

Research Article

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CD5 and CD43 expression are associate with poor prognosis in DLBCL patients

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Abstract: Objective. To investigate the expression and clinical significance of CD5 and CD43 in diffuse large B cell lymphoma (DLBCL) (unspecified). Methods. Sixty - five patients with diagnosed DLBCL were enrolled. The expressions of CD5, CD43, CD10, Bcl-6 and Mun-1 were detected by immunohistochemistry. The relationship between CD5 and CD43 and clinicopathological features and prognosis of DLBCL was analyzed. Results. In sixty - five adult DLBCL patients, 6 cases of DLBCL (9.2%) were CD5 positive, 24 cases of DLBCL (36.9%) were CD43 positive, 5 cases of DLBCL (7.7%) were both CD5 and CD43 positive. 40 cases of DLBCL (61.5%) were CD5 and CD43 negative. CD5 expression was not related to age, sex, clinical stage, type of immunophenotype (Hans typing), location, and whether infected with hepatitis B virus (HBV); CD43 expression was correlated with immunophenotyping and HBV infection, but was not correlated with the age, sex, clinical stage, and site. Median survival time was significantly lower in CD5- and CD43- positive DLBCL patients than CD5- and CD43-negative patients. Conclusion. The prognosis of DLBCL patients may be worse with positive CD5 and CD43 expression.

1 Introduction

Diffuse Large B-Cell Lymphoma (DLBCL) is the most common subtype of non-Hodgkin's lymphoma(NHL) and is clinically highly invasive. Based on the 2008 WHO classification of tumors of hematopoietic and lymphoid tissues, DLBCL was divided into three categories: non-specific (NOS), special subtypes and independent diseases according to morphology, immunophenotype, genetics and clinical features [1]. The majority of DLBCL belongs to DLBCL (NOS). This category has significant heterogeneity, which is reflected in the clinical manifestations, histological features, immunophenotype, genetic features and diverse biological behavior, inconsistent response to treatment, and prognosis differences [2]. Among them, the tumor response to chemotherapy and prognosis is the most concern [3]. In recent years, more scholars have studied the prognosis of this tumor, and put forward relevant prognostic indicators, but its clinical significance is still controversial [4-6]. Therefore, the search for prognostic indicators of DLBCL (NOS) and the further classification of this tumor remains an important clinical issue. With further research, some scholars have suggested that CD5 and CD43 may be adverse prognostic factors of DLBCL [7-9]. Therefore, we planned to study the expression of biomarker CD5 and CD43 and its relationship with the prognosis in 65 patients with DLBCL.

2 Materials and methods

2.1 Patients and samples

There were 65 adult patients enrolled in the research with DLBCL (NOS) from May 2005 to October 2017 at Jiangxi

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Cancer Hospital (Nanchang, China). Three DHL patients were excluded from the research.

Overall, 45 patients were analyzed. Diagnoses of specimens were made by two independent hematopathologists on the basis of the 2008 WHO classification. In the study, all diagnoses of DLBCLs were treated with RCHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) or R-CHOP-based regimens as the first-line therapy. Formalin-fixed and paraffin-embedded tissue blocks were collected, and immunochemical analysis (IHC) for CD5, CD43, CD10, Bcl-6 and Mum-1 was executed when all patients were at initial diagnosis. Computed tomography (CT) scanning and / or PETCT scanning were performed to assess initial therapeutic responses after the initial treatment. This study conformed with principles of the Declaration of Helsinki by the ethics review committee of Jiangxi Cancer Hospital (Nanchang, China).

Informed consent: Informed consent has been obtained from all individuals included in this study.

2.2 Immunochemical staining

Formalin-fixed and paraffin-embedded tissue samples were prepared by standardized procedures. Tissue microarrays were used in the tissue samples and evaluations of IHC. Primary antibodies used for IHC included anti-CD5, anti-CD43, anti-CD10, anti-BCL6 and anti-Mum-1 (Fuzhou, China). Two experienced hematopathologists reviewed the immunohistochemistry results in combination with clinical, morphological and immunophenotypes. DLBCL was diagnosed based upon the protein expression of CD10, Bcl-6 and Mum-1 (Hans typing) respectively, according to previous recommended studies.

2.3 Statistical evaluation

The chi-square test (Graphpad prism 7.0 software) was performed to compare the clinicopathological characteristics of the patients, while the P-values ≤ 0.05 was considered statistically significant. Kaplan–Meier's estimates were used to predict OS (the time from the 1st treatment to the death or the latest follow up) and PFS (the time from the 1st treatment to disease progress) of the patients.

BCL-6 and MUM-1 positive staining localized in the nucleus, while CD5, CD10 and CD43-positive staining localized in the cellular membrane with brownish yellow granules. Semi-quantitative analysis showed that the tumor cells with indices $\geq 30\%$ were correctly located, and clear staining was positive [10].

3 Results

3.1 Patient characteristics

Among the enrolled 65 patients, the age ranged from 17 to 85 years. The median age was 62 years, and the follow up interval was from 2 to 127 months. The median follow-up period was 40 months. The clinical features of DLBCLs are shown in Table 1. In these 65 patients, forty-one (63.07%) were men, and twenty-four (36.93%) were women. Twenty-eight patients (43.08%) examined for lactate dehydrogenase showed high levels, and IPI scores of all patients (100.00%) were 0–2. Moreover, forty-nine patients (75.38%) were at late-stage III–IV (Ann Arbor stage). Thirty-one patients (50.77%) experienced complete response or partial response.

3.2 Immunophenotyping, expression of CD5 and CD43 in DLBCL

There were 19 DLBCL patients (29.23%) diagnosed with germinal center B-cell type, and 16 DLBCL patients (70.77%) were diagnosed with non-germinal center B-cell type in the 65 patients. The positive expression of CD5 and CD43 showed a weakly positive expression of tumor cell membrane in Figure 1. Table 2 shows CD5 positive expression in 6 cases (9.2%), CD43 positive expression in 24 cases (36.9%), and CD5 and CD43 positive expression in 5 cases (7.7%), CD5 and CD43 negative in 40 cases (61.5%).

3.3 The relationship between CD5 and CD43 expression and clinicopathological features

The positive expression of CD5 was not related to age, sex, site, clinical stage, immunophenotype, and HBV infection. The positive expression of CD43 was related to the

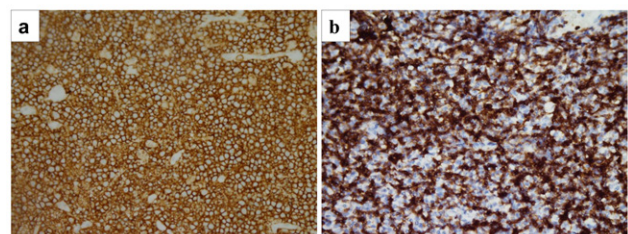


Figure 1: CD5 and CD43 expression in DLBCL patients ($\times 40$). a: CD5 positive expression in DLBCL patients; b: CD43 positive expression in DLBCL patients.

Table 1: Characteristics of patients with diffuse large B-cell lymphoma (n = 65)

Patient characteristic	n	%
Sex		
Male	41	63.07
Female	24	36.93
Age, median (range), years		
≥61	36	55.38
≤61	29	44.62
Stage		
I/II	16	24.62
III/IV	49	75.38
Serum LDH		
Normal	37	56.92
Elevated	28	43.08
ECOG performance status		
0–2	65	100.00
3–5	0	
Extranodal sites		
≥2	1	1.54
<2	25	98.46
IPI score		
IPI 0-2	40	61.54
IPI 3-5	25	38.46
Initial therapy response		
Complete response	7	10.77
Partial response	26	40.00
Stable disease	25	38.46
Progressive disease	7	10.77

ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; LDH, lactate dehydrogenase.

immunophenotyping and HBV infection, but not to the age, sex, site, or clinical stage in Table 3.

3.4 Survival analysis and univariate and multivariate analysis

The median follow-up time of 45 DLBCL (NOS) patients was 33.0 months. The median survival time (MST) of CD5 positive DLBCLs was 11.6 months, and the MST of CD5 negative DLBCLs was 32.8 months. The CD5+ group had a significantly worse MST than the CD5- group ($P=0.001$), as shown in Figure 2a. The MST of CD43-positive DLBCLs was 25.0 months, and the MST of CD43-negative DLBCLs

Table 2: Expression of CD5 and CD43 in diffuse large B-cell lymphoma (n = 65)

	All patients	CD43+	CD43-	P-value
CD5+	5	1	-	
CD5-	19	40	-	
Total	24	41	0.020	

was 34.0 months. the MST of patients with CD43-negative DLBCL was longer than that of CD43-positive ($P = 0.003$), as shown in Figure 2b.

4 Discussion

DLBCL (NOS) has significant heterogeneity. It is one of the more urgent problems to re-classify this tumor to meet the clinical choice of treatment options and prognosis [11]. In recent years, domestic and foreign scholars are actively exploring this problem. The International prognostic index (IPI) is a commonly used clinical reference index for predicting the prognosis of DLBCL. However, patients with the same IPI score have different responses and prognoses to the same chemotherapy regimen [12]. Therefore, to explore the relevant indicators of DLBCL prognosis has always been one of the focuses of scholars at home and abroad.

CD5 is one of the T lymphocyte markers but is expressed in both fetal cord blood and spleen B lymphocytes and 5% -10% of normal adult peripheral blood B lymphocytes. In addition, CD5 is also expressed in some B cell lymphomas, such as partial follicular lymphoma, mantle cell lymphoma, chronic lymphocytic leukemia / small lymphocytic lymphoma, Burkitt's lymphoma and diffuse large B cell lymphoma. It has been reported in the literature that primary CD5-positive DLBCL accounts for 5% -10% of DLBCL (NOS) and has clinical features that are different from those of CD5-negative DLBCL and is one of the unfavorable independent prognostic factors. Primary CD5 positive DLBCL is more common in older women, and has high serum LDH levels, high IPI, high central nervous system recurrence rate and low survival rate [13]. The results of this group of patients showed that the positive expression rate of CD5 in DLBCL was 9.2%. The positive expression of CD5 did not show correlation with age, sex, clinical stage, type of immunophenotype (Hans), location and HBsAg, but may be due to the number of CD5-positive DLBCL cases in this group being too small,. However, the results of the study indicated that the MST of CD5-positi-

Table 3: The relationship between CD5 and CD43 expression and clinicopathological features (n = 65)

	CD5+	CD5-	P-value	CD43+	CD43-	P-value
All patients	6	59	-	24	41	-
<60 years	3	26	0.781	12	17	0.504
≥60 years	3	33		12	24	
Male	5	37	0.314	17	24	0.321
Female	1	22		7	17	
Intranodal sites	2	37	0.162	18	21	0.059
Extranodal sites	4	22		6	20	
I-II Stage	2	14	0.603	6	10	0.956
III-IV Stage	4	45		18	31	
GCB	1	18	0.478	3	16	0.023
non-GCB	5	41		21	25	
HBsAg+	2	10	0.324	9	3	0.002
HBsAg-	4	49		15	38	

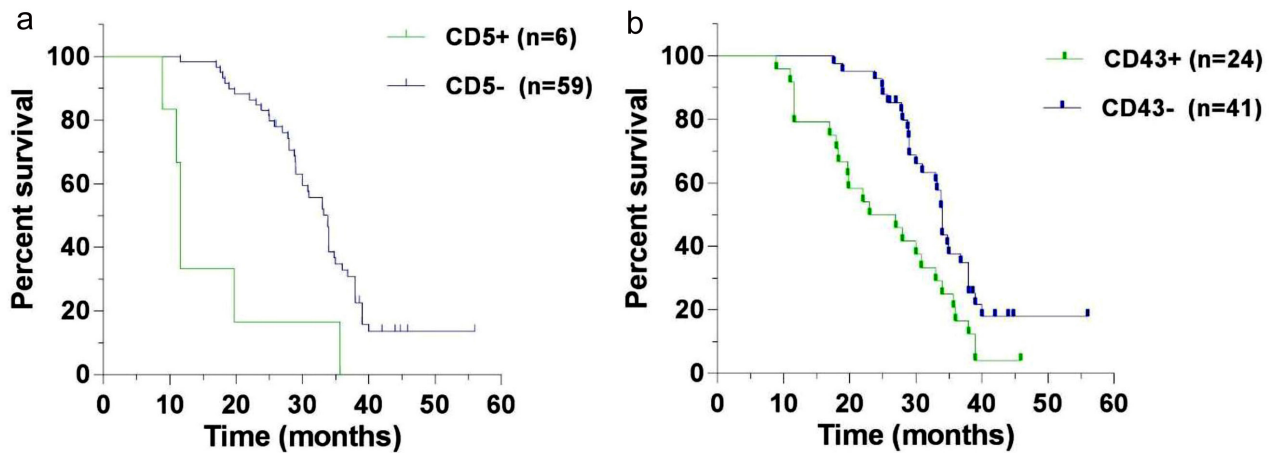


Figure 2: Survival analysis according to the CD5 and CD43 expression in DLBCL patients. a: The MST of CD5 positive DLBCL patients was 11.6 months, and the MST of CD5 negative DLBCL patients was 32.8 months. The difference between the two groups was statistically significant ($P=0.001$); b: The MST of CD43-positive DLBCL patients was 20.2 months, and the MST of CD43-negative DLBCL patients was 36.4 months. The difference was statistically significant ($P=0.003$).

DLBCLs was obviously lower than that of CD5-negative, demonstrating that CD5-positive expression is a poor prognostic marker for patients with DLBCL. CD43 is a transmembrane glycoprotein that is expressed on different lymphoid hematopoietic cells, including certain B lymphocyte subsets. Studies have shown that the positive expression rate of CD43 in DLBCL is between 19.0% and 29.4%, which is correlated with non-GCB and poor prognosis [9, 14]. The results of this group of data show that the positive expression rate of CD43 in DLBCL was 36.9%, higher than the relevant reports. The MST of patients with CD43-positive DLBCL was apparently lower than that of CD43-negative patients, similar to the one reported in the studies 9, 14, 15, and associated with Hans-classified non-GCB and HBsAg + as one of the adverse prognostic

factors in patients with DLBCL, but not with age, gender, clinical stage and location. Reported in the literature, HBsAg positive is more common in non-GCB patients, such as patients with poor prognosis, and the relationship between HBsAg and CD43 expression is not clear [16].

CD5-positive DLBCL always occurs in the elderly, showing an invasive course, prone to central recurrence, with high mortality and poor prognosis. It may represent an independent disease type in DLBCL. The pathogenesis of this disease is still unknown, and standard treatment for CD5-positive DLBCL is uniform. CD5-positive DLBCL patients have moderately aggressive lymphomas, requiring systemic chemotherapy-based combination therapy. Rituximab combined with a stronger chemotherapy regimen may benefit such patients, and targeted therapy

for CD5 may be improved in the future [17]. A potential therapeutic approach to the cure rate of CD5-positive DLBCL, focus on how to improve the clinical cure rate and reduce recurrence of such patients, requires further research. The development of the best treatment model requires confirmation of data from a large sample of prospective randomized controlled trials.

In conclusion, our study indicated that the prognosis of CD5 and CD43 positive in DLBCL may be worse.

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