Received: March 16, 2023
Accepted: June 8, 2023

Acad Med J 2023;3(2):162-171
UDC: 616-006.442-07
DOI:
Original article

CLINICAL CHARACTERISTICS IN DIFFUSE LARGE B-CELL LYMPHOMA

Çavolli Viola[1], Georgievski Borche[2]

[1]Department of Hematology, University Clinical Center of Kosovo, Pristina, Republic of Kosovo
[2]University Clinic for Hematology, Faculty of Medicine, Ss. Cyril and Methodius University, Skopje, Republic of North Macedonia
e-mail: althea_vgj@hotmail.com

Abstract

According to immunohistochemistry, we can divide diffuse large B-cell lymphomas (DLBCL) into 2 major groups - germinal center B-cell-like (GCB) and non-germinal center B-cell-like (non-GCB) lymphoma and 2 minor groups - double positive (DP) and triple negative subgroup (TN). The aim of this study was to analyze clinical characteristics, treatment response and mortality between different groups of diffuse large B-cell lymphoma according to Han's algorithm.

We analyzed the medical records of patients who were diagnosed as *de novo* diffuse large B-cell lymphoma in a cohort during a 12-year period. All patients were treated with RCHOP therapy. Medical records were reviewed for clinical information including age, sex, lactate dehydrogenase (LDH) levels, clinical stages, performance status (PS), extra nodal site involvement (ESI), International Prognostic Index (IPI) and B symptoms. Immunohistochemistry (IHC) was performed on formalin-fixed, paraffin-embedded 4-μm tissue sections. The antibodies used were CD20, CD10, BCL6, and MUM1.

The results showed that patients in the germinal center B-cell-like group had better clinical characteristics, treatment response and mortality than the non-germinal center B-cell-like group. Triple negative and double positive subgroups showed different clinical characteristics compared to the same group of origin.

These factors would provide valuable insights in predicting aggressiveness, redirecting treatment selections, and therefore, benefit in the survival of patients with DLBCL.

Keywords: DLBCL, GCB group, non-GCB group, clinical characteristics, IHC

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoid malignancy, accounting for approximately 25% of all non-Hodgkin's lymphomas[1,2]. The annual incidence of DLBCL is approximately 7 cases per 100,000 persons in the United States[2] and 4.92 cases per 100,000 persons per year in Europe[3]. Like most other NHLs, there is a male predominance and the incidence increases with age[2]. The median age at presentation is about 64 years old, but it appears that younger Blacks are more often affected than Caucasians[4].

Patients with DLBCL typically present with a rapidly enlarging symptomatic mass, most commonly cervical or abdominal lymphadenopathy. Approximately 30% of patients had systemic "B" symptoms (fever, weight loss, night sweats), and more than half had elevated

serum LDH[5,6]. Extra-nodal disease occurs in up to 40% of cases[7]. The most common site of extranodal involvement is the gastrointestinal tract, but the disease can occur in any tissue. Almost 60% of patients will present with advanced Ann Arbor stage (usually stage III or IV disease), while 40% may have more localized diffuse large B-cell lymphoma. The bone marrow in DLBCL is involved in up to 30% of cases[8-13].

The International Prognostic Index (IPI) scoring system was first published in 1993 and is still widely used today[14]. The IPI score assigns 1 point to each negative prognostic factor (age >60 years, serum lactate dehydrogenase (LDH) above the upper limit of normal, Ann Arbor stage III/IV disease, performance status ≥2, and >1 site with extra nodal involvement).

The Eastern Cooperative Oncology Group (ECOG) Scale of Performance status is an important tool used by hematologists to assess patient eligibility for systemic cancer therapy and to predict prognosis in malignancy.

The currently used standard response criteria for lymphoma are the Lugano criteria, which are based on two-dimensional tumor measurements on positron emission tomography (PET) or computed tomography (CT), for non-FDG avid lymphomas, or when PET imaging is not available[15].

The most recent gold standard assay for subtyping DLBCL is gene expression profiling (GEP), used to identify the cell of origin and disease subtype. According to the gene expression profile, DLBCL can be divided into germinal center B-cell-like (GCB), activated B-cell-like (ABC) and not otherwise specified type 3[16-18]. However, this approach has some significant clinical and practical limitations, as it is more expensive in clinical routine, does not classify all DLBCL patients, and is the subject of ongoing research.

Currently, due to the low cost, immunohistochemical (IHC) analysis of lymphoma biopsy specimens appears to be a more widely applicable method for differentiating DLBCL subtypes in clinical practice. For this reason, immunohistochemistry (IHC) algorithms have been proposed to predict GEP subtypes. Among the published IHC algorithms, the Hans algorithm is most frequently used in routine practice[19]. Hans algorithm consists of three antibodies: CD10 (germinal center marker), Bcl6 associated with germinal center and non-germinal center, and MUM1 as a post-germinal center marker[20]. Based on the combination of these three markers, Hans algorithm divides DLBCL into two categories, GCB (B-cell-like germinal center) and non-GCB (B-cell-like non-germinal center).

Although MUM1 is used as a post-germinal center marker, cases with co-expression of CD10 and MUM1 (CD10+MUM1+, double positive or DP), which are classified in the germinal center B-cell group, according to Hans algorithm, do exist.

DLBCL without positive staining for these three markers (CD10-Bcl6-MUM1-, triple negative, or TN) were also detected. These cases were classified into the non-GCB group based on the Hans algorithm. Little is known about differences in clinical characteristics, treatment response, and mortality between GCB and non-GCB groups.

Material and methods

All experimental protocols were approved by the Ethics Committee of the University Clinic of Kosovo and all patients provided informed consent in accordance with all needed requirements. Statistical analyses were performed with SPSS software, version 26. Differences in clinical characteristics between groups and antibodies were performed with Chi-squared and Fisher exact test. P value less than 0.05 was considered statistically significant. This was a longitudinal retrospective cohort study.

Based on the data collected, the total number of patients diagnosed with *de novo* diffuse large B-cell lymphoma at the Hematology Department of the University Clinical Center of Kosovo was 270. Cases of special variants, such as primary central nervous system

lymphoma, primary mediastinal B-cell lymphoma and HIV-positive DLBCL were excluded from the cohort.

Finally, a total of 224 cases were included in this study and analyzed during a period of 12 years, from September 2009 to November 2021. The median follow-up time was 63 months (5 to 146 months).

All patients were treated with RCHOP as first line therapy. Relapsed cases were treated with second or salvage line chemotherapy, like EPOCH, DHAP and R/ICE.

Medical records for clinical information, including age, sex, serum lactate dehydrogenase (LDH) level, clinical stage (Ann Arbor stage), Eastern Cooperative Oncology Group performance status (ECOG PS), involvement of more than one extranodal site (ESI), IPI and B symptoms were reviewed. Immunohistochemistry was performed at the Department of Pathology, University Hospital of Kosovo. Antibodies used were CD20, CD10, Bcl6 and MUM1. Formalin-fixed, paraffin-embedded 4-μm tissue sections from specimens collected at diagnosis were used for immunohistochemical staining. Sections were individually stained positive using a cutoff of 30% positive lymphoma cells.

The Germinal Center and non-Germinal Center classification was determined by the Hans algorithm. If CD10 was positively stained, the sample was included to the Germinal Center-group. If CD10 and Bcl6 both stained negatively, the sample was of the non-Germinal Center-group. If CD10 was negative but Bcl-6 positive, the MUM-1 staining determined the group. MUM-1 negative cases were in the Germinal Center group and MUM-1 positive cases were in the non-Germinal Center group. If the staining was positive for both CD10 and MUM1, the cases were named double positive (DP) and included in the GCB group and cases negative for both CD10, Blcl6 and MUM1 were classified as triple negative (TN) and included in the non-GCB group.

According to these antibodies, patients were divided into 2 major groups - GCB and non-GCB group according to Hans algorithm. In the GCB group, there were 102 patients and in the non-GCB group 122 patients. According to Hans algorithm, there were 21 patients in the double positive subgroup as part of the GCB group and 17 patients in the triple negative subgroup as part of non-germinal center group.

According to the Lugano classification, patients were divided into 2 groups, those with complete response and others with partial response, stable disease or progression disease after first line treatment with RCHOP.

We analyzed the clinical characteristics, mortality and response to treatment between GCB and non-GCB group, between DP and GCB subgroup or between DP and non-GCB group, between TN and non-GCB subgroup or between TN and GCB group, and between TN and DP subgroup.

Results

A total of 224 patients were enrolled in the study. The median age of patients at diagnosis was 64.0 years (range 22 to 92 years), with 40.6% (90/224) of patients being younger than 60 years. There was a slight male predominance, with 51% male and 49% female patients. The annual incidence of diffuse large B-cell lymphoma in Kosovo was nearly 1.24 new cases per 100,000 inhabitants. The annual incidence was slightly highest in man compared to women, 50.9% *versus* 40.1%. The highest incidence was noted between 2016 and 2020 and the lowest incidence in 2010. The prevalence of diffuse large B-cell lymphoma in Kosovo was approximately 6.7 patients per 100,000 inhabitants.

Clinical significance between different groups of DLBCL

According to Hans algorithm, 45.5% of patients (102/224) were in the group of germinal center B-cell-like lymphoma (GCB) and 54.4% of patients (122/224) in the group of non-germinal center B-cell-like lymphoma (non-GCB) (Table1). Non-germinal center B-cell-

like group was associated with slightly higher age at presentation (more than 60 years old) than germinal center B-cell-like group (59.8% and 54.8%) with no significant P value (P=0.45). Male predominance was more characteristic of non-germinal center B-cell-like group, 54% *versus* 47% in germinal center B-cell-like group, but with a no significant P value (P=0.29). Non-germinal B-cell-like group was associated with more advanced Ann Arbor stage than germinal center B-cell-like group, 57.3% *versus* 51.9%, but with no significant P value of 0.41.

Table 1. Clinical characteristics between GCB and non-GCB subtype

Variables	Total No. (%)	GCB no. (%)	Non-GCB no. (%)	P values
Age	224	102	122	
>60y	129(57.5)	56(54.9)	73(59.8)	P=0.45
<=60y	95(42.4)	46(45.0)	49(40.1)	
Gender	224	102	122	
Male	110(49.1)	48(47)	66(54.0)	P=0.29
Female	114(50.8)	54(53)	56(46)	
LDH	224	102	122	
Over ULN	131(58.4)	48(47.0)	83(68)	P=0.002
Normal	93(41.5)	54(52.9)	39(31.9)	
Stage	224	102	122	
I/II	101(45)	49(48)	52(42.6)	P=0.41
III/IV	123(54.9)	53(51.9)	70(57.3)	
ECOG PS	224	102	122	
0-1	135(60.2)	76(74.5)	59(48.3)	
2-4	89(39.7)	26(25.4)	63(51.6)	P=0.001
ESI	224	102	122	
0-1	121(54)	65(63.7)	56(45.9)	P=0.008
>1	103(45.9)	37(36.2)	66(54)	
IPI	224	102	122	
0-2	108(48.2)	61(59.8)	47(35.8)	P=0.002
>2	116(51.7)	41(40.1)	75(61.4)	
B symptoms	224	102	122	
Positive	112(50)	42(41.1)	70(57.3)	P=0.01
Negative	112(50)	60(58.8)	52(42.6)	
Remission	224	102	122	
CR	154(68.7)	73(71.5)	81(63.9)	
PR	70(31.2)	29(28.4)	41(36)	P=0.22
Mortality	224	102	122	
EX	98(43.7)	41(40.1)	57(46.7)	P=0.32
Alive	126(56.2)	61(59.8)	65(53.2)	

Non-GCB patients more often presented with unfavorable clinical variables including elevated LDH level (P<0.002), extranodal disease in more than 1 site (P=0.008), presence of B symptoms (P=0.01), poor PS (P=0.001) and a high-risk IPI (P=0.002) than GCB subtypes (Table 1).

According to Lugano classification, non-GCB patients were characterized by more partial remission, stable disease or progressive disease than GCB patients, but with not a significant P value (P=0.22). Mortality was higher in the group of non-GCB patients (46.7%) than in the group of GCB patients (40.1%), but the P value was not significant (P=0.32).

The differences in clinical features between double positive (DP) and GCB subgroup or non-GCB group are listed in Table 2. The incidence of DP patients was 9.3% (21/224). The DP phenotype was more likely to be characterized by more B Symptoms (P=0.0001), higher LDH values (P=0.01) and higher IPI (IPI>2) (P=0.0001) than GCB subgroup. Complete remission was lower in DP group than GCB subgroup (P=0.003). Additionally, although not

statistically significant, DP subgroup showed higher stage III-IV, higher PS, more than 1 extranodal involvement and higher mortality than GCB subgroup. DP subgroup and non-GCB group did not differ in any clinical parameter expression.

The differences in clinical features between triple negative (TN) and non-GCB subgroup or GCB group are listed in Table 3. The incidence of TN patients was 7.4% (17/224). There was no difference in any of the clinical characteristics between TN and GCB group. Contrary to this, male predominance (P=0.02), higher LDH levels (P=0.01), advanced Ann Arbor stage (III or IV) (P= 0.04), more than one extranodal site disease (P= 0.028) and high-risk IPI (P= 0.003) were significantly more common in non-GCB than in TN subgroup.

Table 2. Differences of clinical characteristics between DP and GCB subgroup or DP and non-GCB group

Variables	Double positive subgroup no. (%)	GCB subgroup (%)	P value	Non-GCB group no. (%)	P value
Age	21	81		122	
>60y	14(66)	42(51.8)	P=0.22	63(51.6)	
<=60y	7(33.3)	39(48.1)		49(40.1)	P=0.55
Gender	21	81		122	
Male	8(38)	40(49.3)	P=0.35	66(54)	
Female	13(61.9)	41(50.6)		56(46)	P=0.17
LDH	21	81		122	
Over ULN	15(71.4)	34(41.9)	P=0.01	83(68.0)	
Normal	6(28.5)	47(58.0)		39(32.0)	P=0.75
Stage	21	81		122	
I/II	8(38)	41(50.6)	P=0.3	52(42.6)	
III/IV	13(61.9)	40(49.3)		70(57.4)	P=0.69
ECOG PS	21	81		122	
0-1	13(61.9)	63(77.7)		59(48.3)	
2-4	8(38)	18(22.2)	P=0.13	63(51.6)	P=0.25
ESI	21	81		122	
0-1	11(52.3)	54(66.6)	P=0.22	56(45.9)	
>1	10(47.6)	27(33.3)		66(54.0)	P=0.58
IPI	21	81		122	
0-2	5(23.8)	56(69.1)		46(37.7)	
>2	16(76.1)	25(30.8)	P<0.0001	76(62.2)	P=0.21
B symptoms	21	81		122	
Positive	16(76.1)	26(32)		70(57.4)	
Negative	5(23.8)	55(67.9)	P<0.0001	52(42.6)	P=0.10
Remission	21	81		122	
CR	11(52.3)	67(82.7)	P=0.003	81(66.3)	
PR	10(47.6)	14(17.2)		41(33.6)	P=0.21
Mortality	21	81		122	
EX	12(57.1)	29(35.8)		57(46.7)	P=0.37
Alive	9(42.8)	52(64.1)	P=0.07	65(53.2)	

The differences of clinical characteristics between TN and DP subgroups are listed in Table 4. Although not statistically significant, DP subgroup showed higher levels of LDH (71.4% *vs.* 41.2%) (P=0.06), higher stage III-IV disease (61.9% *vs.* 35.3%) (P=0.1), male predominance (38% *vs.* 29.4%) (P=0.57), more extra nodal site involvement (47.6% *vs.* 29.4%), more positive B symptoms (73.1% *vs.* 52.9%) (P=0.13) and higher mortality (57.1% *vs.* 35.2%) (P=0.18) than TN subgroup. Additionally, TN subgroup was characterized by significantly lower IPI levels (IPI<2) (P=0.004) and lower but non-significant non-complete remission (P=0.07) than DP subgroup.

Table 3. Differences of clinical characteristics between TN and non-GCB subgroup or TN and GCB group

Variables	Triple Negative no. (%)	Non- GCB subgroup no. (%)	P value	GCB group no. (%)	P value
Age	17	105		102	
>60y	13(76.4)	61(58.1)		56(54.9)	P=0.22
<=60y	4(23.5)	44(41.9)	P=0.33	46(45.1)	
Gender	17	105		102	
Male	5(29.4)	61(58.1)	P=0.02	48(47)	
Female	12(70.6)	44(41.9)		54(53)	P=0.17
LDH	17	105		102	
Over ULN	7(41.1)	76(72.3)	P=0.01	47(46)	
Normal	10(58.8)	29(27.6)		55(54)	P=0.7
Stage	17	105		102	
I/II	11(64.7)	41(39)		49(48)	
III/IV	6(35.2)	64(61)	P=0.04	53(52)	P=0.20
ECOG PS	17	105		102	
0-1	10(58.8)	49(46.6)		78(76.4)	P=0.18
2-4	7(41.1)	56(43.4)	P=0.35	26(23.6)	
ESI	17	105		102	
0-1	12(70.6)	44(41.9)		65(63.7)	P=0.58
>1	5(29.4)	61(58.1)	P=0.028	37(36.3)	
IPI	17	105		102	
0-2	12(70.6)	34(32.3)	P=0.003	61(59.8)	P=0.99
>2	5(29.4)	71(67.7)		41(40.2)	
B symptoms	17	105		102	
Positive	9(52.9)	61(58.1)	P=0.69	42(41.1)	
Negative	8(47.1)	44(41.9)		60(58.9)	P=0.36
Remission	17	105		102	
CR	13(76.4)	68(64.7)		78(76.4)	
PR	4(23.6)	37(35.2)	P=0.34	24(23.5)	P=0.67
Mortality	17	105		102	
EX	6(35.2)	51(48.5)	P=0.30	41(40.2)	
Alive	11(64.7)	54(41.5)		61(59.8)	P=0.7

Table 4. Differences of clinical characteristics between TN and DP subgroup

Variables	Total No. (%)	DP no. (%)	TN no. (%)	P values
Age	38	21	17	
>60y	26(68.4)	14(66.6)	12(70.5)	P=0.79
<=60y	12(31.5)	7(33.3)	5(29.4)	
Gender	38	21	17	
Male	13(34.2)	8(38)	5(29.4)	P=0.57
Female	25(65.7)	13(62)	12(70.5)	
LDH	38	21	17	
Over ULN	22(57.8)	15(71.4)	7(41.2)	P=0.06
Normal	16(42.1)	6(28.5)	10(58.8)	
Stage	38	21	17	
I/II	19(50)	8(38.1)	11(64.7)	
III/IV	19(50)	13(61.9)	6(35.3)	P=0.1
ECOG PS	38	21	17	
0-1	23(60.5)	13(61.9)	10(58.8)	P=0.84
2-4	15(39.4)	8(38.1)	7(41.2)	
ESI	38	21	17	
0-1	23(60.5)	11(52.3)	12(70.5)	
>1	15(39.4)	10(47.6)	5(29.4)	P=0.25
IPI	38	21	17	

0-2	17(44.7)	5(23.8)	12(70.5)	
>2	21(55.2)	16(76.1)	5(29.4)	P=0.004
B symptoms	38	21	17	
Positive	25(65.7)	16(76.1)	9(52.9)	P=0.13
Negative	13(34.2)	5(23.8)	8(38.1)	
Remission	38	21	17	
CR	19(50)	10(47.6)	13(76.4)	P=0.07
PR	19(50)	11(52.3)	4(23.6)	
Mortality	38	21	17	
EX	18(47.3)	12(57.1)	6(35.2)	P=0.18
Alive	20(52.6)	9(42.8)	11(64.7)	

Discussion

Diffuse large B-cell lymphomas have heterogeneous features from a clinical, biological, genetic, and prognostic standpoint of view, requiring special consideration in their treatment. The introduction of chemoimmunotherapy into DLBCL treatment significantly improved the prognosis of these patients compared to chemotherapy alone. The standard of care for diffuse large B-cell lymphoma is R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy.

Because gene expression profile assay is expensive and impractical in the medical routine, IHC algorithms were introduced in order to translate these signatures into protein-based tests. In this study, subtyping DLBCLs according to immunohistochemistry based on the Hans algorithm was considered. The majority of such algorithms were developed in the chemotherapy era and their predictive value in patients treated with chemoimmunotherapy was controversial [21-26]. Among these, the most studied one is Hans algorithm, which uses the immunohistochemical staining of CD10, Bcl6, and MUM1 to classify cases of DLBCL into germinal center B-cell-like or non-germinal center B-cell-like groups[20]. The algorithm has had consistency in some studies and non-compliance in some other studies. IHC algorithm derived by Hans *et al.* to assign DLBCL to GCB and non-GCB groups is considered imperfect and has a misclassification rate of 19.7% when compared to gene expression profiling data.

In the present study, we compared the clinical characteristics of different groups of diffuse large B-cell-lymphoma classified according to Hans algorithm. The results showed that, with a median follow-up of 63 months, 56.2% of patients survived, and 68.7% (150/224) of all patients achieved a complete response after first-line RCHOP treatment. According to clinical significance, non-GCB patients often presented with more unfavorable clinical variables than GCB patients, which were consistent with previous reports[28,30].

In a cohort of almost 12 years, 8.6% (21/244) CD10+MUM1+ (Double Positive) and 7.6% (17/244) CD10-Bcl6-MUM1-(Triple Negative) DLBCLs patients were identified. The reported incidences of DP and TN were 2.3-14.3% and 5.5-19.1%, respectively[20,27,29]. Double positive patients were significantly characterized by more aggressive clinical parameters than the other GCB patients, even if they originated from the same group. DP subgroup and non-GCB group did not differ in any clinical parameter expression. These data demonstrated that patients in DP subgroup had similar clinical characteristics with patients in non-GCB group, even if DP was part of GCB group. However, triple negative DLBCL patients, who were classified in non-GCB group according to Hans algorithm were found to have different clinical characteristics from other non-GCB patients. Beside this, triple negative patients had better clinical characteristics than other non-GCB patients. There was no difference in any of the clinical characteristics between TN and GCB patients. These data raised the possibility that patients in TN subgroup had the same clinical characteristics as patients in GCB group which was consistent with a recent study[28]. Although not statistically significant, triple negative group had better clinical variables than double positive group[28,30].

Conclusion

In summary, although our study has demonstrated that Hans algorithm retains its clinical importance in the era of chemoimmunotherapy, controversies in the literature remain. This is due most commonly to the differences in patient populations, antibodies, and regimens used, and partly due to a lack of homogeneous or large cohorts. In addition, the presence of some special units, such as DP and TN subgroups, may affect the clinical value of Hans algorithm, which is usually ignored in other studies. A more detailed classification of DLBCL based on Hans algorithm could help identify patients with different clinical features, thereby improving patient stratification for risk-adjusted therapy. However, because our study is retrospective, more studies with a larger number of patients who will be treated with rituximab plus standard chemotherapy are needed to confirm our findings.

Conflict of interest statement. None declared.

References

1. Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. *Blood* 2011; 117(19): 5019-5032. doi: 10.1182/blood-2011-01-293050.
2. Morton LM, Wang SS, Devesa SS, Hartge P, Weisenburger DD, Linet MS. Lymphoma incidence patterns by WHO subtype in the United States, 1992-2001. *Blood* 2006; 107(1): 265-276. doi: 10.1182/blood-2005-06-2508.
3. Sant M, Allemani C, Tereanu C, De Angelis R, Capocaccia R, Visser O, *et al.* Incidence of hematologic malignancies in Europe by morphologic subtype: results of the HAEMACARE project. *Blood* 2010; 116(19): 3724-3734. doi: 10.1182/blood-2010-05-282632.
4. Shenoy PJ, Malik N, Nooka A, Sinha R, Ward KC, Brawley OW, *et al*. Racial differences in the presentation and outcomes of diffuse large B-cell lymphoma in the United States. *Cancer* 2011; 117(11): 2530-2540. doi: 10.1002/cncr.25765.
5. Armitage JO, Weisenburger DD. New approach to classifying non-Hodgkin's lymphomas: clinical features of the major histologic subtypes. Non-Hodgkin's Lymphoma Classification Project. *J Clin Oncol* 1998; 16(8): 2780-2795. doi: 10.1200/ JCO.1998.16.8.2780.
6. No authors listed. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. *Blood* 1997; 89(11): 3909-3918. PMID: 9166827.
7. Møller MB, Pedersen NT, Christensen BE. Diffuse large B-cell lymphoma: clinical implications of extranodal versus nodal presentation-a population-based study of 1575 cases. *Br J Haematol* 2004; 124(2): 151-159. doi: 10.1046/j.1365-2141.2003.04749.x.
8. Grosskreutz C, Troy K, Cuttner J. Primary splenic lymphoma: report of 10 cases using the REAL classification. *Cancer Invest* 2002; 20(5-6): 749-753. doi: 10.1081/cnv-120002492.
9. Paryani S, Hoppe RT, Burke JS, Sneed P, Dawley D, Cox RS, *et al*. Extralymphatic involvement in diffuse non-Hodgkin's lymphoma. *J Clin Oncol* 1983; 1(11): 682-688. doi: 10.1200/JCO.1983.1.11.682.
10. Burton GV, Atwater S, Borowitz MJ, Huang AT. Extranodal Head and Neck Lymphoma: Prognosis and Patterns of Recurrence. *Arch Otolaryngol Head Neck Surg* 1990; 116(1): 69-73. doi:10.1001/archotol.1990.01870010073021.
11. Rudders RA, Ross ME, DeLellis RA. Primary extranodal lymphoma: response to treatment and factors influencing prognosis. *Cancer* 1978; 42(2): 406-416. doi: 10.1002/1097-0142(197808)42:2<406::aid-cncr2820420205>3.0.co;2-h.

12. Bunn PA Jr, Schein PS, Banks PM, DeVita VT Jr. Central nervous system complications in patients with diffuse histiocytic and undifferentiated lymphoma: leukemia revisited. *Blood* 1976; 47(1): 3-10. PMID: 1106798.
13. Kuitunen H, Kaprio E, Karihtala P, Makkonen V, Kauppila S, Haapasaari KM, *et al*. Impact of central nervous system (CNS) prophylaxis on the incidence of CNS relapse in patients with high-risk diffuse large B cell/follicular grade 3B lymphoma. *Ann Hematol* 2020; 99(8): 1823-1831. doi: 10.1007/s00277-020-04140-0.
14. International Non-Hodgkin's Lymphoma Prognostic Factors Project. A predictive model for aggressive non-Hodgkin's lymphoma. *N Engl J Med* 1993; 329(14): 987-994. doi: 10.1056/NEJM199309303291402.
15. Cheson BD, Fisher RI, Barrington SF, Cavalli F, Schwartz LH, Zucca E, *et al*. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. *J Clin Oncol* 2014; 32(27): 3059-3068. doi: 10.1200/JCO.2013.54.8800.
16. Morgensztern D, Martin MG, Lossos IS. Gene expression profiling in diffuse large B-cell lymphoma. *Leuk Lymphoma* 2007; 48(4): 669-682. doi: 10.1080/10428190601186176.
17. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, *et al*. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000; 403(6769): 503-511. doi: 10.1038/35000501.
18. Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, *et al*. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med* 2002; 346(25): 1937-1947. doi: 10.1056/NEJMoa012914.
19. Boltežar L, Prevodnik VK, Perme MP, Gašljević G, Novaković BJ. Comparison of the algorithms classifying the ABC and GCB subtypes in diffuse large B-cell lymphoma. *Oncol Lett* 2018; 15(5): 6903-6912. doi: 10.3892/ol.2018.8243.
20. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, *et al*. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood* 2004; 103(1): 275-282. doi: 10.1182/blood-2003-05-1545.
21. Visco C, Li Y, Xu-Monette ZY, Miranda RN, Green TM, Li Y, *et al.* Comprehensive gene expression profiling and immunohistochemical studies support application of immunophenotypic algorithm for molecular subtype classification in diffuse large B-cell lymphoma: a report from the International DLBCL Rituximab-CHOP Consortium Program Study. *Leukemia* 2012; 26(9): 2103-2113. doi: 10.1038/leu.2012.83.
22. Meyer PN, Fu K, Greiner TC, Smith LM, Delabie J, Gascoyne RD, *et al*. Immunohistochemical methods for predicting cell of origin and survival in patients with diffuse large B-cell lymphoma treated with rituximab. *J Clin Oncol* 2011; 29(2): 200-207. doi: 10.1200/JCO.2010.30.0368.
23. Nyman H, Jerkeman M, Karjalainen-Lindsberg ML, Banham AH, Leppä S. Prognostic impact of activated B-cell focused classification in diffuse large B-cell lymphoma patients treated with R-CHOP. *Mod Pathol* 2009; 22(8): 1094-1101. doi: 10.1038/modpathol.2009.73.
24. Gutiérrez-García G, Cardesa-Salzmann T, Climent F, González-Barca E, Mercadal S, Mate JL, *et al*. Gene-expression profiling and not immunophenotypic algorithms predicts prognosis in patients with diffuse large B-cell lymphoma treated with immunochemotherapy. *Blood* 2011; 117(18): 4836-4843. doi: 10.1182/blood-2010-12-322362.
25. Nyman H, Adde M, Karjalainen-Lindsberg ML, Taskinen M, Berglund M, Amini RM, *et al*. Prognostic impact of immunohistochemically defined germinal center

phenotype in diffuse large B-cell lymphoma patients treated with immunochemotherapy. *Blood* 2007; 109(11): 4930-4935. doi: 10.1182/blood-2006-09-047068.
26. de Jong D, Rosenwald A, Chhanabhai M, Gaulard P, Klapper W, Lee A, *et al.* Immunohistochemical prognostic markers in diffuse large B-cell lymphoma: validation of tissue microarray as a prerequisite for broad clinical applications-a study from the Lunenburg Lymphoma Biomarker Consortium. *J Clin Oncol* 2007; 25(7): 805-812. doi: 10.1200/JCO.2006.09.4490.
27. Chang CC, McClintock S, Cleveland RP, Trzpuc T, Vesole DH, Logan B, *et al.* Immunohistochemical expression patterns of germinal center and activation B-cell markers correlate with prognosis in diffuse large B-cell lymphoma. *Am J Surg Pathol* 2004; 28(4): 464-470. doi: 10.1097/00000478-200404000-00005.
28. Lu TX, Miao Y, Wu JZ, Gong QX, Liang JH, Wang Z, *et al*. The distinct clinical features and prognosis of the $CD10^{+}MUM1^{+}$ and $CD10^{-}Bcl6^{-}MUM1^{-}$ diffuse large B-cell lymphoma. *Sci Rep* 2016; 6: 20465. doi: 10.1038/srep20465.
29. Colomo L, López-Guillermo A, Perales M, Rives S, Martínez A, Bosch F, *et al*. Clinical impact of the differentiation profile assessed by immunophenotyping in patients with diffuse large B-cell lymphoma. *Blood* 2003; 101(1): 78-84. doi: 10.1182/blood-2002-04-1286.
30. Ichiki A, Carreras J, Miyaoka M, Kikuti YY, Jibiki T, Tazume K, *et al*, Clinicopathological Analysis of 320 Cases of Diffuse Large B-cell Lymphoma Using the Hans Classifier. *J Clin Exp Hematop* 2017; 57(2): 54-63. doi: 10.3960/jslrt.17029.