# Cyclooxygenase-2 Is an Independent Prognostic Factor in Gastric Carcinoma Patients Receiving Adjuvant Chemotherapy and Is Not Associated with EBV Infection

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**Abstract**

**Purpose:** Cyclooxygenase-2 (COX-2) is believed to be involved in carcinogenesis in patients with chronic gastritis with *Helicobacter pylori* infection. EBV is detected in ~10% of gastric carcinomas and *H. pylori* induces EBV reactivation in the gastric epithelium. We aimed to evaluate significance of COX-2 in gastric carcinoma occurred in EBV and *H. pylori* prevalent area.

**Experimental Design:** Tissue microarray samples from 457 gastric carcinoma patients who underwent gastrectomy and adjuvant chemotherapy were studied with EBER1 *in situ* hybridization for EBV and immunohistochemistry for COX-2 and other gastric carcinoma-related proteins (hMLH1, E-cadherin, c-erbB, and cyclin D1).

**Results:** EBV infection was observed in 10.9% of gastric carcinomas and was associated with proximal tumor location, increased numbers of lymph node, and E-cadherin expression ($P < 0.01$). COX-2 overexpression was closely associated with intestinal histologic type and lower tumor stage ($P = 0.01$). Univariate analysis showed that pT, pN, lymph node ratio, American Joint Committee on Cancer stage, numbers of negative lymph nodes, and resection margin <1 cm were significant prognostic factors. The Cox proportional hazards regression analysis indicated that lack of COX-2 expression and resection margin <1 cm were independent prognostic factors for disease-free survival ($P = 0.008$ and 0.03, respectively) and overall survival ($P = 0.01$ and 0.007, respectively).

**Conclusions:** EBV infection is not associated with COX-2 expression or survival in gastric carcinoma. Lack of COX-2 expression is an independent prognostic factor in both overall and disease-free survival in gastric carcinoma. Our results indicate that COX-2 may play a role in the progression of gastric carcinoma regardless of EBV infection and is closely associated with histologic differentiation and prognosis.

EBV is detected in ~10% of gastric carcinomas, and the association between EBV infection and gastric carcinoma is well established. EBV-associated gastric carcinoma is morphologically characterized as lymphoepithelioma-like carcinoma. It tends to be proximally located, is of diffuse histologic subtype, tends to have lower frequency of lymph node metastasis (1), and often manifests aberrant methylation of multiple genes (2). However, the pathogenic role of EBV in gastric carcinogenesis remains to be elucidated.

Cyclooxygenase-2 (COX-2) is an inducible enzyme and produces prostaglandins in response to various inflammatory stimuli or growth factors (3). COX-2 is frequently undetectable in normal tissue, but it is inducible by cytokines, growth factors, reactive oxygen species, and chemical carcinogens in inflamed or malignant tissues (4). Overexpression of COX-2 has been detected in various human cancers including gastric carcinoma, and it has been suggested that the presence of COX-2 contributes to carcinogenetic mechanisms such as angiogenesis (5), inhibition of apoptosis (6), and invasiveness (7). Chronic atrophic gastritis, principally caused by chronic *Helicobacter pylori* infection, progresses to intestinal metaplasia, dysplasia, and carcinoma (8). In those precursor lesions, expression of COX-2 was detected (9) due to increased COX-2 promoter activity (10). Increased expression of COX-2 has been reported in gastric carcinomas with variable frequencies, but expression is more frequently observed in intestinal-type carcinomas and earlier stages (11–14). However, Mrena et al. (15) and Murata et al. (7) reported that COX-2 expression is associated with poor prognosis and advanced disease stage. Recently, de Maat et al. (16) reported hypermethylation of COX-2 gene promoter and consequent lower expression of COX-2 as an independent prognostic factor in gastric carcinoma patients. Taken together, these observations suggest that COX-2 is involved early in gastric

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### Translational Relevance

With this large-scale study, we newly found that EBV infection is not associated with COX-2 expression or survival in 457 gastric carcinoma patients who underwent gastrectomy and adjuvant chemotherapy. In addition to pN stage and resection margin <1 cm, lack of COX-2 expression is an independent prognostic factor in both overall and disease-free survival. Our results will be applied to the future practice of cancer medicine by easy and quick application of those significant prognostic factors to gastric carcinoma patients.

carcinogenesis and expression of COX-2 can be altered during tumor progression by epigenetic silencing.

EBV is detected in gastric carcinomas worldwide with similar frequency, but *H. pylori* is more frequently found in populations at high risk for gastric carcinoma. Moreover, EBV reactivation in gastric epithelium by *H. pylori* was shown recently (17). Nevertheless, there are insufficient data to show that EBV infection is directly involved in gastric carcinomas. As EBV-associated gastric carcinomas are frequently rich in lymphoid stroma, we hypothesized that EBV infection may promote gastric carcinogenesis by provoking COX-2 activation. Therefore, we studied EBER *in situ* hybridization and COX-2 expression in 457 gastric carcinoma samples, determined correlation with other tumor-related proteins and clinicopathologic variables, and further explored the prognostic role of COX-2 in patients receiving postoperative adjuvant chemotherapy.

## Materials and Methods

***Case selection and tissue microarray.*** The previously reported 467 stage IB to IV ($M_0$) gastric carcinoma patients, all of whom had undergone adjuvant chemotherapy with 5-fluorouracil and leucovorin followed by curative surgery at Samsung Medical Center between 1995 and 2001, were selected for this study (18). Ten samples lost during immunohistochemistry were excluded. The postoperative adjuvant treatment adopted was the same as that used for the INT-0116 (SWOG-9008) trial and the results were reported previously (19). A total of 232 (50.8%) patients received adjuvant radiotherapy. The expression of COX-2 was analyzed in all patients. All patients provided written informed consent according to institutional guidelines and the study was approved by the institutional review board.

Male-to-female ratio was 306:151. The median age was 52 years (range, 23-70). By Lauren classification, 29.8% of gastric carcinomas were intestinal in histologic type. All patients received D2 or greater lymph node (LN) dissection. The mean number of total lymph nodes found was 44 (range, 10-103; median, 49), and the mean number of metastatic nodes was 9.3 (range, 1-82; median, 6). After a mean follow-up duration of 88.6 months [95% confidence interval (95% CI), 83.7-93.4], the 5-year overall survival rate was determined to be 61.9%,

**Table 1.** Correlation between COX-2 expression and clinicopathologic factors

| Variable | *n* | Lack of COX-2 expression, *n* (%) | Overexpression of COX-2, *n* (%) | *P* |
|---|---|---|---|---|
| Sex | | | | |
| Male (*n* = 306) | 306 | 59 (19) | 247 (81) | 0.47 |
| Female (*n* = 151) | 151 | 28 (19) | 123 (81) | |
| Age (y) | | | | |
| <60 (*n* = 340) | 340 | 71 (21) | 269 (79) | 0.05 |
| ≥60 (*n* = 117) | 117 | 16 (14) | 101 (86) | |
| Differentiation | | | | |
| Differentiated (*n* = 128) | 128 | 8 (6) | 120 (94) | <0.0001 |
| Poorly differentiated (*n* = 243) | 243 | 37 (15) | 206 (85) | |
| Signet-ring cell (*n* = 60) | 60 | 24 (40) | 36 (60) | |
| Mucinous (*n* = 26) | 26 | 18 (69) | 8 (31) | |
| Lauren | | | | |
| Intestinal (*n* = 136) | 136 | 10 (7) | 126 (93) | <0.0001 |
| Diffuse (*n* = 321) | 321 | 77 (24) | 244 (76) | |
| Lymphovascular invasion | | | | |
| No (*n* = 21) | 21 | 1 (5) | 20 (95) | 0.06 |
| Yes (*n* = 436) | 436 | 86 (20) | 350 (80) | |
| T stage | | | | |
| 1 | 14 | 0 (0) | 14 (100) | 0.12 |
| 2 | 307 | 55 (18) | 252 (82) | |
| 3 | 123 | 28 (23) | 95 (77) | |
| 4 | 13 | 4 (31) | 9 (69) | |
| N stage | | | | |
| 0 | 29 | 3 (10) | 26 (90) | 0.05 |
| 1 | 201 | 33 (16) | 168 (84) | |
| 2 | 140 | 37 (26) | 103 (74) | |
| 3 | 87 | 14 (16) | 73 (84) | |
| AJCC stage | | | | |
| I | 22 | 0 (0) | 22 (100) | 0.01 |
| II | 162 | 25 (15) | 137 (85) | |
| IIIA | 151 | 36 (24) | 115 (76) | |
| IIIB | 28 | 9 (32) | 19 (68) | |
| IV | 94 | 17 (18) | 77 (82) | |

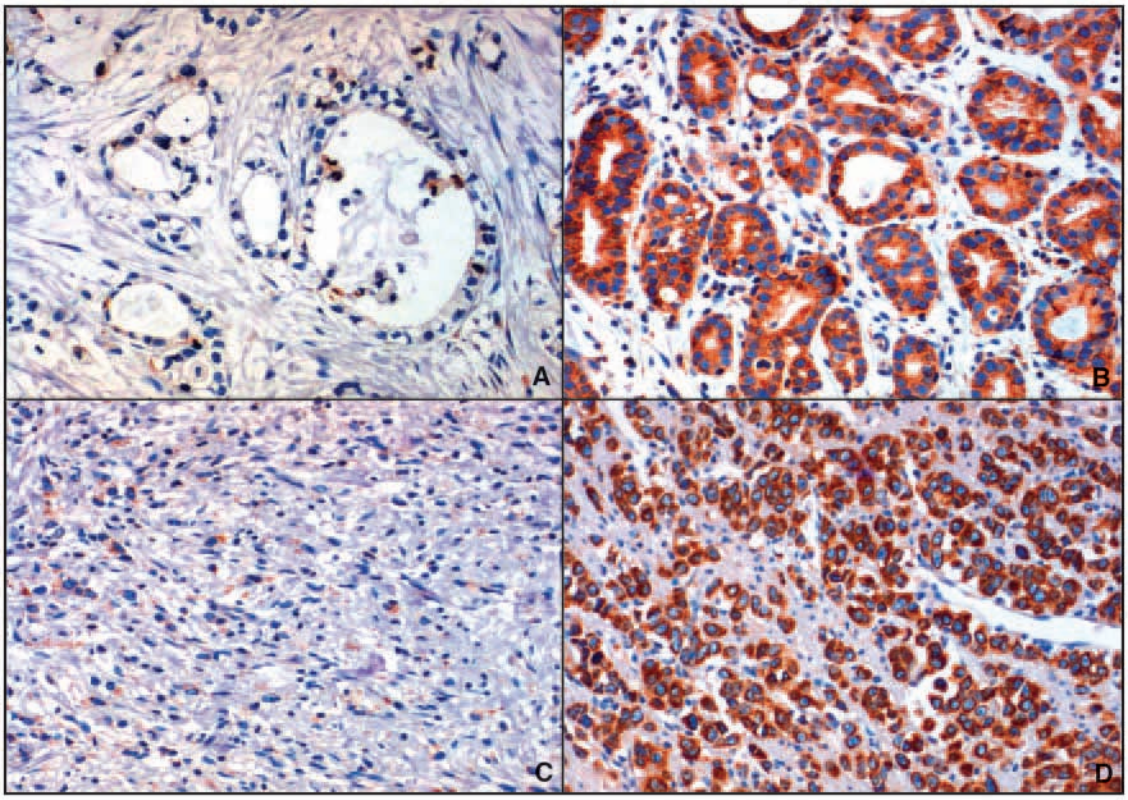


**Fig. 1.** COX-2 expression by Lauren classification. *A,* intestinal-type, negative. *B,* intestinal-type, positive. *C,* diffuse-type, negative. *D,* diffuse-type, positive.

and the 5-year disease-free survival rate was determined to be 60.7%. At the time of analysis, 270 (59.1%) patients were dead and 187 (41.2%) patients were alive. For precise analyses of lymph nodes, in addition to conventional pN stages, numbers of negative lymph nodes were measured and scored as 0 to 23 ($n$ = 117), 24 to 31 ($n$ = 111), 32 to 44 ($n$ = 111), or ≥45 ($n$ = 118). Moreover, the metastatic lymph node ratio (LNR), as determined by dividing the total positive lymph nodes by the total examined lymph nodes, was also divided into four groups based on quartiles: LNR <0.05 ($n$ = 101), 0.05 to 0.19 ($n$ = 164), 0.2 to 0.39 ($n$ = 118), or 0.4 to 1.0 ($n$ = 74) as described previously (20).

***Tissue microarrays.*** All H&E-stained slides were reviewed and representative tumor tissue samples were selected for each case. The corresponding formalin-fixed paraffin-embedded tissue blocks were retrieved. The selected area was circled on the slide with a marker pen for tissue microarray construction. Each 2.0 mm tissue core was taken from the representative region of each paraffin block using the AccuMax (Isu Abxis). Eleven tissue microarrays were constructed, and each tissue microarray contained 45 carcinoma sample cores and 3 nontumor controls.

***EBER* in situ *hybridization.*** Tissue microarray blocks were sectioned at 4 μm thick, digested with proteinase K, dehydrated in 95% ethanol, and dried. Hybridization was done using FITC-labeled EBER1 and EBER2 oligonucleotide probes (Novocastra) complementary to the mRNA portion of the *EBER1* and *EBER2* genes (21). Following the incubation with anti-FITC antibody tagged with alkaline phosphatase, slides were covered with 5-bromo-4-chloro-3-indolyl phosphatase, nitroblue tetrazolium, and 1 mol/L levamisole. Only those cases with strong signals within tumor cell nuclei were considered to be positive.

***Immunohistochemistry.*** Immunohistochemical study was done using the streptavidin-biotin complex method and Tech Mate 1000 automated staining system (DakoChemmate) with COX-2 (1:100; Cayman Chemical), hMLH1 (1:50; clone G168-15; Pharmingen/BD Biosciences), E-cadherin (1:100; Santa Cruz Biotechnology), c-erbB (1:250; Santa Cruz Biotechnology), and cyclin D1 (1:100; DCS-6; Oncogene Research Product) monoclonal antibodies as described previously (18). Staining for COX-2 was considered to be positive when the cytoplasm of ≥50% of the carcinoma cells stained with strong intensity. Staining for E-cadherin, hMLH-1, c-erbB-2, and cyclin D1 were considered positive when tumor cells showed nuclear or cytoplasmic reactivity.

***Statistical analysis.*** Disease-free survival was defined as the time from surgery to the first relapse of cancer, occurrence of a second primary tumor, or death from any cause. Overall survival was measured from the date of surgery to the date of death. Overall survival and disease-free survival were calculated using the Kaplan-Meier method. The log-rank test was used to identify differences between the survival curves of different patient groups. The following independent variables were analyzed: age (<60 versus ≥60 years), sex (male versus female), tumor location (top third versus middle third versus bottom third), tumor stage ($T_1$ versus $T_2$ versus $T_3$ versus $T_4$), histology (differentiated tubular versus undifferentiated tubular versus signet-ring cell versus mucinous carcinoma), Lauren type (intestinal versus diffuse), number of metastatic lymph nodes ($N_0$ versus $N_1$ versus $N_2$ versus $N_3$), LNR (0-0.049 versus 0.05-0.19 versus 0.2-0.39 versus >0.4), radiation therapy (yes versus no), and resection margin (<1 versus ≥1 cm). Analysis of correlation of the expression of COX-2 or EBV with clinicopathologic variables or tumor-related proteins was done using the two-sided $\chi^2$ test, Fisher's exact test, or Breslow test with Bonferroni adjustment. Differences in disease-free and overall survival were analyzed using the log-rank or Breslow test. Multivariate survival analysis was done by Cox's proportional hazards model. Statistical analyses and graphics were obtained using SPSS software (SPSS), and probability values <0.05 were considered statistically significant.

**Table 2.** Disease-free and overall survival by univariate analysis according to clinicopathologic factors

| | Disease-free survival | | Overall survival | |
|---|---|---|---|---|
| | Mean | *P* | Mean | *P* |
| Sex | | | | |
| Male | 87.88 | 0.09 | 91.73 | 0.07 |
| Female | 78.31 | | 81.70 | |
| Age | | | | |
| <60 | 85.16 | 0.91 | 89.28 | 0.63 |
| ≥60 | 82.67 | | 85.04 | |
| Location of tumor | | | | |
| Bottom 1/3 | 89.17 | 0.05 | 92.44 | 0.17 |
| Middle 1/3 | 81.59 | | 84.58 | |
| Top 1/3 | 71.82 | | 81.83 | |
| Lauren classification | | | | |
| Intestinal | 90.78 | 0.09 | 96.31 | 0.03 |
| Diffuse | 82.11 | | 84.97 | |
| Histology | | | | |
| Differentiated | 91.81 | 0.08 | 93.23 | 0.05 |
| Undifferentiated | 89.87 | | 95.61 | |
| Signet-ring | 83.75 | | 86.02 | |
| Mucinous | 71.49 | | 76.44 | |
| COX-2 expression | | | | |
| Positive | 86.90 | 0.11 | 90.40 | 0.11 |
| Negative | 75.40 | | 79.76 | |
| EBV | | | | |
| Positive | 83.51 | 0.80 | 84.50 | 0.56 |
| Negative | 84.50 | | 88.45 | |
| C-erbB | | | | |
| Positive | 92.82 | 0.11 | 97.02 | 0.08 |
| Negative | 83.24 | | 87.81 | |
| Cyclin D1 | | | | |
| Positive | 88.79 | 0.33 | 90.41 | 0.40 |
| Negative | 84.44 | | 88.21 | |
| E-cadherin | | | | |
| Positive | 86.75 | 0.25 | 90.53 | 0.16 |
| Negative | 79.55 | | 82.89 | |
| hMLH1 | | | | |
| Negative | 84.48 | 0.87 | 87.77 | 0.67 |
| Positive | 84.86 | | 88.94 | |

## Results

***Association of COX-2 with clinicopathologic variables in gastric carcinomas.*** Diffuse strong cytoplasmic COX-2 overexpression was observed in 370 of 457 (80.9%) gastric carcinomas. Comparisons between cytoplasmic COX-2 expression and clinicopathologic variables are summarized in Table 1. COX-2 expression was more frequently observed in cases with intestinal-type (93%) than diffuse-type histology (76%; $P < 0.0001$; Fig. 1). Cytoplasmic COX-2 was present in 94%, 85%, 60%, and 31% of differentiated and undifferentiated tubular adenocarcinomas, signet-ring cell carcinomas, and mucinous carcinomas, respectively ($P < 0.0001$). Positive expression of COX-2 correlated significantly with American Joint Committee on Cancer (AJCC) tumor stage ($P = 0.01$). However, COX-2 expression was not related to EBV infection or other clinicopathologic factors.

***Association of EBV infection with clinicopathologic variables in gastric carcinomas.*** EBV infection was observed in 50 of 457 (10.9%) gastric carcinomas. EBV infection was closely related to proximal tumor location ($P < 0.0001$), increased numbers of total lymph nodes (mean, 51.1 versus 43.5; $P = 0.005$), and increased numbers of negative lymph nodes (mean, 40.8 versus 34.3; $P = 0.01$). However, EBV infection was not related to numbers of positive nodes, expression of hMLH1, cyclin D1, or other clinicopathologic variables ($P > 0.05$). Neither the patients' overall or disease-free survival was associated with EBV infection or expression of gastric tumor-related proteins.

***Univariate and multivariate survival analyses.*** The mean survival times in association with clinicopathologic factors, COX-2, or tumor-related protein expression as well as EBV results are summarized in Table 2. On univariate analysis, overall survival was associated with pT stage, pN stage, LNR, and AJCC stage ($P < 0.0001$) and numbers of negative lymph nodes ($P = 0.004$; Fig. 2). The disease-free survival was associated with numbers of negative lymph nodes ($P = 0.01$), LNR, pT stage, pN stage, and AJCC stage ($P < 0.0001$; Fig. 3). Location of carcinoma showed borderline significance in relation to disease-free survival ($P = 0.05$).

Although COX-2 expression [hazard ratio (HR), 1.319; 95% CI, 0.935-1.859; $P = 0.11$] did not show a significant relation with survival in univariate analysis, it was also tested in multivariate Cox regression analysis. In multivariate analysis, lack of COX-2 expression (HR, 1.678; 95% CI, 1.110-2.538; $P = 0.01$) and resection margin <1 cm (HR, 1.829; 95% CI, 1.177-2.841; $P = 0.001$) were independent prognostic factors for overall survival. Lack of COX-2 expression (HR, 1.749; 95% CI, 1.157-2.643; $P = 0.008$), resection margin <1 cm (HR, 1.626; 95% CI, 1.045-2.530; $P = 0.03$), and pT stage ($P = 0.03$) remained significant predictors

**Table 2.** Disease-free and overall survival by univariate analysis according to clinicopathologic factors (Cont'd)

| | Disease-free survival | | Overall survival | |
|---|---|---|---|---|
| | **Mean** | ***P*** | **Mean** | ***P*** |
| Lymphovascular invasion | | | | |
| Yes | 84.60 | 0.64 | 88.26 | 0.65 |
| No | 86.71 | | 90.36 | |
| Perineural invasion | | | | |
| Yes | 82.48 | 0.40 | 87.38 | 0.59 |
| No | 85.68 | | 88.64 | |
| T stage | | | | |
| II | 91.59 | <0.0001 | 95.50 | <0.0001 |
| III | 67.03 | | 69.89 | |
| IV | 35.31 | | 43.69 | |
| N stage | | | | |
| $N_0$ | 86.90 | <0.0001 | 90.65 | <0.0001 |
| $N_1$ | 99.49 | | 102.91 | |
| $N_2$ | 87.03 | | 90.66 | |
| $N_3$ | 36.51 | | 41.41 | |
| Negative nodes (no.) | | | | |
| 0-23 | 71.77 | 0.01 | 74.89 | 0.004 |
| 24-31 | 84.78 | | 88.57 | |
| 32-44 | 97.31 | | 100.50 | |
| >45 | 81.77 | | 86.75 | |
| LNR (positive/examined nodes) | | | | |
| 0-0.049 | 95.99 | <0.0001 | 101.56 | <0.0001 |
| 0.05-0.19 | 92.43 | | 95.07 | |
| 0.2-0.39 | 81.81 | | 85.11 | |
| >0.4 | 38.87 | | 44.32 | |
| AJCC stage | | | | |
| II | 97.82 | <0.0001 | 101.83 | <0.0001 |
| IIIA | 93.18 | | 96.28 | |
| IIIB | 57.29 | | 62.46 | |
| IV | 40.03 | | 44.64 | |
| Radiation therapy | | | | |
| Yes | 76.03 | 0.35 | 81.21 | 0.70 |
| No | 87.26 | | 89.38 | |
| Resection margin (cm) | | | | |
| ≥1 | 86.96 | 0.01 | 90.81 | 0.01 |
| <1 | 62.94 | | 65.37 | |

of recurrence in patients with gastric carcinoma who had received curative surgery and adjuvant chemotherapy (Table 3).

## Discussion

In this large-scale study of COX-2 in gastric carcinoma patient status post-adjuvant chemotherapy, we observed some interesting results. COX-2 was strongly expressed in 80.9% of gastric carcinomas and proven to be an independent prognostic factor in gastric carcinoma. Moreover, it was closely associated with important clinicopathologic factors: histologic type, lymph node metastases, and AJCC stage. This result is consistent with previous studies (15, 16). The current study also confirmed that positive expression of COX-2 was more frequently found in intestinal-type carcinomas and earlier pT and pN stages (11–14). Of the two main histologic types (intestinal and diffuse) of gastric carcinoma, tumorigenesis of the intestinal type is better understood; it is thought to be governed by environmental factors and is preceded by a stepwise precancerous process (22). Previous studies have shown that precursor lesions of intestinal-type carcinoma showed expression of COX-2 (12, 13, 23). These observations strongly support that COX-2 is involved early in the carcinogenesis of intestinal-type gastric cancer.

In ordinary adenocarcinoma, EBV has been detected in 6.9% to 16% of tumors (24), and our study results fell within this range. Based on the proposed hypothesis that EBV is involved in gastric carcinogenesis, we expected to observe an association between EBV infection and other tumor-related proteins or other clinicopathologic variables. However, EBV infection showed no significant clinicopathologic correlation, except for proximal tumor location and E-cadherin expression. Proximal tumor location in EBV-associated gastric carcinomas is consistent with previous studies (25). Alteration of expression of E-cadherin by promoter methylation-mediated silencing was well established and closely associated with the development of EBV-associated gastric carcinomas, although it becomes heterogeneous within a given tumor along its progression (26).

In this single institutional study, we found that, in EBV-associated gastric carcinomas, the numbers of total lymph nodes and negative lymph nodes were significantly increased. As we were observing ordinary adenocarcinoma instead of specific lymphoepithelioma-like carcinoma, this observation was surprising and strongly suggests that EBV infection may incite systemic immunologic reaction in addition to localized attraction of lymphocytes around tumors. Although we failed to show the relationship between EBV infection and low frequency of lymph node metastasis and better prognosis found

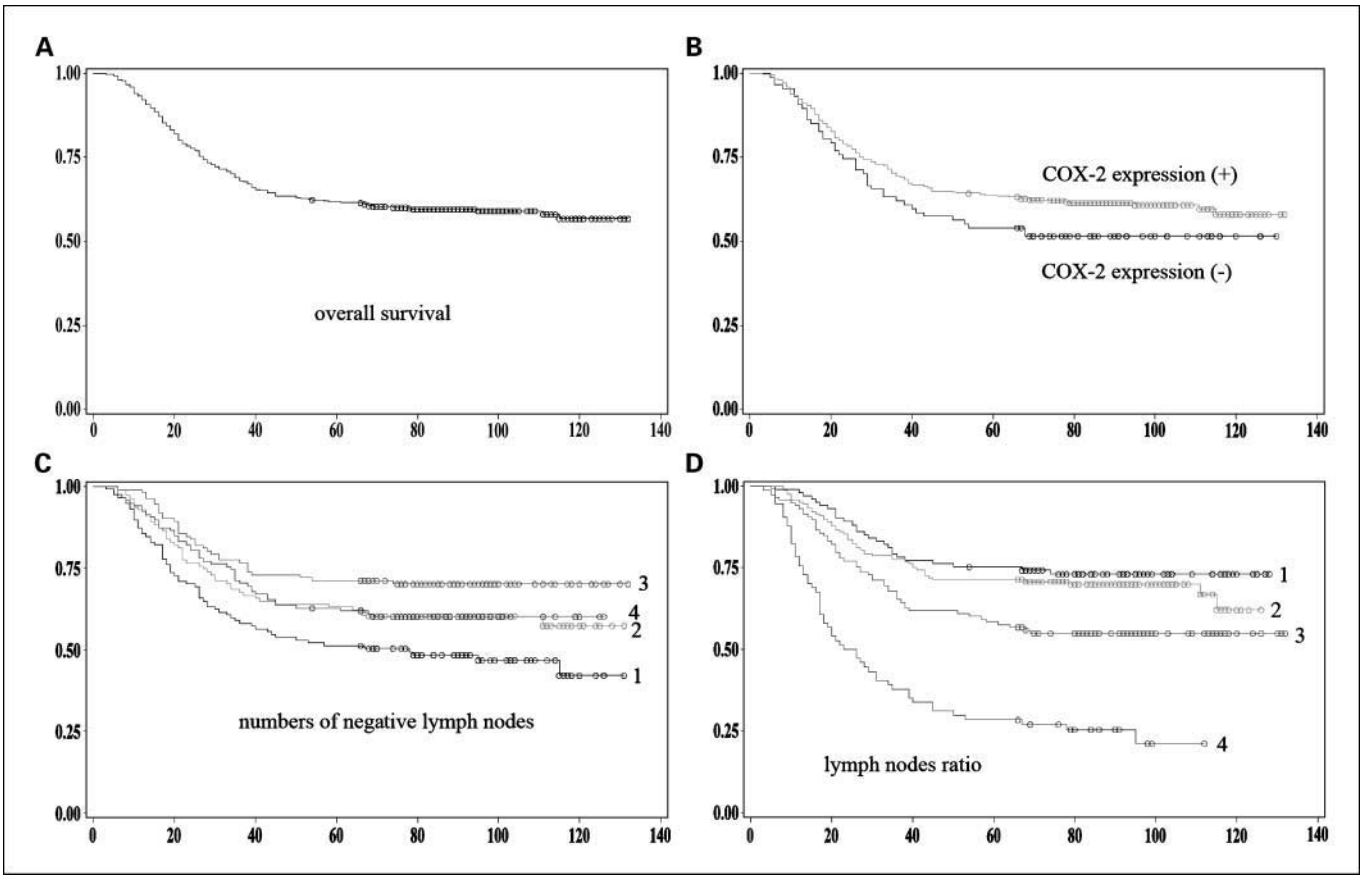


**Fig. 2.** Overall survival by (*A*) overall survival rate, (*B*) COX-2 expression, (*C*) numbers of negative nodes (1, 0-23; 2, 24-31; 3, 32-44; 4, ≥45), and (*D*) LNR (1, 0-0.049; 2, 0.05-0.19; 3, 0.2-0.39; 4, 0.4-1).

in the Dutch trial (25), our results suggest that increased numbers of total lymph nodes found in EBV-related gastric adenocarcinomas do not affect survival in patients with gastric carcinoma. These observations are consistent with previous observations (27–29). Lymph node metastasis is one of the most important factors in determining the prognosis of gastric carcinoma (30). In recent years, based on data from other malignancies, the number of lymph nodes evaluated and the LNR have been highlighted as important predictors of survival (31, 32). However, LNR has rarely been investigated on a large-scale in gastric carcinoma patients in the era of adjuvant chemotherapy and was a multicenter study with different modalities of lymphadenectomy (33). In this large-scale, single institutional study in a unified D2 lymphadenectomy as an adjunct to gastrectomy done in all gastric carcinoma patients, we found that, although LNR, numbers of negative lymph nodes, and pN stage affected overall and disease-free survival on univariate analyses, they have limited effects on both overall and disease-free survival on multivariate analysis. Rather, pT stage significantly affected patients' disease-free survival.

Moreover, the present study has proven that lack of COX-2 expression and resection margin <1 cm are important prognostic factors in curatively resected gastric carcinoma patients with stage IB to IV disease who have undergone adjuvant chemotherapy. In our analysis, COX-2 expression did not significantly influence on survival in univariate analysis, although it was significant in multivariate analysis. One of the plausible explanations for this discrepancy may be due to potential confounding factors. In an attempt to identify confounding variables, we performed further statistical analysis and found that N stage was one of the probable confounders. However, N stage was included in multivariate analysis where COX-2 expression retained its statistical significance. The prognostic significance of COX-2 found in this study is consistent with recent observations by de Maat et al. (16). Although we need to identify the interaction between COX-2 and other tumor-related proteins, COX-2 may serve as a useful biomarker to predict prognosis in gastric carcinoma, and further study may help developing new therapies for intestinal-type gastric carcinoma.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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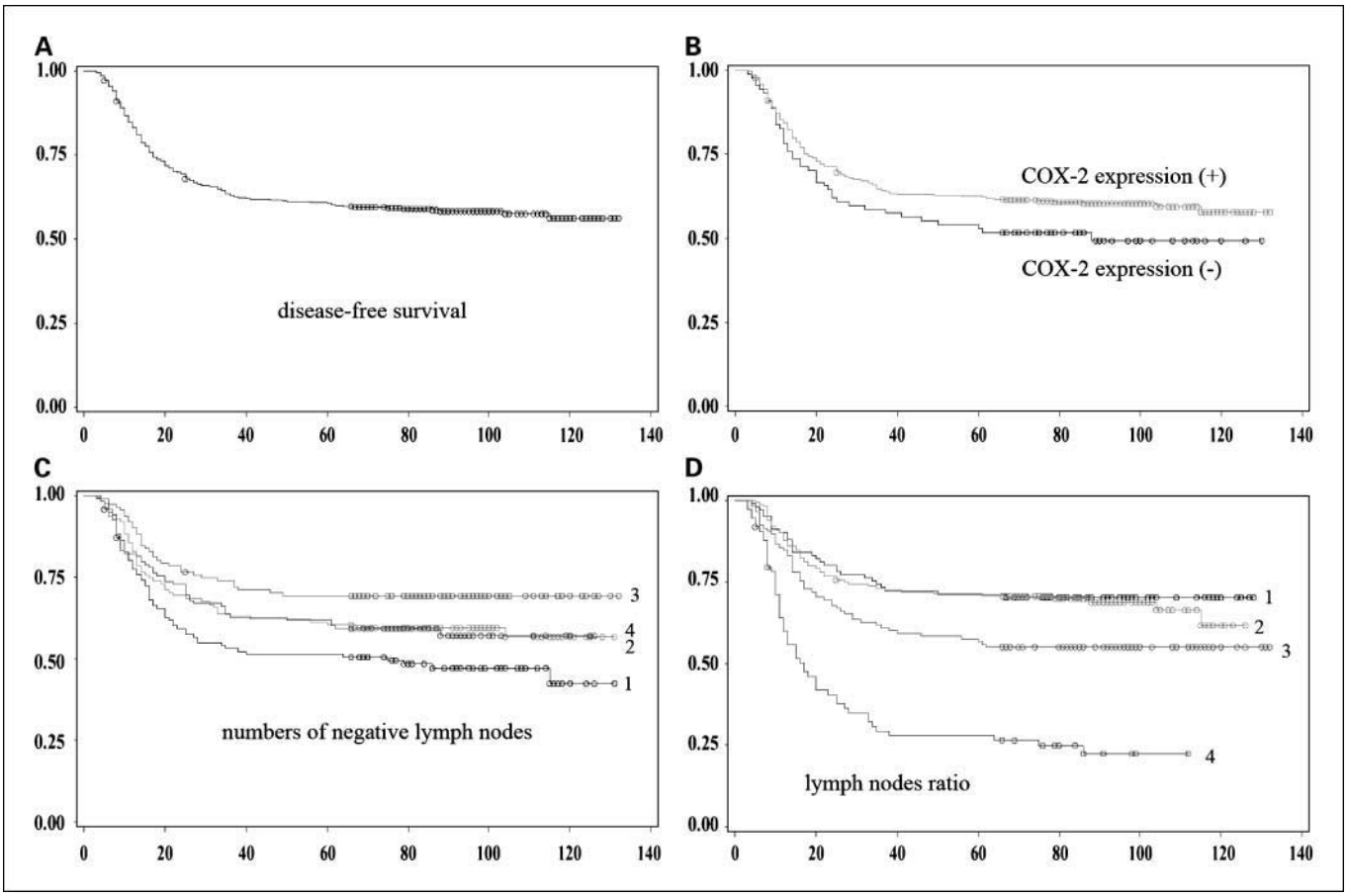


**Fig. 3.** Disease-free survival by (*A*) disease-free survival rate, (*B*) COX-2 expression, (*C*) numbers of negative lymph nodes (1, 0-23; 2, 24-31; 3, 32-44; 4, ≥45), and (*D*) LNR (1, 0-0.049; 2, 0.05-0.19; 3, 0.2-0.39; 4, 0.4-1).


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**Table 3.** Prognostic factors retained at multivariate analysis in 457 patients with gastric carcinoma

| | Disease-free survival | | Overall survival | |
|---|---|---|---|---|
| | HR (95% CI) | *P* | HR (95% CI) | *P* |
| Sex | | | | |
| Male | 1 | 0.18 | 1 | 0.22 |
| Female | 1.241 (0.899-1.714) | | 1.22 (0.886-1.685) | |
| Age (y) | | | | |
| <60 | 1 | 0.49 | 1 | 0.29 |
| ≥60 | 1.131 (0.797-1.605) | | 1.205 (0.850-1.710) | |
| Location of tumor | | | | |
| Middle 1/3 | 1 | 0.60 | 1 | 0.75 |
| Top 1/3 | 1.024 (0.704-1.489) | 1.00 | 1.01 (0.693-1.472 | 1.00 |
| Bottom 1/3 | 1.335 (0.702-2.540) | 0.62 | 0.956 (0.485-1.885) | 1.00 |
| Lauren classification | | | | |
| Intestinal | 1 | 0.48 | 1 | 0.45 |
| Diffuse | 1.704 (0.378-7.679) | | 1.781 (0.394-8.053) | |
| Histology | | | | |
| Differentiated | 1 | 0.49 | 1 | 0.77 |
| Undifferentiated | 2.651 (0.341-20.628) | 0.77 | 2.216 (0.270-18.180) | 1.00 |
| Signet-ring | 1.718 (0.424-6.952) | 1.00 | 1.626 (0.399-6.628) | 1.00 |
| Mucinous | 2.204 (0.499-9.721) | 0.61 | 1.735 (0.383-7.859) | 1.00 |
| COX-2 expression | | | | |
| Positive | 1 | 0.008 | 1 | 0.01 |
| Negative | 1.749 (1.157-2.643) | | 1.678 (1.110-2.538) | |
| EBV | | | | |
| Positive | 1 | 0.44 | 1 | 0.20 |
| Negative | 1.21 (0.741-1.975) | | 1.365 (0.846-2.200) | |
| E-cadherin | | | | |
| Positive | 1 | 0.56 | 1 | 0.75 |
| Negative | 0.896 (0.622-1.292) | | 0.941 (0.649-1.362) | |
| Lymphovascular invasion | | | | |
| Yes | 1 | 0.63 | 1 | 0.49 |
| No | 0.803 (0.328-1.964) | | 0.73 (0.298-1.791) | |
| Perineural invasion | | | | |
| Yes | 1 | 0.61 | 1 | 0.97 |
| No | 0.92 (0.796-1.479) | | 0.99 (0.733-1.379) | |
| T stage | | | | |
| ≤II | 1 | 0.03 | 1 | 0.12 |
| III | 1.520 (0.895-2.579) | 0.15 | 1.511 (0.891-2.565) | 0.16 |
| IV | 3.545 (1.236-10.169) | 0.01 | 2.292 (0.831-6.330) | 0.13 |
| N stage | | | | |
| $N_3$ | 1 | 0.43 | 1 | 0.44 |
| $N_0$ | 0.843 (0.131-5.430) | 1.00 | 1.142 (0.178-7.338) | 1.00 |
| $N_1$ | 0.559 (0.159-1.970) | 0.81 | 0.7 (0.200-2.447) | 1.00 |
| $N_2$ | 0.705 (0.324-1.530) | 0.84 | 0.701 (0.326-1.508) | 0.80 |
| LNR (positive/examined nodes) | | | | |
| >0.4 | 1 | 0.12 | 1 | 0.07 |
| 0-0.049 | 0.519 (0.190-1.416) | 0.35 | 0.437 (0.138-1.068) | 0.75 |
| 0.05-0.19 | 0.627 (0.287-1.370) | 0.46 | 0.578 (0.268-1.252) | 0.27 |
| 0.2-0.39 | 0.561 (0.312-1.012) | 0.57 | 0.711 (0.319-1.024) | 0.66 |
| AJCC stage | | | | |
| II | 1 | 0.29 | 1 | 0.19 |
| IIIA | 1.006 (0.478-2.118) | 1.00 | 1.116 (0.528-2.360) | 1.00 |
| ≥IIIB | 1.682 (0.506-5.597) | 0.66 | 2.062 (0.614-6.913) | 0.36 |
| Radiation therapy | | | | |
| Yes | 1 | 0.52 | 1 | 0.69 |
| No | 1.11 (0.806-1.529) | | 0.937 (0.678-1.295) | |
| Resection margin (cm) | | | | |
| ≥1 | 1 | 0.03 | 1 | 0.007 |
| <1 | 1.626 (1.045-2.530) | | 1.829 (1.177-2.841) | |



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