# Immunohistrochemistry Expression of COX-2 as a Prognostic Factor for Survival in Locally Advanced Cervical Cancer Treated with Concurrent Cisplatin-Radiation Therapy

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## ABSTRACT

**Objective:** Cervical cancer is a major health problem for women. Concurrent Cisplatin-radiation therapy (CCRT) can improve survival, although result of treatment is still not satisfactory. Many prognostic factors have been known, but they cannot explain all the results. Cyclooxygenase-2 (COX-2) is one of the molecular factors related to angiogenesis that can lead to more accurate predictive outcome and modification of the treatment in the future.
**Methods:** Retrospective study to find relationship of COX-2 expression to overall survival (OS), progression free survival (PFS), distant metastasis free survival (DMFS), and loco-regional relapse free survival (LRRFS) of locally advanced cervical cancer in patients who were treated with CCRT at Siriraj Hospital from 2002 to 2007. In this COX-2 IHC study, the interpretations of results were based on both intensity and quantity score.
**Results:** There were 49 patients included into the study. COX-2 was positive in 36 (73%). Median f/u time was 22 months. COX-2 expression was proven to be a significant prognostic factor to predict OS and PFS with HR 19.5 (95% CI 1.1-337.2) and HR 27.1 (95% CI 2.1-355.6) respectively. Total treatment time (>55 days) is the most significant prognostic factor for OS, PFS and DMF.
**Conclusion:** Expression of COX-2 positive is a significant prognostic factor to predict worse OS and PFS compared to COX-2 negative group in multivariate analysis. The COX-2 scoring system used in this study was good to predict prognosis.

**Keywords:** Locally advanced cervical cancer, expression of COX-2, prognosis factor, concurrent chemo-radiotherapy

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## INTRODUCTION

Cervical cancer is the most common cancer in women. In Siriraj Hospital, cervical cancer is the second most common cancer in women with incidence of 546 patients (14.27 %) in the year 2007.

Improved survival rates have been demonstrated because of the advance of surgical and radiation therapy techniques although results of treatment in some stages is still unsatisfactory. Five years overall survival (OS) of early stage I-II cervical cancer is about 75% to 95%, but in stage III-IV cervical cancer, 5 years OS is only about 15% to 50%.

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Many independent prognostic factors have been studied and accepted. FIGO staging is the most significant prognostic factor, but FIGO (International Federation of Gynecology and Obstetrics) staging cannot be the only significant prognosis factor to predict survival outcome. There are other prognosis factors which influence survival outcome.[1,2] However results of treatment of patients with similar prognostic factors are still variable. Molecular biology factors and tumor markers have recently been considered potential markers to predict aggressiveness and natural history of disease.

In this study, we will study immunohistrochemistry of COX-2. Two hypotheses have been associated with angiogenesis which could predict systemic and reduce apoptosis that could effect local control. We were also interested to study this marker because it has related related such as anti-COX-2 inhibitor.[3]

The primary end point in this study was OS as the determinant independent prognosis factor. The secondary end point was progression free survival (PFS), distant metastasis free survival (DMFS), and loco-regional relapse free survival (LRRFS) that can show trends of failure and nature of this disease.

## MATERIALS AND METHODS

### Study design:

This study was a retrospective study to find the relationship of COX-2 expression in the tissue of cervical cancer patients and see whether it has any effects on the results of treatment and prognosis of the patients.

### Patients

Forty nine patients with newly diagnosed cervical cancer FIGO stage IB2-IVA treated with concurrent Cisplatin-radiation therapy (CCRT) at the Radiation Oncology division, Siriraj Hospital from June 2002 to December 2007 and recently, we have been approved by IRB (Institutional Review Board) to study updating data of this research. All available tissue blocks for study were reviewed as adequate tissue specimen (size more than 2 cm.).

### Immunohistochemistry

Scoring and reporting protocol used semi-quantitative method from cytoplasmic and membrane stains by one pathologist. Diffuse weak staining, focally intense staining, and diffuse intense staining were classified as positive for COX-2 expression. Only negative staining and focally weak staining were classified as negative for COX-2 expression.

### Experimental and statistical methods:

Date of start accrual and end accrual was 5 June 2002 and 21 December 2007, respectively. The end of the follow up date was 27 November 2008.

Sample size was calculated by detection of difference in 5 yr, OS between COX-2 positive and negative of 38% (5 yr, OS decreased from 94% to 56% in COX-2 negative and positive group).[4] Type I error was 0.05, 2 sided and power was 0.8. Thus the sample size calculated was 50 patients.

Univariate analysis for Prognostic factor; COX-2 and other clinical prognostic factors used COX regression analysis.

Multivariate analysis used COX regression model with p value from Wald's test and backward elimination method (by likelihood ratio test and percent change of coefficient factors). The final model was performed with systematic approach adding a 2$^{nd}$ degree interaction term. Goodness of fit and statistical test of assumption of proportional hazard was tested.

Survival analysis using Kaplan-Meier curves and 1 year, 2 year and 3 year survival rates were estimated with Kaplan-Meier method for each significant prognostic factors including COX-2.

## RESULTS

of 184 stage IB2-IVA cervical cancer patients treated with concomitant radiotherapy and Cisplatin, only 55 patients had available

paraffin block samples in the Department of Pathology, Siriraj Hospital and 6 patients were excluded due to specimen size < 2 mm, so the number of patients included in the study was 49 patients (26.6%). The comparison of patient characteristics between included and excluded patients showed no different of patient characteristic between our study population and the patient that we had to exclude due to lack of tissue specimens.

Median follow up time was 22.6 months (range 2.4-75.2 months), and estimated follow up rate and loss to follow up rate were 90% and 10%, respectively.

Patient characteristics have been shown in Table 1. There were COX-2 positive in 36 patients (73%) and COX-2 negative in 13 patients (27%). Adenocarcinoma seemed to have very high incidence of COX-2 expression (90%) compared to squamous cell carcinoma which showed COX-2 expression in 68% of the patients.

*Overall survival*

For univariate analysis (Table 2) only overall total treatment time (OTT) was the statistically significant worse prognosis factor to predict survival. The other variables were not statistically significant prognostic factors including COX-2 expression. However for multivariate analysis; COX-2, OTT, tumor size and stage showed statistically significant prognosis to predict OS (Table 3).

Survival curves of COX-2 positive and negative groups and 2 year OS of COX-2 positive and negative groups were 79% and 89%, respectively. In multivariate analysis COX-2 was a significant predictive factor (HR 19.5; 95% CI 1.1-337.2) (Fig 1).

*Progression free survival*

From univariate analysis (Table 2) OTT was the only prognostic factors that showed statistical significance.

For multivariate analysis; COX-2, OTT, tumor size and stage were shown statistically significant poor prognostic factors (Table 3). Two year PFS of COX-2 positive and negative were 63% and 80%, respectively (Fig 2).

*Distant metastasis free survival*

From univariate analysis (Table 2), only OTT was statistically significant worse prognostic factor.

COX-2 positive trended to have more distant metastases, but this was not a statistically significant factor for DMFS with 2 year DMFS of COX-2 positive and negative which were 75% and 91%, respectively.

Multivariate analysis (Table 3) showed that only OTT (HR 42.2, 95% CI 3.5-496.2) was a statistically significant prognostic factor, although COX-2 expression was not significant.

*Loco-regional free survival*

Two year LRRFS of COX-2 positive and

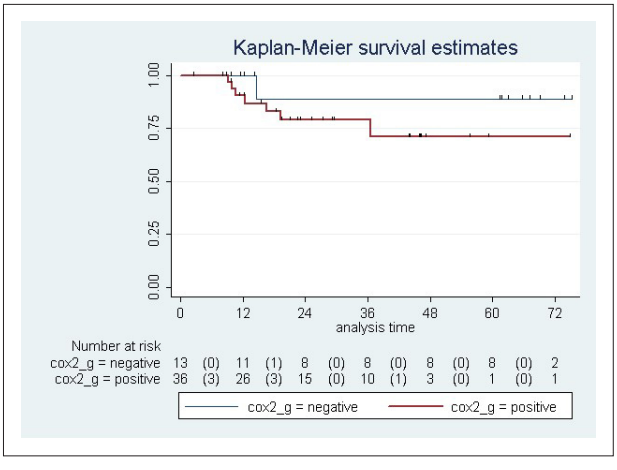


**Fig 1.** Survival curve for overall survival of COX-2.

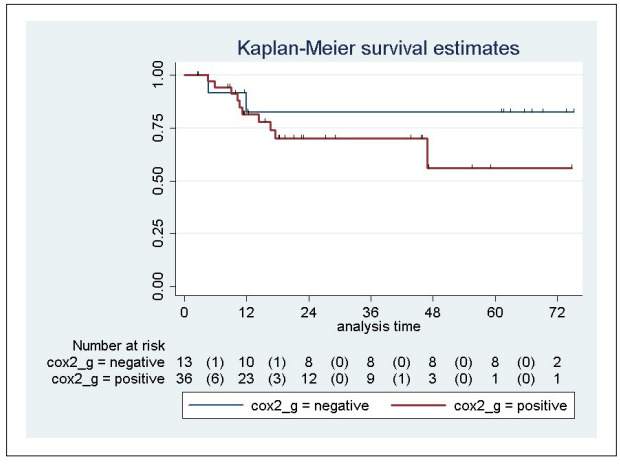


**Fig 2.** Survival curve for progression free survival of COX-2.

**TABLE 1.** Patient characteristics by COX-2 expression.

| Factor | COX-2 positive (n=36) (73%) | COX-2 negative (n=13) (27%) | Total (n=49) |
|---|---|---|---|
| Age (year): <45 | 14 (38.9%) | 8 (61.5%) | 22 (44.9%) |
| >45 | 22 (61.1%) | 5 (38.5%) | 27 (55.1%) |
| Median (range) | 46.5 (34-64) | 44 (38-68) | |
| Mean (SD) | 47.1 (7.5) | 47.8 (9.5) | |
| KPS*: 90 | 29 (80.6%) | 9 (69.2%) | 38 (77.6%) |
| 100 | 7 (19.4%) | 4 (30.8%) | 11 (22.4%) |
| Stage: IB2 | 2 (5.6%) | 0 | 2 (4.1%) |
| IIA | 3 (8.3%) | 0 | 3 (6.1%) |
| IIB | 17 (47.2%) | 7 (53.9%) | 24 (49%) |
| IIIB | 14 (38.9%) | 6 (46.2%) | 20 (40.8%) |
| Histology: Squamous cell carcinoma | 26 (72.2%) | 12 (92.3%) | 38 (77.6%) |
| Adenocarcinoma | 10 (27.8%) | 1 (7.7%) | 11 (22.5%) |
| Tumor size | | | |
| < 4 cm | 9 (25%) | 3 (23.1%) | 12 (24.5%) |
| > 4 cm | 27 (75%) | 10 (77%) | 37 (75.5%) |
| Total treatment time | | | |
| < 55 days | 30 (83.3%) | 10 (76.9%) | 40 (81.6%) |
| > 55 days | 6 (16.7%) | 3 (23.1%) | 9 (18.4%) |
| No. cycle of CMT | | | |
| < 5 cycles | 7 (19.4%) | 4 (30.8%) | 11 (22.5%) |
| > 5 cycles | 29 (80.6%) | 9 (69.2%) | 38 (77.6%) |
| Histology grade | | | |
| Well differentiation | 11 (30.6%) | 5 (38.5%) | 16 (32.7%) |
| Moderate to poor diff. | 23 (63.8%) | 8 (61.5%) | 31 (63.3%) |
| Unspecified | 2 (5.6%) | 0 | 2 (4.1%) |
| Pretreatment Hemoglobin (g/dl) | | | |
| < 12 | 12 (33.3%) | 7 (53.8%) | 19 (38.8%) |
| > 12 | 24 (66.7%) | 6 (46.2%) | 30 (61.2%) |
| Point A dose (Gy) | | | |
| < 80 | 19 (52.8%) | 5 (38.5%) | 24 (49%) |
| > 80 | 17 (47.2%) | 8 (61.5%) | 25 (51%) |
| Pelvic node involvement | | | |
| Negative | 11 (30.6%) | 3 (23.1%) | 14 (28.6%) |
| Positive | 5 (13.8%) | 4 (30.7%) | 9 (18.4%) |
| Unknown | 20 (55.6%) | 6 (46.2%) | 26 (53%) |

*KPS: Karnofsky Performance Status

negative were 83% and 89%, respectively.

None of the factors studied has shown statistically significant predictive value for LRRFS both in the univariate and multivariate analysis (Table 2, 3).

## DISCUSSION

### Compared OS of our study to other studies

Two year OS of our study was 83% comparable to CCRT arm from NCIC study and radiation therapy oncology group trial (RTOG)

**TABLE 2.** Univariate analysis of clinical and molecular factors to predict overall survival (OS), progression free survival (PFS), distant metastasis free survival (DMFS), and loco-regional free survival (LRRFS).

| Factors | OS (HR;95%CI, p value) | PFS (HR;95%CI, p value) | DMFS (HR;95%CI, p value) | LRRFS (HR;95%CI, p value) |
|---|---|---|---|---|
| COX-2 positive | 4.4;0.52-36.2, 0.17 | 2.27;0.48-10.7, 0.3 | 2.96 ;0.36-24.04, 0.31 | 0.54 ;0.09-3.25, 0.5 |
| Age ≥ 45 yr. | 0.32;0.08-1.3, 0.10 | 0.68;0.22-2.12, 0.51 | 1.18;0.28-4.95, 0.83 | 0.46;0.08-2.78, 0.4 |
| Stage IIIA-B | 2.95;0.74-11.8, 0.11 | 3.30;0.99-10.97, 0.052 | 1.59;0.40-6.35, 0.51 | 6.76;0.75-60.53, 0.09 |
| Histology: | 1.6;0.4-6.4, 0.51 | 1.86;0.56-6.17, 0.31 | 1.99;0.47-8.38, 0.35 | 2.45;0.41-14.71, 0.33 |
| Adenocarcinoma | | | | |
| Grading: II-III | 1.03;0.24-4.33, 0.97 | 0.92;0.26-3.28, 0.90 | 1.34;0.24-7.35, 0.74 | 0.56;0.08-3.97, 0.56 |
| Size ≥ 4 cm. | 3.28;0.4-26.3, 0.26 | 4.61;0.60-35.78, 0.14 | 2.81;0.35-22.84, 0.34 | NA* |
| Pelvic node: positive | 4.78;0.43-53.23, 0.20 | 5.52;0.48-63.03, 0.17 | 3.10;0.19-50.67, 0.43 | NA* |
| KPS**: 100 | 0.92;0.74-1.13, 0.92 | 1.01;0.89-1.15, 0.88 | 0.94;0.76-1.16, 0.57 | 0.99;0.80-1.24, 0.94 |
| Initial | 0.47;0.13-1.76, 0.26 | 0.37;0.12-1.17, 0.09 | 0.88;0.21-3.71, 0.87 | 0.13;0.01-1.13, 0.06 |
| Hemoglobin ≥ 12 g/dl | | | | |
| Pt. A dose ≥ 80 Gy | 1.16;0.28-4.91, 0.84 | 1.09;0.33-3.53, 0.89 | 0.76;0.19-3.07, 0.7 | 1.35;0.22-8.09, 0.75 |
| OTT*** ≥ 55 days | 4.64;1.03-20.88,0.046 | 3.82;1.10-13.26, 0.03 | 8.00;1.91-33.50, 0.004 | 1.37;0.15-12.25, 0.78 |
| CMT**** ≥ 5 cycles | 0.87;0.18-4.22, 0.87 | 1.19;0.26-5.45, 0.82 | NA* | 0.93;0.10-8.35, 0.95 |

*NA: Not available

**KPS: Karnofsky Performance Status

***OTT: Overall Treatment Time

****CMT: Chemotherapy

**TABLE 3.** Multivariate analysis for overall survival (OS), progression free survival (PFS), distant metastasis free survival (DMFS), and loco-regional free survival (LRRFS).

| Factors | OS HR (95%CI) | PFS HR (95%CI) | DMFS HR (95%CI) | LRRFS HR (95%CI) |
|---|---|---|---|---|
| COX-2: Positive vs. Negative | 19.5 (1.1-337.2) | 27.1 (2.1-355.6) | 21.8 (0.67-197.7) | 0.28 (0.03-2.54) |
| OTT*: ≥55 d vs. <55 d | 30.9 (2.5-388.2) | 55.4 (4.4-694.0) | 42.2 (3.5-496.2) | - |
| Point A dose:> 80 vs. < 80 | 6.98 (0.9-53.7) | NA** | - | - |
| KPS***:100 vs. 90 | 0.72 (0.54-0.97) | 2.4 (1.2-4.6) | - | - |
| Tumor size:> 4 cm vs. < 4 cm | 52.4 (2.4-1140.4) | 199.3 (4.8-8215.8) | - | - |
| Staging: IIIA-IIIB vs. IB2-IIB | 12.9 (1.6-101.5) | 24.6 (2.7-221.8) | - | - |
| Histology: Adenocarcinoma vs. Squamous cell carcinoma | - | - | - | 7.94 (0.70-90.4) |

*OTT: Overall Treatment Time

**NA: Not available

***KPS: Karnofsky Performance Status

9001 study which had 2 year OS 80% and 82%, respectively.[5, 6] Both studies included cervical cancer patients stage Ib2-IVa. In GOG85[7] and GOG120[8] studies they included only patients stage IIB–IVA, and the patients in the CCRT arm had a little lower OS of 72% and 75%, respectively.

**COX-2 expression and OS/ PFS**

As present knowledge, COX-2 expression is still controversial as a prognostic factor. Studies from Kim YB and Ferrandina G.[4,9-11] showed significantly worse prognosis factors for OS in multivariate analysis, although some other studies did not show COX-2 expression as a prognostic factor.[12-15]

In our study, univariate analysis showed no significance for decrease of OS, although it was significant in multivariate analysis. There were effects of confounding factors between variables when we adjusted to other prognostic factors, and COX-2 positive became a statistically significant poor prognostic factor. Our result, 2 yr, OS was 79% and 89% in COX-2 positive and negative groups, respectively, and the difference was less than the reported calculated sample size which could be the reason why it was non-significant in the univariate analysis.

The result of our studies showed COX-2 was an independent prognostic factor for OS which corresponded with previous positive trials.[4, 9-11] All of these trials were patients treated with CCRT, the same as in our study. Other negative trials were mostly treated by radiation or surgery alone. There is the trend toward more COX-2 expression effect in patients treated with CCRT. Recently, RTOG C0128 has published benefits of added Celecoxib in patients with high COX-2 expression.[16]

*For PFS analysis of COX-2 expression:* three studies reported that Disease Free Survival (DFS) had shown expression of COX-2 related to poor DFS.[4,9,12] In our study we analyzed PFS which showed statistically significant poor prognosis for PFS, similar to the OS result.

**Other prognostic factors and OS/ PFS**

In multivariate analysis, advanced stage, large tumor size and prolonged OTT were poor prognostic factors for overall survival. Large tumor size and advanced stage were poor prognosis for OS and PFS, which corresponded to data from SEER database.

**(Surveillance, Epidemiology, and End Results)** Result from Fyles AW[17], besides FIGO staging; point A dose and OTT were significant prognostic factors.

## COX-2 and DMFS/ LRRF

COX-2 expression affected DMFS and LRRFS, and it showed that COX-2 positive patients showed no statistical significant prognostic factor for both DMFS and LRRFS in both univariate and multivariate analyses in our study.

*For DMFS:* Ishikawa H,[14] studied COX-2 expression in 47 SCC (Squamous Cell Carcinoma) of cervical cancer patients which showed a decrease of DMFS in COX-2 positive patients, but it did not reach statistical significance. Our study also showed a trend of decrease in DMFS in patients with COX-2 positive, but it did not showany statistical significance in both univariate and multivariate analysis.

*For LRRFS*: three studies[4,11,14] found that patients with COX-2 positive had more local failure rate than COX-2 negative groups. Surprisingly in our study, patients with COX-2 positive tended to have better LRRFS than patients with COX-2 negative. This could be due to high incidence of LRR in the COX-2 negative group.

## Clinical application

Prolonged treatment time more than 55 days was a strong prognostic factor for OS, PFS and DMFS in our study. In our practice, we will concern about this point to provide better care for our patients. As molecular tumor marker, COX-2 expression is a prognostic factor for locally advanced cervical cancer.

## REFERENCES


1. Chen RJ, Lin YH, Chen CA, Huang SC, Chow SN, Hsieh CY. Influence of histologic type and age on survival rates for invasive cervical carcinoma in Taiwan. Gynecol Oncol. 1999 May;73(2):184-90.
2. Vinh-Hung V, Bourgain C, Vlastos G, Cserni G, De Ridder M, Storme G, et al. Prognostic value of histopathology and trends in cervical cancer: a SEER population study. BMC Cancer. 2007;7:164.
3. del Campo JM, Prat A, Gil-Moreno A, Perez J, Parera M. Update on novel therapeutic agents for cervical cancer. Gynecol Oncol. 2008 Sep;110(3 Suppl 2):S72-6.
4. Kim YB, Kim GE, Cho NH, Pyo HR, Shim SJ, Chang SK, et al. Overexpression of cyclooxygenase-2 is associated with a poor prognosis in patients with squamous cell carcinoma of the uterine cervix treated with radiation and concurrent chemotherapy. Cancer. 2002 Aug 1;95(3):531-9.
5. Pearcey R, Brundage M, Drouin P, Jeffrey J, Johnston D, Lukka H, et al. Phase III trial comparing radical radio therapy with and without cisplatin chemotherapy in patients with advanced squamous cell cancer of the cervix. J Clin Oncol. 2002 Feb 15;20(4):966-72.
6. Eifel PJ, Winter K, Morris M, Levenback C, Grigsby PW, Cooper J, et al. Pelvic irradiation with concurrent chemotherapy versus pelvic and para-aortic irradiation for high-risk cervical cancer: an update of radiation therapy oncology group trial (RTOG) 90-01. J Clin Oncol. 2004 Mar 1;22(5):872-80.
7. Whitney CW, Sause W, Bundy BN, Malfetano JH, Hannigan EV, Fowler WC, Jr., et al. Randomized comparison of fluorouracil plus cisplatin versus hydroxyurea as an adjunct to radiation therapy in stage IIB-IVA carcinoma of the cervix with negative para-aortic lymph nodes: a Gynecologic Oncology Group and Southwest Oncology Group study. J Clin Oncol. 1999 May;17(5):1339-48.
8. Rose PG, Bundy BN, Watkins EB, Thigpen JT, Deppe G, Maiman MA, et al. Concurrent cisplatin-based radiotherapy and chemotherapy for locally advanced cervical cancer. N Engl J Med. 1999 Apr 15;340(15):1144-53.
9. Kim YB, Kim GE, Pyo HR, Cho NH, Keum KC, Lee CG, et al. Differential cyclooxygenase-2 expression in squamous cell carcinoma and adenocarcinoma of the uterine cervix. Int J Radiat Oncol Biol Phys. 2004 Nov 1;60(3):822-9.
10. Ferrandina G, Ranelletti FO, Legge F, Lauriola L, Poerio A, Zannoni GF, et al. Cyclooxygenase-2 (COX-2) expression in locally advanced cervical cancer patients undergoing chemoradiation plus surgery. Int J Radiat Oncol Biol Phys. 2003 Jan 1;55(1):21-7.
11. Ferrandina G, Ranelletti FO, Legge F, Gessi M, Salutari V, Distefano MG, et al. Prognostic role of the ratio between cyclooxygenase-2 in tumor and stroma compartments in cervical cancer. Clin Cancer Res. 2004 May 1;10(9):3117-23.
12. Gaffney DK, Haslam D, Tsodikov A, Hammond E, Seaman J, Holden J, et al. Epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) negatively affect overall survival in carcinoma of the cervix treated with radiotherapy. Int J Radiat Oncol Biol Phys. 2003 Jul 15;56(4):922-8.
13. Athavale R, Clooney K, O'Hagan J, Shawki H, Clark AH, Green JA. COX-1 and COX-2 expression in stage I and II invasive cervical carcinoma: relationship to disease relapse and long-term survival. Int J Gynecol Cancer. 2006 May-Jun;16(3):1303-8.
14. Ishikawa H, Ohno T, Kato S, Wakatsuki M, Iwakawa M, Ohta T, et al. Cyclooxygenase-2 impairs treatment effects of radiotherapy for cervical cancer by inhibition of radiation-induced apoptosis. Int J Radiat Oncol Biol Phys. 2006 Dec 1;66(5):1347-55.
15. Tangjitgamol S, Ramirez PT, Sun CC, See HT, Jhingran A, Kavanagh JJ, et al. Expression of HER-2/neu, epidermal growth factor receptor, vascular endothelial growth factor,

cyclooxygenase-2, estrogen receptor, and progesterone receptor in small cell and large cell neuroendocrine carcinoma of the uterine cervix: a clinicopathologic and prognostic study. Int J Gynecol Cancer. 2005 Jul-Aug;15(4): 646-56.

16. Doll CM, Winter K, Gaffney DK, Ryu JK, Jhingran A, Dicker AP, et al. COX-2 expression and survival in patients with locally advanced cervical cancer treated with chemoradiotherapy and celecoxib: a quantitative immunohistochemical analysis of RTOG C0128. Int J Gynecol Cancer. 2013 Jan;23(1):176-83.
17. Fyles AW, Pintilie M, Kirkbride P, Levin W, Manchul LA, Rawlings GA. Prognostic factors in patients with cervix cancer treated by radiation therapy: results of a multiple regression analysis. Radiother Oncol. 1995 May; 35(2):107-17. P