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RESEARCH

Correlation Between Immunophenotype Classification and Clinicopathological Features in Chinese Patients with Primary Gastric Diffuse Large B-Cell Lymphoma

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Abstract Recent studies have shown that diffuse large B-cell lymphoma (DLBCL) can be classified into germinal center B-cell–like (GCB) and non-GCB phenotypes by immunohistochemical staining. The aim of this study was to investigate the correlation of immunophenotypic classification with clinicopathological features in Chinese patients with primary gastric DLBCL to further our knowledge of this disease. Seventy-three patients with a histopathological diagnosis of primary gastric DLBCL were studied. Immunohistochemistry was carried out using the EnVision method to detect the expression of CD10, Bcl-6, and MUM1. The clinicopathologic features and follow-up data were analyzed using the Kaplan–Meier method, log-rank test, and χ^2 test. Expression of CD10 was observed in 21.9 % (16/73) of patients, Bcl-6 in 72.6 % (53/73), and MUM1 in 74.0 % (54/73). According to these data, 32.9 % (24/73) of the cases belonged to GCB subtype and 67.1 % (49/73) belonged to non-GCB subtype. There was a significant difference in tumor size and local lymph node metastasis between the GCB and non-GCB groups ($P<0.05$). Complications in the GCB group (4.2 %) occurred less frequently than those in the non-GCB group (18.4 %); however, this difference was not significant ($P>0.05$). Survival analysis revealed that patients in the GCB group had an increased 5-year survival rate compared to those in the non-GCB group (58.5 % vs 35.7 %, $\chi^2=3.939$, $P<0.05$). The 5-year survival rate of patients undergoing R-CHOP chemotherapy was significantly longer than that of patients in the CHOP group (74.7 % vs 37.5 %, $\chi^2=4.185$, $P<0.05$). The immunophenotype classification of primary gastric DLBCL, which is closely related to the tumor size and local lymph nodes metastasis, was found to have prognostic significance.

Keywords Diffuse large B-cell lymphoma · Immunophenotype · Primary gastric lymphoma · Prognosis

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Introduction

Primary gastrointestinal lymphoma is the most common extranodal lymphoma. Recent studies have shown that primary gastric lymphoma and primary intestinal lymphoma differ with regard to their pathological type, modality of treatment, and clinical prognosis [1–3]. All histopathological categories of nodal lymphomas may also arise in the stomach, and the two most common pathological types of primary gastric lymphoma are mucosa-associated lymphoid tissue (MALT) lymphoma and diffuse large B-cell lymphoma (DLBCL). Significant progress has been made in gastric MALT lymphoma research [4–6]. However, the diagnosis, treatment, and prognosis of gastric DLBCL are still subject to controversy.

Several algorithms of immunohistochemistry have been reported to be useful for subclassification and survival prognosis of patients with DLBCL. Among which, the Hans algorithm is the most commonly used methods. In 2004, Hans et al found that DLBCL could be categorized into two subtypes, germinal center B-cell–like (GCB) and non-GCB, according to the expression of CD10, Bcl-6, and MUM-1.

This immunophenotype classification was used to predict the survival of patients with DLBCL [7]; however, it has rarely been used to study primary gastric DLBCL. The main goals of the current study were as follows: (1) to evaluate whether immunohistochemical staining with CD10, Bcl-6, and MUM1 antibodies could define distinct, clinically significant subgroups of gastric DLBCL; and (2) to examine whether these subgroups had any prognostic significance for patients with gastric DLBCL.

Methods

Clinical Data

One hundred patients, diagnosed with primary gastric DLBCL between 1998 and 2010 at Shanghai Ren Ji hospital, were included in this study. Of these, 27 patients did not accept formal chemotherapy because of the advanced age or the other personal reasons. These cases were excluded due to the effects of informal chemotherapy on the prognosis. The remaining 73 patients were enrolled into this study following the approval of the local ethics committee, and attainment of written informed consent from the patients. The median age of the patients was 61 years (range, 18–78), and the study group included 41 men (56.2 %) and 32 women (43.8 %). All cases fulfilled the clinical criteria for primary gastrointestinal lymphoma as presented by Dawson [8]. Among the 73 cases, 45.2 % (33/73) occurred in the gastric antrum, 37.0 % (27/73) in the gastric corpora, 9.6 % (7/73) in the gastric fundus, and 8.2 % (6/73) were multifocal. All patients were pathologically diagnosed by surgery or endoscopic biopsy.

Twenty patients were treated with chemotherapy only, 47 with surgery plus chemotherapy, and six with radiotherapy plus chemotherapy. Fifty-three patients received CHOP-like chemotherapy (CTX 600 mg/m^2, d1; ADM 50 mg/m^2, or EADM 70 mg/m^2, d1; VCR 2 mg, d1; PRED 80 mg/m^2, d1~d5), and 20 patients received rituximab plus CHOP (R-CHOP) therapy. The median follow-up period was 30 months (range, 5–112 months).

Immunohistochemical Investigation

Immunohistochemical staining was carried out using the EnVision two-step method. All tissue biopsies were fixed in 10 % buffered formalin, embedded in paraffin, and cut into 4-μm sections. Following deparaffinization, heat-induced antigen retrieval techniques were used. Then, endogenous peroxidase activity was blocked with 0.5 % H_2O_2. Following rinsing in Tris-buffered saline (TBS), the sections were stained with CD10 (RTU-CD10-270-qh, Novocastra), Bcl-6 (RTU-Bcl-6-564-qh, Novocastra), and MUM1 (GM725902, DAKO) antibodies, and the reaction was carried out at room temperature overnight. Then, after another TBS wash, the secondary antibodies, EnVision™ mouse (K4001, DAKO) or EnVision™ rabbit (K4002, DAKO), were added. The cell nucleus was restained with hematoxylin following positive staining with diaminobenzidine (DAB). Samples were considered positive if 30 % or more of the tumor cells were stained.

Classification of DLBCL

Following determination of CD10, Bcl-6, and MUM1 expression, the 73 cases were classified into two subtypes: GCB and non-GCB. According to the Hans algorithm, cases were assigned to the GCB group if CD10 alone was positive or both Bcl-6 and CD10 were positive. If both Bcl-6 and CD10 were negative, the cases were assigned to the non-GCB group. If Bcl-6 was positive and CD10 was negative, the expression of MUM1 determined the group; if MUM1 was negative, the case was assigned to the GCB group; and if MUM1 was positive, the case was assigned to the non-GCB group.

Statistical Analysis

The analyses were conducted using SPSS16.0 software. The characteristics of the two groups were compared using the χ^2 test. The Kaplan–Meier method was used to estimate the survival distributions. The log-rank test was used to compare the survival distributions. All statistical tests were two-sided, and $P<0.05$ was considered statistically significant.

Results

Immunophenotype Classification and its Correlation with Clinicopathological Parameters

Expression of CD10 was observed in 21.9 % (16/73) of the patients, Bcl-6 in 72.6 % (53/73), and MUM1 in 74.0 % (54/73). According to the immunohistochemical staining results, 32.9 % (24/73) of the cases were classified as GCB subtype and 67.1 % (49/73) as non-GCB subtype.

The clinicopathological parameters of the patients in the study included tumor size; local lymph node metastasis; infiltrate depth; B symptoms; Lugano stage; and complications such as bleeding, obstruction, and perforation. The results of the statistical analysis of the association of immunophenotype classification and clinicopathological parameters are shown in Table 1. Subclassification was not associated with infiltrate depth, Lugano stage, or B symptoms. However, a significant association with tumor size and local lymph node metastasis was observed. The mean

Table 1 Correlation between immunophenotype classification and clinicopathological parameters

	Immunophenotype classification		t/χ2	*P*
	GCB	Non-GCB		
Tumor size				
Mean diameter	4.6±1.9 cm	7.4±3.3 cm	−2.795	0.008
Local lymph node metastasis				
+	4	23	5.232	0.045
−	9	11		
Infiltrate depth				
Mucosa and submucosa	6	8	2.302	0.163
Muscle layer and serosal	7	26		
B symptom				
+	3	5	0.087	1.000
−	21	44		
Lugano stage				
IE	12	14	3.670	0.160
IIE	8	27		
IV	4	8		
Complications				
+	1	9	2.748	0.150
−	23	40		

diameter of the non-GCB tumors (7.4±3.3 cm) was significantly higher than that of the GCB cases (4.6±1.9 cm; t= −2.795, P<0.05). Local lymph node metastasis was present in 30.8 % (4/13) of the GCB cases and 67.6 % (23/34) of the non-GCB cases respectively (χ^2=5.232, P<0.05). When the correlation between subclassification and complications was studied, we found an interesting trend. Complications in the GCB subgroup (4.2 %, 1/24) occurred less frequently than those in the non-GCB subgroup (18.4 %, 9/49); however, this difference was not significant (χ^2=2.748, P>0.05).

Correlation between Immunophenotype Classification and Survival

At the time of analysis, 58.9 % (43/73) of the patients were alive, and 41.1 % (30/73) were deceased. The overall 2-, 3-, and 5-year survival rates of the 73 cases were 69.0 %, 58.4 %, and 44 %, respectively. Univariate analysis of the expression of each immunophenotype biomarker and its relationship to survival is shown in Table 2. The 5-year survival rate was not significantly correlated with the expression of CD10, Bcl-6, or MUM1. However, survival analysis demonstrated that GCB patients had a significantly better 5-year survival rate compared with patients of the non-GCB subtype (58.5 % vs 35.7 %, χ^2=3.939, P<0.05; Fig. 1).

Then, we compared the survival rate of patients following different treatments. Univariate analysis revealed that the 5-year survival rate was not significantly different between patients treated with chemotherapy only and surgery plus chemotherapy (42.7 % vs 42.4 %, χ^2=1.778, P>0.05; Fig. 2). However, R-CHOP chemotherapy was significantly associated with improved survival. The 5-year survival rate of patients in the R-CHOP group was significantly greater than those in the CHOP group (74.7 % vs 37.5 %, χ^2=4.185, P<0.05; Fig. 3).

Discussion

Research has shown that 27–48 % of non-Hodgkin's lymphoma cases are extranodal with the gastrointestinal tract being the most common site. In most of previous studies, primary gastric lymphoma and intestinal lymphoma were together studied as a specific type of lymphoma. However, recent researches have demonstrated that there are considerable differences between gastric and intestinal lymphoma in relation to histological type, optimal treatment, and clinical prognosis [1–3]. The two most common pathological types of primary gastric lymphoma are MALT and DLBCL. In recent years, significant progress has been made in gastric MALT lymphoma research [4–6]. However, the diagnosis, treatment, and prognosis of gastric DLBCL are still subject to controversy. The aim of our study was to investigate the correlation of immunophenotypic classification with clinicopathological features in Chinese patients with primary gastric DLBCL to further our knowledge of this disease.

The criteria for the diagnosis of primary gastric DLBCL are as follows: (1) pathological confirmation of the diagnosis of DLBCL, according to the WHO classification; and (2) clinical confirmation of the diagnosis criterion of primary gastrointestinal lymphoma as specified by Dawson [8]. The 73 cases used in our study fulfilled these criteria.

Table 2 Immunohistochemical stain results and their effect on survival by univariate analysis

		No. (%)	5-year survival rate (%)	χ^2	*P*
CD10	Negative	78.1 % (57/73)	35.3 %	1.157	0.282
	Positive	21.9 % (16/73)	68.8 %		
Bcl-6	Negative	27.4 % (20/73)	35.6 %	3.048	0.081
	Positive	72.6 % (53/73)	66.7 %		
MUM1	Negative	26.0 % (19/73)	41.3 %	2.854	0.091
	Positive	74.0 % (54/73)	41.8 %		

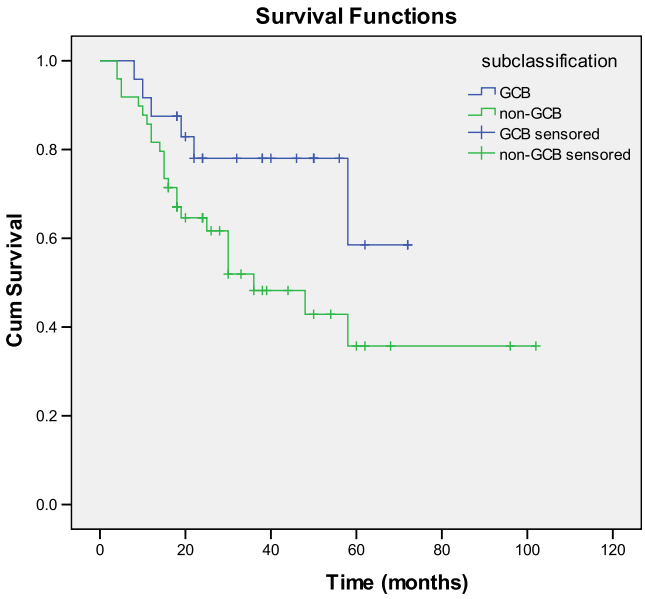

Fig. 1 Kaplan-Meier curve of OS for patients of GCB (*blue*) and non-GCB (*green*). Patients of GCB lived longer than those of non-GCB ($P<0.05$)

DLBCL is defined as diffuse proliferations of large neoplastic mature B cells, and this definition encompasses a group of heterogeneous tumors rather than a uniform entity. Several cDNA microarray studies have shown that DLBCL can be divided into three types according to the differentiation stages of the tumorigenic B-cell: GCB, activated B-cell–like (ABC), and type 3 [9–14]. In recent years, it was

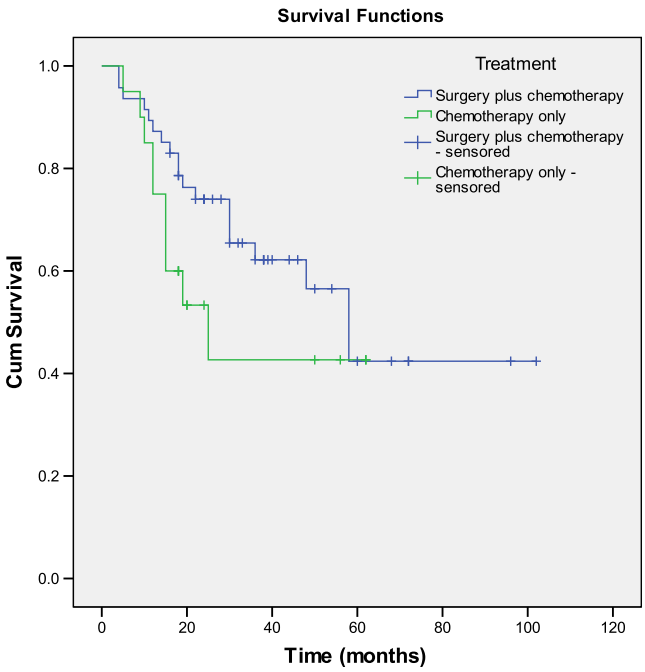

Fig. 2 Kaplan–Meier curve of OS for patients treated with surgery plus chemotherapy (*blue*) and chemotherapy only (*green*). The 5-year survival rate was not significantly different ($P>0.05$)

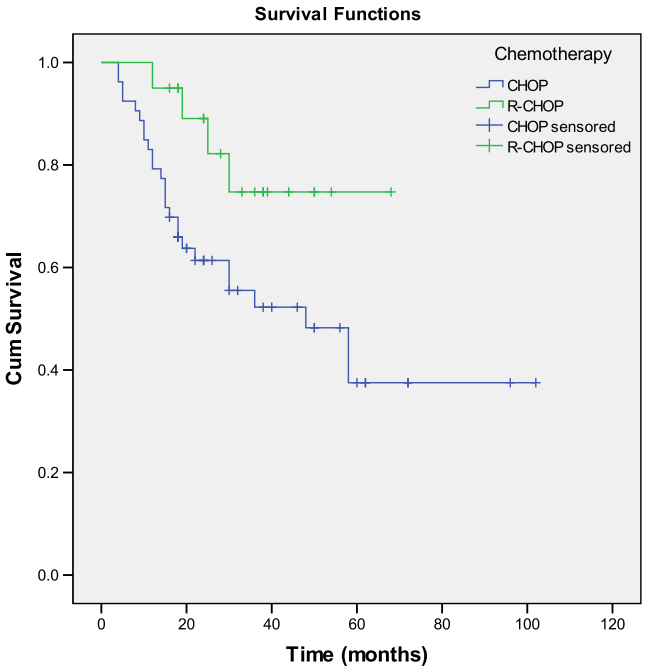

Fig. 3 Kaplan–Meier curve of OS for patients treated with CHOP (*blue*) and R-CHOP (*green*) postoperative chemotherapy. Patients treated with R-CHOP lived longer than those treated with CHOP chemotherapy ($P<0.05$)

proved that immunohistochemistry could be used to classify DLBCL subtypes, and immunophenotype was an important independent prognostic factor [15, 16]. The Hans algorithm, Choi algorithm, and Tally algorithm are examples of methods used in this classification [7, 17, 18]. Among which, the Hans algorithm is one of the most commonly used methods. In 2004, Hans et al found that GCB and non-GCB subtypes could be accurately predicted according to the expression pattern of CD10, Bcl-6, and MUM1. And the non-GCB type may include the ABC type and type 3. The results obtained by immunophenotyping were comparable to those obtained using cDNA microarrays. The gene expression results were reproduced in 71 % of GCB and 88 % of non-GCB cases, and survival was predicted in a similar manner [7].

In our study, we used the Hans algorithm to classify 73 Chinese patients with primary gastric DLBCL. We found that the proportion of samples with the GCB subtype was significantly lower than that reported in Western countries, whereas the proportion of GCB in DLBCL is close to 50 %. This may be due to inherent differences between Eastern and Western DLBCL patients and due to differences between primary gastric DLBCL and nodal DLBCL. Further investigations are warranted to clarify these aspects. We examined the relationship between immunophenotype classification and pathological parameters, and found that the size of the non-GCB tumors was greater than that of GCB tumors. In addition, we discovered that non-GCB patients were more likely to experience local lymph node metastasis than were GCB patients.

However, there was no correlation between these two subtypes and infiltrate depth. Our study also demonstrated that the survival time of patients in the GCB group was longer than that of patients in the non-GCB group. These results were similar to those of Hans who studied both nodal and extranodal DLBCL [7]. Our results indicate that immunophenotype classification could be used to predict the prognosis of primary gastric DLBCL patients.

Our study also found that, in non-GCB DLBCL patients, the frequency of perforation, bleeding, and infarction was higher than that in GCB patients (18.4 % vs. 4.2 %). These results were not statistically significant, probably due to the insufficient sample size. However, this suggests that the indications of surgery for non-GCB patients should be stricter than that for GCB patients to prevent complications.

In the current study, chemotherapy was associated with improved survival while surgical treatment did not significantly affect the survival rate. A number of studies have found that rituximab combined with chemotherapy increases the efficacy of DLBCL treatment [19–22]. Our findings are consistent with these results, since we also found that R-CHOP chemotherapy significantly improved the clinical outcome of patients with gastric DLBCL. However, as our study was a retrospective one, and future studies are warranted, with a larger number of patients with primary gastric DLBCL, to confirm these results.

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Conflicts of Interest The authors declare that they have no conflict of interest.

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