Figure 1. Univariate survival analysis (Kaplan-Meier) of 47 patients with ovarian carcinoma treated with a platinum-based chemotherapy: the median survival of patients with tumors positive for COX-2 was reduced compared to patients with tumors negative for COX-2 (log rank test, $P = 0.03$).

of patients that were treated with platinum-based chemotherapy. In this group of patients, the median survival of patients with tumors negative for COX-2 was 52.7 (± 5.11) months, while it was reduced to 37.9 (± 6.42) months for patients with tumors positive for COX-2 (Figure 1, log rank test, $P = 0.03$). This suggests that COX-2 is a prognostic factor for patients with ovarian carcinomas receiving standard chemotherapy.

The majority of studies showing a prognostic effect of cytoreductive surgery restrict their investigation to FIGO stage III or IV cases.²⁻⁵ Thus, the therapeutic concept of extensive surgical de-bulking is relevant only for therapy of advanced ovarian carcinoma. Since we included 30% of patients with stage I or II ovarian carcinomas in our study, cytoreductive therapy could not be separately assessed as an independent factor in multivariate analysis for the whole study sample. In the subgroup of patients treated with radical surgery, detailed data on the amount of residual tumor after surgery is currently available for 22 cases. Seventeen of these cases (77.3%) had a residual tumor smaller than 2 cm, while 5 (22.7%) had more than 2-cm residual tumor. In this small group of cases, there was no association between expression levels of COX-2 and the amount of residual tumor. We agree, however, that a more detailed and refined analysis studying the role of COX expression and different therapeutic regimens as well as their interaction should be performed in a larger set of patients with advanced ovarian carcinomas.

We appreciate Dr. Agarwal's comment that a multivariate analysis can only give information on the relationship between parameters that are actually included in this analysis. In our study, we have identified COX-2 as an independent prognostic parameter for overall survival with the parameters of FIGO stage, grade, histological type, as well as patient age included in the analysis. The number of possible variables in multivariate analysis is restricted by the number of cases investigated. Therefore, we cannot exclude that factors not analyzed might be associated with increased COX-2 expression as well as survival.

It has been pointed out in a recent commentary⁶ that our study as well as similar studies⁷ on the role of COX-2 in ovarian carcinoma should be seen as hypothesis-generating reports. Prospective and retrospective studies investigating a larger number of cases are needed to determine whether expression of COX-2 may be a clinically relevant prognostic and/or therapeutic marker.

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Rearranged Epstein-Barr Virus Genome in Hodgkin's Disease and Angioimmunoblastic Lymphadenopathy: Swiss Results

To the Editor-in-Chief:

With special interest we studied the article titled, "A Defective, Rearranged Epstein-Barr Virus Genome in EBER-Negative and EBER-Positive Hodgkin's Disease," by Gan and colleagues,¹ published in the March 2002 issue of *The American Journal of Pathology*. The authors assessed the presence of the *Bam*HI W/Z rearrangement, known to disrupt viral latency,² in clinical samples of 56 American and Brazilian children and adolescents suffering from Hodgkin's disease (HD). Applying a sensitive PCR assay on DNA extracted from paraffin-embedded material, Gan et al¹ detected the juxtaposition of the *Bam*HI W and Z fragments in 10 of 32 (31%) EBER-positive and in 8 of 24 EBER-negative HD tumors.

In 1992, we addressed this issue with a sensitive PCR detection system for the *Bam*HI W/Z rearrangement in EBV-positive HD.³ Genomic DNA was purified under sterile conditions from fresh frozen lymph node biopsies of 21 adult Swiss patients. Sixteen patients suffered from EBV-positive HD (LMP1 expressing Reed-Sternberg cells) and five from EBV-associated angioimmunoblastic lymphadenopathy. All biopsy samples contained a high number of EBV-copies, in particular 15 samples $\geq 10^4$ copies per 1 μ g DNA and six samples with 10^3 copies per 1 μ g DNA, when tested with a semi-quantitative graduated dilution method.⁴ The cell line P3J-HR-1, clone HH543-5 (a kind gift from Dr. G. Miller, Yale University), was used as a positive control for *Bam*HI W/Z rearrangements.³ The results were verified by Southern blotting with subsequent specific hybridization including the positive control.

In comparing our findings in EBV-positive HD (all 16 HD cases negative for *Bam*HI W/Z rearrangements) with the results of Gan and colleagues¹ (32% positive), it should be noted that this significant difference occurred even though both groups used a very sensitive PCR detection method; and samples with a high EBV-copy number. What then could make up for this difference? Could it be the geographic origin of the samples? We don't think so, because another polymorphism within the EBV genome, the 30-bp LMP1-deletion variant, is observed at equal frequency (59 to 75%) in infectious mononucleosis or EBV-associated tonsillar hyperplasia of children and adolescents from Brazil, North America, and Switzerland.^{5,6}

What remains, therefore, is the difference of age at diagnosis of HD, which was 40 years (mean) in our series. In adults, a putative loss of defective EBV genomes harboring *Bam*HV W/Z rearrangements over time might be a consequence of a still immunocompetent organism in childhood and early adulthood, and in the years before development of HD which is associated with impaired T-cell immunity.⁷

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Gene Methylation in Non-Neoplastic Mucosa of Gastric Cancer: Age or *Helicobacter pylori* Related?

To the Editor-in-Chief:

We read with interest the article of Waki et al¹ on the promoter methylation status of *E-cadherin*, *hMLH1*, and *p16* genes in non-neoplastic gastric epithelia. We have also recently studied the methylation of the *E-cadherin* gene in non-neoplastic mucosa of patients with gastric cancer.² The methylation of *E-cadherin* was assessed by two different primer sets as designated by Herman et al³ and Graff et al⁴ to confirm the specificity. We observed that *E-cadherin* methylation was associated with age, the presence of chronic gastritis, and *Helicobacter pylori* (*H. pylori*) on univariate analysis. However, *H. pylori* infection was the only independent factor associated with *E-cadherin* methylation on multivariate analysis.

Hypermethylation at the promoter region of tumor suppressor gene has been shown to be an important mechanism in gene silencing. However, gene-acquired methylation may not necessarily be associated with neoplastic transformation. Toyota et al⁵ proposed that two types of methylation might exist: type A methylation (such as the *estrogen receptor* gene) which is age-related and may be present in normal mucosa and type C methylation which is cancer-related and not present in normal mucosa. Furthermore, recent studies have shown that gene methylation may be present in non-neoplastic colorectal mucosa in patients with inflammatory bowel disease,^{6,7} esophageal mucosa in patients with Barrett's esophagitis,^{8,9} and in liver tissues in chronic hepatitis.¹⁰ In the stomach, methylation at *THBS-1* and *TIMP-3* in chronic gastritis was found to be present in 10.1% and 14.5% of cases respectively.¹¹ These findings suggested that gene methylation could be a result of chronic inflammation.

In the stomach, the interplay between age and chronic inflammation is complicated by the presence of *H. pylori* infection. The prevalence of *H. pylori* increases with age. In Western countries, about 50% of people over 60 years of age are infected, but only 20 to 30% below the age of 30 are infected.^{12,13} In addition, the presence of *H. pylori* is almost invariably associated with gastritis. In a meta-