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Original Paper

Multiple Tumour Marker Assays in Advanced Cervical Cancer: Relationship to Chemotherapy Response and Clinical Outcome

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Serum levels of squamous cell carcinoma antigen (SCC), CA 125 and CA 15.3 were measured in 102 patients with locally advanced cervical cancer undergoing neoadjuvant chemotherapy (NACT) and radical surgery. We found a significant correlation between SCC concentration and stage, histotype, cervical tumour size and lymph node status. For CA 125 and CA 15.3, no significant difference in the distribution of marker levels according to histopathological variables was found. In a multivariate analysis, histological type, FIGO stage and SCC positivity (>5 ng/ml) proved to be independent predictors of response to neoadjuvant chemotherapy. Moreover, logistic regression analysis showed that CA 15.3 may be a significant adjunct to SCC in the prediction of chemotherapy response. Of the three markers tested, only CA 125 was significantly related to patient survival. In the multivariate analysis, clinical response to chemotherapy and CA 125 status (>35 U/ml) retained an independent prognostic value. Our data suggest that the tumour markers used in this study could be useful in the management of locally advanced cervical cancer. Pretreatment serum levels of SCC, together with CA 15.3 assay, may be a useful tool in the determination of response to chemotherapy, while CA 125 assay could be evaluated as a prognostic risk factor in these patients.

Key words: serum tumour markers, cervical cancer, gynaecological cancer
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INTRODUCTION

CERVICAL CANCER is the most common malignant disease in women after breast cancer. At present, the spread of the disease, estimated by clinical staging according to FIGO criteria [1], determines treatment and disease outcome.

However, prognostic characterisation based only on clinicopathological parameters is inadequate and additional prognostic factors are needed to select patients for individually programmed therapy. To address this problem, a search for biological characteristics linked to tumour aggressiveness may prove useful. In recent years tumour-associated antigens have gained importance in gynaecological oncology. Several studies have indicated that serum values of squamous cell carcinoma antigen (SCC), a subfraction of the TA-4 antigen, may be a good indicator in the management of cervical cancer [2–8]. In our previous studies we reported that SCC assay might provide useful information for the prognostic characterisation and disease monitoring of patients with locally advanced cervical cancer undergoing neoadjuvant chemotherapy (NACT) and radical surgery. Moreover, SCC expression was found to be closely related to chemoresistance [9, 10]. CA 125, a tumour marker used mainly in epithelial ovarian carcinoma, may be potentially useful in the monitoring of cervical cancer [11, 12]. CA 15.3 antigen, expressed by human breast cancer cells, is used for monitoring breast cancer patients [13,14], but currently there are no data available concerning its application in cervical cancer. This study was aimed at investigating whether the measurement of CA 125 and CA 15.3 could be usefully coupled with SCC in the prognostic characterisation of patients with advanced cervical cancer.

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PATIENTS AND METHODS

Our study included 102 patients (median age 53 years, range 29–72 years) with invasive cervical cancer admitted to the Department of Gynaecology of the Catholic University of Rome. 6 patients had stage Ib-IIa disease (tumour size >4 cm), 47 had stage IIb, 17 had stage IIIa, 29 had stage IIIb, 3 had stage IV, according to the FIGO classification.

Diagnosis was always confirmed by histological examin-

ation. These tumours were histologically classified as squamous in 82 cases and adenocarcinomas in 20 cases. According to the degree of histological differentiation, tumours were found to be well/moderately and poorly differentiated in 62 and 40 patients, respectively. All patients with locally advanced cervical carcinoma received cisplatin based NACT. Cervical tumour size was measured by the combination of gynaecological examination, colposcopy and pelvic ultrasonography. Stability, progression and regression of disease were defined according to World Health Organisation (WHO) criteria. Both protocols of NACT and radical surgery were approved by the Investigational Review Board, and patients gave their informed consent.

Serum samples were obtained before any treatment. Venous blood samples for marker determination were separated by centrifugation and aliquots were stored at −20°C until assay. Marker assays were performed using commercially available kits (CIS, Compagnie ORIS Industrie SAF for CA 125 and CA 15.3, SCC RIA Kit, Abbott Laboratories Diagnostic Division, Abbott Park, Illinois, U.S.A. for SCC).

Intra- and interassay coefficients of variation (CV) were 6.2 and 8.5% for SCC, 8.7 and 7.0% for CA 125 and 5.4 and 6.1% for CA 15.3, respectively.

Since, in accordance with other reports [10, 15–18], the values of 35 and 65 U/ml for CA 125, 30 U/ml for CA 15.3 and 5 ng/ml for SCC proved to be the best discriminators, all the analyses were conducted using these cut-off values.

The Mann–Whitney test was used to evaluate marker levels according to different parameters. Analysis of survival was performed by the product-limit estimate using the Kaplan and Meier method [19] and the curves were examined by means of the log-rank test [20]. Survival was calculated from the day of therapy until the day of death or the date last seen. Cox multivariate regression analysis was used to assess the relative order of importance of prognostic parameters to survival and to clinical response to chemotherapy [21].

Logistic regression in which the response functions are the logits of the categorical dependent variables was used to assess the probability of response to chemotherapy [22].

RESULTS

Table 1 shows the distribution of pretreatment SCC, CA 125 and CA 15.3 antigen levels in relation to the different clinicopathological variables. As previously reported, we found a significant correlation between SCC concentration and stage, histotype, cervical tumour size and lymph node status ($P < 0.05$ by Mann–Whitney test). For CA 125 and CA 15.3 no difference in the distribution of marker levels according to histopathological variables was found. Using the Spearman-test, no correlation between tumour marker values was found (data not shown).

Table 2 shows the univariate and multivariate analysis of the significance of marker levels and clinicopathological parameters as predictive variables of clinical response to chemotherapy. In the univariate analysis, several variables were significantly related to chemotherapy response (Table 2), while multivariate analysis identified histology ($P = 0.05$), FIGO stage ($P = 0.02$) and pretreatment SCC (>5 ng/ml) ($P = 0.01$) as significant independent prognostic factors.

Logistic regression was used to determine probability (P) of response to chemotherapy with a mathematical formula:

$$P = \frac{e^{\lambda}}{1 + e^{\lambda}}$$

where $\lambda = 6.53 - 2.14 \times$ histotype $- 1.27 \times$ grading $- 2.19 \times$ stage $- 0.94 \times$ cervical tumour size $- 2.54 \times$ SCC $- 0.89$ CA 15.3.

Table 1. SCC, CA 125 and CA 15.3 serum levels according to clinicopathological parameters

	No. of cases	SCC (ng/ml)		CA 125 (U/ml)		CA 15.3 (U/ml)	
		Median	Range	Median	Range	Median	Range
Stage							
Ib–IIa	6	4.2†	(1.8–64)	25	(7–121)	20	(16–42)
IIb	47	2.7	(0.1–52)	12	(4–>501)	18	(3.6–96)
IIIa	17	5.4	(0.1–27)	12	(6.5–>501)	25	(7–67)
IIIb	29	5.5	(1.5–34)	26	(6.5–102)	22	(7–160)
IV	3	7.7	(1.5–9)	17	(6.5–221)	31	(15–>220)
Histotype							
Squamous	82	4.3‡	(0.1–64)	14	(4–>501)	20.5	(3.6–>220)
Adenocarcinoma	20	2.5	(0.1–20)	22	(6.5–430)	21	(11–84)
Grading							
Well/moderate	62	4.5	(0.1–36)	12	(5–>501)	20.5	(3.6–96)
Poor	40	3.1	(0.1–64)	25	(4–221)	21	(7–>220)
Cervical tumour size							
<5 cm	60	2.7‡	(0.1–31.7)	13	(4–>501)	20	(3.6–>220)
≥5 cm	42	6.4	(1.5–64)	18	(6.5–221)	22	(7–160)
Cervical infiltration*							
Absent or <5 mm	24	2.8	(0.7–28)	11	(5–>501)	19.5	(7–96)
≥5 mm	50	3.8	(0.1–64)	15.5	(4–430)	20	(8–75)
Lymph node status*							
N−	59	2.7‡	(0.1–64)	14	4–>501)	19	(3.6–96)
N+	15	7.2	(1.5–36)	12	(6.5–80)	22	(12–75)

*Only radically operated patients. †$P < 0.05$ (Ib–II versus stage III–IV). ‡$P < 0.03$.

Table 2. Univariate and multivariate analysis for clinical response to neoadjuvant chemotherapy in the overall population

Variable	No. of cases	CR (%)	PR (%)	NC/P (%)	Univariate *P* value	Multivariate *P* value
Histology						
Squamous	82	12	73	15	n.s.	0.05
Adenocarcinoma	20	15	60	25		
FIGO stage						
I–II	53	19	77	4	0.002	0.02
III–IV	49	6	63	31		
Grading						
Good/moderate	62	14	74	12	n.s.	n.s.
Poor	40	10	65	25		
Cervical tumour size						
<5 cm	60	18	73	8	0.029	n.s.
≥5 cm	42	5	67	28		
Pretreatment SCC						
<5 ng/ml	57	14	77	9	0.02	0.01
≥5 ng/ml	45	11	62	27		
Pretreatment CA 15.3						
<30 U/ml	76	14	74	12	0.02	n.s.
≥30 U/ml	26	8	60	32		

n.s., not significant; CR, complete response; PR, partial response; NC/P, no change/progression.

Table 3. Multivariate analysis for overall survival in cervical carcinoma

Variable	Beta	*P*	Chi-square
Clinical response	1.447	0.002	13.56
Cervical tumour size	—	0.06	3.34
Pretreatment CA 125	0.743	0.03	4.29

The coding of the variables was: Stage I–II = 0, III–IV = 1; histotype: squamous cell carcinoma = 0, adenocarcinoma = 1; grading: differentiated tumours (G1–G2) = 0, undifferentiated tumours (G3) = 1; cervical tumour size: <5 cm = 0, ≥5 cm = 1; SCC: <5 ng/ml = 0, ≥5 ng/ml = 1; CA 15.3: <30 U/ml = 0, ≥30 U/ml = 1.

This mathematical model fitted since the likelihood ratio goodness-of-fit test was not significant ($P = 0.94$). Moreover, when CA 15.3 or CA 15.3 plus SCC were excluded from the formula, the *P* value of the test was reduced to 0.84 or 0.50, respectively, which means a reduction in the accuracy of the model. The addition of CA 125 to the analysis decreased the predictive power of the formula, indicating that CA 125 expression is not associated with chemotherapy response.

We also evaluated the prognostic significance of tumour markers in our patient population. The difference in survival rate between CA 125 positive and CA 125 negative patients was statistically significant, for both cut-off values (35 and 65 U/ml) ($P < 0.05$) (Figure 1a,b). The difference between SCC+ (≥5 ng/ml) and SCC– (<5 ng/ml) patients was not statistically significant, even though those with low SCC values had a better survival than patients with high levels (data

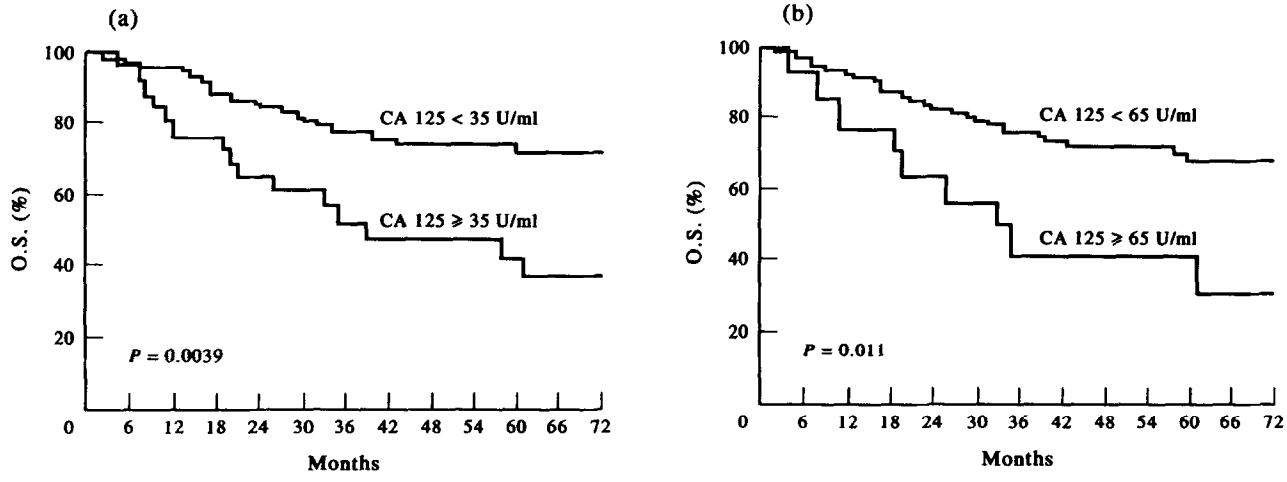

Figure 1. (a) Overall survival rate according to CA 125 status in cervical cancer, using a cut-off value of 35 U/ml. CA 125 positive cases: 26 entered, 15 died with a median of 40 months. CA 125 negative cases: 76 entered, 19 died. (b) Overall survival rate according to CA 125 status in cervical cancer, using a cut-off value of 65 U/ml. CA 125 positive cases: 14 entered, 9 died with a median of 33 months. CA 125 negative cases: 88 entered, 25 died.

not shown). Using a cut-off value of 30 U/ml, 10 of the 26 (38%) patients with high CA 15.3 values died versus 24 of the 76 (32%) patients with low CA 15.3 values ($P = 0.45$) (data not shown).

A multivariate analysis of survival data including only those variables (clinical response to chemotherpy, FIGO stage, grade of differentiation, tumour size and CA 125 levels) which were significant in the univariate analysis was performed. Table 3 shows that among the variables considered, only clinical response to chemotherapy and the pretreatment levels of CA 125 were significantly associated with a worse prognosis.

DISCUSSION

Recently, several studies have suggested that the measurement of multiple markers may be useful in the prognostic characterisation and disease monitoring of cervical carcinoma [11, 12, 23–25].

In this report, which follows our previous study showing that SCC is an independent indicator of chemoresistance, we analysed the clinical usefulness of the combination of SCC, CA 125 and CA 15.3 antigens in the management of patients with locally advanced cervical cancer undergoing NACT.

According to previous data [12, 18, 26], CA 125 and CA 15.3 showed a lower sensitivity than SCC in cervical carcinoma. However, in our series, CA 15.3 levels were significantly related to chemotherapy non-responsiveness. Moreover, CA 15.3 would seem to improve significantly the ability of SCC to predict response to neoadjuvant chemotherapy. We demonstrated that the probability of response to chemotherapy could be estimated by a mathematical formula including SCC and CA 15.3 values and clinical variables. Since the combined use of NACT and radical surgery is becoming a feasible treatment in patients with locally advanced cervical cancer [27, 28], the identification of chemoresponsive patients could be of utmost importance in the clinical management of the disease.

The prognostic value of preoperative serum CA 125 levels in patients with epithelial ovarian carcinomas [29–31] and endometrial carcinomas [17, 32, 33] has been reported. In our study, CA 125 antigen proved to be independently related to the prognosis of patients with cervical cancer. This finding is in accordance with the study by Avall-Lundquist and associates [12], who examined CA 125 serum levels in 142 patients with primary cervical carcinoma, most of whom had stage I–II disease, and were treated by surgery or radiotherapy. Interestingly, in our cases, CA 125 positivity seemed to be a negative prognostic factor independent of response to chemotherapy, which is one of the most important parameters influencing survival in patients undergoing NACT and radical surgery [34, 35].

In conclusion, our data suggest that the tumour markers analysed in this study could be adopted in the management of locally advanced cervical cancer. Pretreatment serum levels of SCC together with CA 15.3 assay may be a useful tool in the determination of response to chemotherapy, while CA 125 assay could be evaluated as a prognostic risk factor in these patients. If our data are confirmed in a larger prospective series, it will be possible to optimise therapeutic planning by identifying patients who need more aggressive and/or experimental therapeutic modalities.

1. Patterson F, ed. Annual report on the results of treatment in gynecological cancer. FIGO, Stockholm, 1988, Vol. 20.
2. Maruo T, Shibata K, Kimura A, Hoshina A, Mochizuki M. Tumour-associated antigen, TA-4, in the monitoring of the effects of therapy for squamous cell carcinoma of the uterine cervix. *Cancer* 1985, **56**, 302–308
3. Kato H, Tamai K, Morioka H, Nagai M, Nagaya, Torigoe T. Tumour antigen TA-4 in the detection of recurrence in cervical squamous cell carcinoma. *Cancer* 1984, **54**, 1544–1546.
4. Senekjian EK, Young JM, Weiser PA, Spencer CE, Magic SE, Herbst AL. An evaluation of squamous cell carcinoma antigen in patients with cervical squamous cell carcinoma. *Am J Obstet Gynecol* 1987, **157**, 433–439.
5. Maiman M, Feuer G, Fruchter RG, Shaw N, Boyce J. Value of squamous cell carcinoma antigen levels in invasive cervical carcinoma. *Gynecol Oncol* 1989, **34**, 312–316.
6. Ngan HYS, Wong LC, Chan SYW, Ma HK. Use of the serum squamous cell carcinoma antigen assays in chemotherapy treatment of cervical cancer. *Gynecol Oncol* 1989, **35**, 259–262.
7. Rose PG, Baker S, Fournier L, Nelson B, Hunter R. Serum squamous cell carcinoma antigen levels in invasive cervical cancer: prediction of response and recurrence. *Am J Obstet Gynecol* 1993, **168**, 942–946.
8. Duk JM, De Brujn HWA, Groenier KH, *et al.* Cancer of the uterine cervix: sensitivity and specifity of serum squamous cell carcinoma antigen determinations. *Gynecol Oncol* 1994, **39**, 186–194.
9. Scambia G, Benedetti Panici P, Baiocchi G, *et al.* The value of squamous cell carcinoma antigen in patients with locally advanced cervical cancer undergoing neoadjuvant chemotherapy. *Am J Obstet Gynecol* 1991, **164**, 631–636.
10. Scambia G, Benedetti Panici P, Foti E, *et al.* Squamous cell carcinoma antigen: prognostic significance and role in the monitoring of neoadjuvant chemotherapy response in cervical cancer. *J Clin Oncol* 1994, **12**, 2309–2316.
11. Duk JM, De Bruijn HWA, Groenier KH, Fleuren GJ, Aalders JG. Adenocarcinoma of the uterine cervix. *Cancer* 1990, **65**, 1830–1837.
12. Avall-Lundquist EH, Sjovall K, Nilsson BR, Eneroth PHE. Prognostic significance of pretreatment serum levels of squamous cell carcinoma antigen and CA 125 in cervical carcinoma. *Eur J Cancer* 1992, **10**, 1695–1702.
13. Hilkens J, Kroezen V, Bonfrer JMG, *et al.* MAM-6 antigen, a new serum marker for breast cancer monitoring. *Cancer Res* 1986, **46**, 2582–2587.
14. Dixon AR, Jackson SY, Chan SY, *et al.* Continuous chemotherapy in responsive metastatic breast cancer: a role for tumour markers? *Br J Cancer* 1993, **68**, 181–185.
15. Bast RC, Klug TL, St John E, *et al.* A radioimmunassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. *N Engl J Med* 1983, **309**, 883–887.
16. Verlooy H, Devos P, Janssens J, *et al.* Clinical significance of squamous cell carcinoma antigen in cancer of the human uterine cervix. *Gynecol Obstet Invest* 1991, **32**, 55–58.
17. Scambia G, Gadducci A, Benedetti Panici P, *et al.* Combined use of CA 125 and CA 15-3 in patients with endometrial carcinoma. *Gynecol Oncol* 1994, **54**, 292–297.
18. Benedetti Panici P, Scambia G, Baiocchi G, *et al.* Circulating tumour markers in cervical cancer. *Tumor Biol* 1989, **10**, 109–116.
19. Kaplan EL, Meier P. Non parametric estimation from incomplete observations. *J Am Stat Assoc* 1958, **53**, 457–481.
20. Hantag BF, Myers MH. Evaluating differences in survival between two groups of patients. *J Chronic Dis* 1971, **24**, 523–531.
21. Cox DR. Regression models and life tables. *J R Stat Soc B* 1922, **33**, 187–220.
22. Hosmer DW, Lemeshow S. *Applied Logistic Regression.* New York, Wiley, 1989.
23. Tomas C, Risteli J, Risteli L, Vuori J, Kauppila A. Use of various epithelial tumor markers and a stromal marker in the assessment of cervical carcinoma. *Obstet Gynecol* 1991, **77**, 566–572.
24. Meier W, Eiermann W, Stieber P, Fateh-Moghadam A, Schneider A, Hepp H. Squamous cell carcinoma and carcinoembryonic antigen levels as prognostic factors for the response of cervical carcinoma to chemotherapy. *Gynecol Oncol* 1990, **38**, 6–11.
25. Yazigi R, Munoz AK, Richardson B, Rissen R. Correlation of

squamous cell carcinoma antigen levels and treatment response in cervical cancer. *Gynecol Oncol* 1991, **41**, 135–138.
26. Goldberg GL, Sklar A, O'Hanlan KA, Levine PA, Runowicz CD. CA 125: a potential prognostic indicator in patients with cervical cancer? *Gynecol Oncol* 1991, **40**, 222–224.
27. Vermorken JB. The role of chemotherapy in squamous cell carcinoma of the uterine cervix: a review. *Int J Gynecol Cancer* 1993, **3**, 129–142.
28. Park RC, Thigpen JT. Chemotherapy in advanced and recurrent cervical cancer. *Cancer* 1993, **71**, 1446–1450.
29. Van der Burg ME, Lammes FB, van Putten WL, Stoter G. Ovarian cancer: the prognostic value of the serum halflife of CA 125 during induction chemotherapy. *Gynecol Oncol* 1988, **30**, 307–312.
30. Sevelda P, Schemper M, Spona J. CA 125 as an independent prognostic factor for survival in patients with epithelial ovarian cancer. *Am J Obstet Gynecol* 1989, **161**, 1213–1216.
31. Makar AP, Kristensen GB, Kaern J, Bormer OP, Abeler VM, Trope CT. Prognostic value of pre- and postoperative serum CA 125 levels in ovarian cancer: new aspect and multivariate analysis. *Obstet Gynecol* 1992, **79**, 1002–1010.
32. Duk JM, Aalders JG, Fleuren GJ, de Bruijn HW. Ca 125: a useful marker in endometrial carcinoma. *Am J Obstet Gynecol* 1986, **155**, 1097–1102.
33. Patsner B, Mann WJ, Cohen H, Losch M. Predictive value of preoperative serum CA 125 levels in clinically localized and advanced endometrial carcinoma. *Am J Obstet Gynecol* 1988, **158**, 399–402.
34. Benedetti Panici P, Scambia G, Baiocchi G, *et al.* Neoadjuvant chemotherapy and radical surgery in locally advanced cervical cancer. *Cancer* 1991, **67**, 372–379.
35. Benedetti-Panici P, Greggi S, Scambia G, *et al.* Locally advanced cervical adenocarcinoma: is there a place for chemosurgical treatment? *Cancer*, in press.

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