

Correlation between Immunohistochemical Subtype and Clinicopathological Features in Patients with Diffuse Large B-cell Lymphoma

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ABSTRACT: The aim of this study was to establish correlations between certain clinical, biological, therapeutic factors and diffuse large B-cell lymphoma (DLBCL) subtypes. For this purpose, between January 2007 and December 2016 a total number of 97 patients with de novo diffuse large B-cell lymphoma were analyzed. Patients with a high prognostic index and non-GCB DLBCL positively correlated and exhibited lower survival rates than low IPI, GCB patients. IPI scoring system and cell-of-origin classification should be used together as a single valid prognostic evaluation tool for DLBCL.

KEYWORDS: diffuse large B-cell lymphoma, international prognostic index, non-Hodgkin lymphoma

Introduction

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous, aggressive group of non-Hodgkin lymphoma, characterized by proliferation of large neoplastic B cells [1]. Of all non-Hodgkin's lymphomas, DLBCL accounts for approximately 30% in the USA and Western Europe. A series of genetic abnormalities including numerical alterations, point mutations, and, more rarely, translocations and gene amplifications are involved in the pathogenesis of this type of lymphoma and may be associated with certain histological and immunophenotypic variants [2]. According to the maturation stage in which the B cell is, and to the type of anomalies that occur during differentiation and maturation, DLBCL presents several variants and subtypes [3].

The gene expression profile can identify the "cell of origin" for certain lymphoma subtypes, however, as this method has numerous limitations both in terms of procedure and cost, certain markers applicable to routine biopsies have been established to match with this method [4,5]. This was possible in either 80% of cases using the Hans algorithm that structured DLBCL in two main subtypes of the germinal center (GCB) and non-germinal center (non-GCB) by analyzing three essential markers: CD10 (marker GCB), MUM1 (multiple myeloma oncogene 1) and bcl-6 (polyclonal B-cell

lymphoma 6, associated with both GCB and ABC subtype) [6,7].

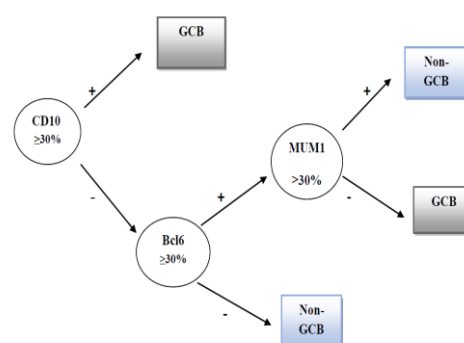


Fig. 1. DLBCL classification according to Hans algorithm [6,7]

This classification has prognostic significance, as germinal center B-cell-like (GCB) subtype has a good prognosis when compare to non-GCB subtype. Another method of risk estimation in DLBCL is the IPI score. Although prognostic estimation has improved significantly, both Hans's algorithm and IPI score can misinterpret the course of the disease, and can induce mistakes in choosing the right treatment [8].

Since the various DLBCL subtypes respond differently to treatment, it is important to know the clinicopathological characteristics and prognostic markers, helping to establish a suitable therapeutic course for each subtype.

The aim of this study was to identify the clinicopathological factors that correlate with an unfavorable prognosis in DLBCL patients, as well as to assess their potential therapeutic implications.

Patients and Methods

Patient inclusion: We selected 97 patients diagnosed with de novo DLBCL in the Filantropia Hospital of Craiova from January 2007 to December 2016. The diagnosis of DLBCL and its two immunohistochemical subtypes relied on the 2008 WHO criteria [9].

The inclusion parameters consisted in the availability of clinical, morphological and therapeutic data for each patient, as well as the immunohistochemical confirmation of GCB/non-GCB subtype using Hans algorithm. We excluded cases with a previously indolent lymphoma that suffered subsequent transformation into a DLBCL and immunodeficiency-associated lymphomas.

In addition to the clinical and histological data, each patient had complete blood cell count, comprehensive metabolic panel (LDH evaluation, liver and renal function) and bone marrow biopsy performed before the initiation of therapy.

For every patient, the IPI score ranging from 0 to 5 was calculated, with one point assigned to each of the following factors: clinical stage III/IV ≥ 2 , age ≥ 60 , the Eastern Cooperative Oncology Group (ECOG) score ≥ 2 , extranodal lesions, and increased lactate dehydrogenase serum levels. The patients were included in the following risk categories: low (0 or 1 points), low-intermediate (2 points), high-intermediate (3 points), high (4 or 5 points). We further divided them into two groups, one group with IPI 0-2 and one group with IPI score 3-5.

We investigated the prognostic value of IPI score in GCB and non-GCB DLBCL in patients treated with conventional chemotherapy regimens according to ESMO guidelines [10].

The protocol of this study was in concordance with the Romanian and European legislation, and had the approval of the Research and Ethics Committee of the University of Medicine and Pharmacy of Craiova.

Statistical analysis: The categorical variables (such as clinicopathological factors, histologic classification according to Hans algorithm, IPI

score etc.) were assessed using Fisher's exact test.

The survival functions for the overall survival (OS), defined as the time (months) between the initiation of treatment until death, and disease-free survival (DFS) were assessed using the Kaplan-Meier method. Log-rank test was applied to conceive a prognostic model for disease-free survival and overall survival.

The descriptive statistics were performed using Microsoft Excel Data Analysis module along with XLSTAT suite (Microsoft Corp, USA). All other statistical tests were performed using GraphPad Prism 7.0 (GraphPad Software Inc. San Diego, USA) with $p \leq 0.05$ being considered statistically significant.

Results

The clinical profile of the patients indicated a M: F sex ratio of 1.32, while mean age (\pm standard deviation) was 56 (± 14.67 years). (Table 1)

Table 1. Clinical features of 97 patients diagnosed with diffuse large B-cell lymphoma

Clinical feature	No. of cases	Percentage (%)
<i>Age</i>		
<60	57	58.76
≥ 60	40	41.23
<i>Gender</i>		
Male	54	55.67
Female	43	44.32
<i>Primary site</i>		
Nodal	85	87.62
Extranodal	12	12.37
<i>Subtype</i>		
Germinal center	44	45.36
Non-germinal center	53	54.63
<i>LDH</i>		
Normal	41	42.26
High	56	57.73

Among the various clinical pathological features of the DLBCL patients, presence of B symptoms, and positive therapeutic response indicated statistically significant levels of correlation with the IPI score, while gender did not seem to influence the prognostic index. (Table 2) The non-GCB subtype is of particular interest, as it shows a very strong correlation with the IPI scoring system ($p < 0.0001$, OR=11.97, CI 95%: 4.527-30.99).

Table 2. Association between clinico-pathological parameters and IPI score in patients with DLBCL

Clinical pathological parameter	No. of cases Percentage (%)	IPI		p (Fisher)	OR	95% CI
		0-2	3-5			
Total	97 (100%)	48	49			
Gender						
Male	54 (55.67%)	26	28	0.83	0.8864	0.4151-2.053
Female	43 (45.33%)	22	21			
Subtype						
GCB	44 (45.36%)	35	9	<0.0001	11.97	4.527-30.99
Non-GCB	53 (54.64%)	13	40			
B symptoms						
Present	64 (65.9%)	24	40	0.0013	0.225	0.096-0.558
Absent	33 (34.1%)	24	9			
Therapy						
Refractory	14 (14.4%)	24	40	0.04	0.2303	0.065-0.865
Responsive	83 (85.6%)	24	9			

Overall survival was compared in four subgroups: low IPI vs. high IPI patients, and GCB DLBCL vs. non-GCB DLBCL.

Patients with a high prognostic index exhibited lower survival rates ($p < 0.001$). Mean survival rate in this subgroup was 23.53 months, while the low IPI patients had a mean survival of 36.87 months. (Fig. 2, 3)

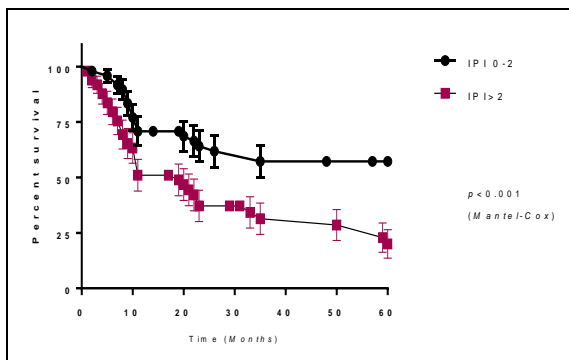


Fig. 2. Overall survival according to IPI score (IPI: 0-2 vs IPI: 3-5) in DLBCL patients. IPI-international prognostic index

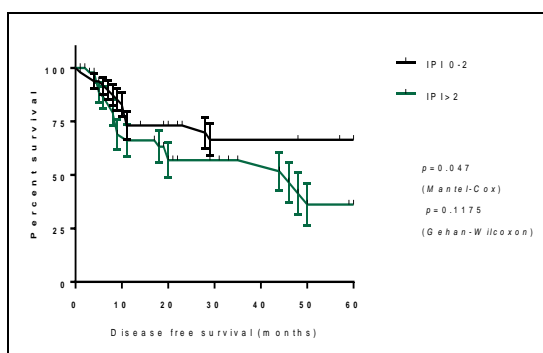


Fig. 3. Disease free survival in DLBCL patients with IPI 0-2 vs. IPI 3-5

Statistical significant difference in overall survival rate and disease-free survival rate was also observed when DLBCL subtype distribution was analyzed. (Fig. 4, 5)

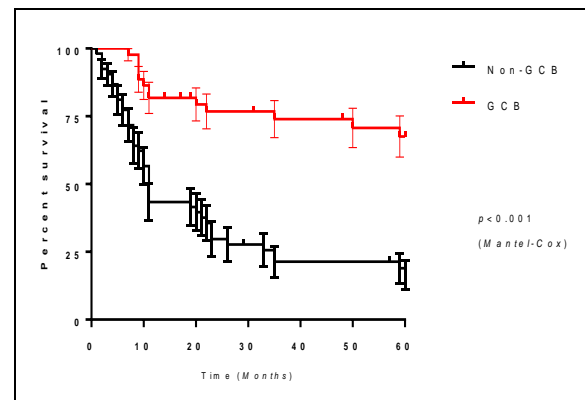


Fig. 4. Overall survival DLBCL patients according to the subtype distribution (GCB vs. non-GCB)

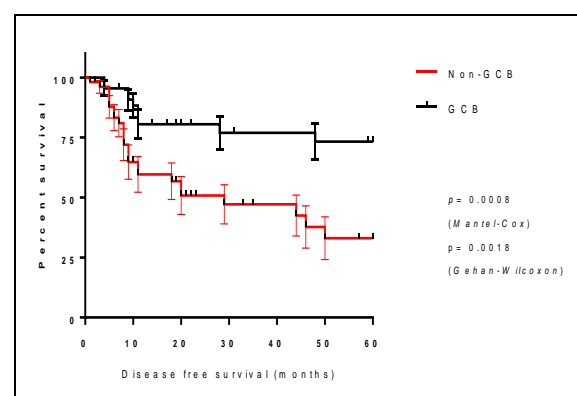


Fig. 5. Disease free survival in GCB vs non-GCB DLBCL patients

Although variables such as age, performance status, stage, tumor burden, proliferating fraction, extranodal involvement, β 2-microglobulin levels, proliferating fraction, therapeutic strategy, and response rate after

chemotherapy have been investigated as common predictors for DLBCL, the results have so far been discrepant [11-12].

Age of the patients is an independent prognostic factor not only for DLBCL patients, but for all non-Hodgkin lymphoma cases, as patients younger than 60 years having a better prognosis, especially when following other therapeutic options [13].

Another method for prognostic assessment is the IPI score, which includes some of the factors listed above, a scoring system that was used long before the introduction of rituximab as therapy for the NHL. In the current practice, it still remains the easiest prognostic score due to the information that can be rapidly obtained but also due to its relative ability to predict the outcome, which has been demonstrated in various studies [14]. Even if the risk stratification by IPI is more accurate than Ann Arbor staging, this scoring system alone is not fully consistent with the disease outcome.

In this study, we have tried to verify the degree of concordance of IPI score with other potential risk factors for disease outcomes. The results point out to a significant correlation between IPI high risk (3-5) subgroup and unfavorable clinical and therapeutic outcomes (display of B-symptoms, refractory disease). Most importantly, we have analyzed the degree of concordance of IPI score and immunohistochemical classification, and our results suggested that there is a strong level of correlation between the IPI scoring system and immunohistochemical subtype ($p < 0.0001$, $OR = 11.97$, $CI\ 95\%: 4.527-30.99$). In addition, IPI values of 0-2 demonstrated better OS than values of 3-5, which was concordant with other studies [14].

One of the potential limitation of our study resides in the heterogeneity of DLBCL patients in terms of treatment regimen. Seventy-eight patients were treated with RCHOP and the rest were treated with R-COEP, R-miniCHOP (young patients with high or intermediate-high risk factors and/or elderly patients).

Since there are several subtypes of DLBCL, each with different clinical evolution, it is necessary to establish the prognostic factors that have the highest accuracy for optimizing patient care. Several studies evaluated the prognostic value of the two major DLBCL subtypes (GCB and non-GCB), with conflicting results [15,16].

A recent study, performed on 601 patients, has shown that patients with GCB subtype had better overall survival and progression-free

survival than non-GCB cases, but depending on the expressed markers, the same subtype may have different prognosis (e.g. GCB CD10 +, MUM1 + subtype, has different prognosis from GCB CD10 +, MUM1 -) [17].

Germinal-center-like (GC) and activated B-cell-like (ABC) DLBCL subtypes display multiple differences in genetic markers expression, activation pathways, and clinical outcomes [18].

Since most studies indicated that the non-GCB subtype has an unfavorable prognosis, the choice of treatment for this subtype should be carefully considered. Some studies have shown that some treatments have greater efficacy in recurrent or refractory patients with non-GCB subtype than those with GCB subtype [19].

The survival rates for non-GCB in our study are comparable with previous studies assessing the implication of this subtype in the disease outcome [20,21]. However, the high level of correlation between IPI scoring system and IHC classification, as well as the similar survival trend for these two type of predictors pleads for their common integration as prognostic evaluation tool for DLBCL.

Conclusion

The routine assessment and implementation of DLBCL cell-of-origin classification according to Hans algorithm and IPI score is essential, as both predictors positively correlate with several clinico-therapeutical parameters, and can serve as a useful instrument in different survival endpoint assessment.

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