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Short Communication

Serum M3/M21 in Cervical Cancer Patients

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Cytokeratins are polypeptides which constitute a subclass of intermediate filaments in epithelial cells. The serum tumour marker M3/M21 is based on monoclonal antibodies against the epitopes M3 and M21 of cytokeratin 18. In the present study, we measured M3/M21 serum levels in 50 patients with FIGO stage IB–IIB cervical cancer and in 50 control subjects using a two-site radiometric immunoassay directed against soluble fragments of cytokeratin 18. Median serum levels of M3/M21 in patients with cervical cancer and in normal controls were 70.6 U/ml (range 0–397.7) and 6.5 U/ml (range 0–205.2), respectively (Mann–Whitney *U*-test, $P = 0.0001$). Median serum levels of M3/M21 prior to therapy and 4 weeks after therapy were 104.2 U/ml (range 24.6–397.7) and 39.3 U/ml (range 0–234.7), respectively (Mann–Whitney *U*-test, $P = 0.004$). We found a significant correlation between elevated M3/M21 serum levels and metastatic disease in pelvic lymph nodes (Mann–Whitney *U*-test, $P = 0.002$). 24 patients relapsed after complete remission. In these patients, elevated M3/M21 serum levels before the detection of relapse by computed tomography was observed in 13 cases. Considering these preliminary results, further studies with an increased number of patients are justified to clarify the prognostic value and the monitoring abilities of M3/M21 in cervical cancer patients.

Key words: tumour marker, M3/M21, cervical cancer

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INTRODUCTION

CYTOKERATINS ARE polypeptides which constitute a subclass of intermediate filaments in epithelial cells. Twenty cytokeratins divided into two types (acidic and neutral–basic) have been identified. These intracellular filaments are part of the cytoskeleton, maintaining the structural integrity of the cell body [1]. Tumours with a strong proliferative rate produce huge amounts of cytokeratins, mainly cytokeratins 8, 18 and 19. In contrast to cytokeratins themselves, cytokeratin fragments are soluble in body fluids and can be used as indicators of tumour activity [2, 3].

The serum tumour marker M3/M21 is based on monoclonal antibodies against the epitopes M3 and M21 of cytokeratin 18. This property supposedly leads to a high specificity in detecting fragments of cytokeratin 18. To the authors' knowledge, no data concerning M3/M21 and cervical cancer have been reported.

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The aim of the present study was to evaluate the clinical usefulness of M3/M21 in patients with cervical cancer, regarding the correlation with tumour burden and the possible prognostic and monitoring potentials.

PATIENTS AND METHODS

This retrospective study includes serological examinations of 50 patients suffering from squamous cell cervical cancer FIGO stages IB ($n = 28$), IIA ($n = 15$) and IIB ($n = 7$). Histologically, 19 tumours were graded as keratinising and 31 tumours as non-keratinising squamous cell carcinomas. The median age of the patients at the time of diagnosis was 57.6 years (range 26–81 years). All patients underwent radical hysterectomy and lymphadenectomy. Patients with histologically verified metastatic disease in pelvic lymph nodes underwent adjuvant irradiation therapy. All patients underwent a follow-up scheme consisting of regular visits in three-month intervals. The range of follow-up was 12–79 months. 24 patients developed recurrent disease after primary therapy with a median disease-free interval of 28 months. 21 patients died of the disease.

In all patients, M3/M21 serum levels were evaluated in serum samples taken prior to therapy and 4 weeks after therapy. In patients with recurrent disease, M3/M21 serum levels were also evaluated in samples taken 3 months before diagnosis of recurrent disease using computed tomography. Serum levels of M3/M21 were additionally evaluated in a panel of 50 healthy blood donors.

Serum assay

Blood samples were collected by peripheral vein puncture, allowed to clot, centrifuged and stored in four aliquots at −20°C. Serum concentrations of M3/M21 were measured using the M3/M21 IRMA (Beki Diagnostics AB, Bromma, Sweden), a two-site radiometric immunoassay with the configuration that both catching antibody (mouse monoclonal antibody M3) and detection antibody (mouse monoclonal antibody M21) are two separate and non-overlapping antibodies directed against a soluble fragment of cytokeratin 18. The intra-assay coefficient of correlation was 5.1% at a concentration of 35 U/l. All tests were run in duplicate according to manufacturer's instructions.

Statistics

Comparison between unpaired groups were made using the Mann–Whitney *U*-test. Survival probabilities were calculated by the product limit method of Kaplan and Meier. Univariate analysis was assessed using the log rank test. Results were analysed for the end points of disease-free and overall survival. The chi-squared test was used where appropriate. $P < 0.05$ was considered statistically significant. We used the SAS statistical software system (SAS Institute Inc., Carey, North Carolina, U.S.A.) to do the calculations.

RESULTS

Median serum levels of M3/M21 in patients with cervical cancer and in normal controls were 70.6 U/ml (range 0–397.7) and 6.5 U/ml (range 0–205.2), respectively (Mann–Whitney *U*-test, $P = 0.0001$). Median serum levels of M3/M21 prior to therapy and 4 weeks after therapy were 104.2 U/ml (range 24.6–397.7) and 39.3 U/ml (range 0–234.7), respectively (Mann–Whitney *U*-test, $P = 0.004$).

When serum levels of M3/M21, taken prior to therapy, were grouped by FIGO stage, pelvic lymph node involvement and grading of tumour cells, we found a statistically significant correlation with pelvic lymph node involvement. Patients with and without metastatic disease in pelvic lymph nodes had median M3/M21 serum levels of 81.2 U/ml (range 0–397.7) and 41.7 U/ml (range 17.5–116.7), respectively (Mann–Whitney *U*-test, $P = 0.002$). FIGO stage and grading of tumour cells were not correlated with M3/M21 levels.

Using the product limit method of Kaplan and Meier, we calculated the probability of pretreatment M3/M21 serum levels to predict the overall and disease-free survival. A cut-off value of 35 U/l was selected according to the 95th percentile of serum concentrations measured in 50 healthy controls. Elevated M3/M21 serum levels prior to therapy were associated with a poorer disease-free survival, but this trend was not statistically significant (log rank test, $P = 0.07$, Figure 1).

We calculated a Receiver–Operator–Characteristics (ROC) curve using serial cut-points ranging from 0 U/l to 400 U/l (Figure 2). M3/M21 reached a sensitivity of 56% and a specificity of 94% at a cut-off level of 35 U/l.

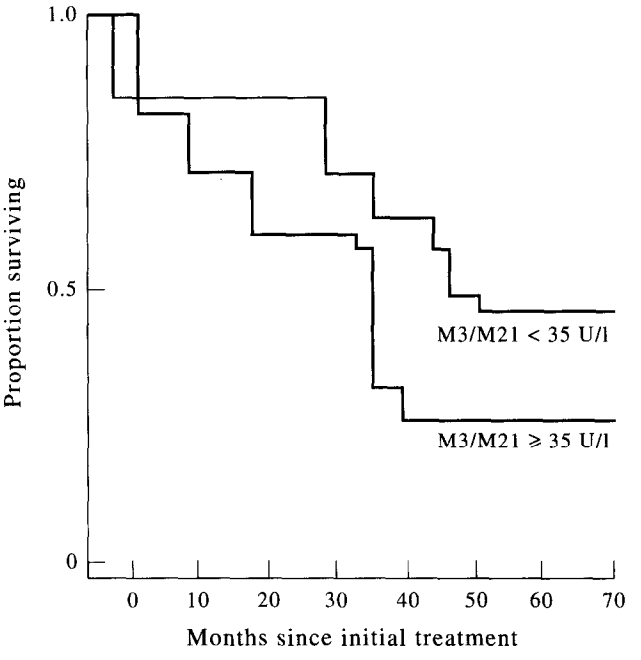

Figure 1. Kaplan–Meier analysis of overall survival of patients with M3/M21 serum levels above the cut-off level (35 U/l) compared to patients with M3/M21 serum levels below the cut-off level.

24 patients relapsed after complete remission. In these patients, elevated M3/M21 serum levels, before the detection of relapse by computed tomography, was observed in 13 cases. All these patients also had elevated M3/M21 serum levels prior to therapy.

DISCUSSION

In the present study, we measured M3/M21 serum levels in 50 patients with FIGO stage IB–IIB cervical cancer. To the authors' knowledge, this is the first report of M3/M21 in squamous cell cancers. We found significantly higher M3/M21 levels in tumour patients compared to control sub-

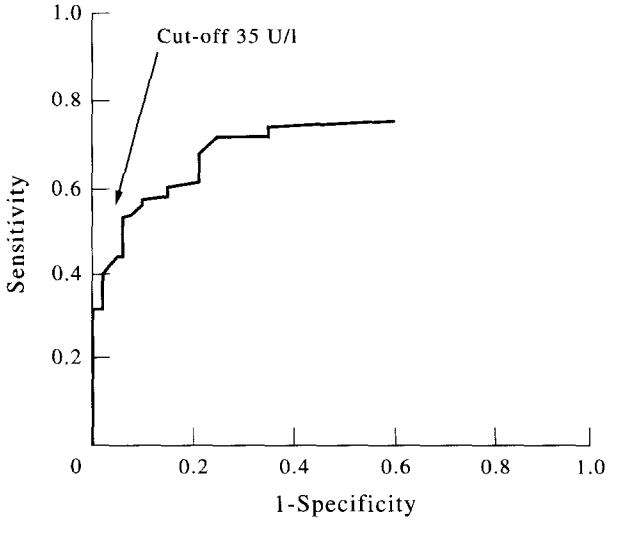

Figure 2. Receiver–Operator–Characteristics (ROC) curve for serum M3/M21, using serial cut points ranging from 0 to 400 U/l.

jects. M3/M21 serum levels decreased after surgical removal of the primary tumour. We found a significant correlation between pre-operative M3/M21 levels and metastatic disease in the pelvic lymph nodes. In 13 of 24 patients, M3/M21 was elevated before clinical detection of relapse. M3/M21 serum levels measured prior to therapy did not compromise overall survival.

SCC (squamous cell carcinoma) antigen is the most useful serum tumour marker in the follow-up of cervical cancer patients. It has been widely described as a reliable tool in assisting clinical diagnosis, especially in keratinising squamous cell carcinoma. SCC is associated with the extent of disease, is predictive of prognosis and is helpful in early detection of recurrent disease [4–6]. The ROC curve for M3/M21 shows that implementing a cut-off level of 35 U/l M3/M21 reached a specificity of 94.5% and a sensitivity of 56.3%. M3/M21 was elevated before clinical detection of relapse in 13 of 24 cases. Considering these preliminary data the value of M3/M21 as a "classical tumour marker", i.e. to evaluate the tumour burden, seems promising.

However, elevated serum levels of M3/M21 have to be interpreted with care since it is known from other cytokeratin markers that benign disease, e.g. diabetes mellitus or benign liver diseases, may be a confounding factor. This should be taken into account when interpreting elevated M3/M21 serum levels.

In our patient sample, survival probabilities were not influenced by M3/M21 serum levels. Although higher serum levels of M3/M21 were found in patients with metastatic disease in pelvic lymph nodes, elevated M3/M21 levels prior to therapy could not predict the patient's outcome. Multivariate analysis involving a bigger series of patients has to be performed to determine definitely the prognostic value of M3/M21.

In summary, our data indicate that, in cervical cancer patients, serum levels of M3/M21 are elevated in pre-operative serum samples, are significantly associated with the presence of tumour, show good sensitivity/specificity characteristics and display lead time effects in the follow-up of cervical cancer patients. Pre-operative serum levels of M3/M21 showed no prognostic impact in our patient collective. However, the sample size of our study may be too small to provide a definitive statement about the usefulness of M3/M21 in the treatment of cervical cancer patients. Considering these preliminary results, further studies with an increased number of patients are justified to clarify the prognostic value and the monitoring abilities of M3/M21 in cervical cancer patients.

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