

Tumor Marker Score for Prognostication of Early-stage Squamous Cell Cervical Cancer

DAN HELLBERG^{1,2} and TIBOR TOT³

^{1,2}*Center for Clinical Research, Falun, Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden;*

³*Department of Pathology and Clinical Cytology, Falun Hospital, Falun, Sweden*

Abstract. *Background/Aim: Histopathological and clinical scores to predict prognosis in cervical cancer have been of limited value. In the present study a tumor marker expression score was evaluated for prognostication in early-stage cervical cancer. Materials and Methods: The entire study population included 128 women with invasive squamous cell cervical cancer followed-up for at least 10 years. Results: Expression of 12 tumor markers (epidermal growth factor receptor (EGFR), Ki-67, c-MYC, p53, p27, E-cadherin, CD44, vascular endothelial growth factor receptor (VEGF), cyclooxygenase-2 (COX2), CD4, and leucine-rich immunoglobulin-like repeats-1 (LRIG1) and LRIG2, considered relevant for cervical cancer prognostication was evaluated by immunohistochemistry. Expression of five markers, LRIG1, LRIG2, p53, COX2 and c-MYC were useful to make a prognostication score, ranging from 0 to 5. Score 0-1 correlated to less than 5% 10-year mortality, while the mortality rate of those with score 4-5 approached 70%; those with score 2 formed an intermediate group. Using different models, a high sensitivity, specificity, positive predictive value and negative predictive value was attained. Conclusion: Tumor marker scoring could be an adjunct to histopathological and clinical parameters in prognostication of early-stage cervical cancer.*

The treatment of cervical cancer includes radiotherapy, surgery and chemotherapy, sometimes in combination, depending on clinical stage, other clinical and histopathological variables, local traditions *etc.* In late stages,

clinical stage III and IV, prognosis is poor, while in stage IB, IIA and IIB, *i.e.* localized cancer, survival is 70-90% (1). In these early stages, it would be a great advantage to identify women with a poor prognosis. All three treatment options above and their combinations are aggressive and associated with severe acute and chronic side-effects. For women with a very good prognosis, it would be very beneficial if unnecessary treatments could be avoided.

In prostate cancer, the Gleason score is established and is used to predict prognosis. This estimation is solely derived from histopathological variables (2). A large number of similar histopathological scores have been constructed for cervical cancer with different clinical stages included. Their success has been moderate or disappointing, and at present none of these scores are in general use (3-10).

Expression of a large number of tumor markers (biological markers) and their possible correlations to prognosis in cervical cancer has been investigated during the last decade. No single marker has proven reliable for prognostication. In a previous study, we attempted to investigate if combinations of expression of two tumor markers that we had found to significantly correlate to prognosis, but with unacceptable sensitivity and specificity when used alone, could improve prognostication. There were slight improvements, but these were still not clinically useful (11).

At present there is no cervical cancer score based on tumor marker expression. If such a score could be constructed, with acceptable sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), it would give the physician an adjunct to clinical and histopathological variables for treatment options. In particular, it would also be beneficial when the patient is treated with radiotherapy and factors such as lymphoglandular metastases are unknown. Thus, it could be an important aid in deciding when to choose aggressive treatment or life expectancy.

The aim of the present study was to construct a tumor marker score and evaluate its potential usefulness for prognostication in early-stage cervical cancer.

Correspondence to: Professor Dan Hellberg, Center for Clinical Research, Nissers vag 3, 79182 Falun, Sweden. Tel: +46 0700915310, e-mail: dan.hellberg@ltdalarna.se and Associate Professor Tibor Tot, Department of Pathology and Clinical Cytology, Falun Hospital, 79182 Falun, Sweden. E-mail: tiber.tot@ltdalarna.se

Key Words: Cervical cancer, tumor markers, prognosis.

Table I. Tumor markers included in the study and their major functions.

Biological marker	Functions	Localization	Clone	Dilution	Reaction time (min)	Source	Antigen retrieval solution ^a
LRIG2	Unknown (promoter?)	Membrane, cytoplasm	Polyclonal	1 µg/ml	60	In-house	10 mM citrate pH 7.3
LRIG1	Tumor suppressor	Membrane, cytoplasm	Polyclonal	0.5 µg/ml	60	In-house	10 mM citrate pH 7.3
c-MYC	Cell cycle progression, Malignant transformation	Nucleus	9E11	1:100	30	Novocastra	TED pH 9 DAKO
p-53	Cell-cycle arrest, apoptosis DNA repair	Nucleus	DO-7	1:200	30	DakoCytomation	TED pH 9 DAKO
Cyclooxygenase-2	Inflammation, angiogenesis, decreased apoptosis	Cytoplasm	SP 21	1:20	30	NeoMarkers, Fremont, California, US	TED pH 9 DAKO

^aAntigen retrieval was carried out for 45 min in a water-bath at 96°C; TED: DAKO TED pH 9 S2367; TRS: DAKO TRS pH 6.

Materials and Methods

The study population consisted of 128 women with invasive squamous cell cervical cancer stage IB to IV who were admitted to the Department of Gynecologic Oncology, Norrlands University Hospital, Umeå during 1984 to 1990. Among those, 75 cases were diagnosed as squamous epithelial cancer stage IB, IIA and IIB. Clinical staging was made according to FIGO classification (12). Thus, stage IB is defined as cancer that exceeds 5 mm depth or 7 mm wide; IIA as cancer spread beyond cervix to upper, but not lower, part of the vagina; and IIB as cancer spread to parametrial tissue next to the cervix. All women were followed-up for at least 10 years.

Three-micrometer sections of the original paraffin blocks were reviewed by one of the authors (TT) and the most representative areas were marked for tissue microarray (TMA). Three-millimeter punch biopsies were taken from the blocks corresponding to the marked area and joined into TMA paraffin blocks, containing 25 punch biopsies on average. Each TMA block also included two controls containing human tissue, as specified by the producer. The microscopic evaluation included the complete TMA.

Immunohistochemistry was performed at the Department of Pathology and Clinical Cytology, as described elsewhere (13). Twelve tumor markers, relevant in cervical cancer, were included, namely epidermal growth factor receptor (EGFR), Ki-67, c-MYC, p53, p27, E-cadherin, CD44, vascular endothelial growth factor receptor (VEGF), cyclooxygenase-2 (COX2), CD4, and leucine-rich immunoglobulin-like repeats 1 (LRIG1) and LRIG2 (Table I).

In brief, 3-µm thick sections from the TMA blocks were cut and rehydrated. Immunohistochemical staining was carried out with a Dako Autostainer (Dakopatts, Stocholm Sweden) which uses biotinylated secondary goat anti-mouse antibody for the detection system and streptavidin-horseradish peroxidase conjugate for visualization of diaminobenzidine (DAB) solution. The slides were weakly counter-stained with hematoxylin and were mounted routinely. All antibody stainings were evaluated by an external senior pathologist (AL; see Acknowledgements), who was blinded to the clinical details. Absence or presence of tumor marker expression was used as the discriminating level both in the univariate and multivariate analyses, and for the final score. Correlations between 10 year survival and tumor marker expression were analyzed with logistic regression to estimate odds ratios 95%

Table II. Expression of tumor markers in squamous cell cervical cancer in clinical stage IB (n=46), IIA (n=14) and IIB (n=15).

Marker	Expression (any) by clinical stage			p-Value
	%	%	%	
LRIG1	56.5	57.1	46.7	0.79
LRIG2	54.4	78.6	33.3	0.04
p53	58.7	57.1	66.7	0.83
COX2	21.7	21.4	20.0	0.99
c-MYC	37.0	35.7	33.3	0.97

LRIG: Leucine-rich immunoglobulin-like repeats; COX2: cyclooxygenase-2; c-MYC:

confidence intervals and *p*-values. Based on these results, a score was constructed and evaluated for sensitivity, specificity, PPV and NPV to discriminate between favorable and poor prognosis. The study was approved by the Research Ethical Committee, Medical Faculty, Umeå University (04-085).

Results

The women were on average 57.7 years of age, 64% of them were post-menopausal and 50% active smokers. Eighty-seven percent had experienced childbirth, with a mean parity of 2.7 and 3.0 pregnancies.

Stepwise exclusion upwards, from lowest odds ratios to highest, revealed five tumor markers for final inclusion in a prognostication score: LRIG1, LRIG2, p53, COX2 and c-MYC, correlated to 10-year survival. Expression of each tumor marker (any) in the different clinical stages was, with few exceptions, similar (Table II). Overall mortality was 28% and did not significantly differ between stages. The impact on prognosis of single-tumor markers, independent of other markers is given in Table III.

Table III. Ten-year mortality in squamous cell cancer stage IB-IIB by expression of tumor markers with prognostic implications in multivariate analyses.

	Mortality (%)	Odds ratio ^a	95% CI ^a	p-Value ^a
Overall	28.0	NA	NA	NA
Clinical stage IB/IB/IIA	23.9/42.9/26.7	0.89	0.88-9.35	0.67
LRIG1, absence ^b	41.2	19.21	4.14-129.58	0.0006
LRIG2, presence	39.0	7.77	1.74-46.38	0.01
p53, absence ^b	40.0	7.89	2.00-40.21	0.006
COX2, presence	43.8	5.12	1.09-29.42	0.048
c-MYC, presence	40.7	3.51	0.89-15.70	0.08

^aAdjusted for expression of all tumor markers in the table; LRIG: leucine-rich immunoglobulin-like repeats; COX2: cyclooxygenase-2; c-MYC.

^bAbsence of expression correlated to poor prognosis for LRIG1 and p-53, while presence of expression for the other tumor markers correlated to poor prognosis.

Tumor marker expression was scored 0 or 1, where 1 indicated a poor prognosis. Expression of LRIG2, COX2 and c-MYC, and a lack of LRIG1 and p-53 expression correlated to poor prognosis. A prognostication score was constructed by adding the expression score of each tumor marker, giving a minimum score of 0 and maximum of 5. There was no case with score 5. Overall mortality was 28%, while 10-year mortality was 0% for patients with score 0 and 80% for these with score 4 (Table IV).

There was a marked border for survival *versus* mortality between score 2 and 3. Comparisons between patients grouped with score 0-2 and 3-4 revealed a sensitivity for predicting mortality of 71.4%, specificity of 85.2%, PPV of 65.2% and NPV of 88.5%.

Another model would be to estimate low-risk (score 0-1), intermediate-(score 2) and high-risk (score 3-4) groups. Mortality rates in the three such groups were 3.7%, 20.0% and 65.2%, respectively. As the overall mortality rate was 28.0%, the high-risk group had a very high, and the low-risk group an extremely low, mortality rate.

The difference in mortality rate between those with score 0-1 *versus* those with score 2 was close to significant ($p=0.057$), and between these with score 2 *versus* those with score 3-4 was highly significant ($p=0.001$). For those with score 2, the mortality rate was only slightly lower than the average of the entire study population. According to these results, it is likely that aggressive treatment or men with score 2 would mainly rely on clinical and histopathological data. Therefore, score 0-1 *versus* 3-4 were compared ($p=0.0001$). The sensitivity and NPV at 93.8% and 96.2%, respectively, were high; specificity and PPV were 76.5% and 65.2%, respectively.

Discussion

A score using tumor marker expression for prognostication in cervical cancer has not been previously assessed. The

Table IV. Early-stage cervical cancer mortality by score.

Score ^a	Mortality n (%)	Survival n (%)
0	0 (0)	3 (100.0)
1	1 (4.2)	23 (95.8)
2	5 (20.0)	20 (80.0)
3	11 (61.1)	7 (38.9)
4	4 (80.0)	1 (20.0)

Calculation of score: 0=No expression of LRIG2, COX2, or c-MYC and expression of LRIG1 or p53 was associated with good prognosis. 1=Expression of LRIG2, COX2 or c-MYC and no expression of LRIG1 or p53 was associated with good prognosis. Score was calculated by adding expression of each tumor marker, 0 or 1. Maximum score was thus 5.

score presented here shows a strong correlation to 10-year mortality in early-stage cervical cancer. The score is, however, not solely intended to direct treatment, but to serve as an adjunct to clinical and histopathological findings. Such a score will aid in identifying women with early-stage cancer who would benefit from aggressive treatment, *i.e.* combined treatments, and those who would probably not. A score for late-stage cancer might be of less interest, as these patients have a very poor prognosis and will receive aggressive therapy.

Sensitivity, specificity, PPV and NPV give information on whether the score is clinically-useful. Thus, the high specificity and NPV when scores 0-2 and 3-4 were compared is satisfactory as women with good prognosis will not have unnecessary aggressive treatment. The sensitivity and PPV were lower and suggest that a low score could also be associated with mortality and that in fact a minority of these women would benefit from aggressive treatment. We believe, however, that sensitivity and PPV are acceptable, as the score

should be used as an adjunct to clinical prognostic factors, such as specified growth, age, positive lymph nodes and general condition of the patient. Taken together, these factors may define the large majority of women with poor prognosis despite low scores.

There is a basis for excluding women with score 2, as their mortality is close to the overall mortality. Hence, when score 0-1 and 3-4 were compared, estimations gave a very high sensitivity that would leave few women with a poor prognosis not treated aggressively. The NPV was similarly high, *i.e.* a low score will also reflect a favorable prognosis. Specificity was, however, slightly lower, compared to comparisons of score 0-2 *vs.* 3-4. Exclusion of the intermediate score seems to be the most attractive model, but should be evaluated in further studies.

The selection of tumor markers in this study serves as a model. Different local traditions, study populations, laboratories, clones and antibodies will influence tumor marker expression. An optimal prognostication score might include our investigated, and other, tumor markers. The tumor markers included here are all biologically-plausible for prognostication and have previously been investigated as single prognostic markers. In the present study, we were able to use our experience from three previous studies to construct a score from five tumor markers that turned out to be clinically useful.

We previously presented the crude correlations with prognosis for 10 out of the 12 tumor markers included in the present study (13). p53, c-MYC, COX2 and CD4 expression appeared promising, and have been widely studied for prognosis in cervical cancer (14-18). In our previous studies, all cancer stages were included, although adjustment for stage was made in multivariate analysis. Models with combinations of tumor marker expression improved prognostication, but were in general clinically unsatisfactory. Subsequently, we were able to evaluate the novel protein LRIG1, and finally LRIG2. In early-stage, but not late-stage cancer LRIG1 expression appeared to correlate to favorable (13), and LRIG2 expression to poor, prognosis (19).

c-MYC is one of the 'classic' oncogenes, and its translocation in Burkitt's lymphoma was first shown in 1982 (20). The functions of c-MYC products are still not completely understood as they bind to hundreds of potential target genes. It is however evident that c-MYC expression contributes to increased proliferation and loss of differentiation in cancer (21). Previous studies have concluded that c-MYC expression is increased and is involved in cervical cancer (17, 18). In our model, c-MYC expression did not significantly correlate to a poor prognosis, but the *p*-value was borderline, and its inclusion was useful.

p53 is a major tumor suppressor and its expression has been studied in a majority of solid cancer types. p53 induces cell-cycle arrest at the G₁ and G₂ checkpoints prior to DNA replication, allowing repair of damaged DNA, but also

induction of apoptosis, thereby hampering development of cancer cells (22). In cervical cancer, the human papillomavirus oncogene E6 is able to promote p53 degradation (23). In contrast, in most cancer types, inactive mutant p53 is found. Results of evaluating mutant p53 in cervical cancer have, therefore, been confusing. In the present study, expression of active wild-type p53 was evaluated (16).

COX2 was included in the study because of its correlation to inflammatory response. COX2 is involved in a number of negative events in cancer, such as angiogenesis and reduced apoptosis (9). COX2 expression has been correlated to poor prognosis in many previous studies on cervical cancer (24).

The *LRIG1* gene is located on chromosome 3p14.3. This region is frequently deleted in cancer. In addition to our study, a correlation with a good prognosis in skin cancer has been reported. LRIG1 has also been shown to be an important determinant of cancer growth and good prognosis in prostate cancer (10), brain tumors (25) and in mediating receptor degradation in breast cancer (26). LRIG1, thus, has a role as a tumor suppressor in a variety of solid, epithelial cancer types (11, 13).

Expression of another member of the LRIG family, LRIG2, in contrast, was recently reported to be strongly associated with glioma risk (27) and poor prognosis in oligodendroglioma (25, 28), as well as in our study with early-stage cervical cancer (19).

All previous scoring models have, with one exception (8), included lymph node metastasis. This necessitates surgery, either explorative, or as treatment. Radiotherapy and radical hysterectomy are both major treatment options for early-stage cancer. When radiotherapy is the treatment of choice, and surgical exploration is not routinely performed, these histopathological scores are thus not useful (4, 5, 7, 9, 10, 29-31). In addition to lymph node status, factors such as tumor size, histopathological type, depth of invasion, lymph vascular invasion, parametrial extension and radical surgical margins have been evaluated. All studies reported that their score correlated to survival. In no study, however, were the sensitivity, specificity, PPV and NPV estimated. Statistical analyses were restricted to *p*-values, risk ratios and Kaplan-Meier curves, which is why it is difficult to evaluate their clinical usefulness.

The present study has presented a novel prognostication score, aimed as an adjunct to clinical and histopathological parameters in choosing treatment of early-stage squamous carcinoma of the uterine cervix. This score is assessable on limited biopsy material before any treatment, whether radiotherapy, surgery or adjunct chemotherapy, and with adequate sensitivity, specificity, PPV and NPV may valuably support decision-making to avoid unnecessary aggressive treatment in women with a favorable prognosis, and to identify women with a poor prognosis requiring for more aggressive therapy.

Conflicts of Interest

There are no conflicts of interest.

Support

Cancer Research Foundation in Northern Sweden.

Acknowledgements

We express our thanks to Håkan Hedman for organizing LRIG staining, Anders Lindgren for expertise in tumor marker expression, Anna Asplund of the Human Proteome Resources project, and Annika Lindström for their contributions to previous studies.

References

- Graflund M, Sorbe B, Bryne M and Karlsson M: The prognostic value of a histologic grading system, DNA profile, and MIB-1 expression in early stages of cervical squamous cell carcinomas. *Int J Gynecol Cancer* 12(2): 149-157, 2002.
- Grisaru DA, Covens A, Franssen E, Chapman W, Shaw P, Colgan T, Murphy J, DePetrillo D, Lickrish G, Laframboise S and Rosen B: Histopathologic score predicts recurrence free survival after radical surgery in patients with stage IA2-IB1-2 cervical carcinoma. *Cancer* 97(8): 1904-1908, 2003.
- Shinohara S, Ochi T, Miyazaki T, Fujii T, Kawamura M, Mochizuki T and Ito M: Histopathological prognostic factors in patients with cervical cancer treated with radical hysterectomy and postoperative radiotherapy. *Int J Clin Oncol* 9(6): 503-509, 2004.
- Smaniotto D, D'Agostino G, Luzi S, Valentini V, Macchia G, Mantini G, Margariti PA, Ferrandina G and Scambia G: Concurrent 5-fluorouracil, mitomycin C and radiation with or without brachytherapy in recurrent cervical cancer: a scoring system to predict clinical response and outcome. *Tumori* 91(4): 295-301, 2005.
- Eggen T, Arnes M, Moe B, Straume B and Orbo A: Prognosis of early cervical cancer (FIGO Stages IA2, IB, and IIA) in northern Norway predicted by malignancy grading score and objective morphometric image analysis. *Int J Gynecol Pathol* 26(4): 447-456, 2007.
- Lindahl B, Ranstam J and Willen R: Prospective malignancy grading of invasive squamous carcinoma of the uterine cervix. Prognostic significance in a long-term follow-up. *Anticancer Res* 27(4C): 2829-2832, 2007.
- Polterauer S, Grimm C, Seebacher V, Rahhal J, Tempfer C, Reinthaller A and Hefler L: The inflammation-based Glasgow Prognostic Score predicts survival in patients with cervical cancer. *Int J Gynecol Cancer* 20(6): 1052-1057, 2010.
- Biewenga P, van der Velden J, Mol BW, Stalpers LJ, Schilthuis MS, van der Steeg JW, Burger MP and Buist MR: Prognostic model for survival in patients with early stage cervical cancer. *Cancer* 117(4): 768-776, 2011.
- Dannenberg AJ and Howe LR: The role of COX-2 in breast and cervical cancer. *Prog Exp Tumor Res* 37: 90-106, 2003.
- Thomasson M, Wang B, Hammarsten P, Dahlman A, Persson JL, Josefsson A, Stattin P, Granfors T, Egevad L, Henriksson R, Bergh A and Hedman H: LRIG1 and the liar paradox in prostate cancer: a study of the expression and clinical significance of LRIG1 in prostate cancer. *Int J Cancer* 128(12): 2843-2852, 2011.
- Tanemura A, Nagasawa T, Inui S and Itami S: LRIG-1 provides a novel prognostic predictor in squamous cell carcinoma of the skin: immunohistochemical analysis for 38 cases. *Dermatol Surg* 31(4): 423-430, 2005.
- Benedet JL, Bender H, Jones H, 3rd, Ngan HY and Pecorelli S: FIGO staging classifications and clinical practice guidelines in the management of gynecologic cancers. FIGO Committee on Gynecologic Oncology. *Int J Gynaecol Obstet* 70(2): 209-262, 2000.
- Lindstrom AK, Ekman K, Stendahl U, Tot T, Henriksson R, Hedman H and Hellberg D: LRIG1 and squamous epithelial uterine cervical cancer: correlation to prognosis, other tumor markers, sex steroid hormones, and smoking. *Int J Gynecol Cancer* 18(2): 312-317, 2008.
- Noordhuis MG, Eijssink JJ, Roossink F, de Graeff P, Pras E, Schuurin E *et al*: Prognostic cell biological markers in cervical cancer patients primarily treated with (chemo)radiation: a systematic review. *Int J Radiat Oncol Biol Phys* 79(2): 325-334, 2011.
- Jung YW, Kim SW, Kim S, Kim JH, Cho NH, Kim JW *et al*: Prevalence and clinical relevance of cyclooxygenase-1 and -2 expression in stage IIB cervical adenocarcinoma. *Eur J Obstet Gynecol Reprod Biol* 148(1): 62-66, 2010.
- Ferenczy A and Franco E: Persistent human papillomavirus infection and cervical neoplasia. *Lancet Oncol* 3(1): 11-16, 2002.
- Protka Z, Arsenijevic S, Dimitrijevic A, Mitrovic S, Stankovic V, Milosavljevic M *et al*: Co-overexpression of bcl-2 and c-myc in uterine cervix carcinomas and premalignant lesions. *Eur J Histochem* 55(1): e8, 2011.
- Riou GF: Proto-oncogenes and prognosis in early carcinoma of the uterine cervix. *Cancer Surv* 7(3): 441-456, 1988.
- Hedman H, Lindstrom AK, Tot T, Stendahl U, Henriksson R and Hellberg D: LRIG2 in contrast to LRIG1 predicts poor survival in early-stage squamous cell carcinoma of the uterine cervix. *Acta Oncol* 49(6): 812-815, 2010.
- Dalla-Favera R, Bregni M, Erikson J, Patterson D, Gallo RC and Croce CM: Human c-myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. *Proc Natl Acad Sci* 79(24): 7824-7827, 1982.
- Pelengaris S and Khan M: The many faces of c-MYC. *Arch Biochem Biophys* 416(2): 129-136, 2003.
- Braithwaite AW, Royds JA and Jackson P: The p53 story: layers of complexity. *Carcinogenesis* 26(7): 1161-1169, 2005.
- Tommasino M, Accardi R, Caldeira S, Dong W, Malanchi I, Smet A *et al*: The role of TP53 in cervical carcinogenesis. *Hum Mutat* 21(3): 307-312, 2003.
- Dannenberg AJ and Howe LR: The role of COX-2 in breast and cervical cancer. *Prog Exp Tumor Res* 37: 90-106, 2003.
- Holmlund C, Haapasalo H, Yi W, Raheem O, Brannstrom T, Bragge H *et al*: Cytoplasmic LRIG2 expression is associated with poor oligodendroglioma patient survival. *Neuropathology* 29(3): 242-247.
- Hamburger AW: The role of ErbB3 and its binding partners in breast cancer progression and resistance to hormone and tyrosine kinase directed therapies. *J Mammary Gland Biol Neoplasia* 13(2): 225-233, 2008.
- Andersson U, Schwartzbaum J, Wiklund F, Sjöström S, Liu Y, Tsavachidis S *et al*: A comprehensive study of the association between the EGFR and ERBB2 genes and glioma risk. *Acta Oncol* 49(6): 767-775, 2010.

- 28 Holmlund C, Nilsson J, Guo D, Starefeldt A, Golovleva I, Henriksson R *et al*: Characterization and tissue-specific expression of human LRIG2. *Gene* 332: 35-43, 2006.
- 29 Stendahl U, Willen H and Willen R: Classification and grading of invasive squamous cell carcinoma of the uterine cervix. *Acta Radiol Oncol Radiat Phys Biol* 18(6): 481-496, 1979.
- 30 Graflund M, Sorbe B, Hussein A, Bryne M and Karlsson M: The prognostic value of histopathologic grading parameters and microvessel density in patients with early squamous cell carcinoma of the uterine cervix. *Int J Gynecol Cancer* 12(1): 32-41, 2002.
- 31 Smaniotto D, D'Agostino G, Luzi S, Valentini V, Macchia G, Mangiacotti MG *et al*: Concurrent 5-fluorouracil, mitomycin C and radiation, with or without brachytherapy, in recurrent endometrial cancer: a scoring system to predict clinical response and outcome. *Tumori* 91(3): 215-220, 2005.

Received November 19, 2013

Revised January 3, 2014

Accepted January 7, 2014