Clinicopathological features of relapsed diffuse large B-cell lymphoma: South Egypt Cancer Institute experience

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Abstract:

Background: Diffuse large B-cell lymphoma (DLBCL) is considered the most common lymphoid malignancy that represented about 35 % of adult non-Hodgkin lymphomas (NHL). Although most patients can be cured with the Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, and Prednisolone (R-CHOP) regimen, many patients die due to relapsed disease. In this study, we aim to assess the clinicopathological characteristics of relapsed DLBCL, not otherwise specified (NOS).

Methods: This is a retrospective study conducted on 16 cases of relapsed DLBCL (relapsed group). Meanwhile, 27 cases with DLBCL who capable of attaining complete remission (CR) for 36 months were used as control (CR group).

Results: clinically, most patients in the relapsed group were male (62.5%). The relapsed group had a significant difference in comparison to the CR group as regard bone marrow involvement (P = 0.003), serum lactate dehydrogenase (LDH) level (P = 0.005), B symptoms (P = 0.007), performance status (PS) (P = 0.008), international prognostic index (IPI) (P = 0.000) and age-adjusted IPI (P = 0.017). Pathologically, relapsed DLBCL was associated with negative CD10 expression (P = 0.001), negative BCL6 expression (P = 0.047), and non-germinal center B-cell (non-GCB) phenotype (P = 0.018).

Conclusions: patients with bone marrow involvement, abnormal serum LDH level, B symptoms, poor PS, intermediate to high IPI & AA-IPI, non-GCB phenotype, lower CD10, and BCL6 expression are more likely to relapse.

Keywords: DLBCL, clinical, pathological, relapse.

Introduction:

Diffuse large B-cell lymphoma (DLBCL) is considered the most common lymphoid malignancy subtype that accounts for 25-40% of adult non-Hodgkin lymphomas (NHL) [1]. It is an aggressive heterogeneous disease with variable pathogenic mechanisms, clinical presentations, and outcomes [2]. The standard treatment for DLBCL is CHOP regimen with or without adding Rituximab [3]. Although about 60% of patients can be cured with this regimen, many patients die due to relapsed disease after a period of remission [4]. Relapsed DLBCL is defined according to Cheson criteria [5] by the appearance of any new lesion or increase by ≥ 50% in the size of previously involved

sites or ≥ 50% increase in greatest diameter of any previously identified node greater than 1 cm in its short axis or in the Sum of product of diameter (SPD) of more than one node. Several clinical and pathological factors such as patient's age, clinical stage, performance status, tumor extension, histological variant, bone marrow involvement, international prognostic index (IPI), molecular phenotype (GCB versus non-GCB), biochemical and immunohistochemical markers can predict the outcome after chemotherapy [6]. This study aims to 1- Assess the clinicopathological characteristics of relapsed DLBCL, NOS. 2-Assess the difference between relapsed DLBCL, NOS, and cases with complete remission (CR) regarding the clinicopathological parameters. 3- Assess the association between relapsed group and CR group as regards the immunohistochemical expression of CD10, BCL6, and MUM1.4- Assess the association between relapsed group and CR group as regard the molecular phenotype (GCB versus non-GCB). This may provide evidence for the clinical and pathological features that may denote further relapse and this is can be used as an early screening test for relapse.

Patients and Methods:

This is a retrospective study performed on 16 cases of relapsed DLBCL. Meanwhile, other 27 cases of DLBCL who had attained complete remission (CR) for 36 months were used as control. Only cases with available clinical data were included in this study. Ethical approval of this study was obtained by the ethical committee of the South Egypt Cancer Institute (Reference number IORG0006563). The diagnosis of DLBCL was confirmed by revising the hematoxylin & eosin and immunohistochemistry (IHC) stained slides by three pathologists. The clinical and pathological data were collected from the database registry at South Egypt Cancer Institute (SECI). The formalin-fixed paraffin-embedded (FFBE) blocks were obtained from the archive of the surgical pathology lab of SECI, Assiut University Hospital, Faculty of Medicine between 2010 and 2018.

Immunohistochemistry

Three µm thick FFBE tissue sections were cut and mounted onto charged glass slides. Sections were dewaxed in Xylene (for half an hour) and rehydrated through graded alcohols (absolute, 90%, 80%, and 70%; 10 seconds each) to distilled water. For antigen retrieval, Dako EnVision™ FLEX Target Retrieval Solution, Citrate buffer, (PH 6.1, 50x) (Code DM829) was used. Then, Dako EnVision™ FLEX peroxidase Blocking Reagent (Code SM801), was applied for ten minutes to block the endogenous peroxidase activity. After that, each of the following mouse monoclonal primary antibodies: CD10 (clone 56C6, ready to use, DAKO), BCL6 (clone PG-B6p, ready to use, DAKO), and MUM1 (clone MUM1p, ready to use, DAKO) was applied and incubated for 20 minutes in a humid chamber. Then the slides were washed 2-3 times using Phosphate-buffered saline (PBS) solution. After washing, the secondary antibody; Dako EnVision™ FLEX HRP (Horseradish peroxidase) (Code SM802) was applied for twenty minutes at room temperature. Diaminobenzidine (DAB solution) was applied to the slides for 5- 10 minutes, and then washed in distilled water. Sections were then counter-stained using Mayer's haematoxylin, washed in tap water, dehydrated in ascending alcohols then cleared in Xylene, and left to dry in air. Staining of positive control was performed on FFPE sections of human tonsil. The positivity was identified as brown membranous staining for CD10 and brown nuclear staining for BCL6 and MUM1. Negative control was obtained by omitting the primary antibodies

Scoring and evaluation of immunohistochemical staining

CD10, BCL6, and MUM1 were evaluated independently by 3 pathologists. The positivity was estimated according to Hans' algorithm. For each marker, more than 30 % was considered positive and less than 30 % was considered negative [7]. The cases were then reclassified accordingly into germinal center B-Cell phenotype (GCB) and Post activated B cell type (non-GCB/ABC) Figure 1.

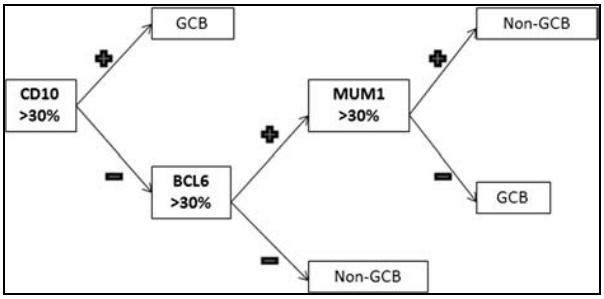

Figure (1) Hans' algorithm for classifying DLBCL subtypes [7]

Statistical analysis

The statistical software package SPSS version 22 was used for all analyses. Clinicopathological data were presented as numbers and percentages. Chi-Square ($\chi 2$) test was used for comparing categorical data. Exact test was used instead when the expected frequency is < 5. All P values were two-tailed and considered statistically significant if ≤ 0.05.

Results:

Clinicopathological characteristics

The study included sixteen cases of relapsed DLBCL representing 37.2 %. Most patients in the relapsed group were males less than 60 years old. As regard tumor size, most cases were less than 10 cm. The most commonly detected histological variant was the centroblastic one. BM involvement was reported in 50 % of cases. Abnormal serum LDH level and poor performance status were found in 81.3 % and 75 % of

cases respectively. The majority of patients in the relapsed group had intermediate to high IPI (81.3%) and AA-IPI (93.8%). The clinicopathological features of the relapsed group in comparison to the CR group are summarized in Table 1.

Immunohistochemical results

CD10 was positive in 44.4% of the CR group while it was negative in all cases in the relapsed group. BCL6 was detected in 74.1% and 43.8% of CR and relapsed groups respectively. Positive MUM1 expression was detected in 70.4% and 68.8% of CR and relapsed groups respectively. The immunohistochemical staining pattern of the three makers is shown in figure (2). The difference between the CR group and relapsed group as regards CD10, BCL6, and MUM1 immunohistochemical expression was summarized in Table 2. The difference between the CR group and relapsed group as regards the molecular phenotype (GCB versus non-GCB) was summarized in Table 3

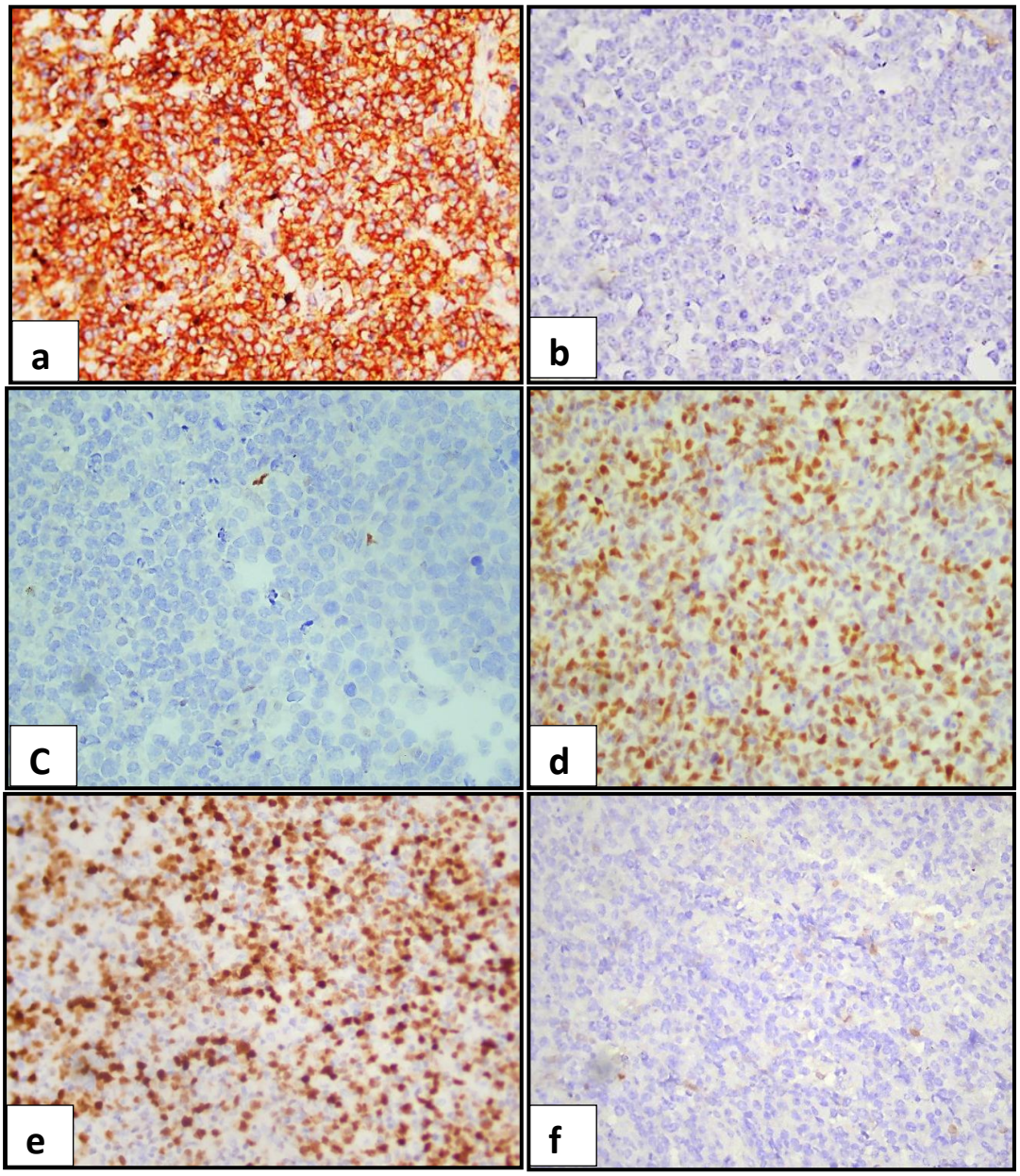

Figure (2): a, d, and e are positive immunohistochemical staining for CD10, BCL6, and MUM1, respectively. b, c, and f are negative immunohistochemical staining for CD10, BCL6, and MUM1, respectively (x400)

Table (1): Differences between CR and relapsed DLBCL cases regarding the clinico-pathological parameters

Variable name		DLBCL, NOS				*P*-value
		CR (n=27)		Relapsed (n=16)		
		N	(%)	N	(%)	
Age groups	<60 years	19	(70.4)	11	(68.8)	
	≥ 60 years	8	(29.6)	5	(31.3)	1.00
Sex	Female	13	(48.1)	6	(37.5)	
	Male	14	(51.9)	10	(62.5)	0.497
Tumor presentation	Nodal	19	(70.4)	9	(56.3)	
	Extranodal	8	(29.6)	7	(43.8)	0.348
Disease bulk	< 10 cm	26	(96.3)	14	(87.5)	
	≥ 10 cm	1	(3.7)	2	(12.5)	0.545
Histological variant	Centroblastic	20	(74.1)	9	(56.3)	
	Immunoblastic	3	(11.1)	2	(12.5)	0.497
	Anaplastic	4	(14.8)	5	(31.3)	
Stage	Early (I, II)	11	(40.7)	3	(18.8)	
	Advanced (III, IV)	16	(59.3)	13	(81.3)	0.137
BM involvement	Free	25	(92.6)	8	(50.0)	
	Involved	2	(7.4)	8	(50.0)	0.003*
Abnormal LDH	< 500	17	(63.0)	3	(18.8)	
	≥ 500	10	(37.0)	13	(81.3)	0.005*
B symptoms	Absent	23	(85.2)	7	(43.8)	
	Present	4	(14.8)	9	(56.3)	0.007*
ECOG-PS	Good (0, 1)	18	(66.7)	4	(25.0)	
	Poor (2, 3, 4)	9	(33.3)	12	(75.0)	0.008*
IPI	L/I (0-2)	21	(77.8)	3	(18.8)	
	I/H (3-4)	6	(22.2)	13	(81.3)	0.000*
Age adjusted IPI	L/I (0-1)	11	(40.7)	1	(6.3)	0.017*
	I/H (2-3)	16	(59.3)	15	(93.8)	

LDH, lactate dehydrogenase. ECOG-PS, Eastern Cooperative Oncology Group - performance status. L/I, low/Intermediate. I/H, Intermediate/High. Chi-square analysis or Fisher Exact test were used for comparing qualitative variables. Significance defined by $p < 0.05$.

Table (2): Differences between CR and relapsed DLBCL cases regarding the immunohistochemical expression of CD10, BCL6, and MUM1

Protein expression		DLBCL, NOS				P-value
		CR (n=27)		Relapsed (n=16)		
		N	(%)	N	(%)	
CD10	Negative	15	(55.6)	16	(100.0)	0.001*
	Positive	12	(44.4)	0	(0.0)	
BCL6	Negative	7	(25.9)	9	(56.3)	0.047*
	Positive	20	(74.1)	7	(43.8)	
MUM1	Negative	8	(29.6)	5	(31.3)	1.0
	Positive	19	(70.4)	11	(68.8)	

Chi-square analysis or Fisher Exact test were used for comparing qualitative variables. Significance defined by $p < 0.05$.

Table (3): Differences between CR and relapsed DLBCL cases regarding the molecular phenotype (GCB and non-GCB)

Molecular phenotype	DLBCL , NOS				P-value
	CR (n=27)		Relapsed (n=16)		
	N	(%)	N	(%)	
GCB	13	(48.1)	2	(12.5)	0.018*
ABC	14	(51.9)	14	(87.5)	

Chi-square analysis or Fisher Exact test were used for comparing qualitative variables. Significance defined by $p < 0.05$.

Discussion:

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of NHL [8]. Relapsed or refractory disease is considered the most common cause of death in DLBCL [4] and so, several clinical and pathological parameters are now widely studied to achieve a better understanding of relapsed DLBCL for screening purposes. In this study, we aimed to assess the clinicopathological characteristics of relapsed DLBCL, NOS and evaluate the difference between relapsed DLBCL and cases with complete remission (CR) regarding the clinicopathological parameters. We also aimed to assess the association between relapsed group and CR group as regards the molecular phenotype (GCB versus non-GCB) and the immunohistochemical expression of each marker of Hans' (CD10, BCL6, and MUM1). In the clinical setting, the relapsed group in our study was associated with bone marrow involvement and poor performance status at the time of diagnosis which is in agreement with a study done by Tsao et al., 2013 [9] and Harrysson et al., 2021 [10]. Moreover, abnormal serum LDH level showed a statistically significant association with the occurrence of relapse which is in concordance with the studies done by Yamamoto etal., 2017, Gong etal., 2020 and Harrysson et al., 2021 [10-12]. High serum LDH level is one of the factors listed in the IPI score and abnormal levels reflect increased tumor bulk and predicts a less favorable outcome [13]. Several researchers conducted the association between the intermediate to high IPI and AA-IPI at the beginning of treatment and relapse in DLBCL which came in line with our study [10, 12, 14]. The assessment of IPI is crucial for therapy selection of relapsed cases. High dose therapy with autologous stem-cell transplants is considered the standard treatment for transplant eligible patients, whereas elderly and frail patients were subjected to more palliative therapy [15]. Pathologically, cell of origin (COO) classification of DLBCL on the basis of Han's algorithm into GCB and non-GCB is confirmed to be a significant prognostic parameter that could be applied in the routine clinical practice using immunohistochemical staining of CD10, BCL6, and MUM1 [16]. In our study, we assessed the difference between relapsed DLBCL group and CR group as regards the molecular phenotype (GCB versus non GCB). We also assessed the association between relapsed DLBCL group and the imunohistochemical expression of each marker of Hans' independently (CD10, BCL6, and MUM1). Our results revealed that most relapsed cases belonged to the non-GCB group which is in concordance with the study done by Gong et al., 2020 [12]. The poor clinical outcome of the non-GCB DLBCL is provided by the expression of several genes that implicated in the proliferation and growth of tumor cells [17]. As regards protein expression, lower CD10 and BCL6 expression were significantly associated with the occurrence of relapse which goes in line with other studies [12,18]. Negative CD10 expression was associated with non-GCB phenotype with expected poorer clinical outcome and more relapse rates [7]. Switching of BCL6 expression from positive at time of diagnosis to negative at the time of relapse was detected in some studies as that done by Todorovic et al., 2014 [19]. Moreover, BCL6 gene rearrangement was included in the triple hit lymphoma (THL) along with MYC and BCL2 with further poorer clinical outcome [20]. This is confirmed that BCL6 could be one of the surrogate markers of the possible molecular triggering mechanisms of relapse. In our study, no association was detected between MUM1 expression and occurrence of relapse [21]. MUM1 expression denotes the terminal B cell differentiation and was reported to be associated with poor clinical response [22]. However, the lack of significance of MUM1 expression in our study may be due to the small number of cases. Also, a higher cutoff value for MUM1 is thought to achieve higher specificity and improve the prognostic performance of MUM1 which was proposed in other algorithms other than Hans' [23].

Conclusion:

Our study confirmed the presence of several clinical and pathological parameters such as bone marrow involvement, abnormal serum LDH level, B symptoms, poor PS, intermediate to high IPI, AA-IPI, non-GCB phenotype, lower CD10, and BCL6 expression that are more likely to be associated with relapse in DLBCL. The limitation of our study includes the lack of clinical data about the late relapse of DLBCL cases. A longer follow-up period is required for comparison between early relapse that occur within 2 years and late relapse that occur after 5 years from diagnosis.

List of abbreviations

DLBCL, diffuse large B-cell lymphoma. NHL, Non-Hodgkin lymphoma. NOS, not otherwise specified. CR, Complete remission. LDH, Lactate dehydrogenase. COO, Cell of origin. IHC, Immunohistochemistry. FFBE, formalin-fixed paraffin-embedded. IPI, International prognostic index. AA-IPI, age adjusted International prognostic index. BM, Bone marrow. ECOG-PS, Eastern Cooperative Oncology Group - performance status. GCB, germinal center B cell. ABC, post activated B cell.

Conflict of interest

The authors declared no conflicts of interest.

Authors' contribution

This work was carried out in collaboration between all authors. Author SSA conduct the study, and wrote the first draft of the manuscript. Author BRZ performed the statistical analysis. Authors AMA, FAMB, MAFA, and EHY wrote the protocol and reviewed the manuscript. All authors read and approved the final manuscript.

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