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ORIGINAL ARTICLE

Disparity Analysis of Clinicopathologic Hallmarks between Transformed DLBCL and Primary DLBCL

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SUMMARY

Background: **A variety of indolent lymphomas, particularly marginal zone lymphoma (MZL) and follicular lymphoma (FL) can be histologically transformed to diffuse large B-cell lymphoma (DLBCL). Little is known about the disparity of clinicopathologic characteristics between transformed DLBCL (tDLBCL) and primary DLBCL (pDLBCL).**
Methods: **This retrospective study analyzed the clinicopathological hallmarks of 10 tDLBCL (7 MZL and 3 FL) and 40 pDLBCL from the Affiliated Hospital of Xuzhou Medical University.**
Results: **Patients of tDLBCL had a higher ECOG score, more B-symptoms, and lower serum albumin level than those in pDLBCL (60.0% vs. 7.50%, 40.0% vs. 10.0%, and 90.0% vs. 10.0%, respectively, $p < 0.01$). Pathologically, tDLBCL had more c-Myc and BCL-2 dual-expression than that in pDLBCL (60.0% vs. 25.0%, $p < 0.01$). The positive rate of CD5 expression and the proportion of high Ki-67 score in tDLBCL were higher than those in pDLBCL (50.0% vs. 7.5%, 50.0% vs. 32.5%, respectively, $p < 0.01$). The median overall survival and progression-free survival were 14 months and 11 months in tDLBCL, 35 months and 28 months in pDLBCL ($p < 0.05$ and $p < 0.001$).**
Conclusions: **Our results demonstrate that tDLBCL manifested aggressive clinical course and pathological features of Myc/BCL-2 expression, CD5 expression, and high Ki-67 score.**
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KEY WORDS

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INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) has heterogeneous clinical, morphologic, immunologic, and cytogenetic characteristics, accounting for 30% - 40% in adult non-Hodgkin lymphomas [1]. Partial DLBCL arising from transformed diffuse large B-cell lymphoma (tDLBCL) with more complexity and heterogeneity. Histological transformation (HT) mostly occurred in chronic lymphocytic leukemia (CLL), follicular lymphoma

(FL), and marginal zone lymphoma (MZL) [2]. Richter's transformation, or Richter's syndrome, was observed in about 5% to 10% of patients with CLL [3]. Annually, approximately 2% to 3% of FL patients will transform to an aggressive lymphoma [4-6]. Moreover, about 3% to 19% per year of MZL patients lead to HT [4,7].

Elevated lactate dehydrogenase (LDH), poor performance status, or new extranodal involvement were more readily observed in the tDLBCL [8-11]. Elevated β2-microglobulin (β2-MG) and erythrocyte sedimentation rate (ESR) were also more readily observed in tDLBCL. Further laboratory examination confirmed that there were 8 patients with significantly elevated LDH levels, 6 patients with elevated β2-MG levels, and 5 patients with varying degrees of elevated ESR levels [12]. Positron emission computed tomography (PET/CT) maximum standardized uptake value (SUVmax) was higher in tDLBCL and aggressive lymphoma than that in indolent lymphoma [13,14]. However, studies on the mechanism of HT of indolent lymphoma to DLBCL have not been fully elaborated.

Overexpression of proto-oncogene (BCL-2), proliferation of c-Myc regulatory genes, unregulated regulation of cell cycle genes (CDKN2A and CDKN2B), TP53 mutation, and abnormal expression of related regulatory genes in the NF-κB signaling pathway such as MyD88 and TNFAIP3 all play pivotal roles in the HT of indolent lymphoma [12,15,16]. The role of cytogenetic abnormalities in tDLBCL, long arm allele replacement on chromosome 9, and mutation on chromosome 13 also lead to the occurrence of tDLBCL [17-19]. The disparity of tDLBCL and primary DLBCL (pDLBCL) are still kept unknown.

In this study, we retrieved clinical and pathological variables and retrospectively analyzed the clinicopathologic hallmarks to explore the disparity of tDLBCL and pDLBCL.

MATERIALS AND METHODS

Patients

A total of 50 patients diagnosed with lymphoma between January 2015 and November 2020 were retrieved from Affiliated Hospital of Xuzhou Medical University. Among all the patients, 10 tDLBCL were consultation cases with 7 MZL and 3 FL, the other 40 pDLBCL patients were selected at random as control group. Patients with primary central nervous system lymphoma and primary testicular lymphoma were excluded. Clinical information was gathered including age, gender, Ann Arbor stage, the Eastern Cooperative Oncology Group (ECOG) score, B symptoms, serum lactate dehydrogenase (LDH), and serum albumin. International Prognostic Index (IPI) was analyzed according to age (> 60 years vs. < or = 60 years), Ann Arbor stage (III - IV vs. I - II), and serum LDH level (above normal vs. normal or below). Follow-up information was obtained from medical records and telephone inquiries. The study was consistent with the Declaration of Helsinki and approved by the ethics committees of the Affiliated Hospital of Xuzhou Medical University.

Pathology review

Hematoxylin and eosin (H&E) staining was performed on formalin-fixed, paraffin-embedded (FFPE) tissues through routine staining protocols for morphological examination. More than 20% positivity of the tumor cells was assumed to indicate positive for the purposes of this study. All cases were reviewed by three independent pathologists according to the WHO classification [19]. Consensus was reached on uncertain cases after discussion.

Treatment and response evaluation

All patients received chemotherapy with a rituximab-based regimen. Each treatment outcome was evaluated 4 cycles later, based on computer tomography or PET/CT. According to Lugano Classifications lymphoma response criteria, they can be divided into complete remission (CR), partial remission (PR), disease progression (PD) and disease stability (SD). According to the revised response criteria for lymphoma, overall survival (OS) was calculated as the interval between the time of diagnosis and death from any cause or the last follow-up; progression free survival (PFS) is defined as the time from diagnosis until lymphoma progression or death as a result of any cause.

Statistical analysis

Data were presented as numbers (percentages) for categorical variables. χ^2 test and Fisher's exact test method were used for intergroup comparison of categorical factors.

Kaplan-Meier analysis was performed to estimate the cumulative survival rate of patients, comparisons between groups were made using the log-rank test. All variables with $p < 0.1$ in univariate analysis were kept in the multivariate analysis by using forward selection. All statistical tests were two-sided and the statistical significance was set at $p < 0.05$. Statistical analysis was conducted with IBM SPSS version 19.0 for Windows software program (IBM Corp., Armonk, NY, USA) and R software (version 4.0.3; http://www.Rproject.org).

RESULTS

Clinical characteristics

A cohort of 50 patients was identified, 10 (20%) developed tDLBCL from 7 MZL and 3 FL, another 40 (80%) from pDLBCL. Survivors of MZL and FL had the highest risk of developing TL. The results revealed that ECOG score ($p < 0.01$), B-symptoms ($p < 0.01$), and serum albumin level ($p < 0.01$) had a significant difference in tDLBCL and pDLBCL. Data of patients were shown in Table 1.

Table 1. Basic clinical information of patients with tDLBCL and pDLBCL.

Clinical Characteristics	tDLBCL (n = 10)	pDLBCL (n = 40)	p
Age			0.189
≥ 65 years	7	23	
< 65 years	3	17	
Gender			0.347
Male	7	26	
Female	3	14	
Stage (Ann Arbor)			0.131
I + II	4	14	
III + IV	6	26	
ECOG PS score			< 0.010
< 2	4	37	
≥ 2	6	3	
Serum LDH			0.747
Normal	6	17	
Elevated	4	23	
IPI			0.641
≤ 3	9	25	
> 3	1	15	
B-symptoms			< 0.010
Absence	6	36	
Presence	4	4	
Serum albumin level			< 0.010
< 35 g/L	9	4	
≥ 35 g/L	1	36	

ECOG PS - Eastern Cooperative Oncology Group Performance Status, LDH - lactate dehydrogenase, IPI - International Prognostic Index.

Table 2. Differences of therapeutic response between tDLBCL and pDLBCL groups.

Groups	tDLBCL (n = 10)	pDLBCL (n = 40)	χ^2	p
CR	3 (30%)	29 (72.5%)	6.727	0.012
PR	3 (30%)	7 (17.5%)	0.781	0.377
PD	3 (30%)	3 (7.5%)	3.835	0.050
SD	1 (10%)	1 (2.5%)	1.172	0.279

CR - complete remission, PR - partial remission, PD - progressive disease, SD - stable disease.

Pathological characteristics

Assessment of board image features of tDLBCL

This study selected representative pathological images of TL under hematoxylin-eosin (HE) staining. Patient 1 had MZL at the time of initial diagnosis and then transformed into DLBCL as the disease progressed. Patient 2 had MZL with DLBCL transformation in the same pathological field at the time of initial diagnosis. Patient 3 had FL at the time of initial diagnosis and then transformed into DLBCL as the disease progressed. Patient 4

Table 3. Univariate analysis for prognostic factors of OS.

Variables	HR	95% CI	p
Age	1.040	0.944 - 1.145	0.427
Gender	2.609	0.573 - 11.879	0.215
Albumin	0.837	0.647 - 1.082	0.173
LDH	1.001	1.000 - 1.002	0.131
B-symptoms	3.623	0.707 - 18.558	0.122
Ann Arbor stage	10.349	1.182 - 90.618	0.035
IPI	4.342	0.800 - 23.556	0.089
c-Myc	1.095	0.009 - 1.068	0.057
CD5	3.561	0.804 - 15.772	0.094
DE2	1.124	0.014 - 1.112	0.062
DE6	1.860	0.200 - 3.698	0.839

DE2 - dual expression (BCL-2, c-Myc), DE6 - dual expression (BCL-6, c-Myc), LDH - lactate dehydrogenase, IPI - International Prognostic Index.

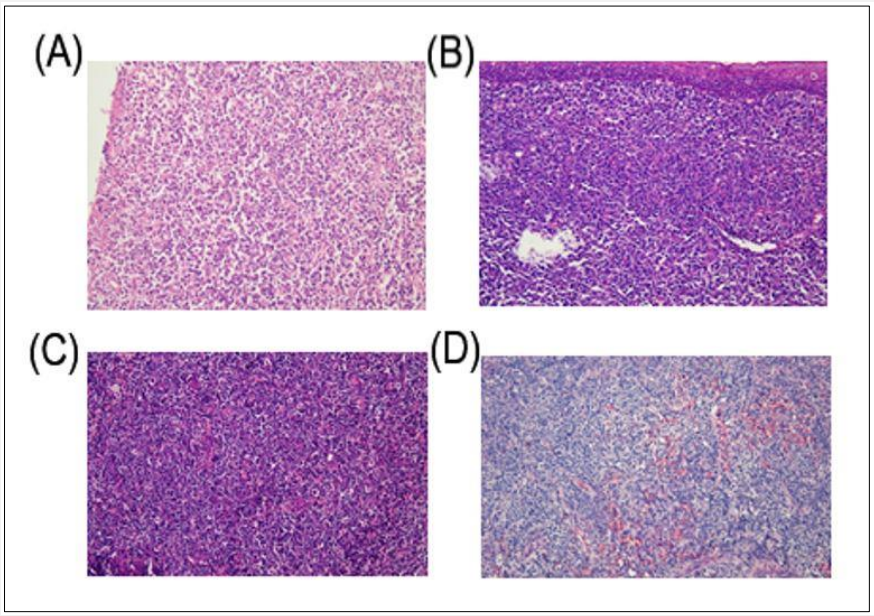

Figure 1. Paraffin sections were used for immunohistochemistry of different patients (× 200). A and C were patient 1 and patient 2, respectively. B and D were patient 3 and patient 4, respectively.

had FL with DLBCL transformation in the same pathological field at the time of initial diagnosis. All of patients were pathologically confirmed at the time of initial diagnosis or secondary pathological biopsy (Figure 1).

Assessment of IHC features of tDLBCL

Immunohistochemistry showed there were 5 (50%) patients with nGCB type, another 5 (50%) patients with GCB subtype in all tDLBCL. In our cohort of 40 pDLBCL patients, there were 8 (20%) patients with nGCB

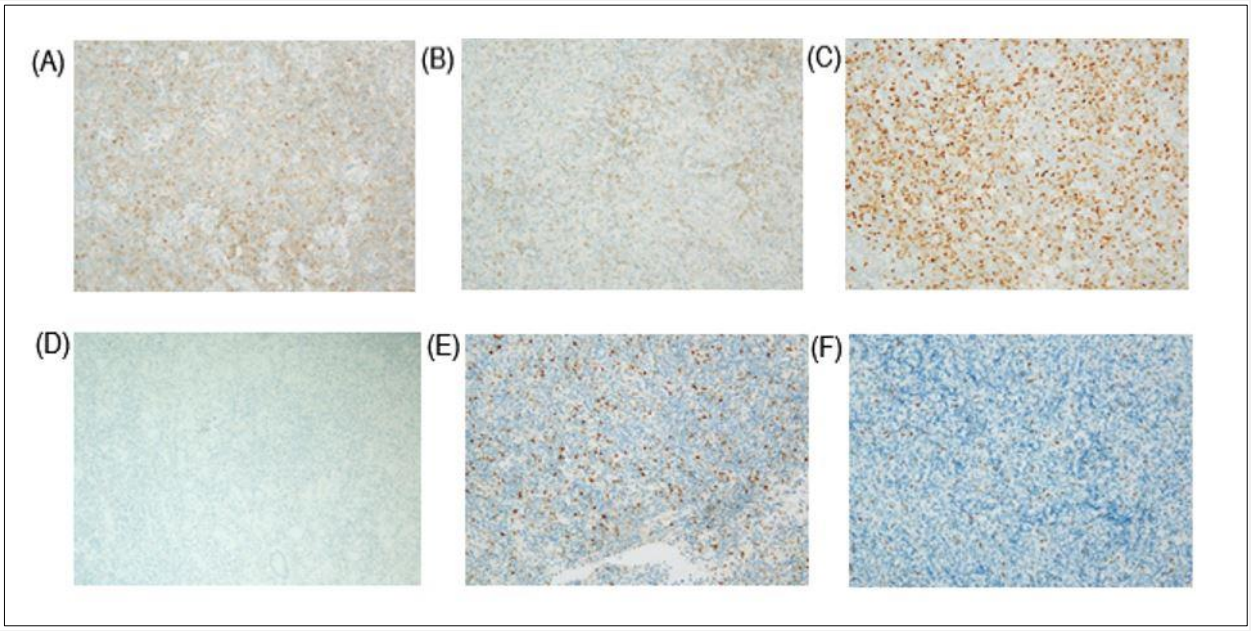

Figure 2. Paraffin sections were used for immunohistochemistry of BCL-2 (A and B), BCL-6 (C and D), and c-Myc (E and F). A, C, and E staining of tDLBCL (× 200). B, D, and F staining of pDLBCL (× 200).

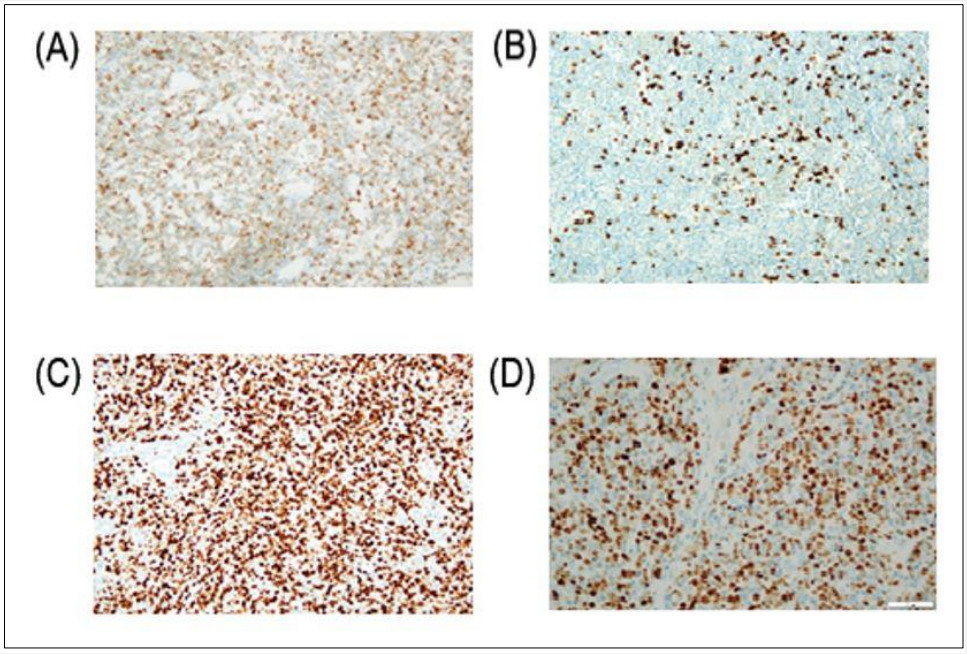

Figure 3. Paraffin sections were used for immunohistochemistry of CD5 (A and B) and Ki-67 (C and D). A and C staining of tDLBCL (× 200). B and D staining of pDLBCL (× 200).

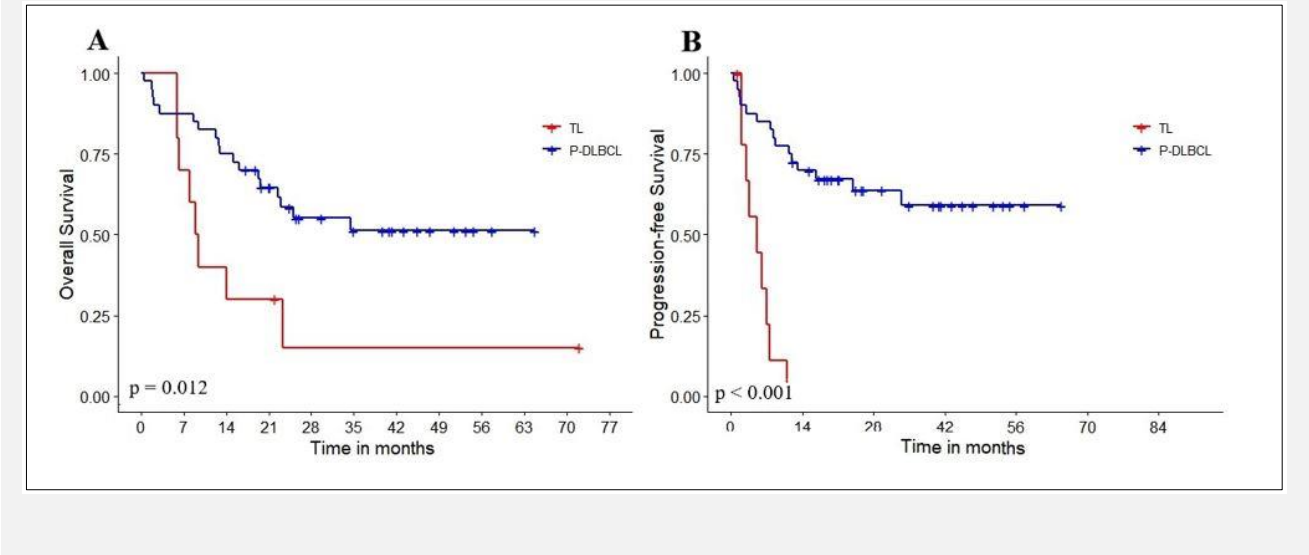

Figure 4. Kaplan-Meier survival curves of the tDLBCL and pDLBCL patients. A. OS in the whole cohort, and B. PFS in the whole cohort.

type, another 32 (80%) patients with GCB subtype ($p < 0.01$). Further studies substantiated that of the 10 patients with histologically tDLBCL, 6 (60%) patients expressed BCL-2 and c-Myc, 3 (30%) patients expressed BCL-6, and 1 (10%) patient expressed c-Myc. In 40 pDLBCL patients, 10 (25%) patients expressed BCL-2 and c-Myc ($p < 0.01$), 12 (30%) patients expressed BCL-6 and c-Myc ($p > 0.05$, Figure 2)

Through the data analysis of 50 patients with lymphoma in this paper, there were 5 (50%) patients with high expression of CD5 molecule in the TL, and there were only 3 (7.5%) patients in pDLBCL ($p < 0.05$, Figure 3). By analyzing the expression level of Ki-67 in the 50 patients we included, the following conclusions could be drawn that the expression level of Ki-67 in 6 (60%) patients was more than 60%, and in 5 (50%) patients was top to 80% in tDLBCL. Otherwise, only 13 (32.5%) patients were more than 80% in the pDLBCL ($p < 0.05$, Figure 3).

Curative effect appraisal

In these tDLBCL patients, 3 (30.0%) patients of whom achieved CR, 3 (30%) achieved PR, 3 (30%) achieved PD, and 1 (10.0%) achieved SD. In the pDLBCL patients, 29 (72.5%) achieved CR, 7 (17.5%) achieved PR, 3 (7.5%) achieved PD, and 1 (2.5%) achieved SD. Responses according to treatment regimen were shown in Table 2. All the patients were treated with standard R-CHOP regimen with an evaluation of efficacy after 4 cycles. CR was very poor in tDLBCL patients ($p < 0.05$). PR, PD, and SD had no statistically significant.

Survival analysis

Univariate and multivariate analyses were conducted to identify potential prognostic factors. The results of univariate analysis showed that Ann Arbor stage, c-Myc, overexpression of BCL-2 and c-Myc, IPI and CD5 affected OS ($p < 0.1$, Table 3). LDH had no statistical significance for the prognosis ($p = 0.131$). In the multivariate model, no other variables provide additional significant information.

The survival analysis showed that the prognosis of tDLBCL patients was worse than pDLBCL. By 2020, 8 tDLBCL patients died due to disease progression, the median time of OS was 14 months, and the median time of PFS was 11 months. In pDLBCL patients, the median OS was 35 months ($p < 0.05$) and the median time of PFS was 28 months ($p < 0.001$, Figure 4).

DISCUSSION

Transformed diffuse large B-cell lymphoma has poor prognosis and short survival time and usually originates in CLL, FL, and MZL [20]. Likewise, as a biologically heterogeneous disease, the disparity of tDLBCL and pDLBCL remain unknown. Thus, this study focused on the clinicopathological variables of tDLBCL and pDLBCL; the clinicopathologic features were retrospectively analyzed, and the aim was to explore the disparity of tDLBCL and pDLBCL.

The clinical characteristics of tDLBCL present elevated lactate dehydrogenase (LDH) and β2-MG, rapid nodal growth, poor performance status, new B-symptoms, hy-

percalcemia, or new involvement in extranodal sites of disease. Our work aligns with previous studies. Among the 10 patients included in this paper, 6 patients had ECOG score ≥ 2, 9 patients had serious lower serum albumin, and 4 patients had B-symptoms in comparison with those in pDLBCL patients, confirming the high degree of malignancy and poor prognosis in tDLBCL. Whether tDLBCL occurrs by direct transformation of the original tumor cells or occurrs gradually as the disease progresses needs to be further investigated [21]. Our work identified that there were 5 MZL and 2 FL at the time of initial diagnosis and then transformed into DLBCL with the disease progression. Likewise, there were 2 MZL and 1 FL with DLBCL transformation in the same pathological field at the time of initial diagnosis.

DLBCL represents a biologically heterogeneous disease with germinal center B-cell-like (GCB) and activated B-cell-like (ABC) subtypes, each arising from different nonmalignant lymphoid counterparts [1]. Transformed DLBCL cell-of-origin (COO) subtypes harbor specific genetic abnormalities and that with more complex heterogeneity [22-24]. This study suggested that compared with pDLBCL, tDLBCL had a variety of relatively specific molecular abnormalities, and 5 out of 10 patients were non-GCB type, far exceeded the incidence of pDLBCL. Further analysis showed dual-expression status of BCL-2 and c-Myc proteins in 6 of the 10 patients, though BCL-6 and c-Myc was only expressed in 3 of the 10 patients. According to the current researchers, CD5 molecule has been widely confirmed as one of the evaluation indexes of malignant degree of lymphoma. In our 10 tDLBCL, there were 5 patients with high expression of the CD5 molecule and was consistent with previous data. A considerable amount of literature has demonstrated that the Ki-67 proliferation index was a predictive factor for the outcome of lymphoma. These studies consistently reported that 6 patients with high Ki-67 (Ki-67 > 60%). Most strikingly, there were 5 patients could be increased to 80% Ki-67, which highlighted the pathological hallmarks of tDLBCL.

It is well known that rituximab-based regimen therapy is now standard for tDLBCL, though previous studies have a poor prognosis in those patients. Our results showed that only 30% tDLBCL patients were CR. After treatment with pDLBCL patients; however, CR was increased to 72.5%, though PR, PD and SD had no statistical significance, indicating that poor outcome could be achieved in tDLBCL patients after the standard treatment. The survival analysis showed that the median time of OS was 14 months, and the median time of PFS was 11 months in tDLBCL, but the median OS was 35 months and the median time of PFS were 28 months in pDLBCL. Meanwhile, the results of univariate analysis found that Ann Arbor stage, c-Myc, dual-express of BCL-2 and c-Myc, IPI and CD5 affected OS.

In conclusion, with retrospectively analyzed the variables, we identified the disparity of the clinicopathologic hallmarks of tDLBCL and pDLBCL. Moving forward, we will be involved in more patients to further study the disparity of the of tDLBCL and pDLBCL.

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Declaration of Interest:

The authors declare that they have no conflicts of interest.

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