Pergamon

European Journal of Cancer Vol. 33, No. 11, pp. 1799–1804, 1997

Printed in Great Britain
0959-8049/97 $17.00 + 0.00

PII: S0959-8049(97)00161-5

## Original Paper

# The Impact of Tumour Angiogenesis, p53 Overexpression and Proliferative Activity (MIB-1) on Survival in Squamous Cervical Carcinoma

E.H. Åvall-Lundqvist,[1] C. Silfverswärd,[2] U. Aspenblad,[2] B.R. Nilsson[3] and G.U. Auer[2]

[1]Department of Gynecologic Oncology; [2]Department of Tumour Pathology; and [3]Department of Cancer Epidemiology and Biostatistics, Karolinska Institute and Hospital, S-171 76 Stockholm, Sweden

**Tumour angiogenesis (antifactor VIII-related antigen antibody), p53 overexpression (DO-1) and proliferative activity (MIB-1) were immunohistochemically analysed for the prediction of long-term survival in 113 patients with squamous cervical carcinoma. The median follow-up time was 82 months (range 72–99). In early stages (IB–IIA), neovascularisation was significantly related to tumour size. Significantly more patients in stage IIA had high tumour vascularity compared to stage IB ($P < 0.01$) but no significant difference was found between early and advanced stages (IIB–IVB) of cervical carcinoma. p53 overexpression was correlated to the stage of disease ($P < 0.01$). No relationship was found between tumour angiogenesis, p53 overexpression or MIB-1 and pelvic lymph node metastases, histological subtype or differentiation. Tumours with more than 50% p53 overexpression was significantly correlated with survival in the univariate analysis, but no independent predictive value was found. It is concluded that immunohistochemically detectable p53 overexpression as measured by DO-1 and proliferative activity as measured by MIB-1 seems of no clinical value for the prediction of long-term survival in squamous cervical carcinoma. The predictive value of tumour angiogenesis for survival outcome has still to be determined in squamous cervical carcinoma.** © **1997 Elsevier Science Ltd.**

**Key words: cervical carcinoma, immunohistochemistry, tumour angiogenesis, MIB-1, p53**
*Eur J Cancer,* Vol. 33, No. 11, pp. 1799–1804, 1997

## INTRODUCTION

IN SWEDEN, cervical carcinoma constitutes 2–3% of newly diagnosed cancers in women and most of the carcinomas are in early stages (IB–IIA). The median age at diagnosis is approximately 50 years [1]. Survival rates for early stages are generally high but treatment may be at the expense of ovarian function and quality of life. Among the 10–20% who relapse, the prognosis is poor. Stage of disease, lymph node metastasis and tumour size are considered to be the most reliable prognostic factors [2]. However, disparate prognosis has been observed in patients with tumours of the same size and stage. Furthermore, information about lymph node status is generally not available at the time for treatment planning.

Correspondence to E.H. Åvall-Lundqvist.
Received 5 Sep. 1996; revised 26 Feb. 1997; accepted 5 Mar. 1997.

There is therefore a need to identify novel markers which more accurately reflect growth rate, progression and metastatic potential for each tumour, facilitating the identification of patients at low risk who could be offered a more conservative therapy and patients at high risk who could be offered an even more intensive treatment.

Tumour microvessels can be highlighted by antifactor VIII immunohistochemical staining in paraffin sections and counted in order to estimate tumour angiogenesis. A correlation between tumour angiogenesis and survival has been demonstrated in several human malignancies [3–5]. The p53 tumour suppressor gene is hitherto the most frequently altered gene in a wide variety of human malignancies [6]. Loss of normal p53 function, either by mutation or inactivation of normal (wild-type) p53, results in entry of cells with damaged DNA into the cell cycle, instead of arresting at the G1 checkpoint, presumably for DNA repair or for apoptosis [7]. Mutant p53 overexpression is of prog-

nostic significance in several human cancers e.g. breast cancer [8].

The Ki-67 proliferation index has also been shown to be of prognostic significance in human cancers e.g. squamous cell carcinoma of the oesophagus [9]. The Ki-67 antigen is expressed in the nuclei of proliferating cells during the cell cycle except during the early G1 and G0 phases. The MIB-1 monoclonal antibody (MAb) has proved to be equivalent to the Ki-67 antibody with the advantage that it is easily applicable to archival material [10].

Recently, we reported an association between serum concentrations of squamous cell carcinoma antigen (SCC) and CA 125 and poor survival in cervical carcinoma, particularly of the squamous cell type [11]. We have now performed immunohistochemical staining for factor VIII-related antigen (M616), p53 overexpression (DO-1) and Ki-67 antigen (MIB-1) in patients with primary squamous cervical carcinoma to assess their predictive value for long-term survival.

## PATIENTS AND METHODS

Tumour specimens were obtained from 117 patients with primary squamous cervical carcinoma, who were referred to the Department of Gynaecological Oncology, Radiumhemmet between February 1986 and March 1989. The patients were previously included in a larger study ($n = 142$) exploring the relationship between serum concentrations of SCC, CA 125, carcinoembryonic antigen (CEA) and tissue polypeptide antigen (TPA) and survival in patients with cervical carcinoma of different histological types. All patients entered the study prospectively and consecutively with periods of breaks [11].

All histological samples were reviewed and evaluated by the same pathologist. Four patients were excluded due to insufficient amount of specimens. The 113 squamous cervical carcinomas were divided according to the Wentz–Reagan classification [12] into large cell keratinising ($n = 22$), large cell non-keratinising ($n = 77$) and squamous cell carcinomas of the small cell type ($n = 11$) (3 unknown). Histological grading revealed 5 well-differentiated, 65 moderately and 43 poorly differentiated carcinomas [13]. Lymph-vascular invasion [14] was found in 14 of 111 evaluable specimens. The median age of the patients was 53 years (range 22–90 years).

Staging of the tumours was done according to the FIGO classification [1] i.e. stage IA ($n = 2$), stage IB ($n = 47$), stage IIA ($n = 24$), stage IIB ($n = 11$), stage IIIA ($n = 5$), stage IIIB ($n = 17$), stage IVA ($n = 4$), stage IVB ($n = 3$). In early stages, a bimanual examination permitted estimation of the tumour size into three categories, i.e. no palpable tumour (group A), enlargement of the cervix (group B) and greatly enlarged cervix (group C). Both patients in stage Ia underwent primary surgery while the patients in stages Ib–IIa received combination treatment with pre-operative intracavitary irradiation followed by radical hysterectomy with pelvic lymphadenectomy. Eight of 51 patients who received surgery for stages IB–IIA had pelvic lymph node metastases. In case of contra-indication for surgery or more advanced stage of disease, the patients received radiotherapy only.

The median observation time for the patients still alive ($n = 63$) at the closing date of the study (March 1995) was 82 (range 72–99) months. All of these women were relapse-free at the closing date of the study. The median observation time for the women who died of their cancer was 19 (range 2–65) months. Forty-one patients died of squamous cervical carcinoma and 8 died of intercurrent disease. In one woman it was histologically unsolved whether her lung tumour was primary or a recurrence of the patient's squamous cervical carcinoma. Two patients were lost to follow-up.

*Immunohistochemistry*

For immunohistochemical staining, an avidin–peroxidase complex technique was used. All tissue sections were 4 μm thick and prepared from formalin-fixed, paraffin-embedded specimens, dewaxed and rehydrated for further procedure.

*Factor VIII-related antigen*

For the detection of factor VIII-related antigen, the MAb M616 (Dako A/S, Glostrup, Denmark) was used. The dewaxed and rehydrated sections were placed on glass slides pretreated with 3-aminopropyltriethoxysilane (APES). The slides were put in 0.05% pronas at 20°C for 15 min. Endogenous peroxidase activity was blocked with 0.5% hydrogen peroxide in distilled water for 20 min. Non-specific staining was blocked with 1% bovine serum albumin for 30 min. The sections were incubated with the primary MAb M616 at a dilution of 1:50 at 4°C overnight. After rinsing in tris-phosphate-buffered saline (TPBS), the biotinylated secondary antibody (antimouse IgG, Vector Laboratories, Burlingham, California, U.S.A.) was applied at a dilution of 1:200, followed by incubation for 30 min with Vectastain Elite (Vector Laboratories). Diaminobenzidine was used as chromogen. The slides were counterstained with Mayer's haematoxylin and thereafter dehydrated and mounted. By scanning the tumour sections at low power (magnification 40×), the area of highest neovascularisation was found and the immunostained microvessels were counted (magnification 200×). Any brown-staining endothelial cell or endothelial cell cluster was considered a single countable microvessel. The results were recored as 0–33, 33–67, 68–100, >100 counts per field. This procedure was chosen in accordance with an evaluation procedure described previously [3]. The tumours stained heterogenously within the same tumour and the quality of staining

*Table 1. Independent variables in the Cox regression analysis*

| Variables | Comparison |
|---|---|
| Stage 1 | stage I versus stages II–IV |
| Stage 2 | stages I–II versus stages III–IV |
| Stage 3 | stages I–III versus stage IV |
| Grade 1 | well versus moderate/poor |
| Grade 2 | well/moderate versus poor |
| p53 1 | –versus 1 + /2 + /3+ |
| p53 2 | –/1+ versus 2 + /3+ |
| p53 3 | –/1 + /2+ versus 3+ |
| LV | invasion versus no invasion |
| SCC (ng/ml) | * |
| CEA (ng/ml) | * |
| CA 125 (U/ml) | * |
| TPA (U/l) | * |
| Microvessel counts | * |
| MIB-1 (%) | * |

Grade, grade of differentiation; LV, lymph-vascular invasion. *Comparison was made between every 10-unit increment of the respective variable.

*Table 2. Microvessel counts (F8), p53 overexpression and MIB-1 in relation to stage, grade, lymph vascular invasion (LV) and the Wentz–Reagan classification i.e. large cell keratinising (L-K), large cell non-keratinising (L-NK) and squamous cervical cell carcinoma of the small cell type (S)*

| | No. of patients with FIGO stage | | | No. of patients with grade of differentiation | | | | | No. of patients with LV | | | Wentz–Reagan classification | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I–II *n* (%) | III–IV *n* (%) | *P* | Well *n* (%) | Moderate *n* (%) | Poor *n* (%) | *P* het. | trend | No *n* (%) | Yes *n* (%) | *P* | L-K *n* (%) | L-NK *n* (%) | S *n* (%) | *P* het. | trend |
| F8 (*n* = 113) | | | | | | | | | | | | | | | | |
| <68 | 64 (76) | 23 (79) | ns | 5 (100) | 52 (80) | 30 (70) | ns | ns | 76 (78) | 9 (64) | ns | 17 (77) | 60 (78) | 8 (73) | ns | ns |
| ≥68 | 20 (24) | 6 (21) | | 0 | 13 (20) | 13 (30) | | | 21 (22) | 5 (36) | | 5 (23) | 17 (22) | 3 (27) | | |
| p53 (*n* = 112) | | | | | | | | | | | | | | | | |
| −/1+ | 60 (72) | 12 (41) | † | 1 (20) | 46 (72) | 25 (58) | * | ns | 60 (63) | 11 (79) | ns | 10 (46) | 54 (70) | 5 (50) | ns | ns |
| 2 + /3+ | 23 (28) | 17 (59) | | 4 (80) | 18 (28) | 18 (42) | | | 36 (37) | 3 (21) | | 12 (54) | 23 (30) | 5 (50) | | |
| MIB1 (*n* = 109) | | | | | | | | | | | | | | | | |
| <50% | 17 (21) | 7 (26) | ns | 1 (20) | 9 (15) | 14 (33) | ns | ns | 22 (23) | 1 (8) | ns | 5 (25) | 15 (20) | 4 (36) | ns | ns |
| ≥50% | 65 (79) | 20 (74) | | 4 (80) | 52 (85) | 29 (67) | | | 72 (77) | 12 (92) | | 15 (75) | 60 (80) | 7 (64) | | |

ns, not significant; *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$. het, heterogeneity.

*Table 3. Microvessel counts (F8), p53 overexpression and MIB-1 in relation to stage, tumour volume and pelvic lymph node metastasis (PLN) in stages IB–IIA squamous cervical carcinoma*

| | No. of patients with FIGO stage | | | No. of patients with tumour volume | | | | | No. of patients with PLN | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | IB *n* (%) | IIA *n* (%) | *P* | A *n* (%) | B *n* (%) | C *n* (%) | *P* het. | trend | Negative *n* (%) | Positive *n* (%) | *P* |
| F8 | | | | | | | | | | | |
| <68 | 41 (87) | 13 (54) | † | 20 (95) | 26 (72) | 8 (62) | * | * | 30 (70) | 7 (88) | ns |
| ≥68 | 6 (13) | 11 (46) | | 1 (5) | 10 (28) | 5 (38) | | | 13 (30) | 1 (12) | |
| p53 | | | | | | | | | | | |
| −/1+ | 33 (70) | 18§ (78) | ns | 15 (71) | 25 (69) | 10§ (83) | * | ns | 31§ (74) | 8 (100) | ns |
| 2+/3+ | 14 (30) | 5§ (22) | | 6 (29) | 11 (31) | 2§ (17) | | | 11§ (26) | 0 | |
| MIB1 | | | | | | | | | | | |
| <50% | 7¶ (16) | 7 (29) | ns | 3¶ (16) | 10 (28) | 1 (8) | ns | ns | 6§ (14) | 1 (12) | ns |
| ≥50% | 38¶ (84) | 17 (71) | | 16¶ (84) | 26 (27) | 12 (92) | | | 36§ (86) | 7 (88) | |

ns, not significant; *$P < 0.05$; †$P < 0.01$. het, heterogeneity. A, no palpable tumour; B, enlargement of the cervix; C, greatly enlarged cervix. §Data unavailable for 1 patient; ¶data unavailable for 2 patients.

varied between tumours. Those judged to be of poor quality were reassessed.

*p53*

The murine MAb DO-1 (Santa Cruz Biotechnology, California, U.S.A.) was used for the immunohistochemical staining of p53. The procedure was the same as that described for factor VIII-related antigen except that the pronas procedure was omitted and the slides were boiled in the microwave oven for 5 min at 800 W and for 5 min at 450 W, in a plastic jar filled with citrate buffer at pH 6.0. They were subsequently cooled for 20 min and rinsed with TPBS. The boiling step preceded the blocking of endogenous peroxidase activity. The dilution of the primary antibody was 1:100. Specimens were recorded as immunoreactive when a distinct staining of the nucleus was observed. A semiquantitative assessment of the staining was performed by estimating the distribution pattern of stained cell nuclei. The staining results were classified on this basis as absent ( ), sporadic >0–10% (1+), sparse/uniform >10–50% (2+) or dense/uniform >50% (3+), as described previously [15].

*Ki-67*

For the immunohistochemical detection of Ki-67, the IgG MAb MIB-1 (Immunotech S.A., Marseille, France) was used. The staining procedure was the same as described for DO-1 except that the boiling time was for $2 \times 5$ min and the dilution of the primary antibody was 1:150. Distinct nuclear MIB-1 staining was recorded as positive. In each preparation, two high-power fields were selected within a tumour-containing area of the section. The number of immunoreactive cells were assessed by using a light microscope equipped with an ocular graticule consisting of $10 \times 10 = 100$ fields. Immunoreactive and non-immunoreactive cells were counted separately within $5 \times 5$ fields of the grid. The percentage of immunoreactive tumour cells in relation to the whole tumour population was calculated.

Human breast cancer cells were used as positive external staining controls. Morphologically normal cells in each specimen served as internal negative controls.

*Serum immunoassays*

As previously described [11], SCC concentrations in serum were measured by a microparticle capture enzyme immunoassay which employs MAbs (Abbott Laboratories, Chicago, Illinois, U.S.A.). CEA levels were determined by means of radioimmunoassay kits using polyclonal antibodies (Diagnostic Productions Corporation, Oxfordshire, U.K.). CA 125 and TPA were determined by means of immunoradiometric assay kits from Byk-Sangtec Diagnostika (Dietzenbach, Germany) and Sangtec Medical (Bromma, Sweden), respectively.

*Statistics*

Comparison between groups were performed by the chi-square exact test (for heterogeneity and/or trend in case of three groups) [16]. All *P*-values are the results of two-sided tests. Survival was calculated from the date of diagnosis to the date of last follow-up or death. Univariate analysis was used to study the impact of potential risk factors on survival. All variables significant at the 5% level were thereafter included in a multivariate analysis based on a forward stepwise procedure [17]. The 8 patients who died of intercurrent disease were considered as censored observations. The independent variables are presented in Table 1.

## RESULTS

The median number of intratumoral microvessels was 44 (range 3–144). The distributions of the microvessel counts were as follows; 34 squamous carcinoma had 0–33 counts per field, 53 had 34–67, 13 had 68–100 and 13 carcinomas had >100 microvessels per 200× field. Overexpression of the p53 protein was observed in 73 of 112 (65%) primary tumours i.e. 33 had sporadic, 21 had sparse/uniform and 19 carcinomas had dense/uniform immunoreactivity and 39 were not immunoreactive. The mean percentage of MIB-1 immunoreactive tumour cells was 67% (range 21–99%) in 109 evaluable specimens.

The results of the microvessel counts, p53 overexpression and MIB-1 were dichotomised for further analyses. The results are summarised in Tables 2 and 3. Although no difference in microvessel counts was found between early and more advanced stage of disease (Table 2), a difference was observed between stage IB and stage IIA (Table 3). In early stages neovascularisation was significantly related to tumour size.

A relation between clinical stage and degree of p53 immunoreactivity was observed. Hence uniform immuno-

*Table 4. Cox univariate regression analysis showing predictors of survival in squamous cervical carcinoma*

| Variables* | RH | 95% CI | $X^2$ | *P* value |
|---|---|---|---|---|
| Stage 1 | 10.5 | 3.75–29.7 | 30.7 | <0.001 |
| Stage 2 | 9.70 | 5.09–18.5 | 69.2 | <0.001 |
| Stage 3 | 9.49 | 4.14–21.7 | 32.0 | <0.001 |
| Grade 1 | 0.33 | 0.10–1.06 | 3.82 | 0.051 |
| Grade 2 | 1.09 | 0.58–2.04 | 0.07 | 0.788 |
| p53 1 | 1.36 | 0.69–2.66 | 0.82 | 0.370 |
| p53 2 | 1.70 | 0.92–3.15 | 2.88 | 0.090 |
| p53 3 | 3.12 | 1.57–6.18 | 11.8 | <0.001 |
| LV | 0.73 | 0.32–1.68 | 11.8 | <0.001 |
| SCC (ng/ml) | 1.26 | 1.17–1.36 | 82.4 | <0.001 |
| CEA (ng/ml) | 1.22 | 0.99–1.51 | 3.76 | 0.052 |
| CA 125 (U/ml) | 1.08 | 1.04–1.11 | 28.5 | <0.001 |
| TPA (U/l) | 1.06 | 1.04–1.08 | 50.0 | <0.001 |
| Microvessel counts | 1.04 | 0.95–1.13 | 0.61 | 0.436 |
| MIB-1 | 0.98 | 0.84–1.14 | 0.10 | 0.751 |

*See Table 1 for explanation of the independent variables. RH, relative hazards; CI, confidence interval; $X^2$, chi-square values; LV, lymph-vascular invasion.

reactivity of p53 overexpression was associated with more advanced stage of squamous cervical carcinoma as compared with sporadic or absent immunoreactivity (Table 2).

No significant association could be found between microvessel counts, p53 overexpression or MIB-1 and grade of differentiation, lymph-vascular invasion, histological type (Wentz–Reagan classification) or pelvic lymph node metastases.

p53 was one of eight potential risk factors significant for predicting survival in the univariate analysis (Table 4). In the following multivariate analysis, however, only SCC and CA 125, apart from stage, had an additional prognostic value (Table 5).

## DISCUSSION

In this study we investigated primary squamous cervical carcinoma from 113 patients followed for at least 72 months. An association between tumour angiogenesis and stage of disease was only found in early stages. Hence, high tumour vascularity was significantly more frequent in stage IIA compared to stage IB. It is possible that neovascularisation is promoted by the less dense tissue in the vagina compared to the cervix. We also found a relationship between neovascularisation and tumour size in stages IB and IIA. Tumour vascularity was not associated with pelvic lymph node involvement nor did we find a correlation between tumour angiogenesis and survival in the regression analyses including patients at all stages.

*Table 5. Cox multivariate regression analysis showing significant predictors of survival in squamous cervical carcinoma*

| Variable* | RH | 95% CI | $X^2$ | *P* value |
|---|---|---|---|---|
| SCC | 1.17 | 1.07–1.28 | 13.0 | <0.001 |
| Stage 2 | 3.63 | 1.74–7.58 | 11.8 | <0.001 |
| Stage 1 | 5.05 | 1.63–15.7 | 7.9 | <0.01 |
| CA 125 | 1.05 | 1.01–1.08 | 6.9 | <0.01 |

*See Table 1 for explanation of the independent variables.
RH, relative hazards; CI, confidence interval; $X^2$, chi-square values.

Our results are in agreement with some previous reports [18, 19] but not all [20]. In the study by Bremer and associates, which included 114 patients with cervical cancer stages IB and IIA, microvessel density was significantly and independently correlated with disease-free survival [20]. In contrast to our study, they included tumours of different histological types i.e. both squamous and adenocarcinomas as well as adenosquamous carcinomas.

Different histological types were also included in the study by Schlenger and associates who recently identified tumour vascularity as the strongest independent prognostic factor in patients with advanced (bulky tumours stages IB–IIa stages IIb–IVa) cervical carcinoma [21]. They used a computer-assisted image analysis system to scan whole biopsy sections immunohistochemically labelled for factor VIII-related antigen and quantified tumour vascularity by measuring the distance from randomly located tumour points to the closest microvessel. They found no correlation between tumour vascularity and tumour size, extent of extracervical spread, pTNstage, lymph node status or lymphatic involvement in a subgroup of 22 patients undergoing primary surgery.

Considering treatment and all stages, patients with squamous cervical carcinomas seem to have better survival compared to patients with adenocarcinomas [14]. Hence, histological type should be taken into account in a statistical analysis to avoid confounding. It is unclear whether this has been considered in the above mentioned studies.

Divergent results have been reported for squamous cell carcinoma in the head and neck region [22, 23]. As pointed out by Leedy and associates, the prognostic significance of tumour angiogenesis has previously been demonstrated mainly in adenocarcinomas and differences in tumour biology as well as in staining characteristics may contribute to differences between tumours of different histology [22].

We found the proportion of tumour cells stained for p53 to be of importance for survival [24]. Thus, in the univariate analysis, only tumours with the most pronounced p53 overexpression (>50%) were significantly correlated with survival compared to tumours with absent or weaker staining. In the multivariate analysis, however, no significant correlation with survival was found for p53 overexpression after stage was included, which is in agreement with other studies [25, 26]. Hence, Oka and associates failed to demonstrate a correlation between p53 expression and prognosis in 192 patients with advanced stages [25] and Kainz and co-workers found no prognostic value of p53 expression in 109 operated patients with stage IB–IIB squamous cervical carcinoma [26].

Like most antibodies used for immunohistochemical staining of p53, the DO-1 MAb cannot discriminate between wild-type and mutant p53. Due to the short half-life of wild-type p53, all immunohistochemically detectable p53 was previously considered to be mutant p53. However, an increase of wild-type p53 can occur in response to DNA damage facilitating detection by immunohistochemistry. Since the majority of squamous cervical carcinomas are human papilloma virus (HPV) positive and the frequency of mutant p53 in these tumours is low, the majority of p53 overexpression found in our study presumably is of the wild type. This assumption is further supported by data from a recent study demonstrating concomitant high expression of p53 and p21 [27]. The role of p53 and HPV in cervical car-

cinoma has recently been summarised by Street and Delgado [28].

A further investigation of the nature of the p53 overexpression would require a more direct mutation identification technique, such as single-strand conformation polymorphism (SSCP) analysis, using the polymerase chain reaction technique or sequence analysis [29], which was not the aim of the study. In a recent study by Waggoner and associates [30], SSCP analysis did not identify any mutant p53 in the tumours with p53 overexpression detected by immunohistochemistry using the DO-7 MAb. Thus, in terms of predicting clinical outcome, p53 overexpression detected by immunohistochemistry appears less useful in squamous cervical carcinoma.

We found no correlation between MIB-1 and the clinicopathological variables or the clinical outcome of the patients with squamous cervical carcinoma, which is in agreement with a previous report [31] but not others [32, 33]. This study also demonstrates that serum concentrations of SCC and CA 125, apart from stage, are of predictive value not only for short-term survival [11] but also for long-term survival in patients with squamous cell carcinoma of the uterine cervix.

In conclusion, no predictive value for long-term survival was found for immunohistochemically detectable p53 overexpression or MIB-1 in squamous cervical carcinoma. The predictive value of tumour angiogenesis for survival outcome has still to be determined in squamous cervical carcinoma.


1. Pettersson F Jr (ed). Annual report on the Results of Treatment in Gynecological Cancer, Stockholm, 21st volume. (suppl) 1991, **36**, 27–130.
2. van Bommell PFJ, van Lindert ACM, Kock HCLV, Leers WH, Neijt JP. A review of prognostic factors in early-stage carcinoma of the cervix (FIGO Ib and IIa) and implications for treatment strategy. *Eur J Obstet Gynecol Reprod Biol* 1987, **26**, 69–84.
3. Weidner N, Semple JP, Welch WR, Folkman J. Tumour angiogenesis and metastasis-correlation in invasive breast carcinoma. *N Engl J Med* 1991, **324**, 1–8.
4. Maeda K, Chung YS, Takatsuka S, *et al.* Tumor angiogenesis as a predictor of recurrence in gastric carcinoma. *J Clin Oncol* 1995, **13**, 477–481.
5. Craft PS, Harris AL. Review. Clinical prognostic significance of tumour angiogenesis. *Ann Oncol* 1994, **5**, 305–311.
6. Levine AJ, Momand J, Finlay CA. The p53 tumour suppressor gene. *Nature* 1991, **351**, 453–456.
7. Lane DP. p53, guardian of the genome. *Nature* 1992, **358**, 15–16.
8. Saitoh S, Cunningham J, De Vries EMG, *et al.* p53 gene mutations in breast cancers in midwestern US women: null as well as missense-type mutations are associated with poor prognosis. *Oncogene* 1994, **9**, 2869–2875.
9. Youssef EM, Matsuda T, Takada N, *et al.* Prognostic significance of the MIB-proliferation index for patients with squamous cell carcinoma of the esophagus. *Cancer* 1995, **76**, 358–366.
10. Cattoretti G, Becker MHG, Key G, *et al.* Monoclonal antibodies against recombinant parts of the Ki-67 antigen (MIB1 and MIB3) detect proliferating cells in microwave-processed formalin-fixed paraffin sections. *J Pathol* 1992, **168**, 357–363.
11. Åvall-Lundqvist EH, Sjövall K, Nilsson BR, Eneroth PE. Prognostic significance of pretreatment serum levels of squamous cell carcinoma antigen and CA 125 in cervical carcinoma. *Eur J Cancer* 1992, **28A**, 1695–1702.
12. Wentz WB, Reagan JW. Survival in cervical cancer with respect to cell type. *Cancer* 1959, **59**, 384–388.
13. Broders AC. Carcinoma grading and practical application. *Arch Pathol* 1926, **2**, 376–385.
14. Shingleton HM, Orr JW. *Cancer of the Cervix*. Philadelphia, PA, JB Lippincott, 1995, 42.
15. Eriksson ET, Schimmelpenning H, Aspenblad U, Zetterberg A, Auer GU. Immunohistochemical expression of the mutant p53 protein and nuclear DNA content during transition from benign to malignant breast disease. *Hum Pathol* 1994, **25**, 1228–1233.
16. Mehta CR, Patel NR, Tsiatis AA. Exact significance testing to establish treatment equivalence for ordered categorical data. *Biometrics* 1984, **40**, 819–825.
17. Cox DR. Regression models and life-tables. *J R Stat Soc* 1972, **34**, 187–200.
18. Kainz C, Speiser P, Wanner C, *et al.* Prognostic value of tumour microvessel density in cancer of the uterine cervix stage IB and IIB. *Anticancer Res* 1995, **15**, 1549–1552.
19. Wiggins D, Granai C, Steinhoff M, Calabresi P. Tumor angiogenesis as a prognostic factor in cervical carcinoma. *Gyn Oncol* 1995, **56**, 353–356.
20. Bremer G, Tiebosch A, van der Putten H, *et al.* Tumor angiogenesis: an independent prognostic parameter in cervical cancer. *Am J Obstet Gynecol* 1996, **174**, 126–131.
21. Schlenger KH, Höckel M, Mitze M, *et al.* Tumor vascularity—a novel prognostic factor in advanced cervical carcinoma. *Gynecol Oncol* 1995, **59**, 57–66.
22. Leedy DA, Trune DR, Kronz JD, Weidner N, Cohen JI. Tumour angiogenesis, the p53 antigen, and cervical metastasis in squamous cell carcinoma of the tongue. *Otolaryngol Head Neck Surg* 1994, **111**, 417–422.
23. Gasparini G, Weidner N, Maluta S, *et al.* Intratumoural microvessel density and p53 protein: correlation with metastasis in head and neck squamous cell carcinoma. *Int J Cancer* 1993, **55**, 739–744.
24. Fisher CJ, Gillett CE, Vjtesek B, Barnes DM, Millis RR. Problems with p53 immunohistochemical staining: the effect of fixation and variation in the methods of evaluation. *Br J Cancer* 1994, **69**, 26–31.
25. Oka K, Nakano T, Arai T. p53CM1 Expression is not associated with prognosis in uterine cervical carcinoma. *Cancer* 1993, **72**, 160–164.
26. Kainz CH, Kohlberger P, Gitsch G, *et al.* Mutant p53 in patients with invasive cervical cancer stages IB to IIB. *Gyn Oncol* 1995, **57**, 212–214.
27. Heselmeyer K, Macville M, Schröck E, *et al.* Advanced stage cervical carcinomas are defined by a recurrent pattern of chromosomal aberrations revealing high genetic instability and a consistent gain of chromosome 3q. *Genes, Chromosomes, Cancer.* **In press.**
28. Street D, Delgado G. The role of p53 and HPV in cervical cancer. *Gynecol Oncol* 1995, **58**, 287–288.
29. Schneider J, Rubio MP, Rodriguez-Escudero FJ, Seizinger BR, Castresana JS. Identification of p53 mutations by means of single strand conformation polymorphism analysis in gynaecological tumours: comparison with the results of immunohistochemistry. *Eur J Cancer* 1994, **30A**, 504–508.
30. Waggoner SE, Anderson SM, Luce MC, Takahashi H, Boyd J. p53 protein expression and gene analysis in clear cell adenocarcinoma of the vagina and cervix. *Gynecol Oncol* 1996, **60**, 339–344.
31. Cole DJ, Brown DC, Crossley E, Alcock CJ, Gatter KC. Carcinoma of the cervix uteri: an assessment of the relationship of tumour proliferation to prognosis. *Br J Cancer* 1992, **65**, 783–785.
32. Garzetti GG, Ciavatti A, Lucarini G, *et al.* MIB 1 immunostaining in stage I squamous cervical carcinoma: relationship with natural killer cell activity. *Gynecol Oncol* 1995, **58**, 28–33.
33. Nakano T, Oka K. Transition of Ki67 index of uterine cervical tumors during radiation therapy. *Cancer* 1991, **68**, 517–523.


**Acknowledgements**—Supported by grants from the Swedish Society of Medicine and the Karolinska Institute.