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Association Study Among Double/Triple Expressor Status, Extranodal Involvement, and Hans Subtype in Diffuse Large B-cell Lymphoma

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Abstract: ***Background: Investigating the Association of Double/Triple Expression with Extranodal Involvement and Hans Classification, and Analyzing the Relationship between Molecular Genetic Characteristics and Clinicopathological Characteristics in DLBCL. Methods: A retrospective analysis of clinical data from 54 patients diagnosed with diffuse large B-cell lymphoma (DLBCL) at Baotou Cancer Hospital between January 2020 and June 2024 was conducted to examine the associations of genetic subtypes with clinical features including extranodal involvement and Hans classification. Results: The double/triple expression group exhibited a significantly higher rate of extranodal involvement than the non-double/triple expression group (76.0% vs. 41.4%; P = 0.014), and the prevalence of double/triple expression was significantly higher in the non-GCB subtype compared to the GCB subtype (60.0% vs. 29.2%; P = 0.024). Conclusions: Double/triple-expressor DLBCL exhibits a more aggressive biological behavior, predisposes to extranodal involvement, and is closely associated with the non-GCB subtype. These findings reveal the molecular heterogeneity of diffuse large B-cell lymphoma (DLBCL) and provide a scientific foundation for its precision treatment and prognostic assessment.***

Keywords: Diffuse large B-cell lymphoma, Double-expression lymphoma, Triple-expression lymphoma, Extranodal involvement, Hans classification, Clinical characteristics**.**

1. Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (NHL), accounting for 30–40% of B-cell non-Hodgkin lymphomas (B-NHL). Its incidence increases with age, with a median age at diagnosis of 70 years. However, DLBCL can occur at any age and exhibits a slight male predominance [1,2]. Most patients present with systemic lymphadenopathy. Extranodal involvement is observed in approximately 30% of patients, most commonly affecting the gastrointestinal tract, bone, testis, spleen, central nervous system (CNS), and other sites [3]. DLBCL constitutes a heterogeneous group of diseases with distinct biological characteristics, clinical manifestations, and treatment responses. R-CHOP remains the cornerstone of first-line therapy, achieving long-term disease control in nearly 90% of patients with limited-stage disease and 60% of those with advanced-stage disease [4]. Nonetheless, a subset of patients experience disease progression, relapse, or refractory disease. Therefore, systematically identifying patients at high risk of adverse outcomes through prognostic assessment tools during the initial disease stage, and formulating individualized precision treatment strategies based on this risk stratification, holds significant clinical importance for improving patient outcomes and enhancing long-term survival rates. This study retrospectively analyzed the clinical data of 54 DLBCL patients to explore the association of double/triple expression with extranodal involvement and Hans classification, and to analyze the relationship between molecular genetic characteristics and clinicopathological features in DLBCL.

2. Materials and Methods

2.1 General Information

A retrospective analysis was conducted on the clinical data of 54 patients diagnosed with diffuse large B-cell lymphoma (DLBCL) at Baotou Cancer Hospital between January 2020 and June 2024. Inclusion criteria comprised: age greater than 18 years at initial diagnosis, pathologically confirmed DLBCL, and treatment with an R-CHOP-based chemotherapy regimen. Exclusion criteria were: receipt of fewer than two treatment cycles and loss to follow-up. The study received approval from the Baotou Cancer Hospital Medical Ethics Committee (Expedited Review Approval No.: 2025-006), with patient informed consent waived. Collected data included patient sex, age, Ann Arbor stage, ECOG performance status score, double-expressor status, lactate dehydrogenase (LDH) level, Hans subtyping classification, International Prognostic Index (IPI) score, extranodal involvement (involved sites and involvement status), and treatment regimen. Cell of origin was classified using the Hans algorithm as germinal center B-cell-like (GCB) or non-germinal center B-cell-like (non-GCB) based on the expression patterns of CD10, MUM1, and BCL6 proteins. Patients were categorized according to protein expression into double-expressor lymphoma (DEL; defined by co-expression of c-MYC and BCL-2), triple-expressor lymphoma (TEL; defined by co-expression of c-MYC, BCL-2, and BCL-6), or non-double/triple-expressor lymphoma.

2.2 Treatment Methods

All diagnosed patients received 3 to 8 cycles of R-CHOP-based chemotherapy. This included R-CHOP combined with agents such as BTK inhibitors (e.g., ibrutinib), lenalidomide, chidamide, or decitabine. Patients with central nervous system (CNS) involvement received additional intrathecal methotrexate. Upon relapse, treatment regimens comprised R-bendamustine, R-DHAP (rituximab, cisplatin, cytarabine, dexamethasone), gemcitabine plus oxaliplatin (GemOx), selinexor plus GemOx, polatuzumab vedotin plus

GemOx, R-ICE (rituximab, ifosfamide, carboplatin, etoposide), autologous stem cell transplantation (ASCT), chimeric antigen receptor T-cell (CAR-T) therapy, or targeted agents including BTK inhibitors and lenalidomide.

2.3 Immunohistochemical Analysis

Tissue specimens underwent processing including paraffin sectioning, hydration, heat-induced epitope retrieval (HIER), and immunohistochemical staining. Interpretation criteria were defined as follows: Positivity thresholds for CD20, CD10, BCL6, MUM1, BCL2, and MYC were all set at ≥30% of tumor cells exhibiting staining. The Ki-67 proliferation index was calculated by counting cells within hotspot areas. Double-expressor lymphoma (DEL) was defined by ≥40% of tumor cells positive for c-MYC protein and ≥50% positive for BCL-2 protein. Triple-expressor lymphoma (TEL) was defined as meeting DEL criteria plus ≥30% of tumor cells positive for BCL6 protein [5]. Germinal center B-cell-like (GCB) subtype was characterized by either: CD10+ (BCL6+/- and MUM1+/-) or CD10- (BCL6+ and MUM1-). Non-germinal center B-cell-like (non-GCB) subtype was characterized by either: CD10- (BCL6+ and MUM1+) or CD10- (BCL6- and MUM1+/-).

2.4 Statistical Analysis

Statistical analysis was performed using SPSS software (version 26.0). Categorical data are expressed as number of cases and percentage [n (%)]. Intergroup comparisons were analyzed using the Chi-square test or Fisher's exact test, as appropriate. A two-sided P-value of less than 0.05 ($P < 0.05$) was considered statistically significant for all analyses.

3. Results

3.1 General Baseline Clinical Characteristics

This study enrolled a total of 54 patients, comprising 33 males (61.1%) and 21 females (38.9%). Body mass index (BMI) classification revealed 2 patients (3.7%) were underweight, 30 patients (55.6%) had normal weight, and 22 patients (40.7%) were obese. Regarding extranodal involvement (ENI), 23 patients (42.6%) had no ENI, while 31 patients (57.4%) exhibited ENI. Among those with ENI, involvement sites included the spleen in 16 patients (29.6%), bone in 13 patients (24.1%), gastrointestinal tract in 8 patients (14.8%), and bone marrow in only 4 patients (7.4%). Furthermore, 30 patients (55.6%) had fewer than 2 ENI sites, whereas 24 patients (44.4%) had involvement in ≥2 sites. Hans subtyping classified 30 patients (55.6%) as non-germinal center B-cell-like (non-GCB) and 24 patients (44.4%) as germinal center B-cell-like (GCB). Disease stage distribution was as follows: Stage I in 9 patients (16.7%), Stage II in 17 patients (31.5%), Stage III in 11 patients (20.4%), and Stage IV in 17 patients (31.5%). Double- or triple-expressor lymphoma status was positive in 25 patients (46.3%) and negative in 29 patients (53.7%). Double- or triple-hit lymphoma (DHL/THL) status was positive in only 2 patients (3.7%) and negative in 52 patients (96.3%). Analysis of c-MYC expression showed low expression in 26 patients (51.0%) and high expression in 25 patients (49.0%). Elevated lactate dehydrogenase (LDH) levels were observed in 32 patients (59.3%), with normal levels in 22 patients (40.7%). International Prognostic Index (IPI) scores were distributed as follows: low risk (0-1) in 13 patients (24.1%), intermediate risk (2-3) in 31 patients (57.4%), and high risk (4-5) in 10 patients (18.5%).

3.2 Relationship of Double- and Triple-Expresser Lymphoma with Extranodal Disease

When classified based on the presence or absence of extranodal involvement (ENI), in the non-double / triple-expresser lymphoma (non-DEL/TEL) group, 17 patients (58.6%) had no ENI while 12 patients (41.4%) exhibited ENI. Conversely, in the DEL/TEL group, 6 patients (24.0%) had no ENI and 19 patients (76.0%) exhibited ENI. A statistically significant difference was observed between the two groups ($P = 0.014$), demonstrating a significant association between DEL/TEL status and extranodal involvement, as detailed in Table 1.

Table 1: Association Between Double/Triple-Expresser Status and Extranodal Involvement

Extranodal Involvement	non-DEL/TEL	DEL/TEL	*P*
Absent	17(58.6)	6(24.0)	0.014
Positive	12(41.4)	19(76.0)	
Total	29(53.7)	25(46.3)	

3.3 Association Between DEL/TEL and Hans Subtyping

When stratified by Hans subtyping, within the non-GCB subtype, 18 patients (60.0%) exhibited DEL/TEL status while 12 patients (40.0%) were non-DEL/TEL. Conversely, in the GCB subtype, 7 patients (29.2%) were DEL/TEL and 17 patients (70.8%) were non-DEL/TEL. A statistically significant difference was observed between the subtypes ($P = 0.024$), demonstrating a significant association between the non-GCB subtype and DEL/TEL status, as presented in Table 2.

Table 2: Association Between DEL/TEL and Hans Subtyping

	non-GCB	GCB	*P*
non-DEL/TEL	12(40.0)	17(70.8)	0.024
DEL/TEL	18(60.0)	7(29.2)	
Total	30(55.6)	24(44.4)	

4. Discussion

Lymphomas, encompassing Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL), represent a heterogeneous group of malignancies arising from clonal proliferation of lymphocytes [6]. Diffuse large B-cell lymphoma (DLBCL) is the most prevalent NHL subtype, accounting for approximately 30% of all NHL cases. Patients typically present with progressive lymphadenopathy, extranodal disease, or both, requiring therapeutic intervention. Although most patients are diagnosed at advanced stages, over 60% achieve cure with R-CHOP immunochemotherapy (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) [7]. The application of gene expression profiling (GEP) in DLBCL research constitutes a major advancement, further elucidating this heterogeneity and providing a molecular basis for subclassification. The most established classification stratifies DLBCL into germinal center B-cell-like (GCB) and activated B-cell-like (ABC) subtypes based on cell-of-origin, with approximately 10-15% of cases remaining unclassifiable.

Patients with the GCB subtype generally exhibit superior prognosis compared to the ABC subtype, while unclassified cases demonstrate intermediate survival [8-10]. Although cell-of-origin provides prognostic insight, significant heterogeneity persists within GCB and ABC subtypes, each encompassing subgroups with varying outcomes [11]. Owing to its expense and complexity, GEP faces challenges for widespread clinical implementation. Consequently, Hans et al. proposed immunohistochemistry (IHC) as an alternative methodology, demonstrating that expression patterns of CD10, BCL-6, and MUM1 proteins could classify DLBCL into GCB and non-GCB subgroups [12]. However, discrepancies between the Hans algorithm and molecular subtyping occur in approximately 20% of cases [13]. Therefore, genomic analysis should be pursued when clinical findings strongly conflict with the Hans subtype. Although first-line R-CHOP therapy cures a substantial proportion of DLBCL patients, 30-40% experience primary refractory disease or relapse [14]. This study employed a retrospective cohort design to analyze the clinical characteristics of 54 DLBCL patients treated at Baotou Cancer Hospital.

In 2016, the World Health Organization designated DLBCL with concurrent MYC and BCL2 protein expression, without specific chromosomal rearrangements, as double-expressor lymphoma (DEL), recognized as a distinct DLBCL subtype associated with poor prognosis [15]. Cases additionally exhibiting high BCL6 expression alongside MYC/BCL2 co-expression are termed triple-expressor lymphoma (TEL). These patients typically display heightened aggressiveness and adverse clinical outcomes. This study demonstrated a significantly higher prevalence of extranodal involvement (ENI) in DEL/TEL patients compared to non-DEL/TEL patients (76.0% vs. 41.4%, $P = 0.014$). This finding suggests that DEL/TEL status may correlate with enhanced tumor aggressiveness and metastatic potential. The overexpression of MYC and BCL2 may facilitate tumor cell dissemination to multiple sites, thereby increasing the risk of secondary extranodal involvement. However, this hypothesis requires validation in larger cohorts due to the current limited sample size. Furthermore, our data revealed that the proportion of DEL/TEL cases was significantly higher within the non-GCB subtype (60.0%) compared to the GCB subtype (29.2%, $P = 0.024$). This observation aligns with the findings of Hu et al., whose study similarly reported a higher frequency of DEL in non-GCB DLBCL, potentially attributable to the more aggressive biological behavior intrinsic to the non-GCB subtype [16]. In the present cohort, DEL/TEL was identified in 25 patients (46.3%), with 60% of non-GCB cases exhibiting this phenotype. These proportions are higher than those reported in certain Western studies, potentially attributable to cohort selection factors (e.g., inclusion of TEL cases) or variations in immunohistochemical positivity thresholds.

In summary, this study demonstrates that patients with double/triple-expressor lymphoma (DEL/TEL) exhibit a higher propensity for extranodal involvement and show a strong association with the non-GCB subtype, highlighting their clinical aggressiveness. Future investigations with expanded multi-center cohorts should validate the following aspects: 1) Clarify the independent prognostic value of BCL6 overexpression in TEL and determine whether it confers additional adverse effects, requiring support from molecular mechanism studies; 2) Explore combination therapeutic strategies, particularly regimens incorporating targeted agents such as the HDAC inhibitor chidamide, which may improve survival in high-risk subgroups; 3) Ultimately guide personalized therapeutic regimens.

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