Int J Clin Exp Med 2019;12(9):11280-11292
www.ijcem.com /ISSN:1940-5901/IJCEM0096647

Original Article

Clinical characteristics, prognostic factors, and histone deacetylase 6 expression in primary gastric diffuse large B-cell lymphoma

Shenhe Jin[1], Hui Liu[2,4], Chunmei Yang[2,4], Liangshun You[2,4,5], Wei Ding[3], Wenbin Qian[2,4,5], Juying Wei[2,4]

[1]Department of Hematology, Sir Run Run Shaw Hospital, College of Medicine, Zhejiang University, Hangzhou, P. R. China; Departments of [2]Hematology, [3]Pathology, [4]Malignant Lymphoma Diagnosis and Therapy Center, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, P. R. China; [5]Institute of Hematology, Zhejiang University, Hangzhou, P. R. China

Received May 7, 2019; Accepted July 11, 2019; Epub September 15, 2019; Published September 30, 2019

Abstract: Primary gastric diffuse large B-cell lymphoma (PG-DLBCL) is a heterogeneous disease. Prognostic factors and treatments of PG-DLBCL, however, remain controversial. In the current study, clinical data of 103 PG-DLBCL patients were retrospectively analyzed. Moreover, 29 available tumor samples were obtained, examining expression levels and prognostic significance of histone deacetylase 6 (HDAC6). The 5-year overall survival (OS) and progression free survival (PFS) rates were 83.4% and 68.4%. B-symptoms, elevated lactate dehydrogenase (LDH), decreased albumin, poor performance status (PS ECOG ≥ 2), high international prognostic index scores (IPI ≥ 3), and advanced stages were associated with poor prognosis. Of these, elevated LDH, poor PS, and advanced stages were shown to be independent prognostic factors. High HDAC6 expression was associated with limited stage and better OS. Rituximab-containing chemotherapy showed better OS and PFS in patients of ECOG ≥ 2, which also showed better PFS in elderly patients (age > 60 years). Rituximab maintenance after 6 cycles of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) chemotherapy improved OS and PFS. Current results showed that elevated LDH, poor PS, and advanced stages were independent prognostic factors. HDAC6 may be a positive prognostic biomarker in PG-DLBCL. Additionally, results showed rituximab-containing chemoimmunotherapy to be the optimal treatment for PG-DLBCL.

Keywords: Primary gastric diffuse large B-cell lymphoma, histone deacetylase 6, prognosis, treatment

Introduction

Primary gastric lymphoma (PGL) is defined as a malignant tumor originating from the stomach, with or without peri-gastric and abdominal lymph node involvement [1]. PGL is an uncommon tumor, accounting for less than 5% of primary gastric neoplasms and 10-15% of all non-Hodgkin's lymphomas (NHL). However, primary gastric lymphoma is still the most common type of extra-nodal lymphoma, representing 30-40% of all extra-nodal NHL cases [2, 3]. Diffuse large B-cell lymphoma (DLBCL) is the predominant pathological subtype of PGL, accounting for 40-70% of cases [4, 5]. Primary gastric DLBCL (PG-DLBCL) is a clinically and biologically heterogeneous disease [6]. Clinical parameters, such as International Prognostic Index (IPI) scores, disease stages, performance status (PS), and serum lactate dehydrogenase (LDH) levels have been reported to be associated with prognosis [7-9]. Treatments for PG-DLBCL include rituximab plus anthracycline-based combination chemotherapy, radiotherapy, surgery, and *H. pylori* (HP) eradication with antibiotic therapy, which is especially used in cases of PG-DLBCL with concomitant mucosa-associated lymphoid tissue (MALT) components. In addition, high-dose chemotherapy, followed by autologous stem cell transplantation, may offer therapeutic benefits for patients with relapsed or refractory disease. However, the roles of surgery and rituximab in the management of PG-DLBCL remain controversial [3, 9-11].

Recently, several studies have showed that serial biological molecular markers are associ-

ated with prognosis of PG-DLBCL. For example, it has been shown that patients with multiple gene amplification and/or copy gains of c-Myc, Bcl-2, and Bcl-6, along with double expression gastric B-cell lymphomas, have poor clinical outcomes [7]. Histone deacetylase 6 (HDAC6), a member of the class IIb HDAC superfamily, is overexpressed in different types of tumors, including lymphomas. It can either trigger tumor development or suppress tumor growth [12]. Some studies have reported that high HDAC6 expression is associated with survival in cancer [13-16]. However, the prognostic roles of HDAC6 in PG-DLBCL have not been reported.

The current study retrospectively investigated clinical characteristics, prognostic factors, values of different treatments, and prognostic roles of HADC6 in PG-DLBCL.

Materials and methods

Patients

The current study retrospectively analyzed 103 patients diagnosed with PG-DLBCL, between May 2008 and March 2016. Each of the cases in this study were negative in the serologic detection of Human immunodeficiency virus (HIV). The current study was approved by the Institutional Review Board of the First Affiliated Hospital, College of Medicine, Zhejiang University. Histopathologic diagnoses were made and reviewed by two experienced pathologists, independently, according to criteria of the World Health Organization (WHO) [17]. Patients with transformations from indolent lymphomas, such as MALT lymphoma, to DLBCL were excluded. The GCB or non-GCB subtype was determined by Han's algorithm based on CD10, BCL-6, and MUM-1/IRF4 [18]. Disease and patient clinical characteristics included age, sex, B-symptoms, LDH, β2-microglobulin (β2-MG) and serum albumin levels, bone marrow involvement, and PS, according to the Eastern Cooperative Oncology Group (ECOG) scale. Presence of HP infections was confirmed by histologic examinations. Stages were accessed according to the Lugano staging system [19], mainly on the basis of physical examinations, computed tomography (CT) or 18F-fluorodeoxyglucose positron emission tomography (18FDG-PET) scans, electronic/ultrasound gastroscopy procedures, and outcomes of intraoperative exploration for those patients that received surgery. Follow-up information was updated through February 2017.

Immunohistochemistry

Immunohistochemical staining for HDAC6 was performed on 29 well-preserved paraffin-embedded sections of pretreatment endoscopic biopsies or surgeries. Briefly, the tissues were cut into 3-μm slices, then dewaxed and rehydrated. The slides were incubated for 1 hour at room temperature with rabbit monoclonal HDAC6 antibody (1:250 dilution, Abcam; ab133493). It was retrieved by boiling in EDTA (PH 8.0) for 20 minutes. This was followed by incubation with a secondary antigen for 15 minutes at room temperature. Finally, immunoreactivity of these slides was developed with diaminobenzidine tetrahydrochloride (DAB). The nuclei were counterstained with hematoxylin.

Evaluation of immunohistochemistry

Immunohistochemistry (IHC) of HDAC6 was evaluated based on the score system comprising the percentage of positive tumor cells and intensity levels of immunoreactivity. The percentage of positive tumor cells was scored 0 for < 5%, 1 for 6-20%, 2 for 21-50%, and 3 for > 50% of stained tumor cells. Intensity of immunoreactivity was scored 0 for no staining, 1 for weak staining, 2 for moderate staining, and 3 for strong staining. Final HDAC6 expression scores were obtained by adding these two individual scores as follows: Low expression (scored 0-2), moderate expression (scored 3-4), and high expression (scored 5-6) [15].

Statistical analysis

Overall survival (OS) is defined as the time from diagnosis to death of any cause or loss to follow-up. Progression free survival (PFS) is defined as the time from diagnosis to disease progression, relapse, death of any cause, or last follow-up. Categorical data were compared with Fisher's exact tests. Kaplan-Meier curves were used to calculate survival outcomes. Log-rank testing was performed to compare differences between the two groups. Main clinical characteristics were analyzed concerning association levels with OS and PFS using Cox's proportional hazard models. Additionally, variables with P values < 0.10 in univariate analysis were

Table 1. Main characteristics of the patients

Characteristics	No. of patients (%)
Age, y (median, range)	(58, 15-85)
> 60	41 (39.8)
≤ 60	62 (60.2)
Gender	
Male	49 (47.6)
Female	54 (52.4)
B symptoms	
Present	19 (18.4)
Absent	84 (81.6)
LDH	
Elevated	30 (29.1)
Normal	73 (70.9)
β2-MG	
Elevated	26 (25.2)
Normal	62 (60.2)
NA	15 (14.6)
Albumin	
Decreased	20 (48.5)
Normal	83 (51.5)
HP	
Positive	23 (22.3)
Negative	25 (24.3)
NA	55 (53.4)
BM involvement	
Yes	6 (5.8)
No	79 (76.7)
NA	18 (17.5)
Subtypes	
GCB	33 (32.0)
non-GCB	51 (49.5)
NA	19 (18.5)
ECOG score	
0-1	68 (66.0)
≥ 2	35 (34.0)
Lugano staging	
I	25 (24.3)
II1	15 (14.6)
II2	7 (6.8)
IIE	7 (6.8)
IV	49 (47.5)
IPI score	
0-2	70 (68.0)
≥ 3	33 (32.0)
Treatment	
Surgery plus chemotherapy	16 (15.5)
Chemotherapy	87 (84.5)
Chemotherapy regimens	
With rituximab	75 (72.8)
Without rituximab	28 (27.2)

Abbreviations: BM, bone marrow; β2-MG, beta 2 microglobulin; ECOG, Eastern Cooperative Oncology Group; GCB, germinal center B-cell like; HP, helicobactor pylori; IPI, International Prognostic Index; LDH, serum lactate dehydrogenase; NA, not available.

included in multivariate analysis, with the backward method in cases of exclusion of some potential prognostic factors. Hazard ratios (HR) and 95% confidence intervals (CI) of significant parameters were also calculated. Two-sided *P* values < 0.05 indicate statistical significance. All analyses were performed in SPSS 19.0.

Results

Clinicopathological characteristics

Characteristics of the 103 patients, consisting of 49 men and 54 women, are listed in **Table 1**. The median age was 58 years (range 15-85). The most common symptom was epigastric pain or discomfort, while 19 (18.5%) patients complained about hematemesis or melena. Main lesions were gastric antrum and body under gastroscopy. According to Lugano staging criteria, stages I/II1/II2/IIE/IV accounted for 24.3%, 16.4%, 6.8%, 6.8%, and 47.5%, respectively. Regarding pathology, all 103 patients were diagnosed with DLBCL. Of the 84 patients with complete immunohistochemical results of CD10, BCL-6, and MUM-1, 33 (32.0%) were GCB subtype, 51 (49.5%) were non-GCB subtype, and the other 19 (18.5%) were indistinguishable, lacking enough specimens for immunohistochemical staining.

Treatment modalities

All 103 patients received chemotherapy. Most of them received at least 4 cycles of chemotherapy except those suffering from bad general conditions or early deaths. The main regimens were CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone) ± R (rituximab). Of the 75 patients that received R-CHOP chemotherapy, 21 patients additionally received at least 2 cycles of rituximab maintenance. Moreover, 16 (15.5%) patients underwent subtotal or total gastrectomy plus lymph node dissections, while 2 patients were treated with radiotherapy.

Table 2. Prognostic factors of univariate and multivariate analyses in patients

Variables		OS				PFS			
		Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
		P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)
Gender	Male	0.237				0.092	1.86 (0.90-3.84)	0.456	
	Female								
Age	> 60 y	0.139				0.221			
	≤ 60 y								
B symptoms	Present	0.026	3.16 (1.14-8.71)	0.848		0.002	3.22 (1.54-6.76)	0.405	
	Absent								
LDH level	Elevated	0.022	3.16 (1.18-8.46)	0.258		0.001	3.57 (1.73-7.38)	0.006	2.84 (1.36-5.94)
	Normal								
β2-MG level	Elevated	0.215				0.117			
	Normal								
Albumin	Normal	0.007	0.25 (0.09-0.69)	0.121		0.010	0.37 (0.17-0.79)	0.058	0.47 (0.22-1.03)
	Declined								
ECOG	0-1	0.001	7.24 (2.32-22.53)	0.002	6.27 (2.00-19.66)	0.001	3.22 (1.58-6.58)	0.006	2.73 (1.33-5.61)
	≥ 2								
Subtypes	GCB	0.816				0.774			
	non-GCB								
Hp infection	Positive	0.837				0.192			
	Negative								
BM involvement	Yes	0.862				0.229			
	No								
Lugano staging	I-II1								
	II2-IIE	0.025		0.005		0.045		0.173	
	IV								
IPI score	0-2	< 0.001	19.22 (4.36-84.84)	-*	-*	<0.001	5.13 (2.45-10.72)	-*	-*
	≥ 3								
Treatment	S+C	0.260				0.080	0.27 (0.61-1.17)	0.342	
	C								
Chemotherapy regimens	With R								
	Without R	0.746				0.235			

Abbreviations: BM, bone marrow; β2-MG, beta-2 microglobulin; C, chemotherapy; HR, hazard ratio; HP, helicobactor pylori; IPI, International Prognostic Index; LDH, serum lactate dehydrogenase; R, rituximab; S+C, surgery plus chemotherapy. *Given that IPI score covers LDH levels, ECOG, and Lugano staging, it was not included in multivariate analysis.

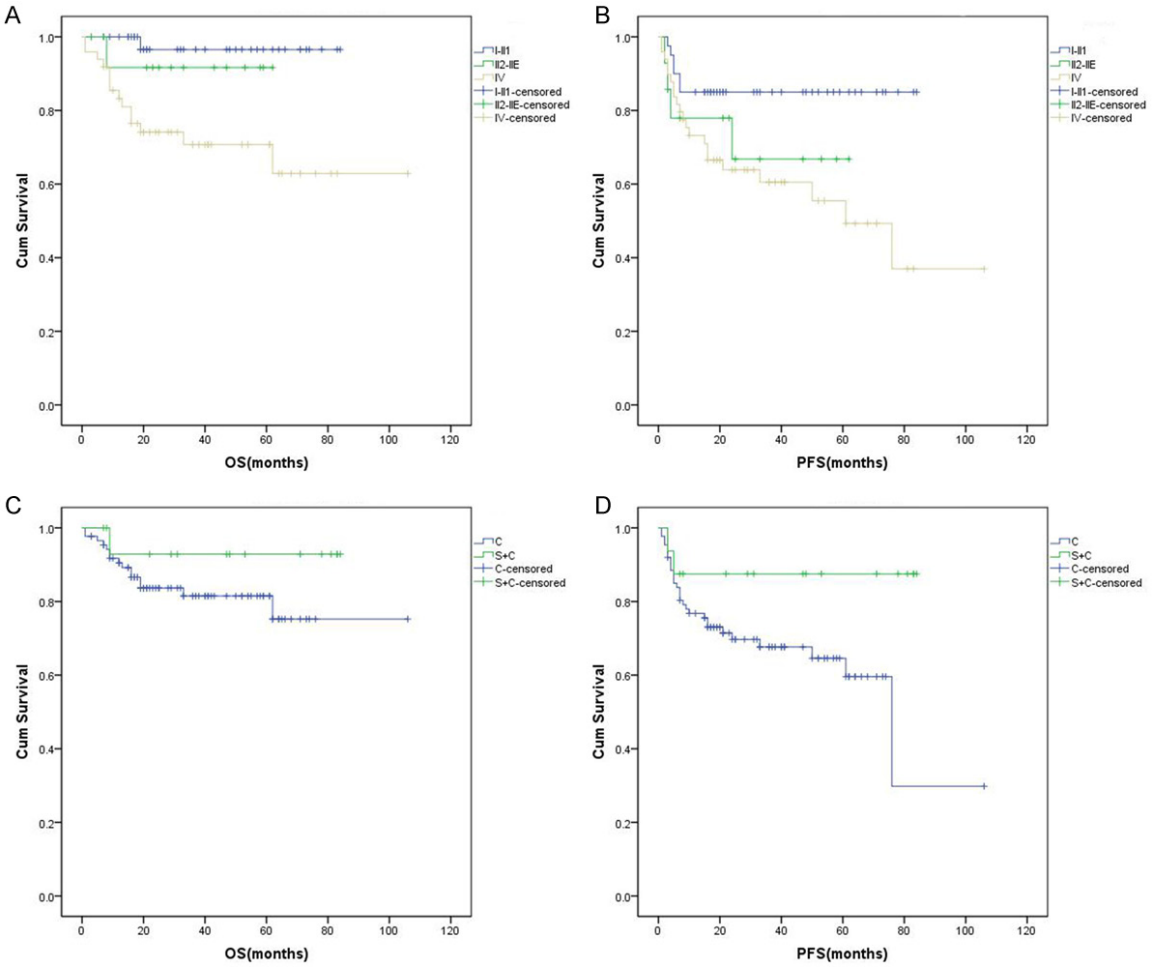

Figure 1. Kaplan-Meier curves according to potential prognostic factors. A, B. OS and PFS of patients according to stage; C, D. OS of patients according to treatments. *C = chemotherapy, S = surgery.

Survival and prognostic analyses

Up through February 2017, 17 (16.5%) of 103 PGL patients died. The median follow-up was 41 months (range 12-106) and the follow-up rate was 89.3%. The 1-year OS and PFS rates were 90.9% and 78.4%. The 5-year OS and PFS rates were 83.4% and 68.4%.

Table 2 summarizes univariate and multivariate analysis of various factors of OS and PFS. According to univariate analysis of potential prognostic factors, B-symptoms, elevated LDH, decreased albumin, poor PS (ECOG ≥ 2), high IPI scores (≥ 3), and advanced stages were associated with poor OS and PFS. In contrast, gender, age, β2-MG levels, Hp infections, and treatment were not associated with survival. According to multivariate analysis, ECOG ≥ 2 (HR = 6.27, 95% CI 2.00-19.66, P = 0.002) and advanced stages (P = 0.005) were independent prognostic predictors of OS. Moreover, stratification analysis of Lugano staging attributed survival differences to stage I-II1 and stage IV (HR = 11.08, 95% CI 1.45-84.40, P = 0.020; **Figure 1A**, **1B**). However, different survival durations between stage I-II1 and stage II2-IIE or stage II2-IIE and stage IV were insignificant. Regarding PFS, elevated LDH (HR = 2.84, 95% CI 1.36-5.94, P = 0.006) and ECOG ≥ 2 (HR = 2.73, 95% CI 1.33-5.61, P = 0.006) were shown to be independent prognostic factors. According to Kaplan-Meier curves, patients receiving surgery plus chemotherapy (S+CT) presented a trend of better OS and PFS, compared with those treated by chemotherapy alone (CT), without statistical significance (OS, P = 0.232; PFS, P = 0.062; **Figure 1C**, **1D**). The survival of patients with distinct chemotherapy regimens (rituximab-containing or not) (OS, P = 0.744;

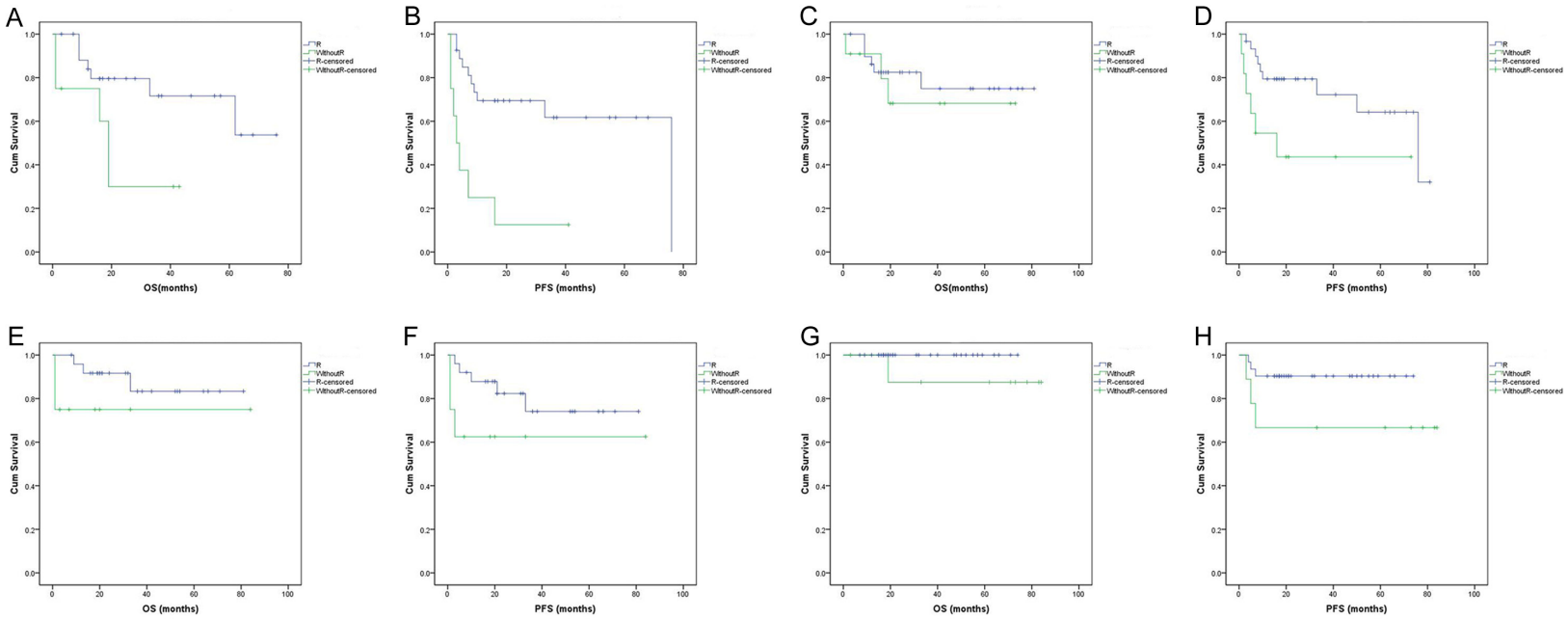

Figure 2. Kaplan-Meier curves in stratified clinicopathological groups according to chemotherapy regimens; A, B. OS and PFS of patients for ECOG ≥ 2 according to chemotherapy regimens; C, D. OS and PFS of patients for age > 60 according to chemotherapy regimens; E, F. OS and PFS of patients for GCB subtype according to chemotherapy regimens; G, H. OS and PFS of patients for stage I-II1 according to chemotherapy regimens. *R, rituximab; GCB, germinal center B-cell.

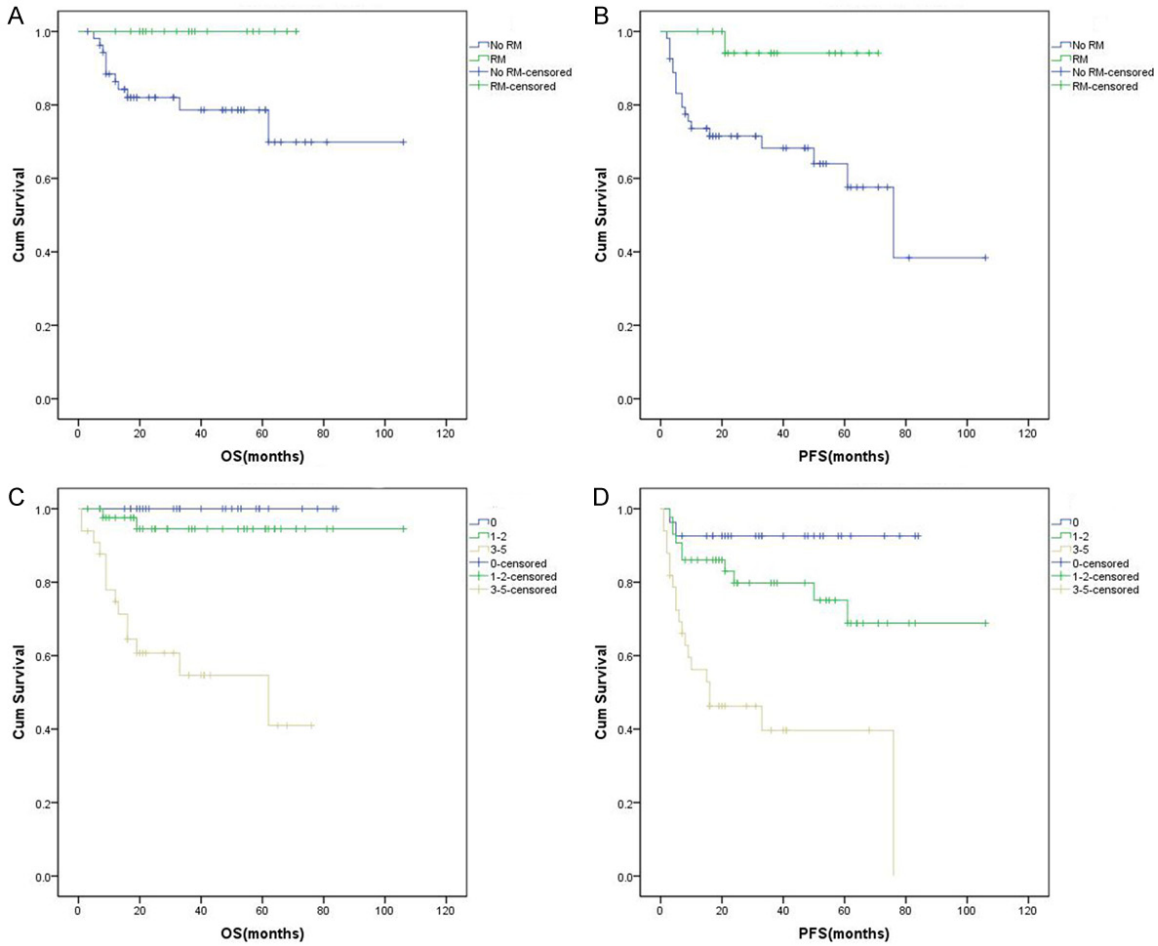

Figure 3. Kaplan-Meier curves according to chemotherapy regimens and R-IPI. A, B. OS and PFS of patients according to rituximab maintenance (OS, P = 0.027; PFS, P = 0.014); C, D. OS and PFS of patients according to R-IPI (OS, P < 0.001; PFS, P < 0.001). *RM, rituximab maintenance; R-IPI, revised IPI score.

PFS, P = 0.228) and pathological subtypes (GCB or non-GCB) (OS, P = 0.815; PFS, P = 0.367) showed no statistically significant differences. However, when stratified with ECOG scores, patients receiving rituximab-containing chemotherapy showed better OS and PFS than the no rituximab group in ECOG ≥ 2 (OS, P = 0.028; PFS, P < 0.001; **Figure 2A**, **2B**). Additional rituximab did not prolong the survival of patients of ECOG < 2 (OS, P = 0.195; PFS, P = 0.886). For elderly patients (age > 60 years), rituximab-containing chemotherapy provided better PFS but similar OS (OS, P = 0.556; PFS, P = 0.029; **Figure 2C**, **2D**). Additional rituximab showed no benefits in OS and PFS of patients ≤ 60 years (OS, P = 0.873; PFS, P = 0.810). Furthermore, patients receiving additional rituximab seemed to achieve better OS and PFS in GCB group and stage I-II1 group, despite insignificant statistical differences (GCB group, OS, P = 0.212; PFS, P = 0.159; **Figure 2E**, **2F**; stage I-II1 group, OS, P = 0.105; PFS, P = 0.073; **Figure 2G**, **2H**). For patients in the non-GCB group, stage II2-IIE, and stage IV, OS and PFS did not differ among different chemotherapy regimens (non-GCB group, OS, P = 0.860; PFS, P = 0.149; stage II2-IIE group, OS, P = 0.398; PFS, P = 0.758; stage IV group, OS, P = 0.724; PFS = 0.430). Moreover, rituximab maintenance after 6 cycles of R-CHOP chemotherapy showed better OS and PFS (OS, P = 0.027; PFS, P = 0.014; **Figure 3A**, **3B**). In addition, revised IPI (R-IPI) scores showed better prediction of survival. Patients scoring zero had the best outcomes. Patients scoring 1 or 2 had moderate outcomes. Patients scoring 3, 4, or 5 had the poorest outcomes, with a 5-year OS ranging from 55%-100% (P < 0.001) and a

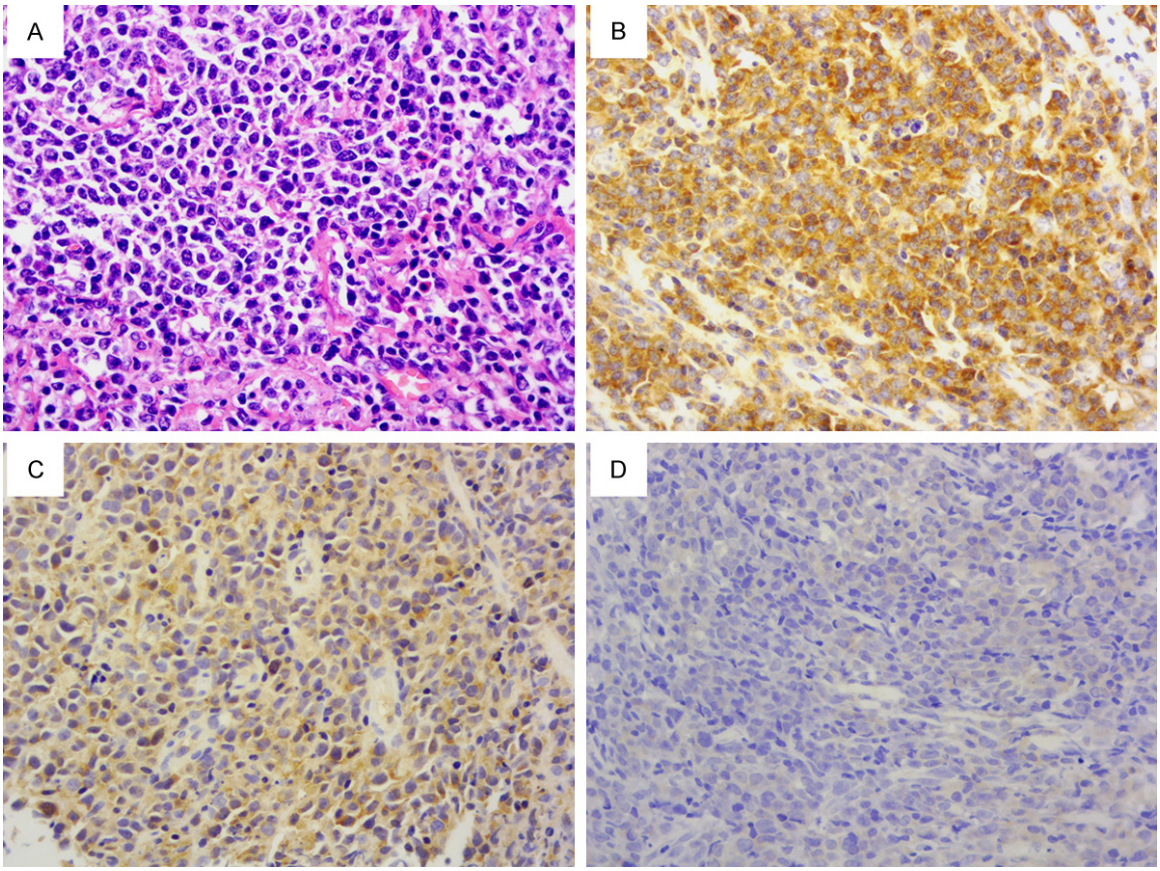

Figure 4. IHC staining of HDAC6 protein expression in PG-DLBCL tissues (Original magnification 400×). A. H&E staining of tumor cells; B. High expression of HDAC6 in tumor cells; C. Moderate expression of HDAC6 in tumor cells; D. Low expression of HDAC6 in tumor cells.

5-year PFS ranging from 40%-93% (P < 0.001; **Figure 3C**, **3D**).

Expression of HDAC6 protein in patients

HDAC6 was mainly expressed in the cytoplasm of tumor cells. Eighteen (62.1%) cases were found with high HDAC6 expression. Cases of low and moderate HDAC6 expression were 5 (17.2%) and 6 (20.7%), respectively (**Figure 4**).

Association of HDAC6 expression with clinicopathological characteristics and survival

High HDAC6 expression was associated with limited stage (P = .008), while differences of HDAC6 expression between gender, age, LDH levels, B-symptoms, Hp infections, PS, IPI scores, and treatment regimens were not significant (**Table 3**). Survival analysis showed that high HDAC6 expression was associated with better OS (P = 0.034; **Figure 5A**), but similar PFS scores (P = 0.106; **Figure 5B**), compared with low-moderate HDAC6 expression.

Discussion

In the current study, common symptoms and the median age of PG-DLBCL were corresponded to reported studies [9, 20]. The male/female ratio was nearly 1:1, which was different from the results of male predominance previously reported. Although, the proportion of patients in stage IV (47.5%) was higher than that in other reported studies [21, 22]. The 5-year OS rate reached up to 83.4%. This was in accord with previous results [6, 23], indicating that the prognosis of PG-DLBCL was favorable.

Based on the access of reported studies, age, LDH, β2-MG, anemia, hypoalbuminemia, B-symptoms, histological subtype, tumor size, bone marrow involvement, stage, PS, and IPI scores were associated with survival of primary gastric

Table 3. Association of histone deacetylase 6 expression with clinicopathological characteristics

Characteristics	Low-moderate expression (n = 11)	High expression (n = 18)	P
Gender			
Male	5 (45.5%)	8 (44.4%)	1.000
Female	6 (54.5%)	10 (55.6%)	
Age, y			
≤ 60	7 (63.6%)	11 (61.1%)	1.000
> 60	4 (36.4%)	7 (38.9%)	
LDH			
Elevated	5 (45.5%)	4 (22.2%)	0.237
Normal	6 (54.5%)	14 (77.8%)	
B symptoms			
Present	5 (45.5%)	2 (11.1%)	0.071
Absent	6 (54.5%)	16 (88.9%)	
HP			
Positive	3 (27.3%)	11 (61.1%)	0.128
Negative	8 72.7%)	7 (38.9%)	
ECOG			
< 2	5 (45.5%)	11 (61.1%)	0.466
≥ 2	6 (54.5%)	7 (38.9%)	
Stage			
I-IIE	1 (9.1%)	11 (61.1%)	0.008
IV	10 (90.9%)	7 (38.9%)	
IPI score			
0-2	4 (36.4%)	13 (72.2%)	0.119
3-5	7 (63.6%)	5 (27.8%)	
Treatment			
RCHOP	6 (54.5%)	10 (55.6%)	1.000
CHOP	5 (45.5%)	8 (44.4%)	

Abbreviations: ECOG, Eastern Cooperative Oncology Group; HP, helicobactor pylori; IPI, International Prognostic Index; LDH, serum lactate dehydrogenase.

lymphoma [24-29]. Current results were similar, indicating that B-symptoms, elevated LDH, decreased albumin, poor PS (ECOG ≥ 2), high IPI scores (≥ 3), and advanced stages (IV) were associated with poor OS and PFS. Furthermore, PS, LDH levels, and stages were considered as independent prognostic factors. There remains no doubt that patients with the GCB subtype had favorable prognosis, compared with non-GCB subtype patients in nodal DLBCL. However, the current study showed no survival differences between GCB and non-GCB subtypes, in accord with Chihara's results. However, results were inconsistent with Zhang's study, in which the GCB subtype of PG-DLBCL showed better survival [30, 31]. Notably, IPI scores are an effective predictive index widely used for prognostic evaluation of patients with non-Hodgkin's lymphoma. Laurie et al. reported that IPI scores identified only 2 risk groups. They proposed R-IPI to identify 3 distinct risk groups in DLBCL [32]. Likewise, the current study verified that R-IPI provided more effective prediction of outcomes, with 5-year OS ranging from 55%-100% in three risk groups in PG-DLBCL.

HDAC6 is crucial for maintaining cell dynamic stabilization, promoting proteasomal degradation, and regulating endocytosis, exocytosis, apoptosis, and transcription. Consequently, HDAC6 expression has been associated with cancer survival and serves as an oncogene or tumor suppressor. Recently, He et al. reported that patients with HDAC6 expression had a longer survival in gastric cancer [13]. Giaginis reported that 41 (63.1%) of 70 pancreatic adenocarcinoma cases detected HDAC6 expression, containing 31 high expression, revealing better survival, compared with low HDAC6 expression [14]. A study of 91 DLBCL cases observed 81% high HDAC6 expression in the cytoplasm of lymphoma cells. However, there were no prognostic differences between high and low expression [15]. Another study of 31 DLBCL cases found that patients with high HDAC6 expression had a significant favorable survival [16]. In the current study, the high HDAC6 expression rate of 62.1% was similar with previous studies. Results suggest that high HDAC6 expression is associated with limited stage and better OS, demonstrating that HDAC6 may inhibit tumor growth in PG-DLBCL. Thus, it can be considered as a novel prognostic biomarker.

Over the last 10-15 years, certain studies have weakened the roles of gastrectomy procedures and extended the function of chemoimmunotherapy in gastric lymphomas [33, 34]. Agustin's large controlled clinical trial of 589 PG-DLBCL patients showed a 10-year OS of 54% in the surgery group. This was lower than CT and S+CT

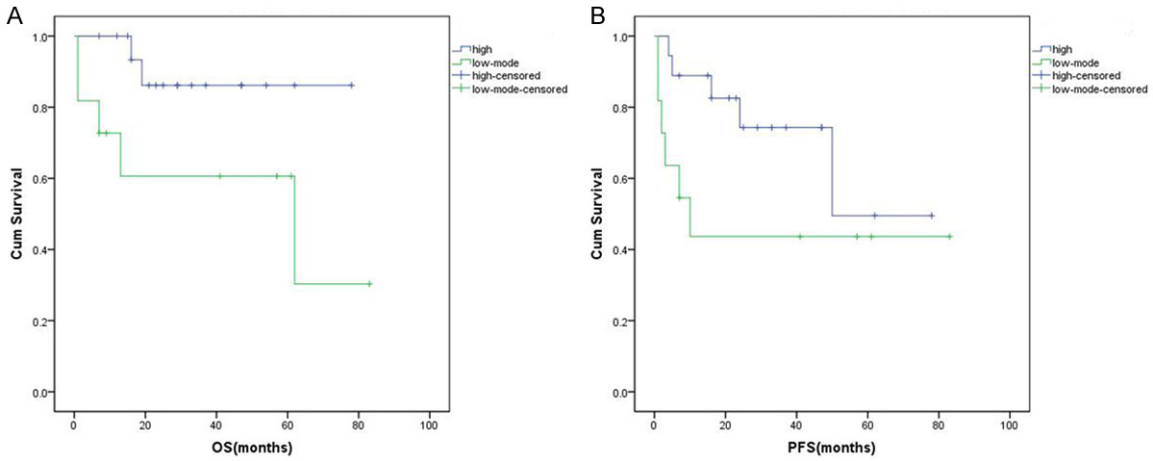

Figure 5. Kaplan-Meier curves according to HDAC6 expression. A. OS of patients for HDAC6 expression; B. PFS of patients for HDAC6 expression. *low-mode, low-moderate HDAC6 expression.

groups, with a 10-year OS of 96% and 91%. Differences between CT and S+CT were insignificant. Therefore, they suggested chemotherapy as the main treatment of PG-DLBCL, since it avoids severe complications and conserves the physiological structure and function of stomach [35]. Similarly, survival of the chemotherapy group was not worse than the surgery group followed by chemotherapy in other studies from France and Korea [36, 37]. A retrospective multicenter clinical study in China reported that the surgery group showed worse OS and PFS than group CT and S+CT groups in PG-DLBCL of early stage (I/IIE). Moreover, they performed a meta-analysis, confirming a lower mortality in the S+CT or CT group than the surgery group in primary gastrointestinal lymphoma of Chinese patients [22]. These results suggest that surgery gave no additional advantages to survival and chemotherapy remained the optimal treatment for gastric DLBCL. Based on current results, the OS and PFS between CT and S+CT were similar, with 5-year survival rates of 81.5% and 92.9%, respectively. Thus, results suggest stomach-preserving chemotherapy as a reasonable therapeutic option for patients of PG-DLBCL. After all, patients obtained remarkably better quality of life levels after conservative nonsurgical treatment, compared with gastrectomy procedures, with a reduced risk of severe malabsorption syndrome, dumping syndrome, anemia, and infections. Moreover, the toxicities of chemotherapy, such as neutropenia and nausea, could be well controlled and solved.

In the rituximab era, R-CHOP has demonstrated a survival benefit in nodal DLBCL. However, its superiority in PG-DLBCL remains contradictory. One series of 75 patients of PG-DLBCL showed a shorter OS duration in chemotherapy without rituximab [30]. Another study observed that rituximab-containing chemotherapy improved CR rates, OS, and PFS, without any additional toxicities [21]. However, the addition of rituximab to CHOP chemotherapy did not improve OS and PFS rates in patients of both localized and advanced stages in Kucukoner's study [38]. According to present data, additional rituximab with chemotherapy showed no survival advantages. This may have resulted from the non-standard chemotherapy regimens, patient tolerance to treatments, and other biases. Excluding these possibilities, the current study demonstrated that the addition of rituximab achieved better OS and PFS in patients of ECOG ≥ 2, as well as prolonging PFS in elderly patients (age > 60). Additional rituximab in GCB and stage I-II1 groups showed a trend of longer OS and PFS. Additionally, it was found that rituximab maintenance showed better OS and PFS. Therefore, results suggest that rituximab-containing chemoimmunotherapy may bring advantages to survival, especially for patients < 60, GCB subtype, ECOG ≥ 2, and limited stage (I-II1). Thus, rituximab maintenance therapy may be beneficial for PG-DLBCL patients.

In conclusion, present results showed that elevated LDH, poor PS, and advanced stages were associated with worse prognosis. R-IPI provid-

ed a more effective prediction of survival. Furthermore, HDAC6 may be an important prognostic biomarker associated with favorable outcomes in PG-DLBCL. It is believed that PG-DLBCL is a highly chemo-sensitive and potentially curable disease, with rituximab-containing chemoimmunotherapy as the optimal therapeutic strategy. Moreover, rituximab maintenance following chemoimmunotherapy may also improve survival. The current study was a retrospective study with a small sample size from a single hospital. Therefore, results should be further confirmed by large sample sizes of future prospective studies, aiming to develop a more comprehensive understanding of this disease.

Acknowledgements

The research was supported by the National Natural Science Foundation of China (No. 81670178), National Key Research and Development Program of China (No. 2016YFC0-90150X), Research Project for Practice Development of National TCM Clinical Research Bases (No. JDZX2015113), Funds of Science Technology Department of Zhejiang Province (No. 2018C03016-1), and Zhejiang Provincial Department of Education (No. Y201635961).

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Wenbin Qian and Juying Wei, Malignant Lymphoma Diagnosis and Therapy Center, The First Affiliated Hospital, College of Medicine, Zhejiang University, 79# Qingchun Road, Hangzhou 310003, Zhejiang, China. Tel: 86-571-56723008; Fax: 86-571-87236702; E-mail: qianwb@zju.edu.cn (WBQ); weijuy@zju.edu.cn (JYW)

References

[1] Huang J, Jiang W, Xu R, Huang H, Lv Y, Xia Z, Sun X, Guan Z, Lin T and Li Z. Primary gastric non-Hodgkin's lymphoma in Chinese patients: clinical chara-cteristics and prognostic factors. BMC Cancer 2010; 10: 358.

[2] Psyrri A, Papageorgiou S and Economopoulos T. Primary extranodal lymphom-as of stomach: clinical presentation, diagnostic pitfalls and management. Ann Oncol 2008; 19: 1992-1999.

[3] Medina-Franco H, Germes SS and Maldonado CL. Prognostic factors in pri-mary gastric lymphoma. Ann Surg Oncol 2007; 14: 2239-2245.

[4] Ghimire P, Wu GY and Zhu L. Primary gastrointestinal lymphoma. World J Gastroenterol 2011; 17: 697-707.

[5] Cui Y, Sun Z, Li X, Leng C, Zhang L, Fu X, Li L, Zhang X, Chang YU, Nan F, Li Z1, Yan J, Zhang M, Li W, Wang G, Zhang D and Ma Y. Expression and clinical significance of cyclooxygenase-2 and interleukin-32 in primary gastric b-cell lymphoma. Oncol Lett 2016; 11: 693-698.

[6] Ferreri AJ and Montalbán C. Primary diffuse large B-cell lymphoma of the stomach. Crit Rev Oncol Hematol 2007; 63: 65-71.

[7] He M, Chen K, Li S, Zhang S, Zheng J, Hu X, Gao L, Chen J, Song X, Zhang W, Wang J and Yang J. Clinical significance of "double-hit" and "double-p-rotein" expression in primary gastric b-cell lymphomas. J Cancer 2016; 7: 1215-1225.

[8] Rotaru I, Găman GD, Stănescu C and Găman AM. Evaluation of parameters with potential prognosis impact in patients with primary gastric diffuse large b-cell lymphoma (PG-DLBCL). Rom J Morphol Embryol 2014; 55: 15-21.

[9] Wang YG, Zhao LY, Liu CQ, Pan SC, Chen XL, Liu K, Zhang WH, Yang K, Chen XZ, Zhang B, Chen ZX, Chen JP, Zhou ZG and Hu JK. Clinical charac-teristics and prognostic factors of primary gastric lymphoma: a retrospective stud-y with 165 cases. Medicine 2016; 95: e4250.

[10] Chang MC, Huang MJ, Su YW, Chang YF, Lin J and Hsieh RK. Clinical outcome of primary gastric lymphoma treated with chemotherapy alone or surgery followed by chemotherapy. J Formos Med Assoc 2006; 105: 194-202.

[11] Selçukbiricik F, Tural D, Elicin O, Berk S, Ozgüroğlu M, Bese N and Ferha-noglu B. Primary gastric lymphoma: conservative treatment modality is not inferior to surgery for early-stage disease. ISRN Oncol 2012; 2012: 951816.

[12] Seidel C, Schnekenburger M, Dicato M and Diederich M. Histone deacetyla-se 6 in health and disease. Epigenomics 2015; 7: 103-118.

[13] He Q, Li G, Wang X, Wang S, Hu J, Yang L, He Y, Pan Y, Yu D and Wu Y. A decrease of histone deacetylase 6 expression caused by helicobacter pylori infection is associated with oncogenic transformation in gastric cancer. Cell Physiol Biochem 2017; 42: 1326-1335.

[14] Giaginis C, Damaskos C, Koutsounas I, Zizi-Serbetzoglou A, Tsoukalas N, P-atsouris E, Kouraklis G and Theocharis S. Histone deacetylase (HDAC)-1, -2, -4 and -6 expression in human pancreatic adenocarcinoma: associations with cl-inicopathological parameters, tumor proliferative capacity and patients' survival. BMC Gastroenterol 2015; 15: 148.

[15] Lee SH, Yoo C, Im S, Jung JH, Choi HJ and Yoo J. Expression of histone deacetylases in dif-

fuse large b-cell lymphoma and its clinical significance. Int J Med Sci 2014; 11: 994-1000.
[16] Marquard L, Poulsen CB, Gjerdrum LM, de Nully Brown P, Christensen IJ, Jensen PB, Sehested M, Johansen P and Ralfkiaer E. Histone deacetylase 1, 2, 6 and acetylated histone h4 in b- and t-cell lymphomas. Histopathology 2009; 54: 688-698.
[17] Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H and Jaffe ES. The 20-08 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood 2011; 117: 5019-5032.
[18] Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, Müller-Hermelink HK, Campo E, Braziel RM, Jaffe ES, Pan Z, Farinha P, Smith LM, Falini B, Banham AH, Rosenwald A, Staudt LM, Connors JM, Armitage JO, Chan WC. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004; 103: 275-282.
[19] Rohatiner A, d'Amore F, Coiffier B, Crowther D, Gospodarowicz M, Isaacson P, Lister TA, Norton A, Salem P, Shipp M, et al. Report on a workshop conven-ed to discuss the pathological and staging classifications of gastrointestinal tract lymphoma. Ann Oncol 1994; 5: 397-400.
[20] Li M, Zhang S, Gu F, Xiao W, Yao J, Chao K, Chen M, Li J and Zhong B. Clinicopathological characteristics and prognostic factors of primary gastrointes-tinal lymphoma: a 22-year experience from South China. Int J Clin Exp Pathol 2014; 7: 2718-2728.
[21] Leopardo D, Di Lorenzo G, De Renzo A, Federico P, Luponio S, Buonerba C, Matano E, Merola G, Imbimbo M, Montesarchio E, Rea A, Merola MC, De Placido S, Palmieri G. Efficacy of rituximab in gastric diffuse large b cell l-ymphoma patients. World J Gastroenterol 2010; 16: 2526-2530.
[22] Chen Y, Chen Y, Chen S, Wu L, Xu L, Lian G, Yang K, Li Y, Zeng L, Huang K. Primary gastrointestinal lymphoma: a retrospective multicenterclinic-al study of 415 cases in Chinese province of guangdong and a systematic review containing 5075 Chinese patients. Medicine 2015; 94: e2119.
[23] Fischbach W. Long-term follow-up of gastric lymphoma after stomach con-serving treatment. Best Pract Res Clin Gastroenterol 2010; 24: 71-77.
[24] Ferreri AJ, Cordio S, Paro S, Ponzoni M, Freschi M, Veglia F and Villa E. Therapeutic management of stage I-II high-grade primary gastric lymphomas. Oncology 1999; 56: 274-282.
[25] Koch P, del Valle F, Berdel WE, Willich NA, Reers B, Hiddemann W, Grothaus-Pinke B, Reinartz G, Brockmann J, Temmesfeld A, Schmitz R, Rübe C, Probst A, Jaenke G, Bodenstein H, Junker A, Pott C, Schultze J, Heinecke A, Parwaresch R, Tiemann M; German Multicenter Study Group. Primary gastrointest-inal non-Hodgkin's lymphoma: I. anatomic and histologic distribution, clinical fea-tures, and survival data of 371 patients registered in the German Multicenter Study GIT NHL 01/92. J Clin Oncol 2001; 19: 3861-3873.
[26] Hung YS, Lin TL, Kuo MC, Tang TC, Dunn P, Wang PN, Wu JH, Chang H, Kuo TT and Shih LY. Primary gastric diffuse large b-cell lymphoma. Chang Gung Med J 2008; 31: 159-166.
[27] Nakamura S, Matsumoto T, Iida M, Yao T and Tsuneyoshi M. Primary gast-rointestinal lymphoma in Japan: a clinicopathologic analysis of 455 patients with special reference to its time trends. Cancer 2003; 97: 2462-2473.
[28] Ding D, Pei W, Chen W, Zuo Y and Ren S. Analysis of clinical characteri-stics, diagnosis, treatment and prognosis of 46 patients with primary gastrointe-stinal non-Hodgkin lymphoma. Mol Clin Oncol 2014; 2: 259-264.
[29] Delamain MT, da Silva MG, Miranda EC, Desterro J, Luminari S, Fedina A, Merli F, Chiattone CS, Pagnano KB, Federico M and de Souza CA. Age-adjusted international prognostic index is a predictor of survival in gastric diffuse b-cell non-hodgkin lymphoma patients. Rev Bras Hematol Hemoter 2016; 38: 247-251.
[30] Chihara D, Oki Y, Ine S, Kato H, Onoda H, Taji H, Kagami Y, Yamamoto K and Morishima Y. Primary gastric diffuse large b-cell lymphoma (DLBCL): analyses of prognostic factors and value of pretreatment FDG-PET scan. Eur J Haematol 2010; 84: 493-498.
[31] Zizhen Z, Hui C, Yanying S, Danping S, Jiahua L, Chao H and Xingzhi N. Correlation between immunophenotype classification and clinicopathological feat-ures in chinese patients with primary gastric diffuse large b-cell lymphoma. Pathol Oncol Res 2013; 19: 317-322.
[32] Sehn LH, Berry B, Chhanabhai M, Fitzgerald C, Gill K, Hoskins P, Klasa R, Savage KJ, Shenkier T, Sutherland J, Gascoyne RD and Connors JM. The revised International prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large b-cell lymphoma treated with R-CHOP. Blood 2007; 109: 1857-1861.
[33] Cuccurullo R, Govi S and Ferreri AJ. De-escalating therapy in gastric aggressive lymphoma. World J Gastroenterol 2014; 20: 8993-8997.
[34] Ferrucci PF and Zucca E. Primary gastric lymphoma pathogenesis and trea-tment: what has changed over the past 10 years? Br J Haematol 2007; 136: 521-538.
[35] Avilés A, Nambo MJ, Neri N, Huerta-Guzmán J, Cuadra I, Alvarado I, Castañeda C, Fernández R, González M. The role of surgery in primary

gastric lymphoma: results of a controlled clinical trial. Ann Surg 2004; 240: 44-50.

[36] Binn M, Ruskoné-Fourmestraux A, Lepage E, Haioun C, Delmer A, Aegerter P, Lavergne A, Guettier C and Delchier JC. Surgical resection plus chemother-apy versus chemotherapy alone: comparison of two strategies to treat diffuse l-arge B-cell gastric lymphoma. Ann Oncol 2003; 14: 1751-1757.

[37] Kim SJ, Cheong JW and Hahn JS. Therapeutic comparison of chemotherapy and surgery for early stage diffuse large b-cell gastric lymphoma. Yonsei Med J 2007; 48: 942-948.

[38] Mehmet K, Sener C, Uyeturk U, Seker M, Tastekin D, Tonyali O, Balakan O, Yazici OK, Urakci Z, Isikdogan A, Ozdemir N, Inal A, Kaplan MA, Suner A, Dal S, Uncu D, Gumus M, Boruban MC, Oksuzoglu B, Ayyildiz O, Benekli M. Treatment modalities in primary gastric lymphoma: the effect of rituximab and surgical treatment. A study by the anatolian society of medical oncology. Contemp Oncol (Pozn) 2014; 18: 273-278.