VOLUME 22 · NUMBER 4 · FEBRUARY 15 2004

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

# EBV-Positive Gastric Adenocarcinomas: A Distinct Clinicopathologic Entity With a Low Frequency of Lymph Node Involvement

*Josine van Beek, Axel zur Hausen, Elma Klein Kranenbarg, Cornelis J.H. van de Velde, Jaap M. Middeldorp, Adriaan J.C. van den Brule, Chris J.L.M. Meijer, and Elisabeth Bloemena*

From the Department of Pathology, VU University Medical Center, Amsterdam; and the Department of Surgery, Leiden University Medical Center, Leiden, the Netherlands.

Submitted August 8, 2003; accepted December 3, 2003.

Supported by Dutch Cancer Society grant VU99-1990.

Authors' disclosures of potential conflicts of interest are found at the end of this article.

Address reprint requests to Elisabeth Bloemena, MD, PhD, Department of Pathology, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, the Netherlands; e-mail: e.bloemena@vumc.nl.



0732-183X/04/2204-664/$20.00

DOI: 10.1200/JCO.2004.08.061

## ABSTRACT

**Purpose**
Epstein-Barr virus (EBV) is detected in a substantial subgroup of gastric adenocarcinomas worldwide. We have previously reported that these EBV-positive gastric carcinomas carry distinct genomic aberrations. In the present study, we analyzed a large cohort of EBV-positive and EBV-negative gastric adenocarcinomas for their clinicopathologic features to determine whether they constitute a different clinical entity.

**Patients and Methods**
Using a validated polymerase chain reaction/enzyme immunoassay–based prescreening method in combination with EBER1/2-RNA in situ hybridization, EBV was detected in the tumor cells of 7.2% (n = 41) of the gastric carcinomas from the Dutch D1D2 trial (N = 566; mean follow-up, 9 years). EBV status was correlated with clinicopathologic features collected for the Dutch D1D2 trial.

**Results**
EBV-positive gastric carcinomas occurred significantly more frequently in males ($P < .0001$) and in younger patients ($P = .012$). Most were of the intestinal type according to the Laurén classification ($P = .047$) or tubular according to the WHO classification ($P = .006$) and located in the proximal part of the stomach ($P < .0001$). A significantly lower tumor-node-metastasis system-stage ($P = .026$) was observed in the patients with EBV-carrying carcinomas, which was solely explained by less lymph node (LN) involvement ($P = .034$) in these cases. In addition, a better prognosis, as reflected by a longer disease-free period ($P = .04$) and a significant better cancer-related survival ($P = .02$), was observed for these patients, which could be explained by less LN involvement, less residual disease, and younger patient age.

**Conclusion**
EBV-carrying gastric adenocarcinomas are a distinct entity of carcinomas, characterized not only by unique genomic aberrations, but also by distinct clinicopathologic features associated with significantly better prognosis.

*J Clin Oncol 22:664-670. © 2004 by American Society of Clinical Oncology*

## INTRODUCTION

The Epstein-Barr virus (EBV) is known to cause benign and malignant diseases of both lymphoid and epithelial origin [1]. In the last decade, the association of this human γ-herpes virus with a subgroup of gastric cancer has generally been accepted—that is, in 10% of the gastric adenocarcinomas not otherwise specified (NOS) [2], in more than 80% of the relatively rare lymphoepithelioma-like carcinomas (LELCs) of the stomach [3,4], and in approximately 35% of the stump carcinomas [5,6]. Given that 876,000 new cases of gastric cancer occur per year worldwide [7], the absolute number of EBV-positive gastric cancer cases is considerable and is thus the largest group of EBV-associated malignancies.

An etiologic association between EBV and gastric carcinomas is based on its uniform expression in all tumor cells [8] and its absence in normal epithelium or dysplastic lesions [9]. In addition, the virus is present

**Table 1.** Large* Studies of Clinicopathologic Features of EBV-Carrying Gastric Carcinomas

| Investigators | Reference No. | Year of Publication | No. of Patients With EBV | Total No. of Patients | % Patients With EBV | Country | Patient Sex | Patient Age | Tumor Histology | Tumor Location | LN Metastasis | Survival |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Kijima et al | 18 | 2001 | 23 | 313 | 7.3 | Japan | NS | ND | Tub 2, por1† | Cardia | NS | |
| Chang et al | 21 | 2001 | 17 | 306 | 5.6 | South Korea | Male | NS | Poorly differentiated; diffuse | ND | NS | NS |
| Takano et al | 26 | 1999 | 33 | 513 | 6.4 | Japan | Male (NS) | NS | moderate to poor differentiation (NS) | NS | NS | ND |
| Tokunaga et al | 9 | 1993 | 67 | 970 | 6.9 | Japan | Male | ND | Tub2, por1† | Cardia | ND | ND |
| Tokunaga et al | 27 | 1993 | 120 | 1795 | 6.7 | Japan | Male | Younger (NS) | Laurén (NS) | Upper middle | ND | No effect on mortality |

Abbreviations: EBV, Epstein-Barr virus; LN, lymph node; NS, not significant; Tub2, moderately differentiated tubular adenocarcinoma; Por1, poorly differentiated solid adenocarconoma; ND, not determined.
*Studies with more than 300 consecutive patients in study hospitals.
†According to Japanese Research Society for Gastric Cancer classification.

in a monoclonal and episomal form [10,11] and is transcriptionally active [12,13]. Third, there is serologic evidence of high antiviral titers, especially of EBV viral-capsid antigen IgA and EBV early antigen IgG, many years before the diagnosis of EBV-positive gastric cancer [10,11,14]. However, the exact pathophysiologic mechanism by which EBV contributes to the development of gastric carcinomas remains to be established. Recent studies from our laboratory and others have shown that EBV-carrying gastric carcinomas carry distinct chromosomal aberrations compared with EBV-negative carcinomas [15,16] and that transcripts of the EBV-encoded epithelial oncogene *BARF1* are expressed in these gastric carcinomas [13].

Previous studies on clinicopathologic features of EBV-positive gastric cancer showed a male predominance and location in the upper part of the stomach [8,9,17,18]. EBV-carrying gastric adenocarcinomas are also characterized by a prominent CD8+ activated lymphocyte infiltrate [19]. It has been suggested that the EBV-carrying adenocarcinomas NOS do not differ in prognosis compared with their EBV-negative counterpart [20,21]. However, a significantly better prognosis has been established for LELC of the stomach compared with gastric adenocarcinomas not otherwise specified [22-25].

To further evaluate these findings for a European population, we have performed the first large study outside Japan (> 500 cases; Table 1). We studied a Dutch patient population (n = 566) for clinicopathologic features to determine whether EBV-carrying carcinomas form a distinct clinicopathologic entity. Patients with gastric cancer were originally selected for the randomized D1D2 trial performed in the Netherlands between 1989 and 1993, which aimed to determine whether extensive lymph node dissection influenced long-term survival after surgery with curative intent. In that study, a standard limited D1 dissection was compared with an extended D2 lymph-node dissection in terms of mortality, postoperative mortality, and cumulative risk of relapse after surgery [28].

The aim of the present study was to analyze in a large, well documented patient population with uniform treatment and long-term follow-up, whether EBV-carrying and EBV-negative gastric adenocarcinomas display distinct clinicopathologic features and have a different prognosis.

## PATIENTS AND METHODS

### *Subjects*

We studied the patient population of the Dutch D1D2 trial (N = 711), which has extensively been described by Bonenkamp et al [28]. For the original analysis and all subsequent analyses, permission was obtained from the medical ethical committees of the participating hospitals. This large, randomized surgical study, performed between August 1989 and July 1993, included 80 Dutch hospitals and 52 affiliated pathology departments. In the original study, the extent of lymph node dissection (D1 *v* D2) in patients with gastric cancer was evaluated. To be eligible for the study, patients had to have a histologically confirmed adenocarcinoma of the stomach without evidence of distant metastasis or coexisting malignancies nor a previous gastrectomy for benign disease. They had to be younger than 85 years and physically suitable for D1 and D2 dissection. Surgery was performed with curative intent. During this study, pathology data and surgical data were collected using standardized forms. Tumor-node-metastasis system classification has been revised according to the 1997 guidelines of the WHO [29]. In this revision, the number of tumor-positive lymph nodes (LNs) determines the *N*-stage instead of the location of the tumor-positive LN and one LN station (station 12) is now defined as M1. Collection of data is still in progress, with at present a mean follow-up of 9 years. For our study, patients (n = 145) were excluded if tumor sections were no longer available. Statistical analyses of the original patient population and our study population confirmed the absence of bias by availability of patient material. Data described in the Results section is based on 566 patients unless otherwise specified. In addition, no differences in correlations were observed when analyzing the separate D1 and D2 groups for the different parameters.

**Table 2.** Characteristics of the Patient Populations

| | Total Population (N = 566) | | EBV Status | | | | |
|---|---|---|---|---|---|---|---|
| | | | Negative (N = 525) | | Positive (N = 41) | | |
| | No. of Patients | % | No. of Patients | % | No. of Patients | % | *P* |
| Sex | | | | | | | < .0001* |
| Male | 324 | 57.2 | 286 | 54.5 | 38 | 92.7 | |
| Female | 242 | 42.8 | 239 | 45.5 | 3 | 7.3 | |
| Mean age, years | 63.9 | | 64.3 | | 59.6 | | .012† |

Abbreviation: EBV, Epstein-Barr virus.
*Pearson $\chi^2$.
†Mann-Whitney *U* test.

### Histology

Paraffin-embedded tissue sections of the primary tumor were selected based on the original reports from the Dutch pathology database. Presence of tumor in the sections was confirmed by hematoxylin and eosin staining, and histologic typing of the tumors was performed according to both the Laurén classification and WHO guidelines by two pathologists (A.z.H. and E.B.) [30,31]. The pathologists were blinded for the EBV status of the tumors.

LELCs were not distinguished as a separate entity in either classification system. Due to their morphology, they constitute either a diffuse or intestinal type tumor in Laurén, depending on the extent of glandular differentiation and cell size, and a (poorly differentiated) tubular type according to the WHO classification. Early gastric carcinomas were classified as a separate entity defined by not invading the external muscle layer in both classification systems. Because of the inclusion criteria of the D1D2 study, no gastric stump carcinomas were included in the present study [28].

### EBV Detection

EBV status of the tumor was determined by a polymerase chain reaction (PCR)/enzyme immunoassay (EIA)–based prescreening method as recently described by us [32]. Briefly, crude DNA extractions were tested by a combination of beta-globin PCR and a sensitive PCR for the BamHI W repeat in the EBV genome. Hybridization of the PCR products in an EIA allowed the semiquantitative detection of the specific PCR products. EBER1/2 RNA in situ hybridization was performed to confirm the presence of EBV in the tumor cells of those cases with a strong hybridization signal after overnight substrate incubation or those cases in which EBV presence could not be ascertained by PCR-EIA because of poor DNA quality, even after DNA purification.

### Statistical Analysis

Pearson $\chi^2$ tests were applied, as indicated in the results. Because the tables contained empty cells, a permutation procedure was used to compute the *P* value. The number of permutations was set at 100,000. Age was analyzed using the Mann Whitney *U* test. Survival analysis was performed using the Kaplan-Meier method, log-rank testing, and Cox regression. For overall survival analyses, time to event was defined as time of surgery until death, irrespective of cause of death. For cancer-related survival, an event was defined as time of surgery until death due to gastric carcinoma recurrence. If the patient died as a result of a non–cancer-related cause, survival was the same as the disease-free period since surgery. An event was defined as time until relapse to determine the disease-free period. *P* values less than .05 were considered statistically significant. All statistical analyses were performed with the SPSS 10.0 statistical software program for Windows (SPSS Inc, Chicago, IL).

## RESULTS

### EBV Prevalence

In this large Dutch cohort of gastric carcinoma patients, operated on both in academic hospitals (13% of the study population) and in local hospitals, the overall EBV prevalence was 7.2% (41 of 566 patients).

### Patient Population

Although the overall study population consisted of 57% of males and 43% females, 92.7% of the EBV-positive tumors (38 of 41 tumors)occurred in men, and only 7.3% of the EBV-positive tumors arose in women, resulting in a significant male predominance ($P < .0001$; Table 2).

The mean age of the patients in the study population was 63.9 years. The mean age of the patients with EBV-positive tumors was 59.6 years, significantly younger than patients with EBV-negative tumors (64.3 years; $P = .012$; Table 2).

### Tumors

According to the Laurén classification [30], 50.7% of the tumors in the overall population was of the intestinal type, whereas 20.1% had a diffuse-type histology, 2.7% were of mixed histology, and 26.5% were early gastric carcinomas (Table 3). The same distribution was observed in the EBV-negative tumor group (49.7%, 21.3%, 2.9%, and 26.1%, respectively). In contrast, in the population with EBV-positive tumors, only two tumors (4.9%) were of the diffuse histologic type, indicating a significantly smaller number of tumors with this type of histology ($P = .047$).

In addition, all tumors were classified according to the WHO guidelines for both histology and differentiation [31]. According to the WHO classification, 51.2% of the EBV-positive tumors were of the tubular type, in contrast with 29.7% of the EBV-negative carcinomas. The other EBV-positive tumors comprised two papillary, two signet ring cell carcinomas, and three tumors of undifferentiated

**Table 3.** Tumor Presentation

| | Total Population (N = 566) | | EBV Status | | | | |
|---|---|---|---|---|---|---|---|
| | | | Negative (n = 525) | | Positive (n = 41) | | |
| | No. of Patients | % | No. of Patients | % | No. of Patients | % | *P* |
| Histology, Laurén | | | | | | | .047* |
| Early | 150 | 26.5 | 137 | 26.1 | 13 | 31.7 | |
| Intestinal | 287 | 50.7 | 261 | 49.7 | 26 | 63.4 | |
| Mixed | 15 | 2.7 | 15 | 2.9 | 0 | 0 | |
| Diffuse | 114 | 20.1 | 112 | 21.3 | 2 | 4.9 | |
| Histology, WHO | | | | | | | .006* |
| Early | 150 | 26.5 | 137 | 26.1 | 13 | 31.7 | |
| Papillary | 44 | 7.8 | 42 | 8.0 | 2 | 4.9 | |
| Tubular | 177 | 31.3 | 156 | 29.7 | 21 | 51.2 | |
| Mucinous | 39 | 6.9 | 39 | 7.4 | 0 | 0 | |
| Signet-ring cell carcinomas | 132 | 23.3 | 130 | 24.8 | 2 | 4.9 | |
| Undifferentiated | 24 | 4.2 | 21 | 4.0 | 3 | 7.3 | |
| Differentiation of tubular type carcinomas (n = 177) | | | | | | | .002* |
| Good | 15 | 8.5 | 15 | 9.6 | 0 | 0 | |
| Moderate | 92 | 52.0 | 87 | 55.8 | 5 | 23.8 | |
| Poor | 70 | 39.5 | 54 | 34.6 | 16 | 76.2 | |
| Location | | | | | | | < .0001* |
| Proximal | 202 | 35.7 | 168 | 32.0 | 34 | 82.9 | |
| Distal | 321 | 56.7 | 315 | 60.0 | 6 | 14.6 | |
| Whole stomach | 43 | 7.6 | 42 | 8 | 1 | 2.4 | |
| TNM stage, 1997 | | | | | | | .026* |
| IA | 114 | 20.1 | 103 | 19.6 | 11 | 26.8 | |
| IB | 134 | 23.7 | 117 | 22.3 | 17 | 41.5 | |
| II | 134 | 23.7 | 128 | 24.4 | 6 | 14.6 | |
| IIIA | 86 | 15.2 | 84 | 16.0 | 2 | 4.9 | |
| IIIB | 38 | 6.7 | 37 | 7.0 | 1 | 2.4 | |
| IV | 60 | 10.6 | 56 | 10.7 | 4 | 9.8 | |
| T stage, 1997 | | | | | | | .94* |
| T1 | 150 | 26.5 | 137 | 26.1 | 13 | 31.7 | |
| T2 | 270 | 47.7 | 249 | 47.4 | 21 | 51.2 | |
| T3 | 136 | 24.0 | 131 | 25.0 | 5 | 12.2 | |
| T4 | 9 | 1.6 | 7 | 1.3 | 2 | 4.9 | |
| N stage, 1997 | | | | | | | .034* |
| N0 | 229 | 40.5 | 203 | 38.7 | 26 | 63.4 | |
| N1 (1–6 nodes) | 209 | 36.9 | 198 | 37.7 | 11 | 26.8 | |
| N2 (7–15 nodes) | 76 | 13.4 | 74 | 14.1 | 2 | 4.9 | |
| N3 (≥16 nodes) | 38 | 6.7 | 36 | 6.9 | 2 | 4.9 | |
| M1 | 14 | 2.5 | 14 | 2.7 | 0 | 0 | |
| M stage, 1997 | | | | | | | |
| M0 | 543 | 92.8 | 503 | 92.6 | 40 | 93.7 | .58 |
| M1 | 23 | 7.2 | 22 | 7.6 | 1 | 4.3 | |

Abbreviations: EBV, Epstein-Barr virus; T, tumor; N, node; M, metastasis.
*Permutation Pearson $\chi^2$.

histology. Therefore, EBV-positive tumors were significantly more often of tubular type and less often of signet-ring cell type ($P = .006$).

Tumors of tubular type were further analyzed according to grade of differentiation. The EBV-positive tumors had a poorer grade of differentiation (described as good, moderate, or poor) compared with the EBV-negative tumor group ($P = .002$).

In the D1D2 study, standardized forms were used to indicate the localization of the tumor in the stomach. For our analysis, cardia and cardia-middle were defined as proximal (35.7% of the tumors), whereas middle-antrum and antrum were considered to be the distal part of the stomach (56.7% of the tumors; Table 3). In 7.6% of the tumors, the whole stomach was involved. Of the EBV-positive tumors, 82.9% were located in the proximal part of the stom-

ach, and only 32.0% of the EBV-negative tumors were located in the proximal part of the stomach. In addition, only one EBV-positive tumor involved the whole stomach ($P < .0001$).

WHO tumor-node-metastasis system classification was determined according to the guidelines of 1997 [29]. As shown in Table 3, a significantly lower stage was observed in the EBV-positive tumor group, with 68.3% in stage I versus 41.9% of the EBV-negative tumor population ($P = .026$). This significant difference in tumor-node-metastasis system stage could solely be attributed to a difference in N stage and not to T stage ($P = .094$) or M stage ($P = .58$). A significantly lower N stage was observed in the EBV-positive tumor group. In 63.4% of the patients with EBV-positive tumors, no lymph node metastases were found, compared with 38.7% of the EBV-negative carcinomas ($P = .034$).

Sixty-one of the EBV-negative cases showed either presence of tumor in the resection margin or positive cytology of ascites or abdominal washing during surgery (R1). This was not observed in any of the EBV-positive cases ($P = .021$). No correlation was observed between either histology or tumor stage and the R1 cases.

### Survival and Disease-Free Period

Consistent with the original data published by Bonenkamp et al [28], we observed no significant difference in overall survival and disease-free period after surgery between the D1 and D2 study arms in the subset of 566 patients analyzed by us during an extended follow-up time with a mean of 9 years.

No difference was observed in the overall survival of the EBV-carrying tumor population (n = 41) compared with patients with an EBV-negative tumor (n = 525; $P = .6$; Fig 1A). However, a significant difference ($P = .02$) was observed when the population was analyzed for cancer-related survival

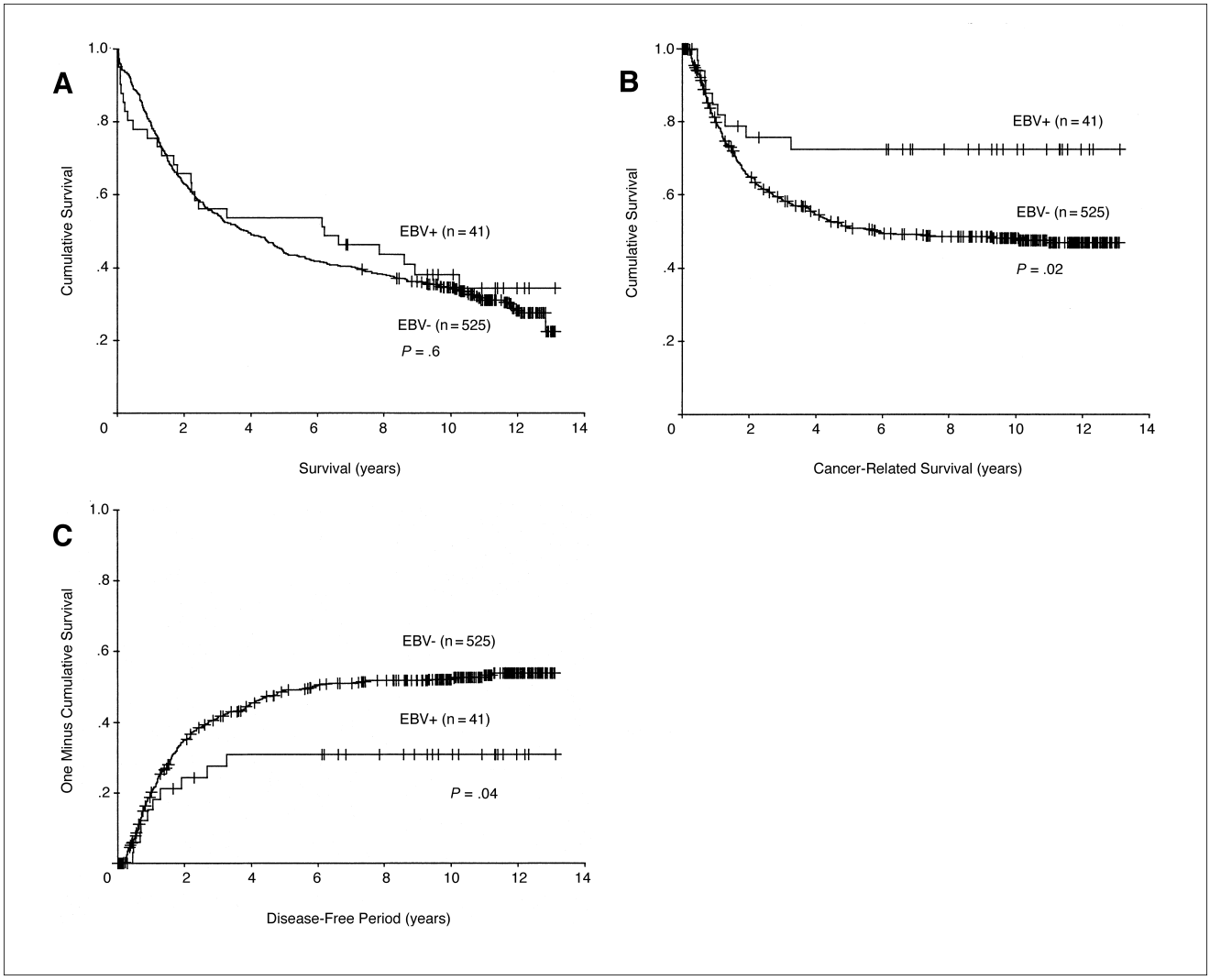


**Fig 1.** Kaplan-Meier method log-rank testing for overall survival. (A) Cancer-related survival and (B) disease-free period after surgery for (C) EBV-positive (n = 41) versus EBV-negative (n = 525) gastric carcinomas.

(Fig 1B). In addition, a longer disease-free period was observed for the EBV-carrying tumor population ($P = .04$; Fig 1C).

Exclusion of patients who died within 30 days after surgery did not affect the differences observed in the cancer-related survival or disease-free period ($P = .03$; $P = .04$ respectively). Once a recurrence occurred, no significant difference was observed in the location of the recurrence.

When the effects were adjusted for other predictors by multivariate Cox regression analyses, LN involvement, residual disease, and age were the most important factors for both cancer-related survival and disease-free period, whereas EBV, resection type, and sex did not contribute as independent variables.

## DISCUSSION

In the present study, we demonstrate in a Dutch multicenter trial (N = 566) that EBV-carrying gastric adenocarcinomas are a distinct entity, with different clinicopathologic features in addition to previously described differences in molecular aberations. This is the first large study (> 500 patients) performed outside Japan. In our cohort, the EBV-carrying tumors are observed more often in males and in younger patients. The tumors are localized in the proximal part of the stomach. Histologically, a lower prevalence of diffuse type of tumor (Laurén classification). In this first paper in which the WHO classification for histology is used, a higher prevalence of tubular type tumor is observed. Patients present with less LN involvement and have less residual disease, which results in a better prognosis for patients with EBV-positive carcinomas.

The Netherlands is a country with a low gastric cancer incidence of approximately 2,000 new cases per year [33]. In this population, an EBV prevalence in gastric cancer of 7.2% is observed. This is comparable with the EBV prevalence detected in gastric adenocarcinomas worldwide, including countries with high gastric cancer prevalence, such as Japan [8,9,34], indicating that the overall prevalence of EBV in gastric carcinomas is independent of geographic regions [9,10,26,35].

Our observations of male predominance [8,9], younger patient age, and localization of the tumors in the proximal part of the stomach [9,18] are in agreement with those of several previous studies. However, this is the first large study (> 500 patients) performed outside Japan. Our male to female ratio of 9.8 is the highest described so far, followed by a sex ratio of 7.8 in a Korean study [21], 7.0 in a study from the United States [8], and 6.2 in a Russian cohort [17]. Only in two studies from South America were there relatively more females among patients with EBV-positive gastric cancer [36,37].

The difference in age is significant but small. A trend toward younger age has been observed previously [20,38]; however, absence of a correlation with age has been described as well [9,26].

The localization of the EBV-carrying tumors in the proximal part of the stomach may reflect the physiologic interaction with infectious EBV in saliva and, probably, previously damaged upper gastric epithelium.

Of major importance was the observation of less LN involvement in EBV-positive patients, especially given that no difference was observed in the tumor size of the two groups. This has been described before for LELC [9,39,40]. Less LN involvement was also reflected in a better prognosis of the patients with the EBV-positive tumors in our cohort. Overall survival did not correlate with the EBV status of the tumors, which is most likely a result of the age of the cohort (mean age at intake, 64 years). However, a significantly better cancer-related survival ($P = .02$) and a longer disease-free period ($P = .04$) was observed in the EBV-carrying group.

We hypothesize that EBV induces an immune response that prevents the outgrowth of micrometastases in the LNs. This immune response is also reflected by the large CD8+ infiltrate characteristically surrounding the EBV-carrying gastric carcinomas [19]. The target for the immune response could either be an EBV antigen or a cellular EBV-induced antigen.

Molecular analysis of EBV-positive gastric adenocarcinomas versus EBV-negative carcinomas has already shown distinct characteristics for EBV-carrying gastric carcinomas as reflected by different chromosomal aberrations [15], different and lower frequencies of microsatellite instability [41] and allelic loss [16], and more CpG methylation [42]. In addition, specifically in EBV-carrying gastric carcinomas, p53 does not seem to be affected [16], whereas p16 is downregulated by promotor methylation [43]. In the present study, we show that these molecular differences are also reflected in distinct clinicopathologic features indicating that EBV-positive gastric carcinomas are a distinct entity with a unique pathway of carcinogenesis.

EBV-positive gastric cancer can be viewed as an immunotherapeutic experiment of nature in which EBV provides growth advantage of tumor cells but also induces a putative antitumor response that prevents outgrowth of metastases, as reflected by the lower number of lymph node metastases in EBV-positive gastric carcinomas, resulting in a better clinical outcome. As such, it provides a rationale for clinical tumor vaccination trials.

### Acknowledgment

We thank Bas van Beek, Muriel Verkuijten, and Ralph Warring for excellent technical assistance and Saskia Bulk and Hans Berkhof for statistical advice.

### Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

## REFERENCES


**1.** International Agency for Research on Cancer: Epstein-Barr virus and Kaposi sarcoma herpes virus/human herpes virus 8. IARC Monogr Eval Carcinog Risks Hum 70 1997

**2.** Takada K: Epstein-Barr virus and gastric carcinoma. Mol Pathol 53:255-261, 2000

**3.** Burke AP, Yen TS, Shekitka KM, et al: Lymphoepithelial carcinoma of the stomach with Epstein-Barr virus demonstrated by polymerase chain reaction. Mod Pathol 3:377-380, 1990

**4.** Shibata D, Tokunaga M, Uemura Y, et al: Association of Epstein-Barr virus with undifferentiated gastric carcinomas with intense lymphoid infiltration: Lymphoepithelioma-like carcinoma. Am J Pathol 139:469-474, 1991

**5.** Yamamoto N, Tokunaga M, Uemura Y, et al: Epstein-Barr virus and gastric remnant cancer. Cancer 74:805-809, 1994

**6.** Baas IO, van Rees BP, Musler A, et al: Helicobacter pylori and Epstein-Barr virus infection and the p53 tumour suppressor pathway in gastric stump cancer compared with carcinoma in the non-operated stomach. J Clin Pathol 51: 662-666, 1998

**7.** Parkin DM: Global cancer statistics in the year 2000. Lancet Oncol 2:533-543, 2001

**8.** Shibata D, Weiss LM: Epstein-Barr virus–associated gastric adenocarcinoma. Am J Pathol 140:769-774, 1992

**9.** Tokunaga M, Land CE, Uemura Y, et al: Epstein-Barr virus in gastric carcinoma. Am J Pathol 143:1250-1254, 1993

**10.** Imai S, Koizumi S, Sugiura M, et al: Gastric carcinoma: Monoclonal epithelial malignant cells expressing Epstein-Barr virus latent infection protein. Proc Natl Acad Sci U S A 91:9131-9135, 1994

**11.** Fukayama M, Hayashi Y, Iwasaki Y, et al: Epstein-Barr virus–associated gastric carcinoma and Epstein-Barr virus infection of the stomach. Lab Invest 71:73-81, 1994

**12.** Sugiura M, Imai S, Tokunaga M, et al: Transcriptional analysis of Epstein-Barr virus gene expression in EBV-positive gastric carcinoma: Unique viral latency in the tumour cells. Br J Cancer 74:625-631, 1996

**13.** zur Hausen A, Brink AA, Craanen ME, et al: Unique transcription pattern of Epstein-Barr virus (EBV) in EBV-carrying gastric adenocarcinomas: Expression of the transforming BARF1 gene. Cancer Res 60:2745-2748, 2000

**14.** Levine PH, Stemmermann G, Lennette ET, et al: Elevated antibody titers to Epstein-Barr virus prior to the diagnosis of Epstein-Barr-virus–associated gastric adenocarcinoma. Int J Cancer 60:642-644, 1995

**15.** zur Hausen A, van Grieken NC, Meijer GA, et al: Distinct chromosomal aberrations in Epstein-Barr virus–carrying gastric carcinomas tested by comparative genomic hybridization. Gastroenterology 121:612-618, 2001

**16.** van Rees BP, Caspers E, zur Hausen A, et al: Different pattern of allelic loss in Epstein-Barr virus–positive gastric cancer with emphasis on the p53 tumor suppressor pathway. Am J Pathol 161:1207-1213, 2002

**17.** Galetsky SA, Tsvetnov VV, Land CE, et al: Epstein-Barr-virus–associated gastric cancer in Russia. Int J Cancer 73:786-789, 1997

**18.** Kijima Y, Hokita S, Takao S, et al: Epstein-Barr virus involvement is mainly restricted to lymphoepithelial type of gastric carcinoma among various epithelial neoplasms. J Med Virol 64:513-518, 2001

**19.** Saiki Y, Ohtani H, Naito Y, et al: Immunophenotypic characterization of Epstein-Barr virus-associated gastric carcinoma: Massive infiltration by proliferating CD8+ T- lymphocytes. Lab Invest 75:67-76, 1996

**20.** Gulley ML, Pulitzer DR, Eagan PA, et al: Epstein-Barr virus infection is an early event in gastric carcinogenesis and is independent of bcl-2 expression and p53 accumulation. Hum Pathol 27:20-27, 1996

**21.** Chang MS, Lee HS, Kim CW, et al: Clinicopathologic characteristics of Epstein-Barr virus–incorporated gastric cancers in Korea. Pathol Res Pract 197:395-400, 2001

**22.** Watanabe H, Enjoji M, Imai T: Gastric carcinoma with lymphoid stroma: Its morphologic characteristics and prognostic correlations. Cancer 38:232-243, 1976

**23.** Lertprasertsuke N, Tsutsumi Y: Gastric carcinoma with lymphoid stroma: Analysis using mucin histochemistry and immunohistochemistry. Virchows Arch A Pathol Anat Histopathol 414:231-241, 1989

**24.** Minamoto T, Mai M, Watanabe K, et al: Medullary carcinoma with lymphocytic infiltration of the stomach: Clinicopathologic study of 27 cases and immunohistochemical analysis of the subpopulations of infiltrating lymphocytes in the tumor. Cancer 66:945-952, 1990

**25.** Nakamura S, Ueki T, Yao T, et al: Epstein-Barr virus in gastric carcinoma with lymphoid stroma: Special reference to its detection by the polymerase chain reaction and in situ hybridization in 99 tumors, including a morphologic analysis. Cancer 73:2239-2249, 1994

**26.** Takano Y, Kato Y, Saegusa M, et al: The role of the Epstein-Barr virus in the oncogenesis of EBV(+) gastric carcinomas. Virchows Arch 434:17-22, 1999

**27.** Tokunaga M, Uemura Y, Tokudome T, et al: Epstein-Barr virus–related gastric cancer in Japan: A molecular patho- epidemiological study. Acta Pathol Jpn 43:574-581, 1993

**28.** Bonenkamp JJ, Hermans J, Sasako M, et al: Extended lymph-node dissection for gastric cancer: Dutch Gastric Cancer Group. N Engl J Med 340:908-914, 1999

**29.** Sobin LH, Wittekind CH UICC TNM Classification of Malignant Tumours. New York, NY, Wiley-Liss, 1997

**30.** Lauren P: The two histologic main types of gastric carcinoma: Diffuse and so-called intestinal type carcinoma—An attempt at a histoclinical classification. Acta Pathol Microbiol Scand 64:31-49, 1965

**31.** Hamilton SR, Aaltonen LA: Pathology and Genetics: Tumours of the Digestive System. Lyon, France, IARC Press, 2000

**32.** Van Beek J, zur Hausen A, Klein Kranenbarg E, et al: A rapid and reliable enzyme immunoassay PCR-based screening method to identify EBV-carrying gastric carcinomas. Mod Pathol 15:870-877, 2002

**33.** Association of Comprehensive Cancer Centers: Incidence of cancer in the Netherlands 1998. Utrecht, Netherlands, 2002

**34.** Rowlands DC, Ito M, Mangham DC, et al: Epstein-Barr virus and carcinomas: Rare association of the virus with gastric adenocarcinomas. Br J Cancer 68:1014-1019, 1993

**35.** Koriyama C, Akiba S, Itoh T, et al: Prognostic significance of Epstein-Barr virus involvement in gastric carcinoma in Japan. Int J Mol Med 10:635-639, 2002

**36.** Corvalan A, Koriyama C, Akiba S, et al: Epstein-Barr virus in gastric carcinoma is associated with location in the cardia and with a diffuse histology: A study in one area of Chile. Int J Cancer 94:527-530, 2001

**37.** Herrera-Goepfert R, Reyes E, Hernandez-Avila M, et al: Epstein-Barr virus-associated gastric carcinoma in Mexico: Analysis of 135 consecutive gastrectomies in two hospitals. Mod Pathol 12:873-878, 1999

**38.** Koriyama C, Akiba S, Iriya K, et al: Epstein-Barr virus–associated gastric carcinoma in Japanese Brazilians and non-Japanese Brazilians in Sao Paulo. Jpn J Cancer Res 92:911-917, 2001

**39.** Wu MS, Shun CT, Wu CC, et al: Epstein-Barr virus–associated gastric carcinomas: Relation to H. pylori infection and genetic alterations. Gastroenterology 118:1031-1038, 2000

**40.** Ohfuji S, Osaki M, Tsujitani S, et al: Low frequency of apoptosis in Epstein-Barr virus–associated gastric carcinoma with lymphoid stroma. Int J Cancer 68:710-715, 1996

**41.** Chang MS, Lee HS, Kim HS, et al: Epstein-Barr virus and microsatellite instability in gastric carcinogenesis. J Pathol 199:447-452, 2003

**42.** Kang GH, Lee S, Kim WH, et al: Epstein-Barr virus–positive gastric carcinoma demonstrates frequent aberrant methylation of multiple genes and constitutes CpG island methylator phenotype-positive gastric carcinoma. Am J Pathol 160:787-794, 2002

**43.** Osawa T, Chong JM, Sudo M, et al: Reduced expression and promoter methylation of p16 gene in Epstein-Barr virus–associated gastric carcinoma. Jpn J Cancer Res 93:1195-1200, 2002