Prognostic factors in Epstein-Barr virus-associated stage I-III gastric carcinoma: Implications for a unique type of carcinogenesis

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Abstract. Epstein-Barr virus-associated gastric carcinoma (EBVaGC) has distinct clinicopathological features. However, the prognostic factors remain unclear, particularly in UICC/AJCC stage I-III cancer. We retrospectively enrolled 1,020 patients with stage I-III gastric cancer that received radical gastrectomy with lymphadenectomy. Formalin-fixed, paraffin-embedded surgical specimens were retrieved to construct tissue microarrays. EBV positivity was identified by *in situ* hybridization with EBV-encoded small RNA, and the histological classification was reviewed. Fifty-two cases of EBVaGC were identified, exhibiting a male predominance (p=0.003), a higher prevalence in stump cancer (p<0.001), and poorly differentiated carcinoma (p=0.010) compared with the controls. The survival analysis revealed no difference in survival between the EBVaGC cases and the EBV-negative cases (p=0.977). The multivariate analysis showed that EBVaGC cases with a tumor size >5 cm, non-lymphoepithelioma-like carcinoma (LELC), or a lymph node ratio >0.15 had a worse overall survival (hazard ratio 2.884, 12.178 and 19.352; p=0.027, 0.005 and <0.0001, respectively). The depth of tumor invasion and the number of lymph node metastases did not reach statistical significance (p=0.834 and 0.833, respectively). These prognostic factors, tumor size, LELC classification and lymph node ratio, may reflect a unique type of carcinogenesis of EBVaGC and may be considered when selecting high-risk patients for adjuvant treatment.

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Introduction

Epstein-Barr virus (EBV) is a ubiquitous γ-herpes virus that maintains a life-long latent infection in B lymphocytes in over 90% of adults following salivary transmission during childhood or adolescence (1). Since its discovery in tumor cells of Burkitt's lymphoma 40 years ago, EBV has been associated with various types of cancers, including lymphoid neoplasms, nasopharyngeal and gastric epithelial malignancies, and a subset of mesenchymal tumors (1,2). EBV-associated gastric carcinoma (EBVaGC) accounts for ~10% of gastric cancer cases (3-5). Gastric cancer has the fourth highest incidence of all types of cancers worldwide, and the burden of EBVaGC is estimated at 75,000-90,000 new cases annually, representing the largest subpopulation among EBV-related tumors (1,6).

From the viewpoint of the clinical distribution, EBVaGC presents the distinct characteristics of a male preponderance, proximal location and high incidence in stump cancer (3-5,7,8). In fact, EBVaGC is a heterogeneous histological group consisting of lymphoepithelioma-like carcinoma (LELC) and conventional adenocarcinoma (3,4,7). More than 80% of LELC cases are associated with EBV infection, while only 5-10% of ordinary adenocarcinoma cases are positive for EBV infection. The LELC histotype has been demonstrated to present a significantly favorable prognosis as a result of extensive infiltration of $CD8^+$ T cells and mature dendritic cells within these tumors (9-11). Nevertheless, controversy still exists regarding the prognostic significance of EBV infection itself. Several studies reported a better prognosis in EBVaGC, but a favorable outcome may not be observed after adjusting for other clinicopathological features (11,12). Other researchers have failed to show that EBVaGC differs from EBV-negative gastric cancer in terms of survival (8,13-15). There is a paucity of studies regarding the impact of clinicopathological factors on survival in EBVaGC. A recent study showed that advanced stage and histological classification were meaningful indicators of survival (11).

Since stage I-III gastric cancer patients comprise a population with diverse outcomes, there is considerable interest in understanding the predictors of this cancer's behavior beyond

the Union for International Cancer Control/American Joint Committee on Cancer (UICC/AJCC) stage. These factors, including the tumor size and lymph node (LN) ratio, defined as the ratio of metastatic to retrieved lymph nodes, have not yet been examined specifically in EBVaGC. Therefore, the present study was conducted to investigate the prognostic significance of the relevant clinicopathological parameters in stage I-III EBVaGC and to determine the predictive factors that would aid in the identification of high-risk patients who may benefit from adjuvant therapy.

Materials and methods

Case selection. We retrospectively enrolled gastric cancer cases according to the following criteria: radical resection with lymph node dissection and final pathologic stage I to III according to the 2010 UICC/AJCC staging system. From January 1999 to December 2006, 1,020 consecutive surgically resected specimens of gastric carcinoma were retrieved from the archives of the Department of Pathology at Chang Chung Memorial Hospital, Linkou, Taiwan. Clinical information concerning the patient characteristics and outcome was collected from the medical records. The survival period was traced until December 31, 2012. The study was approved by the institutional review board at our hospital.

Tissue microarrays and EBV-encoded small RNA in situ hybridization. The formalin-fixed and paraffin-embedded tissue samples were arrayed using an automated tissue-arraying machine (Beecher ATA-27; Beecher Instruments, Sun Prairie, WI, USA). All hematoxylin and eosin-stained slides were reviewed to choose representative tumor areas. Three 1.0-mm tissue cores were taken from tissue blocks and transferred to receipt blocks. The tissue microarrays were used for EBV-encoded small RNA (EBER) *in situ* hybridization.

EBER *in situ* hybridization was performed on tissue microarray slides using the EBV Probe ISH kit from Leica Microsystems in an automated immunostaining machine (BOND-MAX™; Leica, Wetzlar, Germany). The procedures were conducted according to the manufacturer's instructions. Only cases with universal and strong nuclear staining within almost all tumor cells were interpreted as EBV-positive.

Pathological analysis. More than two hematoxylin and eosin-stained slides of each EBVaGC were reviewed blindly by two pathologists (Dr S.C. Huang and Dr T.C. Chen) to determine the tumor type. We subclassified the EBVaGC specimens into LELC, tubular adenocarcinoma, poorly cohesive adenocarcinoma and mucinous adenocarcinoma. LELC was defined by the Watanabe's and Shibata's criteria: well circumscribed, undifferentiated carcinoma, non-desmoplastic stroma with dense lymphoplasmacytic infiltration (10,16). Tubular adenocarcinoma, poorly cohesive adenocarcinoma, and mucinous adenocarcinoma were defined as tumors with >50% of the tumor cells growing in a tubular pattern, poor cohesion and mucin pools, respectively, according to WHO classification (17). Mixed carcinoma was defined as a mixture of tubular adenocarcinoma and poorly cohesive carcinoma if either component accounted for >10% of the tumor.

Immunohistochemistry of HER2. We used a HER2 monoclonal antibody (A485, 1:200; Dako, Carpinteria, CA, USA) for immunostaining. Tissue sections of EBVaGC were prepared at a thickness of 3-μm and deparaffinized in xylene and rehydrated in a graded ethanol series. The slides were submitted to antigen retrieval, antibody incubation and chromogen counterstaining in an automated immunostainer (BOND-MAX™). Optimal positive and negative controls were also performed at the same time. Two experienced pathologists interpreted the HER2 status according to the recommendation for gastric cancer (18).

Statistical analysis. Statistical analysis was performed on an SPSS platform (version 17; SPSS, Chicago, IL, USA). The associations between the clinicopathological characteristics and the EBV status were evaluated by independent t-test, Pearson's χ^2 or Fisher exact test, according to the variable type. A multivariate logistic regression model was applied to the variables with a p-value <0.05 in the univariate analysis. For the comparison of LELC and non-LELC EBVaGC, the Mann-Whitney U test was employed for some continuous variables, including age, size and LN ratio. The overall survival was measured from the date of surgery to the date of death. Kaplan-Meier estimate was performed to calculate the overall survival, and the statistical significance of different variables was examined by the log-rank test. The Cox proportional hazard regression model was undertaken to determine the independent prognostic factors. Two-sided p-values were calculated, and $p<0.05$ was considered to be significant for all statistical analyses.

Results

Patient characteristics and pathological findings. A total of 52 EBVaGCs (5.1%) were identified by EBER *in situ* hybridization from 1,020 stage I-III gastric cancers. The clinical and pathologic features of the cases of EBVaGC and EBV-negative gastric cancer are summarized in Table I. The EBVaGC cases included 43 men and 9 women with a mean age of 64.83 years. The multivariate logistic regression analysis demonstrated that EBVaGC had a male predominance [odds ratio (OR) 3.120, 95% confidence interval (CI) 1.473-6.612, p=0.003] and a higher incidence in stump cancers (OR 5.957, 95% CI 2.423-14.648, p<0.0001) and poorly differentiated adenocarcinoma (OR 2.494, 95% CI 1.249-4.981, p=0.010) in comparison to EBV-negative gastric cancer. The EBVaGCs tended to occur more frequently in the proximal and middle portion than EBV-negative gastric cancer (57.7 vs. 32.0%), although the multivariate analysis failed due to the occurrence of non-convergence in the logistic regression models. No significant difference was found in regards to patient age, Lauren's classification, depth of invasion, metastatic node number, UICC/AJCC stage, lymphovascular permeation, perineural invasion and *Helicobacter* infection status between the EBVaGCs and EBV-negative gastric cancer. The tumor size and LN ratio were also not significantly different.

The 52 cases of EBVaGC were further subclassified into 19 LELC cases (36.5%) and 33 non-LELC cases (63.5%) (Fig. 1). The 33 non-LELC EBVaGC cases consisted of 29 cases of tubular adenocarcinoma (87.9%), 1 case of poorly cohesive carcinoma (3.0%), 1 case of mucinous adenocarcinoma (3.0%)

Table I. Clinicopathological findings of stage I-III gastric cancer patients classified by EBV status.

Parameters	EBV status		Univariate analysis	Multivariate analysis		
	Negative (n=968)	Positive (n=52)	P-value	Odds ratio	95% CI	P-value
Age (years), mean ± SD	63.65±13.51	64.83±11.02	0.536			
Gender			0.001			
Male	567 (58.6)	43 (82.7)		3.120	1.473-6.612	0.003
Female	401 (41.4)	9 (17.3)		1		
Stump cancer			<0.0001			
Yes	21 (2.2)	10 (19.2)		5.957	2.423-14.648	<0.001
No	947 (97.8)	42 (80.8)		1		
Localization[a]			<0.001			
Upper	149 (15.4)	20 (38.5)				
Middle	161 (16.6)	10 (19.2)				
Lower	620 (64.0)	21 (40.4)				
Diffuse	38 (3.9)	1 (1.9)				
Tumor size, mean ± SD (cm)	4.43±3.17	4.44±2.36	0.966			
Differentiation			0.010			
Well/moderate	397 (41.0)	12 (23.1)		1		
Poor	571 (59.0)	40 (76.9)		2.494	1.249-4.981	0.010
Lauren's classification			0.120			
Intestinal	489 (50.5)	25 (48.1)				
Diffuse	365 (37.7)	16 (30.8)				
Mixed	114 (11.8)	11 (21.2)				
Depth of invasion			0.039			
T1/T2	360 (37.2)	12 (23.1)		1		
T3/T4	608 (62.8)	40 (76.9)		1.233	0.604-2.520	0.565
Nodal status			0.753			
N0	384 (39.7)	17 (32.7)				
N1	133 (13.7)	7 (13.5)				
N2	154 (15.9)	10 (19.2)				
N3	297 (30.7)	18 (34.6)				
Stage			0.179			
I	283 (29.2)	9 (17.3)				
II	177 (18.3)	11 (21.2)				
III	508 (52.5)	32 (61.5)				
LN ratio, mean ± SD	0.21±0.27	0.19±0.21	0.702			
Lymphatic invasion[b]			0.204			
No	475 (49.4)	21 (40.4)				
Yes	486 (50.6)	31 (59.6)				
Vascular invasion[b]			0.863			
No	838 (87.7)	46 (88.5)				
Yes	118 (12.3)	6 (11.5)				
Perineural invasion[b]			0.892			
No	505 (52.9)	28 (53.8)				
Yes	450 (47.1)	24 (46.2)				
HP infection			0.462			
No	771 (80.5)	44 (84.6)				
Yes	187 (19.5)	8 (15.4)				

Data are numbers with percentages in parentheses, unless otherwise stated. EBV, Epstein-Barr virus; SD, standard deviation; LN ratio, lymph node ratio; HP, *Helicobacter pylori*; CI, confidence interval. [a]There is the non-convergence in logistic regression models when the data are quasi-completely separated. The maximum likelihood estimation does not exist. [b]Not all data are available in EBV-negative cases.

Table II. Clinicopathological findings of stage I-III EBV-associated gastric cancer patients classified by tumor histology.

Parameters	Tumor type		P-value
	LELC (n=18)	Non-LELC (n=33)	
Age (years), median (range)	62 (44-81)	70 (39-80)	0.253
Gender			0.703
Male	14 (77.8)	28 (984.8)	
Female	4 (22.2)	5 (15.2)	
Stump cancer			0.727
Yes	4 (22.2)	6 (18.2)	
No	14 (77.8)	27 (81.8)	
Localization			0.322
Upper	5 (27.8)	15 (45.5)	
Middle	3 (16.7)	7 (21.2)	
Lower	10 (55.6)	10 (30.3)	
Diffuse	0	1 (3.0)	
Tumor size (cm), median (range)	3.8 (1.0-6.0)	4.5 (0.8-14.0)	0.161
Differentiation			0.004
Well/moderate	0	12 (36.4)	
Poor	18 (100.0)	21 (63.6)	
Lauren's classification			0.106
Intestinal	5 (27.8)	19 (57.6)	
Diffuse	7 (38.9)	9 (27.3)	
Mixed	6 (33.3)	5 (15.2)	
Depth of invasion			0.304
T1/T2	6 (33.3)	6 (18.2)	
T3/T4	12 (66.7)	27 (81.8)	
Nodal status			0.282
N0	9 (50.0)	8 (24.2)	
N1	2 (11.1)	5 (15.2)	
N2	3 (16.7)	6 (18.2)	
N3	4 (22.2)	14 (42.4)	
Stage			0.189
I	5 (27.8)	4 (12.1)	
II	5 (27.8)	6 (18.2)	
III	8 (44.4)	23 (69.7)	
LN ratio, median (range)	0.03 (0-0.57)	0.19 (0-0.81)	0.116
Lymphatic invasion			0.244
No	9 (50.0)	11 (33.3)	
Yes	9 (50.0)	22 (66.7)	
Vascular invasion			0.078
No	18 (100.0)	27 (81.8)	
Yes	0	6 (18.2)	
Perineural invasion			0.147
No	12 (66.7)	15 (45.5)	
Yes	6 (33.3)	18 (54.5)	
HP infection			0.686
No	15 (83.3)	29 (87.9)	
Yes	3 (16.7)	4 (12.1)	

Figures are numbers with percentages in parentheses, unless otherwise stated. EBV, Epstein-Barr virus; LELC, lymphoepithelioma-like carcinoma; LN ratio, lymph node ratio; HP, *Helicobacter pylori*.

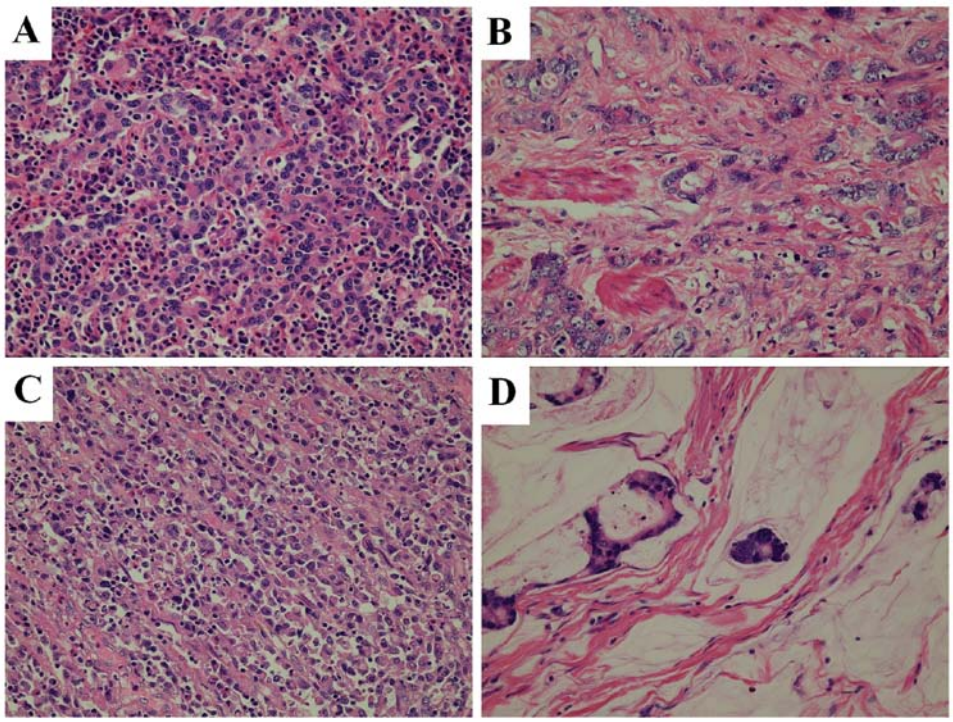

Figure 1. Histological classification of Epstein-Barr virus-associated gastric cancer: (A) lymphoepithelioma-like carcinoma, (B) tubular adenocarcinoma, (C) poorly cohesive carcinoma and (D) mucinous adenocarcinoma (magnification, x400).

and 2 cases of mixed carcinoma (6.1%). After excluding 1 case that suffered from surgical mortality, the clinicopathological parameters of LELC and non-LELC in the 51 cases of EBVaGC were not significantly different, with the exception of tumor differentiation (p=0.004) (Table II).

HER2 immunohistochemistry. Only one case of the 52 EBVaGCs (1.9%) demonstrated HER2 overexpression (strong intensity, score 3^+).

Survival analysis. According to the available survival data, the mean survival duration for the 51 cases of EBVaGC and the 943 cases of EBV-negative gastric cancer was 87.74 (95% CI 70.37-105.11) and 88.1 (95% CI 84.09-92.12) months, respectively. The follow-up duration for the 51 cases of EBVaGC ranged from 1.2 to 166 months (median 45.04 months). EBV infection itself was not a prognostic factor in stage I-III gastric cancer (p=0.977) (Fig. 2). According to the survival analysis of EBVaGC, the log-rank analysis revealed that tumor location, tumor size >5 cm, depth of tumor invasion, number of lymph node metastasis, LN ratio, lymphovascular invasion, perineural invasion and LELC subtype reached statistical significance as prognostic factors (Table III). In the Cox proportional hazard model, tumor size >5 cm [hazard ratio (HR) 2.884, 95% CI 1.129-7.365, p=0.027], LN ratio >0.15 (HR 19.352, 95% CI 4.383-85.441, p<0.0001), and non-LELC subtypes (HR 12.178, 95% CI 2.135-69.474, p=0.005) had an unfavorable effect on survival. However, the depth of tumor invasion and nodal status lost their statistical significance (p=0.834 and 0.844, respectively). Tumor location was excluded due to the occurrence of the monotone likelihood, and the maximum likelihood estimation did not exist. The median survival for cases with a tumor size >5 cm was 22.1 months with a 5-year survival rate of 13.3% compared to 74.9% in tumors with a size ≤5 cm (Fig. 3A). LELC EBVaGC had a 5-year survival rate of 87.5%, which was superior to the non-LELC cases with a 5-year survival rate of 38.4% and a median survival of 32.7 months (Fig. 3B). Cases with an LN ratio >0.15 had a median survival of 28.3 months and a 13.4% 5-year survival rate, whereas cases with an LN ratio ≤0.15 had a 96.0% 5-year survival rate (Fig. 3C).

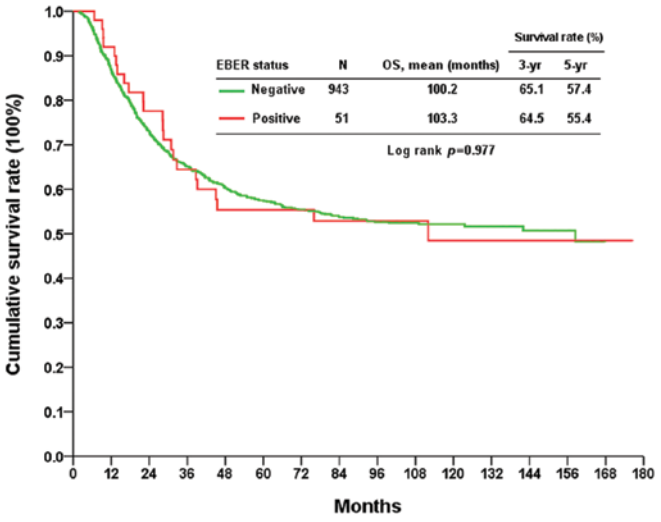

Figure 2. Comparison of Kaplan-Meier estimated survival curves of gastric cancer according to the results of EBV-encoded small RNA (EBER) *in situ* hybridization.

Table III. Survival analysis of patients with stage I-III EBV-associated gastric cancer.

	Univariate analysis			Multivariate analysis		
Clinicopathological factors	Mean survival (months)	95% CI	P-value	Hazard ratio	95% CI	P-value
Age (years)			0.116			
≤65 (n=25)	120.81	91.59-150.03				
>65 (n=26)	79.73	51.57-107.90				
Gender			0.859			
Male (n=42)	101.62	77.56-125.68				
Female (n=9)	80.24	49.32-111.15				
Location[a]			<0.0001			
Upper (n=20)	79.68	49.76-109.61				
Middle (n=10)	102.77	60.51-145.02				
Lower (n=20)	119.82	86.44-153.21				
Diffuse (n=1)	6.67	6.67-6.67				
Tumor size (cm)			<0.0001			
≤5 (n=35)	135.28	112.41-158.14		1		
>5 (n=16)	30.58	17.45-43.71		2.884	1.129-7.365	0.027
Differentiation			0.892			
Well/moderate (n=12)	86.48	51.27-121.69				
Poor (n=39)	105.19	80.19-130.20				
Histological classification			<0.0001			
LELC (n=18)	158.97	136.27-181.68		1		
Non-LELC (n=33)	67.04	44.61-89.47		12.178	2.135-69.474	0.005
Depth of invasion			0.032			0.834
T1/T2 (n=12)	145.23	118.37-172.09				
T3/T4 (n=39)	90.10	65.54-114.65				
Nodal status			<0.0001			0.844
N0 (n=17)	160.30	145.92-174.69				
N1 (n=7)	116.73	86.43-147.03				
N2 (n=9)	79.00	27.26-131.93				
N3 (n=18)	37.42	20.87-53.98				
LN ratio			<0.0001			
≤0.15 (n=27)	151.83	134.94-168.72		1		
>0.15 (n=24)	44.72	22.92-66.51		19.352	4.383-85.441	<0.0001
Lymphatic invasion			0.001			0.699
No (n=20)	146.21	123.60-168.81				
Yes (n=31)	72.10	46.34-97.87				
Vascular invasion			0.003			0.935
No (n=45)	114.57	91.82-137.31				
Yes (n=6)	36.97	6.17-67.77				
Perineural invasion			0.002			0.464
No (n=27)	132.40	106.92-157.88				
Yes (n=24)	63.63	36.68-90.58				
HP infection			0.846			
No (n=44)	101.76	79.04-124.47				
Yes (n=7)	104.29	42.14-166.44				

EBV, Epstein-Barr virus; LELC, lymphoepithelioma-like carcinoma; LN ratio, lymph node ratio; HP, *Helicobacter pylori*; CI, confidence interval. [a]This factor was excluded from the Cox proportional hazard model due to occurrence of the monotone likelihood and the maximum likelihood estimation did not exist.

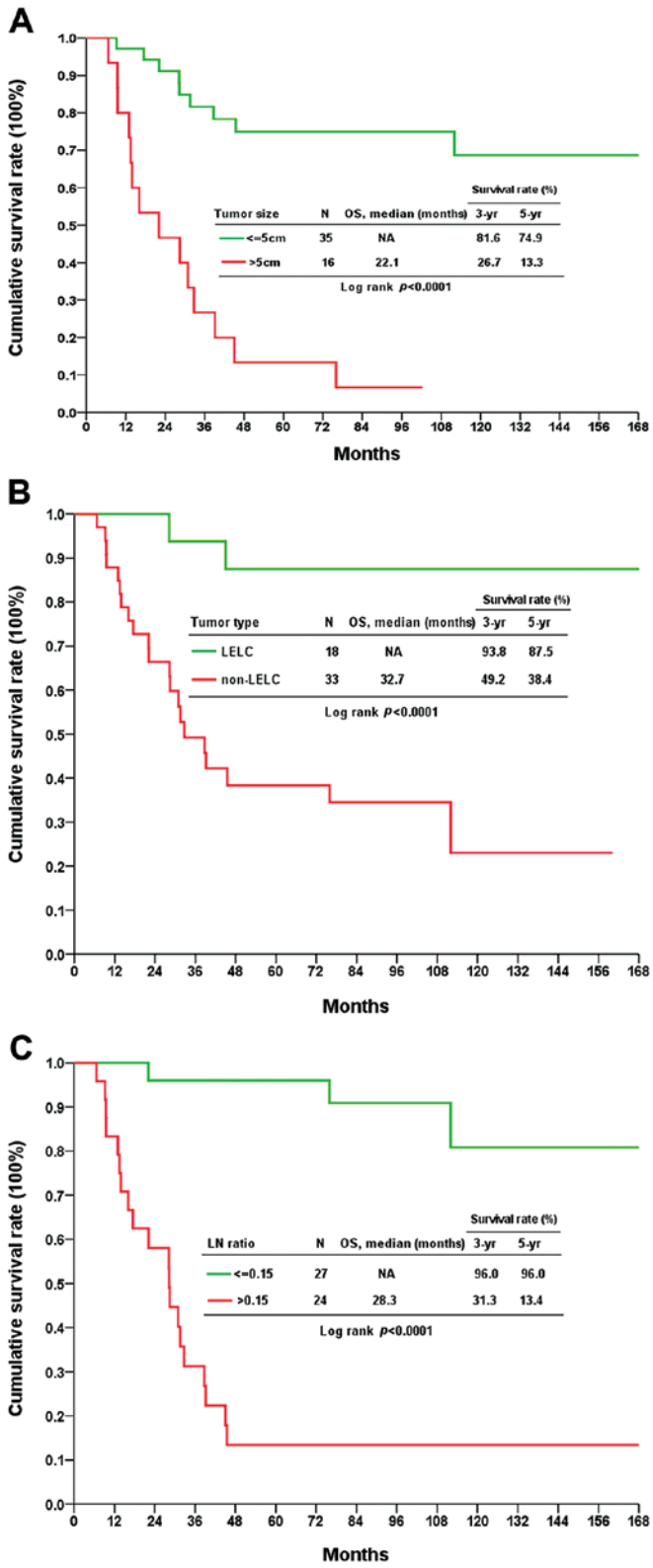

Figure 3. Overall survival analysis of 51 Epstein-Barr virus-associated gastric cancer cases according to different prognostic factors: (A) tumor size, (B) histological lymphoepithelioma-like carcinoma (LELC) classification and (C) lymph node ratio (LN ratio).

Discussion

In the present study, EBVaGC constituted 5.1% of the cases of stage I-III gastric cancer and was associated with demographic features that have been described in previous studies. According to a large-scale meta-analysis of 70 studies including 15,952 cases of gastric cancer, EBVaGC had a prevalence estimate of 8.7% (95% CI 7.5-10.0%, range 1.33-19.9%) (5). The meta-analysis revealed that EBVaGC presented a 2-fold higher incidence in males (11.1%) than in females and occurred twice as often in the gastric cardia or corpus than in the antrum (13.6 or 13.1 vs. 5.2%) compared to EBV-negative gastric cancers. Stump cancer had a 4-fold higher incidence of EBV positivity (35.1%). The above results are similar to the finding of our study, which revealed a male preponderance and a higher incidence in stump cancer. Regarding the location in the stomach, EBVaGC seems to occur more frequently in the upper third and less frequently in the lower third than EBV-negative gastric cancer. No significant difference was observed in prevalence between the intestinal, diffuse and mixed tumor types. In addition, the present study revealed that poorly differentiated carcinoma has a higher likeliness of EBV positivity. Taiwan is an endemic area for nasopharyngeal cancer, which is also associated with EBV infection. The data gathered at our institute indicated that EBVaGC was a rare (1/6, 16.6%) event of secondary cancer in patients with nasopharyngeal cancer in Taiwan (19). Furthermore, the incidence of EBVaGC is similar worldwide, with 8.3% in Asia, 9.2% in Europe and 9.9% in America (5). EBV-associated gastric cancer and nasopharyngeal cancer express latency I and latency II protein, respectively (1,2). EBVaGC may be a universal and distinct variant of gastric cancer with characteristic clinical features that are independent from nasopharyngeal cancer.

In addition, our results showed that EBV infection itself seems not to be a prognostic factor, and risk stratification by tumor size, histological classification, and LN ratio may help identify high-risk patients. According to the literature, a favorable outcome was observed only in those cases categorized as LELC, and EBV infection itself was not associated with any survival advantage (10,11,13-15). Our results also support this prevailing opinion. LELC-type EBVaGC presents a striking benefit for both the 3- and 5-year survival rate. This unique histological subtype is distinguished by the presence of a non-desmoplastic stroma infiltrated with an abundance of lymphocytes and plasma cells (10,16). These extensive intratumoral inflammatory components are composed of activated cytotoxic T, nature killer and mature dendritic cells (9,15,20). The T cell infiltration has been correlated to intratumoral FoxP3-positive regulatory T cells (21). Some authors hypothesized that the intratumoral inflammatory reaction represents an effective host immune reaction against tumor cells (9,20,22). In colon, breast and lung malignancies, tumor-infiltrating lymphocytes also express a cytotoxic T-cell phenotype and are related to a survival advantage (22).

Likewise, the intratumoral inflammation pattern and intensity have been reported to influence the survival of EBVaGC patients (9,11,22). Song *et al* purported that a typical Crohn's disease-like lymphocytic reaction may share a similar morphology with LELC on the basis of the similar survival benefit in both groups (11). Grogg *et al* found that increased lymphocyte infiltration of the tumor indicated a better prognosis as EBVaGC had a higher lymphocyte count (450/10 HPF) than EBV-negative gastric cancer (21/10 HPF) (22). The amount of intratumoral T and dendritic cells was also more plentiful

in EBVaGC without lymph node metastasis than in EBVaGC with node metastasis (9). Although the underlying mechanism of the antitumor immune reaction is not well elucidated, the accurate classification of EBVaGC into LELC or non-LELC is not only associated with patient prognosis but also implies a different tumor-host interaction.

Tumor size has been regarded as an important prognostic factor due to its close relationship to histological grade, UICC/AJCC stage, vascular invasion and neural permeation (23). We observed that a tumor size >5 cm is an independent parameter of poor prognosis in stage I-III EBVaGC instead of the depth of tumor invasion. EBVaGC frequently grows in ulcerated or saucer-like tumors featured by well-delineated and pushing borders (7,10,16). This macroscopic pattern corresponds to a microscopic expanding front rather than to infiltrative invasion. EBVaGC may follow a different encroachment fashion from EBV-negative gastric cancer. That may be why tumor size is a powerful predictor. Indeed, tumor size is a paramount component for UICC/AJCC tumor stage in some malignancies, including lung and breast cancers (24). For gastric cancer, some authors have found that tumor size is a simple prognostic indicator and could even improve the accuracy of UICC/AJCC staging for gastric cancer (25,26). Due to the heterogeneity of gastric cancer, tumor size may be a significant outcome indicator in some subgroups, such as EBVaGC, as shown by the present study.

The LN ratio is an emerging parameter that may be more useful than UICC/AJCC lymph node stage due to its consistent prognostic power whenever the type of lymphadenectomy or total number of resected nodes varies (27-30). This study suggests that an LN ratio >0.15 is an independent and powerful predictor that is superior to the UICC/AJCC nodal status in stage I-III EBVaGC. The mean number of harvested lymph nodes from the EBVaGC group in our study was 30.5 with 6 cases having a total of <15 lymph nodes (11.8%) (data not shown). An LN ratio >0.15 retained its statistical significance in the Cox regression multivariate analysis and had the strongest hazard ratio compared with the other factors. Most previous studies have divided LN ratio into categories corresponding to 0, 1-9, 10-25 and >25% to stratify patient risk, which is intended to imitate the AJCC/UICC node status (27,28). Our data demonstrated that a simple threshold of 0.15 could achieve considerable discernability for patient outcome. Metastatic foci of EBVaGC in lymph nodes still maintain an EBV genome (9). The occurrence of node metastasis does not signify tumor escape by virus deletion, but it may imply alteration of the tumor to lose the target antigen or to attenuate the immune defense. Indeed, a better prognosis observed in EBVaGC has been attributed to less lymph node involvement (12). The relationship between tumor molecular alteration and lymph node metastasis still requires further study, but the LN ratio may be used as another simple approach to predict this host-tumor interaction and its effect on patient outcome.

EBV is thought to play a critical role, not only in stimulation of an immunologic reaction, but also in gastric carcinogenesis. Through the presentation of an undefined antigen, EBV attracts strikingly numerous lymphocytes and plasma cells that are intimately admixed with tumor cells by upregulation of major histocompatibility complex class II molecules and IL1-β cytokines (9,31). Aside from the differences in protein expression and chromosomal aberrations compared with EBV-negative counterparts, EBVaGC exhibits characteristic molecular features, including a global and non-random CpG island methylation epigenotype, which is activated by DNMT1 through the phosphorylation of STAT3 from viral LMP2A effect (7,32-36). Considering the unique host-tumor interaction and the molecular alterations detected in EBVaGC, our data suggest that the use of the histological classification, tumor size, and LN ratio could discriminate the prognosis of patients with stage I-III EBVaGC. For therapeutic options that utilize virus-host interactions in EBV-associated tumors, demethylating agents, such as 5-azacytidine, can induce lytic infection of EBV, leading to lysis of the infected tumor cells, by restoring the expression of the BMRF1, BZLF1 and BRLF1 genes after removing promoter methylation (37,38). These novel antitumor drugs may provide a maximal survival benefit to those high-risk patients with a large tumor size, non-LELC tumors or a high LN ratio.

It is important to characterize the tumor pathway and processes for personalized medicine. The present study is one of our serial investigations aimed at the molecular classification of gastric cancers. EBVaGC and HER-2-overexpressing gastric cancers may represent distinct subsets since only one EBVaGC case had HER-2 overexpression. The incidence of 1.9% was much lower than that of 6.1% in the general population of gastric cancer, as demonstrated in our previous study (39). In addition, the low incidence of HER2 amplification is also consistent with the results reported by a Korean group (1/123, 0.8%) (11).

In conclusion, we conducted a large-scale study involving 1,020 stage I-III gastric cancer cases from a single institute. We identified 52 cases of EBVaGC. EBVaGC showed a male preponderance and a higher incidence in stump cancer and poorly differentiated carcinoma. The survival analysis suggested that tumor size, LELC classification, and LN ratio were important prognostic factor. These influential factors most likely reflect EBVaGC's unique mode of carcinogenesis and host-tumor interaction and should be considered in the identification of high-risk patients who may benefit from adjuvant regimens or virus-specific treatments.

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