly positive association between CA125 and serum progesterone ($P < 0.00032$), SHBG ($P < 0.00008$) and progesterone-albumin ratio ($P < 0.000015$) for blood samples taken prior to surgery [10]. Extrapolating from the published bar charts, it would appear that only those patients with stage IV disease, 31% (15 patients), had raised levels of progesterone, and only those with stage III or stage IV disease, 65% (31 patients), had CA125 significantly greater than the normal controls. In our study, we had 32% (14 patients) with raised progesterone and 59% (26 patients) with raised CA125. Thus, although the values that we obtained for these parameters were measured in blood samples taken after surgery, our two populations were probably similar in both disease distribution and bulk of disease at the time of blood sampling. Therefore, these patients' characteristics do not appear to explain the discrepancy between Mahlck's results and those we now present.

Another explanation for this low and maybe negative correlation between hormone parameters and CA125 was sought. Looking at the data presented by Mahlck, they did not have more patients with raised levels, but perhaps the actual levels were higher than among our patients, and that this was due to a greater number of well differentiated tumours in the Mahlck group—these well differentiated cells would be capable of maintaining their ability to secrete steroids. There were 42% (20 patients) with type I pathology in their report, while we had only 14% with well differentiated non-clear cell carcinomas. This may be the explanation for the discrepancy between the two series.

This study does raise a number of issues: firstly, approximately 36% of postmenopausal women with epithelial ovarian cancer had detectable levels of progesterone and, in particular, 10/16 patients with raised levels of progesterone had normal levels of CA125, indicating that progesterone may have a role as a tumour marker in CA125-negative disease. In addition, the patient group with raised serum progesterone would be an interesting subgroup in which to test endocrine therapy in the maintenance of remission or in the treatment of relapsed disease. The relationship between grade of tumour and secretion of steroid hormones needs further investigation.

Table 3. Correlation of progesterone levels with CA125 values

	Progesterone		
	<0.34 nmol/l	>0.34 nmol/l	Total
CA125			
<20 U/ml	5	9	14
Normal 20–35 U/ml	3	1	4
Abnormal >35 U/ml	20	6	26
Total	28	16	44

χ^2 6.92, degrees of freedom: 2, $P = 0.03$. Trend 6.38, degrees of freedom: 1, $P = 0.01$. Residual 0.54, degrees of freedom: 1, $P = 0.46$.

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

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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.
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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

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