The Egyptian Journal of Hospital Medicine (January 2025) Vol. 98, Page 341- 351

Clinical and Laboratory Relevance of MYD88 L265P Mutation in B-cell Neoplasm: A Focus on Diffuse Large B-cell Lymphoma Subtype

Sally S. Mandour*[1], Rasha Adel Mohamed Abdelmoneum[2], Fatma A. Khalaf[3], Fatma Omar Khalil[4], Heba Yahia Alsayed[5], Hatem Rabie[1]

Departments of [1]Clinical Pathology, [3]Clinical Biochemistry and [4]Clinical Microbiology and Immunology, National Liver Institute, Menoufia University, Egypt

Departments of [2]Clinical Oncology and Nuclear Medicine and [5]Internal Medicine, Faculty of Medicine, Menoufia University, Egypt

***Corresponding author:** Sally S. Mandour, **Mobile:** (+20) 01004824962,

E-mail: Sallysaad@liver.menofia.edu.eg, sallysaad2012@gmail.com

ABSTRACT

Background: Diffuse large B-cell lymphoma (DLBCL), the most common subtype of non-Hodgkin lymphoma (NHL), is characterized by clinical and molecular heterogeneity. MYD88 L265P mutation is a major driver mutation with significant diagnostic, prognostic, and therapeutic implications and has been extensively studied in multiple populations. However, its prevalence and clinical relevance in our cohort remain to be fully investigated.

Objective: This study aimed to determine the frequency of the MYD88 L265P mutation in patients with B-cell neoplasms, particularly those with DLBCL and its association with laboratory and clinical parameters as well as its potential association with disease progression.

Patients and methods: This prospective study was conducted on 123 individuals (63 B cell neoplasms & 60 healthy controls). MYD88 L265P mutation was detected using polymerase chain reaction (PCR) techniques. DLBCL patients were classified according to MYD88 mutation status and subgroup analysis was performed.

Results: The MYD88 L265P mutation was detected exclusively in 17% of DLBCL cases (n = 8). According to subgroup analysis, beta-2 microglobulin (B2M) levels were substantially higher in MYD88-mutated DLBCL patients than in the wild-type group (p = 0.005). MYD88-mutated individuals had more advanced disease (stage IV represent 37.5% vs. 17.9% in wild-type group), presence of extra nodal involvement (50% vs. 23.1 % in wild-type group) and high IPI score (50% vs. 17.9% in wild type group). The MYD88 L265P mutation was found to be an independent predictor of both progression-free survival (HR 16.543, p=0.001) and overall survival (HR 17.538, p=0.007) by multivariate analysis.

Conclusion: The MYD88 mutation can be considered as an important biomarker that has impact on the prognosis and course of treatment for DLBCL patients, which suggests that MYD88 L265P might be a target for risk assessment and response to treatment.

Keywords: Non-Hodgkin lymphoma (NHL); MYD88; Gene mutation; B-cell, DLBCL; Treatment outcomes.

INTRODUCTION

Clonal tumors of B cells at different phases of development are known as B-cell neoplasms. These neoplasms are categorized and named based on how closely they mimic various phases of healthy B-cell development. More than 90% of lymphoid neoplasms are mature B-cell neoplasms, which include DLBCL, follicular lymphoma (FL), chronic lymphocytic lymphoma (CLL), mantle cell lymphoma (MCL), Burkitt lymphoma (BL), FL, lymphoplasmacytic lymphoma (LPL)/Waldenstroem's macroglobulinemia (WM), nodal marginal zone lymphoma (NMZL), splenic marginal zone lymphoma (SMZL), and hairy cell leukemia (HCL) [1].

Globally, NHL is the most common hematologic cancer. In Egypt, it is the third most common kind of cancer. DLBCL, which accounts for over one-third of NHL diagnoses each year, is the most common lymphoma subtype in adults globally, according to Globocan 2020 [2]. DLBCL is a highly heterogeneous lymphoid tumor with diverse gene expression patterns, genetic characteristics, and clinical manifestations [3].

Being a soluble adaptor protein for inflammatory signaling pathways downstream of members of the Toll-like receptor (TLR) and interleukin (IL)-1, the myeloid differentiation main response gene 88 (MYD88) is crucial for innate immunity. The cellular signal transduction of the TLR, IL-1 receptor (R), and IL-18R is principally mediated by MYD88 [4, 5].

The L265P mutation is located at position 794 of the MYD88 coding gene sequence. This mutation causes a missense change from leucine to proline at 265 location in the MYD88 protein's coding region, which results in abnormal activation of MAPK, JAK, and STAT3 signaling pathways by IL-1R and causes tumorigenesis [6, 7].

The pathophysiology and emergence of treatment resistance in B-cell neoplasms have been linked to mutations in this gene [8].

Furthermore, research has revealed that the MYD88 L265P mutation may have therapeutic significance since it promotes signaling through the Bruton's tyrosine kinase (BTK) and Interleukin-1 Receptor Associated Kinase (IRAK1/IRAK4) pathways, which culminates in nuclear factor kappa B (NF-kB) pathway activation [9].

Received: 14/09/2024
Accepted: 14/11/2024

The significance of MYD88 L265P in prognosis and therapeutic responsiveness in mature B-cell neoplasms remains controversial. MYD88 L265P mutation-free individuals were reported to have lower International Prognostic Scoring System score, and a poorer response to treatment [10]. Conversely, some findings indicate that the MYD88 L265P mutation associated with positive outcomes [11]. Therefore, further research was necessary to determine the prognostic importance of the MYD88 L265P mutation.

Molecular genetic methods such as Sanger sequencing, real-time PCR, PCR-RFLP, and allele specific PCR can identify the MYD88 L265P mutation [10]. In the current study, we used amplification-refractory mutation system PCR (ARMS-PCR) to investigate the prevalence of MYD88 L265P mutation among patients of B-cell neoplasm with focus on DLBCL and its correlation to clinicopathological parameters, laboratory profiles and treatment outcomes.

PATIENTS AND METHODS

Study subjects (Figure 1):

The current research is a prospective study that including 123 participants divided into two groups: 63 adult patients diagnosed with B cell neoplasm, recruited from the Department of Clinical Oncology Menoufia University Hospitals between March 2020 and December 2020, and 60 healthy control participants, matched with cases by age and sex, whose samples were collected from the National Liver Institute blood bank unit. The 2017 WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues was used to determine the diagnosis of various forms of B-cell neoplasms based on morphological and immunophenotypic characteristics [12].

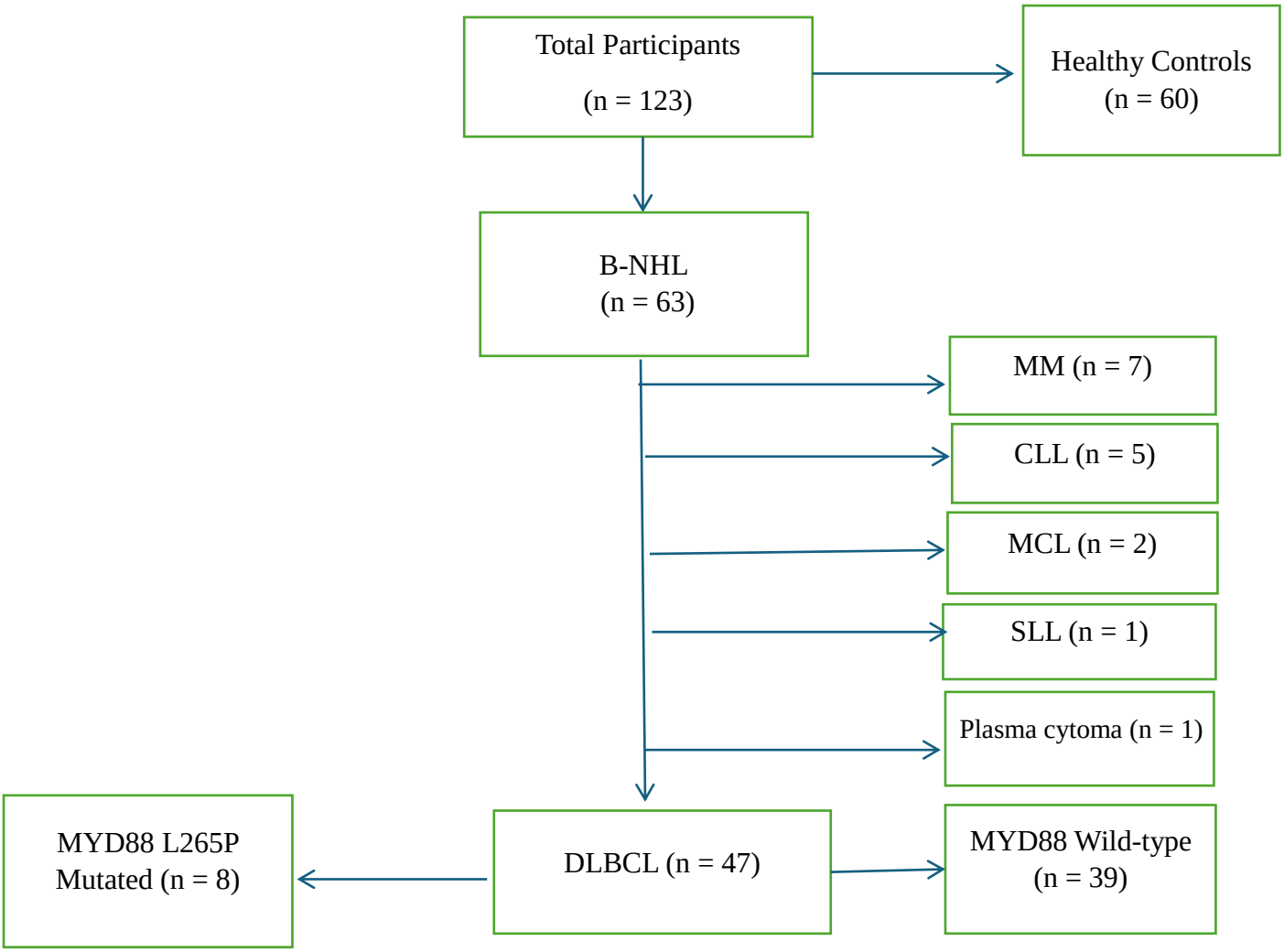

Figure (1): Flowchart of the study participants.

Inclusion criteria: Patients with histopathological evidence of B-cell neoplasm, age >18 years & < 65 years, ECOG performance status (PS) ≤ 2 and with normal Echo.

Exclusion criteria: Patients with solid or other hematological malignancies, cardiovascular or renal illness, liver diseases (chronic or acute, excluding HCV and HBV virus), age <18 years & > 65 years, and ECOG performance status (PS) < 2 were excluded from the present study.

METHODS

All study individuals were subjected to complete history taking (age, gender, family history, comorbidity, B symptoms, lymphadenopathy, splenomegaly and medically significant history) and physical examinations. Venous blood (7 ml) was collected and separated into 3 aliquots. The first part (2 ml) was put in EDTA vacutainers for the MYD88 gene mutation by ARMS-PCR. The second EDTA tube containing (2 ml) of blood was allowed for a CBC test, while the third portion (3 ml) was transferred to a standard plain vacutainer and subsequently subjected to clotting then centrifugation. Then, serum was isolated for LDH, β2 microglobulin (B2M), and kidney functions, specifically creatinine and uric acid levels estimation.

Genomic DNA extraction: The genomic DNA was obtained from EDTA-preserved blood from the patient and controls by QIAamp DNA Blood Kit (Cat. No. 51104; Qiagen, Ltd., Sussex, UK), following the manufacturer's instructions for the Generation DNA Purification Capture Column Kit. The isolated genomic DNA was kept at -80 °C until analysis when the NanoDrop 2000 spectrophotometer was used to determine its purity and concentration.

Amplification Refractory Mutation System (ARMS) PCR genotyping assay: The MYD88 gene mutation was evaluated by the expeditious, straightforward, and economically efficient ARMS technique. Extracted DNA was amplified by Applied Biosystems 2720 Thermal Cycler-Thermo Fisher Scientific. Total volume for each reaction was 25 µL comprising 4 µL DNA, 4 µL of each primer and 13 µL 2x master mix (PCR SuperMix High Fidelity, Fisher Scientific). Each sample was subjected to two separate PCR reactions: one targeting the wild-type allele and the other the mutant allele.

To distinguish between the mutant and wild-type alleles of MYD88 L265P, two reverse primers were designed. The reverse primer of mutant-type: 5′-CCT TGT ACT TGA TGG GGA aCG-3′. The reverse primer of wild-type: 5′-GCC TTG TAC TTG ATG GGG AaC A-3′. The common forward primer was: 5′-AAT GTG TGC CAG GGG TAC TTA G-3′. Thermal cycling conditions were: 2 minutes at 94 °C, followed by 40 cycles of 94 °C for 30 seconds, 57 °C for 30 seconds, and 68 °C for 30 seconds, with a final extension at 68 °C for 5 minutes. Amplified PCR products (159 bp) for wild and mutant types were separated by agarose gel electrophoresis. 1.0% agarose slab gel electrophoresis was used to evaluate DNA samples. After immersing the gel containing DNA samples in 1× Tris-Phosphate-EDTA buffer (TPE), it was electrophoresed for 20 minutes at 200 volts. Ethidium bromide staining was used to visualize the DNA under a UV lamp, and a digital camera was used to photograph the gel.

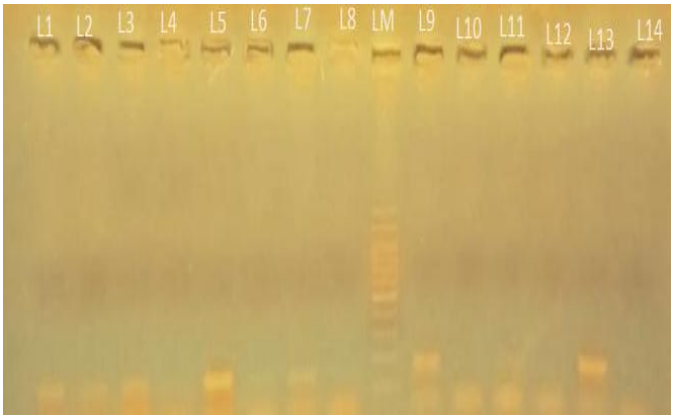

Figure (2): The gel electrophoresis image displays PCR products obtained using the ARMS method. Each pair of lanes represents the PCR reactions for a single patient: one lane corresponds to the wild-type MYD88 L265P genotype, and the other to the mutant genotype. Lanes (1,2) represent patient with mutant genotype, lanes (3 ,4 ; 5,6; 7,8; 9,10; 11,12; and13,14) represent wild genotype. Lane M Indicate ladder 100bp (thermo scientific).

Following the detection of MYD88 L265P mutation, all mutant cases were identified within the DLBCL group (47 patients). Hence, a subgroup analysis was conducted among patients with DLBCL based on the presence or absence of mutation. Subsequently, **Group I** (MYD88 L265P mutant cases included 8 patients) and **Group II** (MYD88 L265P wild cases included 39 patients).

Regarding disease characteristics among studied groups the following data were collected concerning medical history, physical examination, laboratory investigations, and bone marrow infiltration. The patients were staged depending on the Ann Arbor staging system [13], the presence of extra-nodal involvement and the International Prognostic Index (IPI) were also recorded [14].

Treatment and follow up strategy: The DLBCL patients in both studied groups were treated according to stage. Regarding stages I–II, patients underwent three to six cycles of chemo-

immunotherapy(R-CHOP; rituximab 375 mg/m^2, doxorubicin 50 mg/m^2, cyclophosphamide 750 mg/m^2, vincristine 2 mg and prednisolone 100 mg for 5 days), with or without involved site radiation targeting the bulky sites, patients in stages III-IV had six cycles of chemo-immunotherapy followed by radiotherapy for the original bulky sites and regions of extranodal expansion. The presence or lack of toxicity was documented using the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, which was adopted [15].

In context of assessment, clinical assessment was done prior to each cycle as well as hematological and laboratory evaluation for both kidney and liver function. Additionally, radiological evaluation was performed with positron emission tomography-computed tomography (PET-CT) or computed tomography (CT) every three to six months depending on the Lugano categorization response criteria [13]. The patients have been monitored, and their outcomes (response, progression-free survival and overall survival) were documented.

Overall survival (OS) was measured from the date of diagnosis to the date of death or the date of final follow-up, whereas progression-free survival (PFS) was calculated from the date of diagnosis to the date of progression or the last follow-up.

Ethical approval: All cases signed written consents. Ethical approval from National Liver Institute Menoufia University Ethical Committee was obtained prior to starting study (No 00548/2023). The Helsinki Declaration was followed throughout the study's procedures.

Statistical analysis:

Version 20.0 of the IBM SPSS software suite was used to analyze the data. Numbers and percentages were used to represent categorical data. Continuous data were examined for normalcy using the Kolmogorov-Smirnov test. Mean ± SD was used to characterize variables with a normal distribution, while median (interquartile range, IQR) was used to characterize variables with a non-normal distribution. A 5% significance level was applied.

RESULTS

At diagnosis NHL patients comprised 47 cases of DLBCL, 7 cases of multiple myeloma (MM), 5 cases of CLL, 2 cases of MCL, 1 case each of small lymphocytic lymphoma (SLL) and 1 case of plasma cytoma, in addition to 60 healthy controls. Regarding age, the majority of DLBCL, MM, and CLL patients were over 50 years. While females were more common in CLL (80%) and MCL (100%), males predominated in DLBCL (57.4%) and MM (85.7%). Performance status (PS) analysis revealed that most DLBCL and MM patients had PS 1, with a considerable proportion showing PS 2. Comorbidities were common in DLBCL (40.4%) and MM (42.9%), with diabetes and hypertension as the most frequent conditions. Laboratory tests showed that MM patients had the least mean hemoglobin concentration, while MCL and CLL patients had the highest LDH levels. The highest lymphocytic count was observed among CLL patients. The greatest levels of beta-2 microglobulin (B2M), which indicate disease activity, were found in DLBCL and MM. The diagnostic significance of the MYD88 L265P mutation was highlighted by the fact that it was found exclusively in 17% of DLBCL cases. The lymphocytic infiltration of bone marrow was evident in 40.4% of DLBCL patients (Table 1).

Table (1): Patients characteristics at diagnosis

	DLBCL (n = 47)	MM (n = 7)	CLL (n = 5)	MCL (n = 2)	SLL (n = 1)	Plasmacytoma (n = 1)	Control (n = 60)
Age (years)							
<50	24 (51.1%)	1 (14.3%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	42 (70.0%)
>50	23 (48.9%)	6 (85.7%)	5 (100.0%)	2 (100.0%)	0 (0.0%)	1 (100.0%)	18 (30.0%)
Mean ± SD.	47.7 ± 13.2	57.1 ± 10.3	58 ± 4.74	61 ± 1.41	31	65	43.20 ± 13.06
Gender							
Male	27 (57.4%)	6 (85.7%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	47 (78.3%)
Female	20 (42.6%)	1 (14.3%)	4 (80.0%)	2 (100.0%)	1 (100.0%)	1 (100.0%)	13 (21.7%)
PS							
0	12 (25.5%)	2 (28.6%)	1 (20.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	–
1	22 (46.8%)	3 (42.9%)	2 (40.0%)	2 (100.0%)	0 (0.0%)	0 (0.0%)	–
2	13 (27.7%)	2 (28.6%)	2 (40.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	–
Family history	5 (10.6%)	1 (14.3%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	–
Comorbidity	**19 (40.4%)**	**3 (42.9%)**	**3 (60.0%)**	**0 (0.0%)**	**0 (0.0%)**	**0 (0.0%)**	**0 (0.0%)**
DM	8 (17.0%)	2 (28.6%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
HTN	8 (17.0%)	1 (14.3%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
B Symptoms	20 (43.5%)	0 (0.0%)	0 (0.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	–
Lymphadenopathy	42 (89.4%)	0 (0.0%)	1 (20.0%)	1 (50.0%)	1 (100.0%)	0 (0.0%)	–
Splenomegaly	14 (29.8%)	0 (0.0%)	3 (60.0%)	1 (50.0%)	1 (100.0%)	0 (0.0%)	–
Hemoglobin (g/dL)							
Mean ± SD.	12.4 ± 1.92	9.84 ± 1.97	12 ± 1.18	10.3 ± 0.85	10.0	10.40	14.89 ± 1.14
Lymphocyte ($\times10^9$/L)							
Median (Min. – Max.)	1.60(0.40 – 4.50)	1.10(0.80 – 3.70)	32 (12.9 – 45.3)	1.50(0.90 – 2.10)			1.15 (0.8 – 3.5)
Platelets ($\times10^9$/L)							
Median (Min. – Max.)	306 (87 – 546)	261 (63 – 364)	239 (185 – 314)	295.5(230 – 361)			253 (142 – 362)
LDH (U/L)							
Median (Min. – Max.)	362 (119 – 647)	243 (124 – 575)	527 (143 – 591)	454 (346 – 562)	514.0	547.0	164.5(121 – 241)
B2M (mg/L)							
Median (Min. – Max.)	3.70 (1.40 – 26)	4 (1.50 – 25.3)	3.70 (2 – 5.10)	2.75(2.40 – 3.10)			2.1 (1.5 – 3.3)
Uric acid (mg/dL)							
Median (Min. – Max.)	7.40 (2.60 – 46)	8.50 (3.70 – 22)	8.70 (8.10 – 14)	7.25(5.10 – 9.40)			4.85 (2.1 – 7.1)
B.M infiltration	19 (40.4%)	7 (100.0%)	5 (100.0%)	1 (50.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)
MYD88 L265P							
Mutant	8 (17.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Wild	39 (83.0%)	7 (100.0%)	5 (100.0%)	2 (100.0%)	1 (100.0%)	1 (100.0%)	60 (100.0%)

Median (Min. – Max.): Non-parametric test.

PS: performance status, SD: Standard deviation, DM: diabetes mellitus, HTN: hypertension, LDH: lactate dehydrogenase, B2M: beta 2 microglobulin, BM: bone marrow, DLBCL: diffuse large B cell lymphoma, MM: multiple myeloma, CLL: chronic lymphocytic lymphoma, MCL: mantle cell lymphoma, SLL: small lymphocytic lymphoma.

The comparison of baseline demographic characteristics between MYD88 L265P-mutated and MYD88 wild-type subgroups within the DLBCL patient cohort (n = 47) showed that the age distribution of the 47 DLBCL patients did not differ significantly between both groups. Meanwhile, there was a little male predominance in mutated group (62.5% vs. 56.4%, p=1.000). Clinical characteristics such lymphadenopathy and B symptoms, as well as family history and comorbidities, did not differ significantly between the two groups. The MYD88-mutated group's beta-2 microglobulin (B2M) levels were significantly higher than those of the wild-type group (p=0.005). MYD88-mutated patients had a higher rate of bone marrow lymphocytes infiltration (62.5% vs. 35.9%), although this difference was not statistically significant (p=0.240) (Table 2).

Table (2): Comparison of the baseline demographic characteristics between MYD88 L265P mutated and MYD88 Wild-type in DLBCL patients (n = 47)

	MYD88 L265P		Test of Sig.	p
	Mutated (n = 8)	Wild (n = 39)		
Age (years)				
<50	4 (50.0%)	20 (51.3%)	χ^2=	^{FE}p=
>50	4 (50.0%)	19 (48.7%)	0.004	1.000
Mean ± SD.	48.6 ± 14.4	47.5 ± 13.2	t=	0.954
Median (Min. – Max.)	48.5 (31 – 65)	49 (24 – 65)	0.058	
Sex				
Male	5 (62.5%)	22 (56.4%)	χ^2=0.101	^{FE}p=1.000
Female	3 (37.5%)	17 (43.6%)		
Family history	1 (12.5%)	4 (10.3%)	χ^2=0.035	^{FE}p=1.000
Comorbidity	**3 (37.5%)**	**16 (41.0%)**	χ^2=0.034	^{FE}p=1.000
DM	0 (0.0%)	8 (20.5%)	χ^2=1.978	^{FE}p=0.318
HTN	1 (12.5%)	7 (17.9%)	χ^2=0.140	^{FE}p=1.000
B Symptoms	3 (37.5%)	17 (44.7%)	χ^2=0.141	^{FE}p=1.000
Lymphadenopathy	7 (87.5%)	35 (89.7%)	χ^2=0.035	^{FE}p=1.000
Splenomegaly	0 (0.0%)	14 (35.9%)	χ^2=4.090	^{FE}p=0.084
Hemoglobin (g/dL)				
Mean ± SD.	11.6 ± 2.61	12.6 ± 1.74	t= 0.215	0.831
Lymphocyte (10^9/L)				
Median (Min. – Max.)	1.40(0.40 – 2.40)	1.60(0.70 – 4.50)	U= 127.0	0.427
Platelets (10^9/L)				
Median (Min. – Max.)	305 (87 – 457)	306 (146 – 546)	U= 155.50	0.989
B2M (mg/L)				
Median (Min. – Max.)	4.90 (4.0 – 7.90)	3.10 (1.40 – 26)	U= 59.00*	0.005*
Uric acid (mg/dL)				
Median (Min. – Max.)	8.25 (2.60 – 17)	7.30 (2.60 – 46)	U= 132.00	0.513
B.M infiltration	5 (62.5%)	14 (35.9%)	χ^2= 1.951	^{FE}p=0.240

Median (Min. – Max.): Non-parametric test. *: Significant. t: Student t-test, U: Mann Whitney test, χ^2: Chi square test, FE: Fisher Exact. p: p value for comparing between MYD88 L265P Mutated and MYD88 Wild-type.

Although MYD88 L265P-mutated group showed more advanced disease (stage IV represent 37.5% vs. 17.9% in wild-type group), presence of extra nodal involvement (50% vs. 23.1 % in wild-type group) and high IPI score (50% vs. 17.9% in wild type group), No significant differences regarding those prognostic indicators were observed between both groups (Table 3).

Table (3): Comparison of prognostic indicators between MYD88 L265P mutated and MYD88 Wild-type in DLBCL patients (n = 47)

		MYD88 L265P		χ^2	p
		Mutated (n = 8)	Wild (n = 39)		
PS	0	1 (12.5%)	11 (28.2%)	2.287	^{MC}p= 0.361
	1	3 (37.5%)	19 (48.7%)		
	2	4 (50.0%)	9 (23.1%)		
Stage	I	1 (12.5%)	8 (20.5%)	2.228	^{MC}p= 0.586
	II	1 (12.5%)	12 (30.8%)		
	III	3 (37.5%)	12 (30.8%)		
	IV	3 (37.5%)	7 (17.9%)		
LDH (U/L)	Normal	2 (25.0%)	18 (46.2%)	1.215	^{FE}p= 0.437
	High	6 (75.0%)	21 (53.8%)		
	Presence of extra nodal involvement	4 (50.0%)	9 (23.1%)	2.405	^{FE}p= 0.191
IPI score	0	0 (0.0%)	8 (20.5%)	3.960	^{MC}p= 0.107
	1 – 2	4 (50.0%)	24 (61.5%)		
	3 – 5	4 (50.0%)	7 (17.9%)		

χ2: Chi square test, MC: Monte Carlo, FE: Fisher Exact. p: p value for comparing between MYD88 L265P Mutated and MYD88 Wild-type.

Regarding treatment toxicity, both studied groups exhibited similar toxicity profiles (p=0.059). However, significant statistical difference (^{MC}p <0.001) was observed between mutated and un-mutated groups in relation to treatment response. Complete response at the end of treatment was higher in wild-type group (87.2% vs 12.5% in mutant group). The rate of relapse and disease progression was notably elevated in the mutant cases (87.5% vs 12.8%), the mortality rate was also significantly higher in MYD88 L265P-mutated group (62.5% vs 5.1%). These indicated the difference in treatment outcomes between both study groups (Table 4).

Table (4): Comparison between MYD88 L265P mutated and MYD88 wild-type in DLBCL patients (n = 47) regarding toxicity, response and survival

	MYD88 L265P		χ^2	p
	Mutated (n = 8)	**Wild (n = 39)**		
Toxicity of treatment	7 (87.5%)	19 (48.7%)	4.039	^{FE}p=0.059
Response at the end of treatment				
Progressive disease	5 (62.5%)	3 (7.7%)	17.456*	^{MC}p <0.001*
Partial response	2 (25.0%)	2 (5.1%)		
Complete response	1 (12.5%)	34 (87.2%)		
Progression status	7 (87.5%)	5 (12.8%)	19.472*	^{FE}p<0.001*
Survival status				
Alive	3 (37.5%)	37 (94.9%)	17.238*	^{FE}p=0.001*
Dead	5 (62.5%)	2 (5.1%)		

*: Significant.

The overall survival among patients was studied and revealed that MYD88 L265P-wild group outperformed the MYD88 L265P-mutated group, with a mean duration of 37.12 months versus 22.50 months respectively (p-value=0.001) (Figure 3). Moreover, the other factors associated with significant shorter OS among DLBCL cases included poor performance status (P=0.010), advanced stages III & IV (P=0.017)**,** extra nodal extension (p=0.038) and a high IPI score (P=0.002) (Table 5).

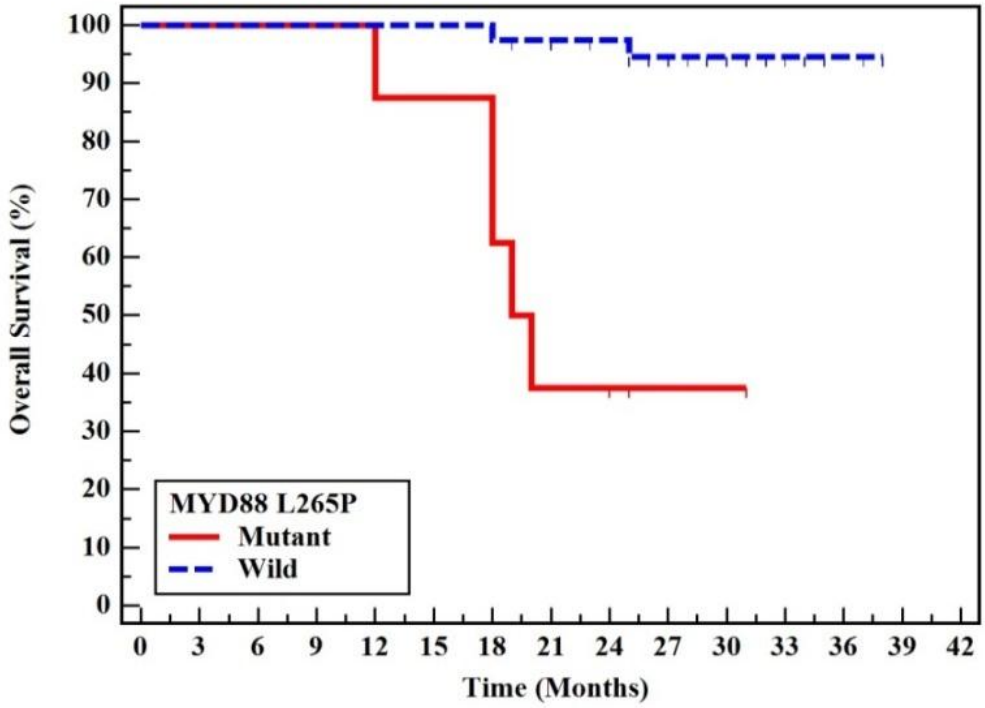

Figure (3): Kaplan-Meier survival curve for overall survival with MYD88 L265P in DLBCL cases (n = 47).

In addition, the Progression-Free Survival (PFS) was significantly extended in the MYD88 L265P-wild group, with a mean value of 34.23 months, compared to 8 months in the MYD88 L265P-mutated group (p-value=0.001) (Figure 4). Interpreting risk association of different parameters among patients with DLBCL in the univariate analysis showed that PS, the stage of disease, elevated LDH levels, presence of extra nodal extension and high IPI score are considered risk factors (Table 5).

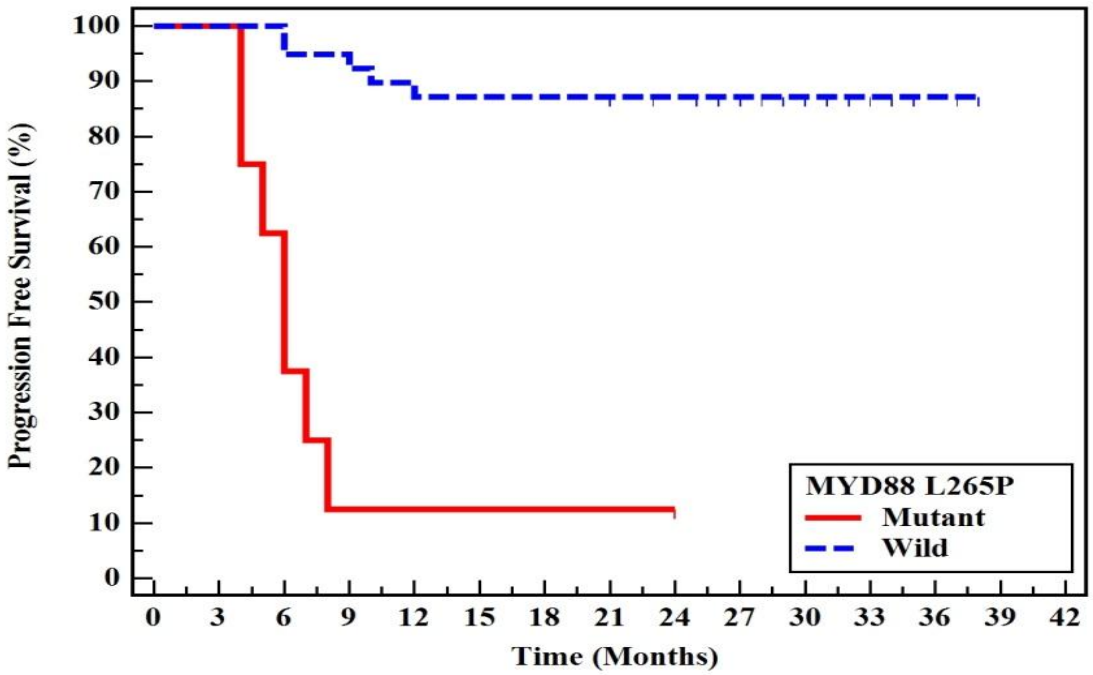

Figure (4): Kaplan-Meier survival curve for progression free survival with MYD88 L265P in DLBCL cases (n = 47).

Table (5): Log rank for overall survival and progression free survival with different parameters in DLBCL cases (n = 47)

	Overall Survival					Progression Free Survival				
	Mean	Median	%End of study	Log rank χ^2	p	Mean	Median	%End of study	Log rank χ^2	p
Age (years)										
<50	35.33	–	87.5%	0.201	0.654	32.50	–	83.3%	1.580	0.209
>50	32.32	–	81.8%			25.57	–	65.2%		
PS										
0	38.00	–	100.0%			38.00	–	100.0%		
1	33.55	–	90.9%	9.281*	0.010*	31.00	–	86.4%	19.621*	<0.001*
2	27.80	–	59.3%			14.00	9.00	30.8%		
Stage										
I	35.00	–	100.0%			35.00	–	100.0%		
II	38.00	–	100.0%	10.251*	0.017*	35.46	–	92.3%	12.330*	0.006*
III	34.51	–	79.0%			27.87	–	66.7%		
IV	27.00	–	60.0%			16.00	8.00	40.0%		
LDH (U/L)										
Normal	37.05	–	95.0%	2.677	0.102	36.40	–	95.0%	7.361*	0.007*
High	33.56	–	77.0%			25.37	–	59.3%		
Extra nodal involvement										
Absent	36.47	–	90.8%	4.310*	0.038*	32.56	–	82.4%	4.183*	0.041*
Present	28.62	–	69.2%			20.31	–	53.8%		
IPI score										
0	38.00	–	100.0%			38.00	–	100.0%		
1 – 2	36.68	–	92.9%	12.622*	0.002*	34.46	–	89.3%	26.497*	<0.001*
3 – 5	26.68	–	53.0%			11.45	8.00	18.2%		
MYD88 L265P										
Mutated	22.50	19.00	37.5%	22.326*	<0.001*	8.00	6.00	12.5%	35.688*	<0.001*
Wild	37.12	–	94.6%			34.23	–	87.2%		

The multivariate analysis identified that detection of MYD88 L265P mutation was considered as an independent prognostic factor affecting OS with p=0.007 & HR 17.538 (2.185 – 140.795). Furthermore, detection of mutation and PS were independent prognostic factors affecting PFS with (p=0.048 & HR 7.176) and (p=0.001& HR 16.543) respectively (Table 6).

Table (6): Multivariate Cox regression analysis for overall survival and progression free survival with different parameters in DLBCL cases (n = 47)

	Overall Survival		Progression Free Survival	
	p	**HR (LL – UL 95% C.I)**	**p**	**HR (LL – UL 95% C.I)**
PS (2)	0.334	2.716 (0.358 – 20.605)	0.048*	7.176 (1.018 – 50.565)
Stage (III/IV)	0.950	144464.7 (9.4×10^{-157} – 2.197×10^{166})	0.311	3.544 (0.306 – 41.013)
LDH (High)			0.199	5.802 (0.397 – 84.826)
Presence of **Extra nodal involvement**	0.132	5.044 (0.616 – 41.324)	0.539	1.500 (0.412 – 5.466)
IPI score (3 – 5)	0.806	0.749 (0.075 – 7.501)	0.682	0.597 (0.051 – 7.031)
MYD88 L265P (Mutated)	0.007*	17.538 (2.185 – 140.795)	0.001*	16.543 (2.945 – 92.914)

HR: Hazard ratio, C.I: Confidence interval, LL: Lower limit, UL: Upper Limit *: Significant.

DISCUSSION

NHL includes a diverse group of B lymphoid clonal disorders with specific clinical and laboratory characteristics. Accurate diagnosis as well as prognostic classification and treatment decision are very crucial in NHL disease management [16]. Several prognostic mutations and numerous genetic abnormalities have recently been identified in B-cell neoplasm. Hence, understanding the biological characteristics of B-cell neoplasm with poor outcomes is crucial. It was shown that the effect of MYD88 mutations differs across distinct lymph-proliferative illnesses and cellular pathways they regulate, with their role in cell survival influenced by the stage of B-cell maturation. [17].

The majority of our DLBCL, MM, and CLL patients were over 50 years, representing the typical age distribution for these malignancies. In line with our findings, **Shekhar *et al.*** [18] reported a median age of 63 years for their B-cell neoplasm cohort**.** Male predominance in DLBCL (57.4%) and MM (85.7%) was also observed in their population, though with a higher male-to-female ratio (2.9:1) across all B-cell neoplasms. Interestingly, despite reporting no MYD88 mutations in their DLBCL cases, their participants' clinical presentation as lymphadenopathy and B symptoms did not show statistical difference between MYD88-mutated and wild-type DLBCL cases aligning with our study [18].

We found that the MYD88 L265P mutation was exclusively present in 17% of DLBCL cases. This is similar to the prevalence in earlier research that revealed low MYD88 L265P mutation frequency ranging from 6.5% to 29.6% [6, 19, 20] and the meta-analysis conducted by **Lee *et al.*** [21] who reported an overall mutation frequency of 16.5%, excluding CNS and testicular subtypes in 2285 DLBCL patients. In contrast to the previous frequencies, a study by **Hanbal *et al.*** [22] identified a high MYD88 L265P mutation frequency of 59.8% among DLBCL patients**.** Another tissue-based study for MYD88 L265P mutation analysis by droplet digital PCR reported prevalence of 29% of DLBCL cases especially among extranodal subtypes, such as primary CNS lymphoma (66.7%) and testicular DLBCL (68%). These findings highlighted the potential site-specific differences in mutation percentage, which peripheral blood analysis couldn't capture. [23]. The variation in MYD88 L265P frequencies across studies may be explained by difference in the ethnic and genetic backgrounds of the study populations, the methods employed for mutation detection and the heterogenicity of the disease.

Consistent with the observations of other cohorts, our results showed that the MYD88 L265P mutation had no significant effect on the clinical and laboratory characteristics of DLBCL patients [22, 24, 25, 26]**.** Our investigation demonstrated an elevated incidence of bone marrow infiltration in MYD88-mutated individuals (62.5%) relative to wild-type cases, but this disparity lacked statistical significance. In contrast, Beta-2 microglobulin (B2M) levels were significantly elevated in DLBCL patients with MYD88 mutations compared to those in wild-type group (5.55 ± 1.56 mg/L vs. 4.03 ± 3.89 mg/L, p = 0.005). Several studies including those by **Niu *et al.*** [23]**, Mohamed *et al.*** [25]**, Choi *et al.*** [27] **and Kraan *et al.*** [28] found that the MYD88 mutation was linked to poor prognosis, including advanced Ann-Arbor stages, high β2-microglobulin and LDH levels, extranodal involvement, and a higher IPI score. Patients with high ECOG scores (72.4%) have shown higher MYD88 mutation rates compared to those with low score (27.6%). However, in contrast to these finding the current results demonstrated no significant difference in terms of age, LDH, and extranodal participation between the mutant and wild type groups.

In term of aggressiveness of the disease MYD88-mutated group had more advanced disease (stage IV represented 37.5% vs. 17.9% in wild-type group), presence of extra nodal involvement (50% vs. 23.1 % in wild-type group) and high IPI score (50% vs. 17.9% in wild type group). These findings are in alignment with a recent study by **Ebid *et al.*** [26] who revealed MYD88 mutation rate of 18.37% among DLBCL patients and combining both MYD88 and TP53 mutational analysis role in risk stratification and tailored therapeutic approaches**.** Both **Niu *et al.*** [23] and **Ebid *et al.*** [26] clinical findings are in accordance with

our data patterns, confirming that MYD88 mutations contribute in defining a more aggressive clinical phenotype in DLBCL**.**

The MYD88 L265P mutation significantly impacted treatment response. Complete response at the end of treatment was notably higher in the wild-type group (87.2%) compared to the mutant group (12.5%), while relapse and disease progression were significantly more frequent in mutant cases (62.5% vs. 7.7%, p = 0.001). In contrast, a study by **Hanbal *et al.*** [22] reported a higher rate of complete response in the mutant group (60.9% vs. 39% in the wild-type group) and a higher incidence of progressive disease in the mutant group (72.7% vs. 27.2%), though this difference was not statistically significant (p = 0.872). This discrepancy may be attributed to the lower prevalence of the mutation in our study population (17% vs. 59.8%).

The prognostic significance of the MYD88 L265P mutations and their relationship with survival has been a topic of controversy. Some studies have identified a substantial relationship between MYD88 L265P mutations and decreased survival rates [19, 29, 30]**.** Similarly, our findings exhibited that MYD88 L265P-mutated group had lower OS compared to wild type group (22.50 months versus 37.12 months, p-value=0.001). However, other studies reported no impact on the survival outcomes [22, 31, 32]**.**

The current study identified that MYD88 L265P mutation was an independent predictor of worsening both progression-free survival (HR 16.543, p=0.001) and overall survival (HR 17.538, p=0.007) by multivariate analysis. This is consistent with numerous investigations by **Vermaat *et al.*** [20]**, Ebid *et al.*** [26] and **De Groen *et al.*** [33]**.** A meta-analysis by **Lee *et al.*** [21] did not support this (HR = 2.029; 95% CI: 0.873–4.713; p = 0.100). Additionally, there was no statistically significant correlation between the MYD88 mutation and overall survival in a different research by **Xu *et al.*** [34].

Limitations: The tiny sample size was one of the study's main limitations, particularly in the MYD88-mutated group. To validate these findings and further explore the prognostic impact of MYD88 mutations in DLBCL, larger multicenter studies are needed including DLBCL's GCB and ABC subtyping to improve our comprehension of MYD88's pathophysiological function. Also, integrating advanced diagnostic techniques, such as ddPCR and DNA sequencing could enhance the sensitivity and specificity of mutation detection.

CONCLUSION

The MYD88 L265P mutation plays a critical role in DLBCL, as it was linked to more advanced illness, greater B2M levels, higher frequencies of extra nodal involvement, and elevated IPI scores. Moreover, low complete response rates, high relapse and mortality rates were among the worst treatment outcomes seen by patients with the MYD88 mutation. It can be considered as an important biomarker that has impact on the prognosis and course of treatment for DLBCL patients, which suggests that MYD88 L265P may be a target for risk assessment and treatment.

Authors' contributions: Sally S. Mandour and Hatem Rabie made the study design and the laboratory analysis. Heba Yahia Alsayed and Rasha Adel Mohamed Abdelmoneum collected participants' samples. Fatma A. Khalaf and Fatma Omar Khalil performed the statistical analysis. All authors helped writing the manuscript.

Conflict of interest: None.
Financial disclosures: None.

REFERENCES

1. **Swerdlow S, Campo E, Pileri S *et al.* (2016):** The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood, 127: 2375–90.
2. **Siegel R, Miller K, Jemal A (2019):** Cancer statistics, 2019. CA Cancer J Clin., 69 (1): 7–34.
3. **Sehn L, Salles G (2021):** Diffuse Large B-Cell Lymphoma. N Engl J Med., 384: 842–858.
4. **Motshwene P, Moncrieffe M, Grossmann J *et al.* (2009):** An oligomeric signaling platform formed by the Toll‐like receptor signal transducers MyD88 and IRA K‐4. J Biol Chem., 284: 25404‐25411.
5. **Gay N, Symmons M, Gangloff M *et al.* (2014):** Assembly and localization of Toll‐like receptor signalling complexes. Nat Rev Immunol., 14: 546‐558.
6. **Ngo V, Young R, Schmitz R *et al.* (2011):** Oncogenically active MYD88 mutations in human lymphoma. Nature, 470: 115‐119.
7. **Jardin F (2014):** Next generation sequencing and the management of diffuse large B‐cell lymphoma: From whole exome analysis to targeted therapy. Discov Med., 18: 51‐65.
8. **Nagao T, Oshikawa G, Ishida S *et al.* (2015):** A novel MYD88 mutation L265RPP, in Waldenström macroglobulinemia activates the NF-kB pathway to upregulate Bcl-xL expression and enhances cell survival. Blood Cancer J., 5: 314. doi: 10.1038/bcj.2015.36
9. **Yang G, Zhou Y, Liu X *et al.* (2013):** A mutation in MYD88 (L265P) supports the survival of lymphoplasmacytic cells by activation of Bruton tyrosine kinase in Waldenstro¨m macroglobulinemia. Blood, 122: 1222–32.
10. **Patkar N, Subramanian P, Deshpande P *et al.* (2015):** MYD88 mutant lymphoplasmacytic lymphoma/Waldenstro¨m macroglobulinemia has distinct clinical and pathological features as compared to its mutation negative counterpart. Leuk Lymphoma, 56: 420–25.
11. **Treon S, Xu L, Hunter Z (2015):** MYD88 mutations and response to ibrutinib in Waldenstro¨m's macroglobulinemia. N Engl J Med., 373: 584–6.

12. **Campo E, Pileri S, Jaffe E *et al.* (2017):** Nodal marginal zone lymphoma. In: WHO classification of tumors of haematopoietic and lymphoid tissues. Swerdlow S, Campo E, Harris N (editors), (Revised 4th edition). Lyon: IARC, Pp: 263–5.
13. **Cheson B, Fisher R, Barrington S *et al.* (2014):** Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. Journal of Clinical Oncology, 32 (27): 3059-68.
14. **International Non-Hodgkin's Lymphoma Prognostic Factors Project (1993):** A predictive model for aggressive non-Hodgkin's lymphoma. New England Journal of Medicine, 329 (14): 987-94.
15. **Atkinson T, Ryan S, Bennett A *et al.* (2016):** The association between clinician-based common terminology criteria for adverse events (CTCAE) and patient-reported outcomes (PRO): a systematic review. Supportive Care in Cancer, 24: 3669-76.
16. **Jaffe E, Barr P, Smith S (2017):** Understanding the New WHO Classification of Lymphoid Malignancies: Why It's Important and How It Will Affect Practice. Am Soc Clin Oncol Educ Book, 37: 535-546.
17. **Martínez-Trillos A, Pinyol M, Navarro A *et al.* (2014):** Regular Article LYMPHOID NEOPLASIA Mutations in TLR/MYD88 pathway identify a subset of young chronic lymphocytic leukemia patients with favorable outcome. Blood, 123 (24): 3790–6.
18. **Shekhar R, Naseem S, Binota J *et al.* (2021):** Frequency of MYD88 L256P mutation and its correlation with clinico-hematological profile in mature B-cell neoplasm. Hematology/Oncology and Stem Cell Therapy, 14 (3): 231-239.
19. **Rovira J, Karube K, Valera A *et al.* (2016):** MYD88 L265P mutations, but no other variants, identify a subpopulation of DLBCL patients of activated B-cell origin, extranodal involvement, and poor outcome. Clin Cancer Res., 22 (11): 2755–64.
20. **Vermaat J, Somers S, De Wreede L *et al.* (2020):** MYD88 mutations identify a molecular subgroup of diffuse large B-cell lymphoma with an unfavorable prognosis. Haematologica, 105 (2): 424–34.
21. **Lee J, Jeong H, Choi J *et al.* (2017):** Clinicopathologic significance of MYD88 L265P mutation in diffuse large B-cell lymphoma: a meta-analysis. Sci Rep., 7: 1785. https://doi.org/10.1038/s41598-017-01998-5.
22. **Hanbal A, El-Ashwah S, Eladl A *et al.* (2024):** Utility of clinical, laboratory, and lymph node MYD88 L265P mutation in risk assessment of diffuse large B-cell lymphoma patients. J Egypt Natl Canc Inst., 36 (1): 31. doi: 10.1186/s43046-024-00237-z.
23. **Niu J, Ma Z, Nuerlan A *et al.* (2020):** Prognostic value of MYD88 L265P mutation in diffuse large B cell lymphoma via droplet digital PCR. Mol Med Rep., 22 (2): 1243-1256.
24. **Kim Y, Ju H, Kim D *et al.* (2014):** CD79B and MYD88 muta tions in diffuse large B-cell lymphoma. Hum Pathol., 45 (3): 556–64.
25. **Choi J, Kim Y, Lee J *et al.* (2013):** MYD88 expression and L265P mutation in diffuse large B-cell lymphoma. Hum Pathol., 44 (7): 1375–81.
26. **Ebid O, Ezz El Arab L, Saad A *et al.* (2023):** Prognostic impact of MYD88 and TP53 mutations in diffuse large B Cell lymphoma. Ann Hematol., 102: 3477–3488.
27. **Mohamed A, Elfeky M, Elshorbagy S *et al.* (2022):** Prognostic values of myeloid differentiation factor 88 (MYD88) and transducin (β)-like receptor 1 (TBLR1) expression in tissues of diffuse large B-cell non-Hodgkin lymphoma patients - an immunohistochemical study. Contemp Oncol., 26 (1): 49-58.
28. **Kraan W, Horlings H, van Keimpema M *et al.* (2013):** High prev alence of oncogenic MYD88 and CD79B mutations in diffuse large B-cell lymphomas presenting at immune-privileged sites. Blood Cancer J., 3 (9): e139. doi: 10.1038/bcj.2013.28.
29. **Fernández-Rodríguez C, Bellosillo B, García-García M *et al.* (2014):** MYD88 (L265P) mutation is an independent prognostic factor for outcome in patients with diffuse large B-cell lymphoma. Leuk., 28 (10): 2104–2106.
30. **Hattori K, Sakata-Yanagimoto M, Okoshi Y *et al.* (2017):** MYD88 (L265P) mutation is associated with an unfavorable outcome of primary central nervous system lymphoma. Br J Haematol., 177(3): 492–494.
31. **Takano S, Hattori K, Ishikawa E *et al.* (2018):** MyD88 mutation in elderly predicts poor prognosis in primary central nervous system lymphoma: multi-institutional analysis. World Neurosurg., 112: 69–73
32. **Lee Y, Liu J, Fricano K *et al.* (2017):** Lack of a prognostic impact of the MyD88 L265P mutation for diffuse large B cell lymphoma patients undergoing autologous stem cell transplantation. Biol Blood Marrow Transplant., 23 (12): 2199–2204.
33. **De Groen R, Schrader A, Kersten M *et al.* (2019):** MYD88 in the driver's seat of B-cell lymphomagenesis: from molecular mechanisms to clinical implications. Haematologica, 104 (12): 2337-48.
34. **Xu P, Zhong H, Huang Y *et al.* (2017):** B-cell function gene mutations in diffuse large B-cell lymphoma: a retrospective cohort study. EBioMedicine, 16: 106–114.