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Predictive Value of Serial CA 125 Antigen Levels in Ovarian Cancer Evaluated by Second-Look Laparotomy

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Abstract—*Serial serum CA 125 levels were measured before definitive surgery and during chemotherapy for 12 months or more in 64 patients with ovarian cancer. In the 42 patients who had a complete clinical remission and thus were subjected to a second-look laparotomy, an absence of disease was not predicted by patterns of CA 125 levels. Whilst rising or persistently high levels indicated the presence of tumour in 92% of patients, declining levels to negative predicted the absence of tumour in only 50%. Although the majority of these patients showed microscopic foci or a tumour mass < 1 cm, 3 patients had a larger amount of disease. In the follow-up of 49 patients, the accuracy of prediction of a good outcome was better than that of a poor outcome on the basis of CA 125 patterns, with rates of 92% and 79%, respectively. Our findings indicate that CA 125 lacks sensitivity in detecting small tumour masses (< 1 cm dia.) but rising or persistently high levels suggest a strong likelihood of a residual tumour to be found at a second-look laparotomy.*

INTRODUCTION

WITH ovarian cancer, as with other 'hidden' intra-abdominal cancers, difficulties are often encountered in evaluating the effectiveness of adjuvant treatment following definitive surgery because of the relative inaccessibility of the tumour to examination. The increasing use of computerized tomography (CT) and ultrasound scan has not added significantly to the detection of small tumour recurrences. It is in this area of tumour assessment that the potential use of circulating tumour markers is considered to be important. An earlier marker, carcinoembryonic antigen (CEA), although valuable in tumour monitoring, was found to have limitations [1].

A newer marker—CA 125—has now been described [2] which appears to have a better specificity for ovarian cancer. A monoclonal antibody has been raised to a serous cystadenocarcinoma of the ovary which reacts to an antigenic determinant (CA 125) that is found to be expressed by more than 80% of non-mucinous epithelial tumours of the ovary [3]. The development of sensitive radioimmunoassays has led to a wide-scale study of CA 125 in serum. At a level of greater than 35 U/ml, CA 125 positivity was reported in 82% of women with ovarian carcinoma and in only 1% of healthy subjects [4]. This clear-cut discrimination has, however, been blurred by more recent reports in patients with non-malignant diseases: CA 125 was elevated (> 35 U/ml) in 33% of women with acute pelvic inflammatory disease and 24% of pregnant women [5].

Despite the lack of specificity of the marker, it was felt that the serum level of CA 125 may be a reliable predictor of the presence of clinically impalpable intraabdominal tumour in ovarian cancer. The purpose of the present prospective study was to determine the predictive value of CA 125 in unselected patients with advanced ovarian cancer treated by cytotoxic chemotherapy. Particular attention was directed to a group of patients in whom complete clinical remission was considered to be present (often with the aid of CT scan) and documentation of the presence of tumour was checked by a careful and comprehensive second-look evaluation by laparotomy. The conditions under which serial CA 125 levels could be used

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to identify patients with subclinical disease were examined.

MATERIALS AND METHODS

Clinical design

There were 64 patients with histologically-confirmed ovarian cancer admitted to the study (serous cystadenocarcinoma, 43; endometrioid carcinoma, 8; mucinous cystadenocarcinoma, 6; anaplastic carcinoma, 5; and clear cell carcinoma, 2). The disease was classified as Stage 2 in 15 patients and Stage 3 in 49 patients.

Peripheral blood samples were collected at the time of the patient's admission to hospital for initial surgery, within 4 weeks after the operation, and then at intervals of 2–3 months in the follow-up clinic for 12 months or more; in some patients, samples were taken after 18 and 24 months. Aliquots of serum were stored at −20°C and assayed in batches.

CA 125 antigen was measured by a solid phase immunoradiometric assay (ELSA CA 125™ CENTOCOR assay, Australian Atomic Energy Commission [4]). Briefly, the solid phase (ELSA) coated with mouse monoclonal antibody to CA 125 was incubated with the test sample, standard or control. During this incubation, the unknown CA 125 in the test sample was bound to the solid phase, in competition with its binding to the same anti-CA 125 antibody labelled with ^{125}I. After the unbound tracer had been washed out, the radioactivity bound to the solid phase (which was proportional to the amount of CA 125 present) was counted. The sensitivity range of the assay was between 6.5 U/ml and 500 U/ml. Although a positive level has been taken arbitrarily at 35 U/ml on the basis of previous experience with a healthy population [4], for the purposes of the present study where serial levels were compared with each other in the same patient, the threshold for positivity was 7 U/ml, the limit of the assay's sensitivity. The results were not made available to the attending clinicians.

Treatment programme

Cytotoxic chemotherapy was considered for all patients after initial surgery at which maximal tumour excision was attempted. After the first definitive surgery, the patients were classified as follows: no residual tumour (21 patients), maximal tumour mass < 1 cm dia. (25 patients) and tumour mass > 1 cm dia. (16 patients). In general, patients with minimal or no residual disease were assigned to oral chlorambucil (7.5 mg/m^2 body area daily for 14 days of every month) with or without i.v. cisplatin (50 mg/m^2 body area every 4–6 weeks). In the absence of response, or with larger residual disease and high-grade tumour, the patients received a combination of drugs (PAC): cisplatin, adriamycin (50 mg/m^2 body area) and cyclophosphamide (500 mg/m^2 body area) every 4 weeks. The manipulations in chemotherapy strategy were made independent of the CA 125 results.

Follow-up and tumour behaviour

The patients attended the follow-up clinic at monthly intervals. At each visit they were examined carefully for evidence of tumour and a decision was made concerning continuation of chemotherapy. Relevant investigations were performed if tumour presence was suspected. The tumour status of the patient was recorded by consensus agreement of the consultative staff of the clinic after 12 months of follow-up unless the patient had died of the disease before then. In the presence of complete remission (often after a negative CT scan), the patient was recommended to have a second-look laparotomy to evaluate the abdomen and pelvis so that a decision on further chemotherapy could be made. In this series, findings from the second-look procedure were available in 42 patients; the procedure had yet to be performed in another 15 patients who had shown complete clinical remission and became unnecessary in another 7 because of obvious clinical progression. In addition, clinical progression had also been observed in 11 patients subsequent to the second-look procedure. In the 18 patients with clinical progression, 12 had already died of the disease.

Second-look procedure

The procedure was performed by one of us to ensure uniform evaluation. Comprehensive exploration of the abdomen and pelvis including inspection and palpation was made through an adequate vertical incision. In addition to taking biopsies from suspicious nodules anywhere in the abdomen and pelvis, steps were taken to obtain peritoneal washings for cytology from the pelvis and both paracolic gutters, random multiple biopsies from the peritoneum over the bladder, vaginal vault, both infundibulo pelvic pedicles, rectosigmoid, and the undersurface of the right leaf of diaphragm, and sampling of the para-aortic nodes at the level of the renal vessels as well as the obturator, common and external iliac nodes.

Patterns of serial CA 125 levels

Although there was a tendency for CA 125 levels to fluctuate, a clear trend became evident after 6 months of follow-up. Four patterns were observed in this study.

(*a*) *Negative results throughout.* The levels remained < 7 U/ml throughout the follow-up period.

Table 1. Relationship of pattern of CA 125 levels to tumour status and findings at second-look surgery

Pattern CA 125		No. of patients	Findings at second-look surgery: Tumour absent	Tumour present: Microscopic	Tumour present: Macroscopic < 1 cm	Tumour present: Macroscopic > 1 cm
(a)	Negative values throughout	20	14	2	2	2
(b)	Complete disappearance	10	5	2	2	1
(c)	Reappearance after initial disappearance	8	1	2	3	2
(d)	Persistently high or rising values	4	—	—	—	4

(*b*) *Complete disappearance.* There was a decline of positive CA 125 values to below 7 U/ml, often rapidly within 4–8 weeks after surgery, and the levels remained negative thereafter.

(*c*) *Reappearance after initial disappearance.* After the rapid disappearance of CA 125 during the early phase of observation, negative levels were maintained for a variable period of time before a rise again, usually to significant levels exceeding 35 U/ml.

(*d*) *Persistently high or rising levels.* There were 2 subtypes: one in which there was a sustained elevation of CA 125 levels throughout the follow-up period, and in the other, there was a rapid rise. A terminal fall in CA 125 levels was sometimes observed.

RESULTS

Relationship of pattern of CA 125 levels to tumour status and findings at second-look surgery

Table 1 shows the results in patients in whom the findings at second-look procedure were available—20 of 42 patients (48%) showed no undetectable CA 125 (< 7 U/ml) throughout the period of study. There were 6 patients with mucinous tumours in this group.

There were 20 patients in whom there was no histological evidence of disease. CA 125 was undetectable throughout the period of observation in 14 and in another 5 the positive values (as high as 350 U/ml in 1 patient) returned to negative values (Fig. 1). However, there was 1 patient in whom the

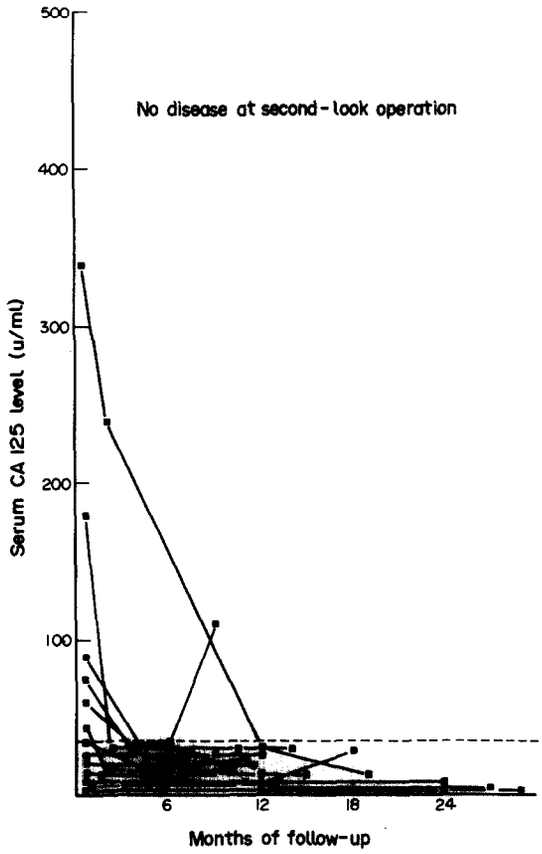

Fig. 1. Serial serum CA 125 levels of individual patients with ovarian cancer who showed no evidence of tumour at second-look laparotomy.

CA 125 became negative after an initial value of 74 U/ml, but increased to 110 U/ml after 9 months. She had had a radical hysterectomy, bilateral salpingo-oophorectomy and infracolic omentectomy for a poorly-differentiated cystadenocarcinoma, Stage III. Residual disease after surgery was minimal and PAC chemotherapy was administered for 7 cycles.

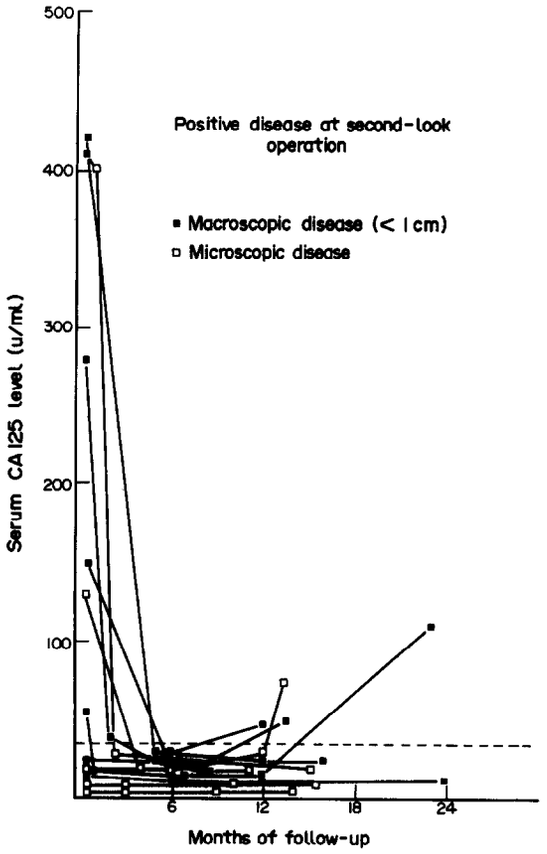

Fig. 2. Serial serum CA 125 levels of individual patients who were found to have microscopic (□—□) or macrocopic disease with tumour masses < 1 cm dia. (■—■) at second-look laparotomy.

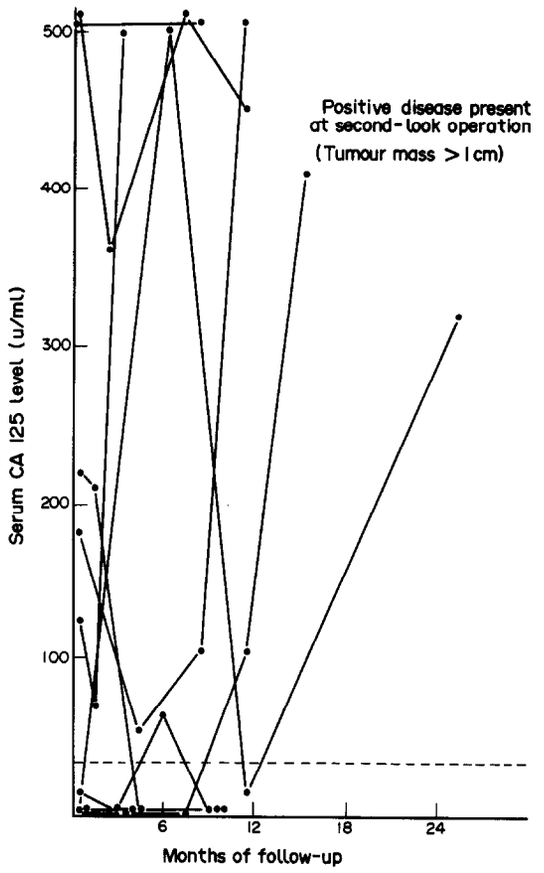

Fig. 3. Serial serum CA 125 levels of individual patients who showed more extensive disease at second-look laparotomy, with tumour masses > 1 cm dia.

At the second-look procedure, there was no microscopic or macroscopic evidence of disease; to date (3 months later), she remains clinically free of disease.

Microscopic tumour was found in 6 patients and their individual patterns of CA 125 are shown in Fig. 2. CA 125 values were negative throughout in 2 patients who had foci of tumour in the para-aortic nodes only. In another 2 patients, CA 125 rapidly became negative despite the detection of microscopic tumour foci diffusely over the abdominal peritoneum at the second-look procedure; these patients had remained clinically free of disease 14 and 18 months, respectively, after the second-look procedure. The remaining 2 patients in this group who showed a reappearance of CA 125 had tumour foci in random biopsies taken from the infundibulopelvic pedicle and vaginal vault. Thus, of the 6 patients with microscopic disease, CA 125 was falsely negative in 4 and truly positive in 2.

Macroscopic disease was found in 16 patients, the tumour mass was < 1 cm dia. in 7, and > 1 cm dia. in another 9. As shown in Fig. 2, when the tumour mass was < 1 cm, the CA 125 patterns showed negative values throughout the period of observation (2 patients) or a rapid disappearance (2 patients); in 3 of these patients there was disease solely in 1 organ (spleen, liver, lymph node). In 3 patients there was a reappearance of CA 125 before the second-look procedure; all had tumour nodules scattered over the peritoneum of the pelvis and abdomen. Thus in the detection of macroscopic disease (< 1 cm dia.) CA 125 gave a true-positive result in 3 of 7 patients.

More extensive disease (macroscopic tumour mass > 1 cm dia.) was found in 9 patients. As shown in Fig. 3, the CA 125 patterns in this group were characterised by very high and fluctuating levels; in 4 patients, the levels exceeded 400 U/ml before the second-look procedure. In the patient in whom there was a return to negative CA 125 levels, a moderate amount of tumour (> 5 cm dia.) was found in the cervical stump; the subsequent course of the disease was slow and she died after 33 months. Patterns of highly elevated CA 125 levels or reappearance of CA 125 (usually to levels > 400 U/ml) were observed in 6 patients, all of whom had progressive disease after the second-look procedure and had died within 24 months. A true-positive result for CA 125 in the detection of clinically-impalpable but macroscopic disease > 1 cm dia. was obtained in 6 of 9 patients.

Excluding the CA 125 pattern which showed

Table 2. Relationship patterns of CA 125 levels to disease outcome in patients with ovarian cancer

Pattern CA 125		No evidence of disease	Stable disease (clinically) undetectable)	Progressive disease	Death
(a)	Negative values throughout	14	5	2	4
(b)	Complete disappearance	5	4	—	1
(c)	Reappearance after initial disappearance	1	2	3	3
(d)	Persistently high or rising values	—	—	1	4

negative values throughout the follow-up, the prediction on the basis of patterns of CA 125 levels was correct as follows: for absence of tumour, 5/10 (50%), and for presence of tumour, 11/12 (92%).

Relationship of patterns of CA 125 levels to disease outcome

Table 2 shows the relationship of CA 125 patterns to disease outcome in 49 patients who had been followed for at least 24 months. This analysis included an additional 7 patients in whom the disease was clinically progressive before the usual interval of time had elapsed for the consideration of the second-look procedure. The patterns in patients who showed no evidence of disease were predominantly those of negative values throughout (14 patients) or those of complete disappearance (5 patients). However, there was 1 patient in whom CA 125 reappeared and to date, no disease was detectable after a follow-up period of 18 months.

Of 11 patients who have remained clinically well and free of disease subsequent to a second-look procedure which revealed the presence of tumour, 9 showed CA 125 patterns of negative values throughout or complete disappearance—these patterns being considered to indicate a favourable outcome. However, 2 patients showed a reappearance of CA 125, but have remained well. In the third group of 18 patients, clinical disease was present and 12 have died. Among these latter patients with a poor outcome, 6 showed negative CA 125 values throughout. There was 1 patient with terminal disease who showed a precipitous fall in CA 125 levels; she died in 4 months. The predominant CA 125 patterns in this group were those of reappearance or highly elevated levels; these were observed in 11 patients of whom 7 had already died.

For the purposes of this analysis, a poor outcome was taken as the presence of clinical disease (progression and death) and a good outcome as either no evidence of disease or presence of clinically stable and undetectable disease. The predictive accuracy of pattern (b) was 9/10 (90%) to indicate a good outcome and that of patterns (c) and (d) was 11/14 (79%) to indicate a poor outcome. It was difficult to interpret pattern (a) but if it were to predict a good outcome, the accuracy rate was 19/25 (76%).

DISCUSSION

Because of caution in the interpretation of single isolated levels of CA 125, the present study attempted to reduce the problem of false-positive and false-negative results by using serial serum levels over 12 months or more. Blood sampling was performed at intervals of 2–3 months rather than monthly to reduce the number of venepunctures. In general, the sequential changes of CA 125 values in patients with ovarian cancer appeared to correlate with the amount of tumour as determined by second-look operative evaluation and with the clinical outcome. However, there were exceptions, discrepancies and limitations.

One limitation is that nearly 50% of patients in the present study had negative CA 125 results at the level of < 7 U/ml throughout the period of follow-up. However, if mucinous tumours were excluded, the incidence fell to 38%. On the basis of a single preoperative sample, the incidence of CA 125 negativity at a level > 35 U/ml was 57% in our series; this compared with 39% in a series of consecutive patients with primary ovarian cancer [6] but the incidence was lowest (20%) in selected patients with ovarian cancer [4]. The possibility of the hook-effect as a cause for falsely low CA 125 levels in some patients has to be considered but seems unlikely because most patients in the present study had a small tumour burden.

The significance of the pattern showing CA 125 levels < 7 U/ml throughout is uncertain. It could be related to failure of CA 125 synthesis by the tumour; values in patients with mucinous tumours were found to be consistently negative [3, 7]. There

may also be failure of significant release of CA 125 into the circulation and this has a direct relationship to the site and size of the tumour. Other factors can also operate to influence the blood levels of CA 125, such as the rate of synthesis, the vascularity of the tumour, and the rate of metabolism of the patient. Our findings would tend to associate this pattern with a good prognosis and an absence of disease. However, the prediction of a good outcome on the basis of negative CA 125 values throughout the follow-up was incorrect in 24% of cases. Furthermore, 6 of 20 patients (30%) were found at second-look procedure to have microscopic or macroscopic disease.

One of the 2 objectives in the present study was to determine whether the use of CA 125 levels could be a guide to the timing and performance of the second-look procedure—an invasive procedure not without its attendant morbidity. Based on single CA 125 estimations, positive results have been reported to correlate strongly with the presence of tumour. In a retrospective study, Niloff *et al.* [8] reported a positive result > 35 U/ml in 7 of 8 patients with intraperitoneal tumour, however, not all the second-look evaluation was by laparotomy. In a later study, the same group of workers found that none of 24 patients with a negative second-look operation had a positive CA 125 level (> 35 U/ml), compared with 6 of 20 patients (30%) with < 1.5 cm disease, and 6 of 10 patients (60%) with > 1.5 cm disease [9]. When 25 U/ml was used as the threshold of CA 125 positivity, such positive levels were frequently associated with surgically demonstrable disease but bowel obstruction was a cause of false-positive results [10]. In fact, high CA 125 levels (> 1000/U/ml) were found to indicate advanced disease, a poor response to cytotoxic chemotherapy and poor operability [11]. However, a negative CA 125 level was not predictive of the absence of tumour—false-negative rates of 43–46% have been reported [9, 10].

We were able to confirm that patterns of CA 125 levels, as with single estimations in the previous studies, were also not predictive of the absence of tumour when the evaluation was uniformly based on a comprehensive second-look laparotomy. In the present study, whereas rising or persistently high CA 125 levels indicated the presence of tumour in 92% of patients, declining levels to negative predicted the absence of tumour in only 50%. We found false-negative results in 11 of 22 patients. Although the majority of these patient showed microscopic foci or a tumour mass < 1 cm dia., 3 patients had a larger amount of disease and there was no site of the tumour in the abdomen and pelvis which was consistently associated with negative CA 125 levels.

The pattern of persistently high or rising CA 125 levels—even in the absence of clinically-detectable disease—was found to be associated with more extensive disease. However, there was 1 patient with high fluctuating levels in whom the second-look procedure revealed no disease; final assessment has to be guarded because of the known problem of later recurrence subsequent to negative findings at surgery [12]. There may be a long latent interval between CA 125 positivity and clinical development of tumour [10, 11].

The other objective was to assess the value of CA 125 in predicting the clinical outcome in ovarian cancer. In this respect, the accuracy of prediction of a good outcome was better than that of a poor outcome on the basis of CA 125 patterns, as was shown in the present study with rates of 90 and 79%, respectively. As noted previously [13], a significant increase (more than 50% of previous value) in CA 125 levels within the 'normal' range (6.5–35 U/ml) may also be representative of tumour progression, an accurate prediction was found in 41 of 43 patients (95%) [10]. Furthermore, a precipitous—regarded as paradoxical—fall in CA 125 levels has been observed in the terminal phase of the disease. However, it remains difficult to explain many of the false-positive results in our series, especially in the absence of the known non-tumour causes of elevated CA 125 levels. It is possible that the tumour, although present, is not detectable, even by a detailed second-look procedure. A longer follow-up period in these patients should resolve this problem.

Despite the limitations and restrictions, the use of CA 125 estimation should be encouraged in the management of patients with ovarian cancer. They are especially valuable when used in conjunction with clinical and other investigative parameters. Because of the strong likelihood of a significant residual tumour, a second-look procedure should be deferred or cancelled when the patterns of CA 125 levels show a reappearance of the marker or pesistently high values. However, the CA 125 lacks sensitivity in detecting small tumour masses. Therefore, a pattern showing negative values should not be regarded as indicative of an absence of tumour to obviate the need for a second-look procedure. With our present knowledge of CA 125, its use in the monitoring of the effectiveness of chemotherapy remains debatable. There are problems that the expression of OVCA 433, a cell surface antigen, may be altered by cytotoxic drugs and thus give falsely low levels, and intercurrent disease during treatment such as bowel obstruction, inflammatory conditions and hepatic dysfunction [14] may contribute to the level of CA 125 in the blood.

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REFERENCES

1. Khoo SK, Whitaker S, Jones I, Mackay E. Predictive value of serial carcinoembryonic antigen levels in long-term follow-up of ovarian cancer. *Cancer* 1979, **43**, 2471–2478.
2. Bast RC Jr, Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC. Reactivity of a monoclonal antibody with human ovarian carcinoma. *J Clin Invest* 1981, **68**, 1331–1337.
3. Kabawat SE, Bast RC Jr, Welch WR, Knapp RC, Colvin RB. Immunopathologic characterization of a monoclonal antibody that recognizes common surface antigens of human ovarian tumors of serous, endometrioid and clear cell types. *Am J Clin Pathol* 1983, **79**, 98–104.
4. Bast RC Jr, Klug TL, St John E, *et al.* A radioimmunoassay using a monoclonal antibody to monitor course of epithelial ovarian cancer. *N Engl J Med* 1983, **309**, 883–887.
5. Halila H, Stenman U-H, Seppala M. Ovarian cancer antigen CA 125 levels in pelvic inflammatory disease and pregnancy. *Cancer* 1986, **57**, 1327–1329.
6. Einhorn N, Bast RC Jr, Knapp RC, Tjernberg B, Zurawaski VR. Preoperative evaluation of serum CA 125 levels in patients with primary epithelial ovarian cancer. *Obstet Gynecol* 1986, **67**, 414–416.
7. Klug TL, Bast RC Jr, Niloff JM, Knapp RC, Zurawaski VR. Monoclonal antibody immunoradiometric assay for an antigenic determinant (CA 125) associated with human epithelial ovarian carcinomas. *Cancer Res* 1984, **44**, 1048–1053.
8. Niloff JM, Bast RC Jr, Schaetzl EM, Knapp RC. Predictive value of CA 125 antigen levels in second-look procedures for ovarian cancer. *Am J Obstet Gynecol* 1985, **151**, 981–986.
9. Berek JA, Knapp RC, Malkasian GD, *et al.* CA 125 serum levels correlated with second-look operations among ovarian cancer patients. *Obstet Gynecol* 1986, **67**, 685–689.
10. Krebs HB, Goplerud DR, Kilpatrick SJ, Myers MB, Hunt A. Role of CA 125 as tumour marker in ovarian carcinoma. *Obstet Gynecol* 1986, **67**, 473–477.
11. Kivinen S, Kuoppala T, Leppilampi M, Vuori J, Kauppila A. Tumor-associated antigen CA 125 before and during treatment of ovarian carcinoma *Obstet Gynecol* 1986, **67**, 468–472.
12. Cain JM, Saigo PE, Pierce VK, *et al.* A review of second-look laparotomy for ovarian cancer. *Gynecol Oncol* 1986, **23**, 14–25.
13. Atack DB, Nisker JA, Allen HH, Tustanoff ER, Levin L. CA 125 surveillance and second-look laparotomy in ovarian carcinoma. *Am J Obstet Gynecol* 1986, **154**, 287–289.
14. Bergmann JF, Beaugrand M, Labadie H, Bidart JM, Bohuon C. CA 125 (ovarian tumour-associated antigen) in ascitic liver diseases. *Clin Chim Acta* 1986, **155**, 163–166.