Prognostic Significance of the Angiogenic Factors Angiogenin, Endoglin and Endostatin in Cervical Cancer

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Abstract. *Background/Aim: Angiogenesis plays a key role in tumour growth and metastasis. Expression of angiogenic factors has been suggested as a marker for tumour malignity, and it may help to identify those patients with a poorer prognosis, aiding patient stratification for more aggressive and/or angiogenesis-targeted therapy. The present study examines the relationship between concentration of circulating angiogenic factors and clinical tumour criteria as well as patient survival. Patients and Methods: A total of 125 patients with cervical cancer who underwent follow-up examinations between October 2002 and June 2005 were enrolled, and serum samples were examined for angiogenin, endoglin and endostatin by means of an ELISA. Concentrations were statistically correlated with clinical and outcome parameters. Results: Concentrations of all examined angiogenic factors were on average within the manufacturer-provided normal range. Both angiogenin and endostatin increased from non-invasive tumours through invasive lesions to recurrent disease, and endoglin showed an equally steady inverse trend; differences between non-invasive, invasive and recurrent stages of the disease were statistically significant. However it was not possible to determine a sufficiently selective cut-off point for either factor by receiver operating characteristic analysis, and there was no significant correlation with survival. Conclusion: Angiogenic factors angiogenin, endoglin and endostatin show a definite relationship with disease stage in uterine cervical cancer, but are presently not suitable for use in risk stratification.*

Cervical cancer is one of the most frequent malignant diseases in women worldwide and causes substantial mortality and health care expenditure (1-4). The prognosis of the disease is generally favourable in regions or social strata where PAP smear screening and comprehensive modern therapy are available(4); however, there is a subgroup of patients with comparatively poor prognosis that is currently only defined based on empirical rather than biological data (5).

On the other hand, there has been an intense development of novel targeted treatment modalities for malignant diseases (6-8), mostly aiming at the angiogenesis that is crucially involved in tumour growth, invasion, and metastasis (6, 7, 9). Based on this research, the monoclonal antibody bevacizumab has evolved as an established therapy modality for several types of cancer including cervical cancer (7, 10, 14). The undeniable benefit of this treatment notwithstanding, the response to novel targeted treatment modalities appears to be less predictable than in conventional chemotherapy (15, 16), and aggressive combinatorial treatment approaches are associated with an increased risk of toxicity (17, 18). The resulting caution concerning the application of angiogenesis-targeted treatment in unselected populations is reinforced by the comparatively high cost of targeted modalities at present (19).

Since angiogenesis is the target for most of the novel agents currently under investigation, it is also a plausible goal for individualized prognosis assessment and prediction of treatment response. The actual process of angiogenesis on tissue and cellular level can be investigated, but with rather complex methods of histomorphometrical microvessel density (MVD) assessment (9, 20, 21) or biological assays such as tube formation (22). More convenient, however, would be a method based on simple serum concentration analysis, and

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indeed a rather convincing correlation of serum angiogenic factor concentrations and biological and/or clinical tumour features has been shown in several studies (9, 23-27).

While vascular endothelial growth factor (VEGF) plays a prominent role – probably due to the fact that bevacizumab is a monoclonal VEGF antibody –, there are several more factors under investigation, *e.g.* endoglin (28, 29). Endoglin (CD105) is a transforming growth factor (TGF)-β1 receptor that is highly expressed on endothelial cells during tumour angiogenesis and inflammation but sparsely occurring in normal vascular endothelium (30), making it a plausible candidate for a prognostic marker. Whereas CD105-positive MVD has been related to prognosis in cervical cancer (28) and other malignancies (31, 32), conflicting evidence has also been published (9). A prognostic significance of circulating concentrations has been suggested for endoglin (33, 34), angiogenin (35, 36) and endostatin (36-39), but not proven conclusively so far.

In the present paper, the utility of circulating endoglin, angiogenin and endostatin concentrations as a prognostic marker in cervical cancer is assessed. Marker concentrations are compared between pre-invasive, invasive and recurrent disease; meaningful cut-off points for patient stratification are sought and a possible correlation between serum concentrations and patient survival is examined.

Patients and Methods

Patients. The study participants represent a sample of patients from the ongoing cervical cancer monitoring database of the Charité, University Hospital Berlin, Germany. Data acquisition, storage and processing in this database required written informed consent hence no specific ethical requirements were considered for the present investigation. Patients who underwent diagnostic or follow-up examinations for cervical uterine neoplasms between October 2002 and June 2005 were enrolled into the study. A total of 125 patients were included, and their serum samples were obtained prior to therapy and stored at –80˚C immediately after collection.

Data acquisition. Information obtained from the database included tumour stage, histology, presence of nodal metastases, lymphatic and venous vessel invasion, as well as patient age, menopausal and smoking status. The sample characteristics regarding the aforementioned criteria are shown in Table I.

The serum concentrations of angiogenin, endoglin and endostatin were determined by ELISA (R&D Systems, Minneapolis, MN, USA) as part of the clinical routine, and the respective values were obtained from the database.

All previously collected serum samples were employed for an endothelial-cell proliferation assay in November/December, 2005.

Statistical data evaluation. Statistical data evaluation was performed with the SPSS™ 15.0 software package (SPSS™ Inc., Chicago, IL). Non-parametric methods were employed for inference testing, and statistical significance was considered when $p<0.05$.

Group differences were analysed with the Mann-Whitney *U*-test (metric variables, 2 groups), Kruskal-Wallis test (metric variables, <2 groups) and χ^2 test (discrete variables), and the Bonferroni-Dunn post hoc test was employed to identify significant pair differences. Differences in survival time were analysed with the Kaplan-Meier method and multivariate Cox regression, and sensitivity and specificity of different cut-off points for angiogenesis were determined with the receiver operating characteristic (ROC) method.

Table I. *Baseline characteristics of patients enrolled in this study.*

Criterion	Number	Percentage
Tumour stage		
Non-invasive	50	40.0
CIN I	7	5.6
CIN II	8	6.4
CIN III	35	28.0
Invasive	51	40.8
FIGO I	22	17.6
FIGO II	13	10.4
FIGO III	13	10.4
FIGO IV	3	2.4
Recurrent disease	24	19.2
Tumour histology (only invasive tumours)		
Squamous cell carcinoma	58	76.3
Adenocarcinoma	8	10.7
Adenosquamous carcinoma	5	6.7
Not classifiable	4	5.3
Prognostic criteria		
Nodal metastasis	29	23.2
Lymph vessel invasion	20	16.0
Blood vessel invasion	8	6.4
Grading		
G 1	2	1.6
G 2	38	30.4
G 3	31	24.8
None available	54	43.2
Age, years (average)	42.9±13.5	
Menopausal status		
Pre-menopausal	88	70.4
Menopausal	2	1.6
Post-menopausal	35	28.0
Smoking status		
Smoker	45	36.0
Non-smoker	47	37.6
Not available	33	26.4

Results

Overview. On average, all three tested markers were within the normal range provided by the manufacturer of the assay (Table II).

Correlation of angiogenic factors and clinical criteria. There was a marked and significant difference in all three markers depending on the tumour stage: Both angiogenin and endostatin increased from non-invasive tumours through invasive lesions to recurrent disease, and endoglin showed an equally steady inverse trend (Table II). Moreover, both endostatin and

Table II. *Angiogenic factors (mean±std. dev.) and their correlation with clinical criteria.*

Group	Angiogenin (ng/ml)	Endoglin (ng/ml)	Endostatin (ng/ml)
Entire sample	326.5±101.6	4.18±1.11	120.7±47.5
Normal (mean/range)	360/196-437	3.96/2.54-7.06	122/58-232
Tumour stage	**	**	**
Non-invasive	276.7±69.6	4.52±1.02	102.8±17.3
Invasive	336.1±101.7	4.21±1.15	115.8±51.1
Recurrent disease	410.1±100.0	3.35±0.78	168.2±52.2
Invasive	**	n.s.	n.s.
FIGO I	292.1±163.0	4.48±0.56	102.2±118.8
FIGO II	330.2±192.2	3.89±0.93	108.3±125.4
FIGO III	379.9±110.9	4.31±1.87	116.5±138.8
FIGO IV	494.3±142.6	3.32±0.94	245.0±145.8
Tumour histology	*	n.s.	n.s.
Squamous cell ca.	367.6±112.6	3.95±1.19	135.9±62.5
Adenocarcinoma	277.1±33.9	3.90±0.87	110.5±24.1
Adenosquamous ca.	413.8±46.5	3.41±0.56	132.8±17.2
Prognostic criteria†			
Nodal metastasis	369.6±113.2**↑	3.95±0.98n.s.↓	138.9±53.6*↑
Lymph vessel invasion	330.7±93.6n.s.↑	3.97±1.07n.s.→	110.5±32.1n.s.↓
Blood vessel invasion	305.4±71.9n.s.↓	3.87±0.77n.s.↓	107.5±29.1n.s.↓
Grading	n.s.	n.s.	*
G 1	317.0±110.3	4.44±0.95	87.2±5.7
G 2	360.8±110.6	3.93±1.32	122.9±45.1
G 3	348.1±196.7	3.86±0.87	135.8±50.7
Menopausal status	*	n.s.	*
Pre-menopausal	312.7±34.8	4.24±1.01	117.5±19.2
Post-menopausal	365.6±91.8	4.06±1.30	130.9±34.2
Smoking status	n.s.	n.s.	n.s.
Smoker	302.5±104.1	4.31±1.22	112.1±28.9
Non-smoker	340.3±114.7	3.83±0.80	128.1±44.2

n.s. Not significant, ca. carcinoma, *$p<0.05$, **$p<0.001$, ***$p<0.0001$. †Significance and direction of difference indicated for positive *vs*. negative.

angiogenin increased within the group of invasive tumours with FIGO stage, the latter with statistical significance ($p<0.001$).

Only angiogenin showed a significant dependency on tumour histology, but this was not very plausible since values for squamous cell carcinoma fell between those for adenocarcinoma and adenosquamous carcinoma (Table II).

Patients with nodal metastases had higher concentrations of endostatin and angiogenin and lower concentrations of endoglin, whereas vessel invasion was not appreciably correlated. Grading and menopausal status were loosely correlated with expression of angiogenic factors, but displayed similar tendencies, with the exception of endoglin, which was inversely correlated with menopausal status.

All in all, higher concentrations of endostatin and angiogenin and lower concentrations of endoglin reflected increased tumour malignancy.

ROC analysis of sensitivity and specificity. Regardless of the chosen cut-off points, sensitivity and specificity of the angiogenesis factors for stage discrimination did not exceed ~70% at best, thus being insufficient for clinical application. The respective most accurate concentrations were: Angiogenin: 300 ng/ml with a sensitivity of 69.3%, specificity of 72.0%, positive predictive value of 78.8% and negative predictive value of 61.0%; Endoglin: 4.0 ng/ml with a sensitivity of 53.3%, specificity of 70.0%, positive predictive value of 72.7% and negative predictive value of 50.0%; Endostatin: 120 ng/ml with a sensitivity of 54.7%, specificity of 82.0%, positive predictive value of 82.0% and negative predictive value of 54.7%.

Whereas the combination of 2-3 markers over the aforementioned thresholds nearly ruled out CIN stages (*i.e.* had a high specificity of 92.0% for the detection of invasive stages), the sensitivity remained low, *i.e.* a substantial percentage of patients with advanced stage disease had one or no elevated markers.

Correlation of angiogenesis factors and survival. A subgroup of 67 patients (53.6% of the original sample) was included in a follow-up questionnaire. Thirty (44.8%) of these had died in the meantime, and the median survival time of the examined subgroup was 51.8 months (95% confidence interval, 29.2-74.4 months).

The established prognostic factors (*i.e.* invasive tumour stage, lymph node and distant metastasis) were verified in the sample group.

The sample group with information about survival was dichotomized according to the mean concentration of the respective factors in order to create subgroups of approximately identical size. The only factor for which this yielded an appreciable difference in survival times was endoglin: Both the percentage of women who had died in the subgroups (≥3.94 ng/ml 41.7%, <3.94 ng/ml 48.2%) and the median survival time [≥3.94 ng/ml 79.1 (95% CI 40.6-117.7) months, <3.94 ng/ml 39.9 (95% CI 19.5-60.3) months] indicated some advantage for women with higher serum endoglin concentrations. However, the difference failed to be statistically significant.

Discussion

There is a growing body of evidence for the prognostic significance of circulating angiogenic factors in uterine cervical cancer (7, 22, 23, 40), and the present study basically corroborates this. However, the clinical utility of circulating angiogenic factor testing for survival prediction, risk stratification and treatment decision making appears to be limited according to the present study. Without any reasonable doubt, circulating angiogenesis factors are elevated in patients with malignancies, and their concentrations are correlated to clinical criteria of disease severity; the literature provides sufficient evidence for this. As for the clinical utility of serum concentration measurement, it may serve one or both of two purposes: Supporting the treating oncologist in an individual assessment of a particular patient's prognosis and/or helping with the selection of patients for novel treatment modalities according to their likely response.

Whereas the basic correlation of circulating angiogenin and endoglin concentrations with clinical tumour stage were confirmed in the present study, the results fall well short of suggesting an early fulfilment of either of the aforementioned goals. The ROC analysis failed to result in a meaningful cut-off point with a reasonable degree of sensitivity and specificity for the prediction of advance disease, and there was no appreciable relationship between the markers' concentrations and patient survival.

Clinical studies correlating circulating endoglin concentrations with survival are relatively sparse, but the reported results for cervical (9) and gastric cancer (41) are equally negative.

Other results of our group obtained in the same patient sample suggest that the lack of meaningful correlations with clinical prognosis is not exclusive to the molecules under investigation in the present paper, but applies to circulating angiogenic factors in general (23, 40).

The overall loose and inconclusive – albeit in principle undeniable – relationship between circulating angiogenic factors and prognosis/treatment response is hardly surprising, considering the current biological understanding of the processes involved. Pre-treatment concentrations of angiogenic factors are only loosely related to those under conventional chemotherapy, which mobilises endothelial precursor cells (EPC) and their progenitors; this in turn may lead to tumour neovascularisation (42), and the rationale for early antiangiogenic therapy in combination with chemotherapy is this very process, as well as the angiogenesis involved in the primary tumour growth; only the latter is present previous to therapy and hence accessible via pre-treatment examinations.

Therefore, biological modelling of the actual process of tumour angiogenesis may be a more meaningful approach to prognosis assessment and patient stratification, and indeed our group has obtained promising results employing a vascular tube formation assay (22).

In conclusion, we consider the potential of circulating angiogenic factor determination for the assessment of prognosis and targeted treatment response in cervical cancer to be doubtful.

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