Overall Survival Study and Prevalence of MYD88 Protein Expression by Immunohistochemistry in Diffuse Large B Cell Lymphoma: A Retrospective Cohort Study

Wanlapa Rattanasawat, MD[1], Wasakorn Bunyayothin, MD[2], Sirisanpang Yodavudh, MD[2]

[1] Division of Medicine, Charoenkrung Pracharak Hospital, Bangkok, Thailand; [2] Division of Pathology, Charoenkrung Pracharak Hospital, Bangkok, Thailand

Abstract

Background: Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma (NHL), characterized by clinical and molecular heterogeneity. Genetic alterations in signaling pathways, especially the MYD88 gene, have been implicated in disease progression and prognosis. Although MYD88 protein expression is frequently observed in the activated B-cell (non-GCB) subtype, its prevalence and prognostic significance in all DLBCL remain controversial.

Objective: To investigate the prevalence of MYD88 protein expression, its associated factors, and its prognostic role in patients with DLBCL.

Materials and Methods: This retrospective study analyzed eighty-six DLBCL tissue samples collected between January 1, 2014, and December 31, 2023. These samples were evaluated for MYD88 protein expression using immunohistochemistry (IHC) to determine the expression, associated factors, and impact on overall survival (OS).

Results: The prevalence of MYD88 protein expression in the present study was 87.2%, being highest in the non-GCB subtype (61.3%). The expression was significantly associated with advanced Ann Arbor stage (III-IV) (74.7%), high lactate dehydrogenase (LDH) levels (84%), high intermediate-risk NCCN-International Prognostic Index (IPI) (scores 4 to 5) (49.3%), and a high Ki-67 proliferation index ≥70% (74.7%). Significantly poor prognostic variables for OS included ECOG status ≥2 (p=0.03), advanced Ann Arbor stage (III-IV) (p=0.018), and high-risk NCCN-IPI (scores ≥6) (p=0.001), but not MYD88 protein expression (hazard ratio [HR] 1.07, p=0.869). Only a high serum LDH level was an independent risk factor (adjusted HR 8.324, p=0.042).

Conclusion: High prevalence of MYD88 protein expression was found in DLBCL, especially the non-GCB subtype. No significant association between MYD88 protein expression and OS in DLBCL patients.

Keywords: Diffuse large B-cell lymphoma; Immunohistochemistry; MYD88 protein expression

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Malignant lymphoma (ML) is a significant contributor to cancer-related mortality globally. Among Thai males, non-Hodgkin lymphoma (NHL) is the fifth most frequently diagnosed cancer[1]. According to the 2016 World Health Organization classification, based on histomorphology, immunohistochemistry (IHC), and molecular genetic features, ML is categorized into more than five main types: mature B and T-cell NHL, Hodgkin lymphoma (HL), and other rare categories[2]. NHL is the most frequent subtype (92.7%), predominantly of B-cell origin (75.0%)[3].

Among the B-cell NHL, diffuse large B-cell

Correspondence to:

Rattanasawat W.
Department of Medicine, Charoenkrung Pracharak Hospital, Thanon Tok Road, Bang Kho Laem District, Bangkok 10120, Thailand.
Phone: +66-81-3024657
Email: wanlapa28504@gmail.com
ORCID: 0009-0002-7504-3622

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What is already known about this topic?

About the multivariate OS study in DLBCL, MYD88 protein expression by IHC showed controversial results. Niu et al. reported MYD88 expression was an independent prognostic factor, affecting the OS, Choi et al. declared a conflicting outcome.

What does this study add?

In terms of prevalence, factor association, and prognostic significance of MYD88 protein expression in DLBCL by IHC, our study is the first to be reported in Thailand. The prevalence of 87.2% was higher than previously studied (38% and 38.7%) by Niu et al. and Choi et al., respectively. IHC is broadly accessible in a surgical pathology laboratory and is low-cost, compared with the PCR technique. Also discovered, the high serum LDH and age ≤63 years exhibited a trend for factor association with MYD88 protein expression, but not statistical significance. Finally, we found that there was no significant association between MYD88 protein expression and OS in DLBCL, but multivariate analysis revealed a trend toward poorer outcome in positive MYD88 protein expression. Future research with the correlated PCR method, larger sample sizes, and conducted in other populations should be investigated for good, reliable results.

lymphoma (DLBCL) is most commonly found. DLBCL is further classified into two distinct molecular subtypes by the Hans algorithm: germinal center B-cell (GCB) and activated B-cell (non-GCB), which have distinctive morphologic features, different genetic expression, and clinical activities[4,5].

Mutations in the MYD88 gene play a key role in mediating responses to signals from Toll-like receptors (TLRs), which are one of several families of so-called pattern recognition receptors (PRRs) that typically activate innate immunity, but also operate in B lymphocytes[6,7]. A mutation in B-cell NHL is the MYD88 leucine 265 to proline in TLRs produce signal through interleukin-1 receptor-associated kinase (IRAK), which stimulates NF-kappa B (transcription anti-apoptosis factor) and leads to tumorigenesis[8] and contributes to resistance against targeted therapies[9]. However, the MYD88 leucine 265P variation does not seem to be a significant prognostic indicator, while MYD88 protein expression may be significantly associated with tumor recurrence and shortened disease-free survival (DFS)[10].

MYD88 protein expression can be determined by either droplet digital polymerase chain reaction (ddPCR) or IHC. However, previous studies reported inconsistent findings from the two techniques. One study demonstrated a high agreement rate of 73% between the 2 methods[11], whereas another study found only fair correlation with a Spearman's rank correlation coefficient of –0.074[12]. The prevalence of the MYD88L265P mutation by ddPCR in DLBCL among diverse patient cohorts reported in one meta-analysis study varied widely between 0% and 94%, with a poor prognosis for patients with the MYD88L265P mutation[12,13]. Others used IHC to determine and reported approximately 38% prevalence of MYD88 protein expression in DLBCL[11].

Based on molecular studies, the MYD88L265P mutation in B-cell lymphoma was reported to be strongly linked with several unfavorable clinical features. These include a poorer Eastern Cooperative Oncology Group (ECOG) performance status of the patients, extranodal locations, advanced Ann Arbor stage, high-risk National Comprehensive Cancer Network International Prognostic (NCCN-IPI) scores, and a high Ki-67 index (≥60%)[14,15]. By the IHC study, inconsistent findings of the prognostic role of MYD88 protein expression were reported, as either it was associated with shortened DFS in DLBCL patients[12] or no such association[11].

Although IHC is a simple, cost-effective method and a less time-consuming technique with a lower risk of contamination compared to the polymerase chain reaction (PCR) method, only a limited number of studies were available[11,12].

The objective of the present study was to assess the prevalence, clinicopathological associations, and prognostic significance of MYD88 protein expression by IHC in a cohort of DLBCL patients.

Materials and Methods

This retrospective descriptive study was performed at Charoenkrung Pracharak (CKP) Hospital. Ethical approval for the study was granted by the Bangkok Metropolitan Administration Human Research and Ethics Committee (No. S006hc/67_EXP). Informed consent was waived by the ethical policy.

Study population

A list of patients with DLBCL treated at the hospital between January 1, 2014, and December 31, 2023, was searched from the hospital's archive. Inclusion criteria were individuals who were aged greater than 15 years and had a diagnosis of DLBCL. Exclusion criteria were those who had no available tissue blocks or insufficient tissue for the IHC study.

As a usual practice in the hospital for patients who have a diagnosis of DLBCL, Ann Arbor staging is assigned with data from laboratory investigations, computed tomography of the chest and abdomen, and bone marrow studies. The treatment options are rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) or cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) regimens or palliative care according to the performance status of the patient and discretion of the attending hematologist-oncologist. Clinico-pathological data of the patients were searched and checked for inclusion criteria before the immunohistochemical study. All DLBCL patients were observed for a one-year duration from diagnostic time to the last follow-up or date of death due to it was a high aggressive disease.

Histopathology and immunohistochemistry

The academic standard of the hospital pathology laboratory has been accredited by the Royal College of Pathologists of Thailand for meeting (cert. No. 67-01-01). The formalin-fixed paraffin-embedded (FFPE) tissue blocks of the patients with DLBCL were sectioned at 4-μm using an automated microtome and stained with hematoxylin and eosin (H&E). The unstained slides were heated in a hot air oven at 80°C for 30 minutes before the IHC process. The IHC staining procedure was performed on the Ventana

automated staining platform. Deparaffinization was conducted using EZ Prep (Lot K29354), and the samples underwent antigen retrieval with the Cell Conditioning CC1 solution (Lot K29354) for 36 minutes. The primary antibody, a rabbit polyclonal anti-MYD88 antibody (GeneTex, Lot 445527), was then incubated at 36°C for 44 minutes. Following primary antibody incubation, a chromogenic reaction was initiated by applying an amplified signal kit consisting of ultraView Universal HRP Multimer and DAB reagents to generate a brown precipitate. Following the primary staining, the slides underwent a counterstain with Ventana Hematoxylin II for 12 minutes, followed by bluing reagent for 4 minutes. Subsequently, the specimens were dehydrated with a graded series of alcohol and xylene and were mounted with a coverslip.

To ensure objectivity, all H&E-stained slides were reviewed by two pathologists before the study of MYD88 protein expression, which was independently evaluated for expression with blinded clinical information.

The agreement between the two pathologists was used to evaluate the inter-observer reliability of MYD88 protein expression. To ensure consistent scoring, the first 20 slides were jointly assessed, yielding a high kappa value of 0.9 (18/20), indicating strong agreement. An initial discrepancy was found in the staining results for 8 of the 86 slides. The two pathologists resolved these discordant outcomes through a joint review, ultimately reaching a consensus for all cases.

MYD88 protein expression was assessed using an Olympus BX43 bright field microscope equipped with a 40x objective lens and 10x eyepieces (field number=22), yielding a total magnification of 400x. The field of view had a diameter of 0.65 mm and an area of 0.32 mm^2. Using human lung papillary cancer as a positive external control. Regarding of false positive assessment, the pathologist evaluated the background and normal lymphoid cell staining to determine if it was a false positive for MYD88 protein expression due to horseradish peroxidase conjugates in the commercial kit. These slides will be restained by a pretreatment process reduction time from 32 to 16 minutes; the present study had no false positive staining.

Moreover, in intensity evaluation, the pathologist used a microphotograph to compare scores 0-3. MYD88 protein expression was assessed using a modified scoring method based on the protocol described by Niu et al., which employed both intensity and extent[11]. The expression was determined as positive with cytologic staining. The intensity was graded from 0 to 3 (0: negative, 1: weak, 2: moderate, 3: intense) (Figure 1). The extent was defined by the area of positive cytologic staining (0: negative, 1: <10%, 2: 10% to 50%, 3: >50%). The combined scores for intensity and extent ranged from 0 to 6. MYD88 protein expression was divided into two groups: negative for scores 0 to 1 and positive for scores 2 to 6.

Statistical analysis

Data collected were age, sex, tumor location, Hans algorithm, Ann Arbor stage, B symptom, ECOG performance status, serum lactate dehydrogenase (LDH), NCCN-IPI score, Ki-67, MYD88 protein expression, treatment regimen, date of diagnosis, last follow-up, and living status.

A statistical analysis was performed with IBM SPSS Statistics, version 26.0 (IBM Corp., Armonk, NY, USA) to determine the prevalence of MYD88 protein expression, its association with key clinicopathological factors, other previous CKP hospital IHC results, and overall survival (OS) status. Descriptive statistics were presented as numbers and percentages, mean with standard deviation, or median with range. Statistical comparisons of categorical variables were performed using the Pearson chi-square and Fisher's exact tests as appropriate. Cox proportional hazards regression was used to determine the association of MYD88 protein expression and other factors. OS was obtained from the date of diagnosis to the date of last follow-up at the hospital or date of death. Survival data of the patients in each group were analyzed by Kaplan-Meier analysis and compared by the log-rank test. All tests considered a p-value less than 0.05 as the threshold for significance.

Results

During the study period, 92 DLBCL cases were identified. Six cases were excluded due to unavailable FFPE tissue blocks (4 cases) or inadequate tumor tissue for IHC staining (2 cases). A total of 86 cases were included in the study.

The median age of the patients was 63 years (range 22-87). Slightly over half were male (59.3%). Nearly half of the patients had a poor performance status, with 49.4% having an ECOG score ≥2. B symptoms were present in 64.9%. Disease distribution was predominantly extranodal (72.9%), with involvement of the central nervous system (CNS) in 9.7% of cases. According to the Hans algorithm, 60.5% of patients were of the non-GCB subtype, and the majority presented with advanced Ann Arbor stage (III-IV)

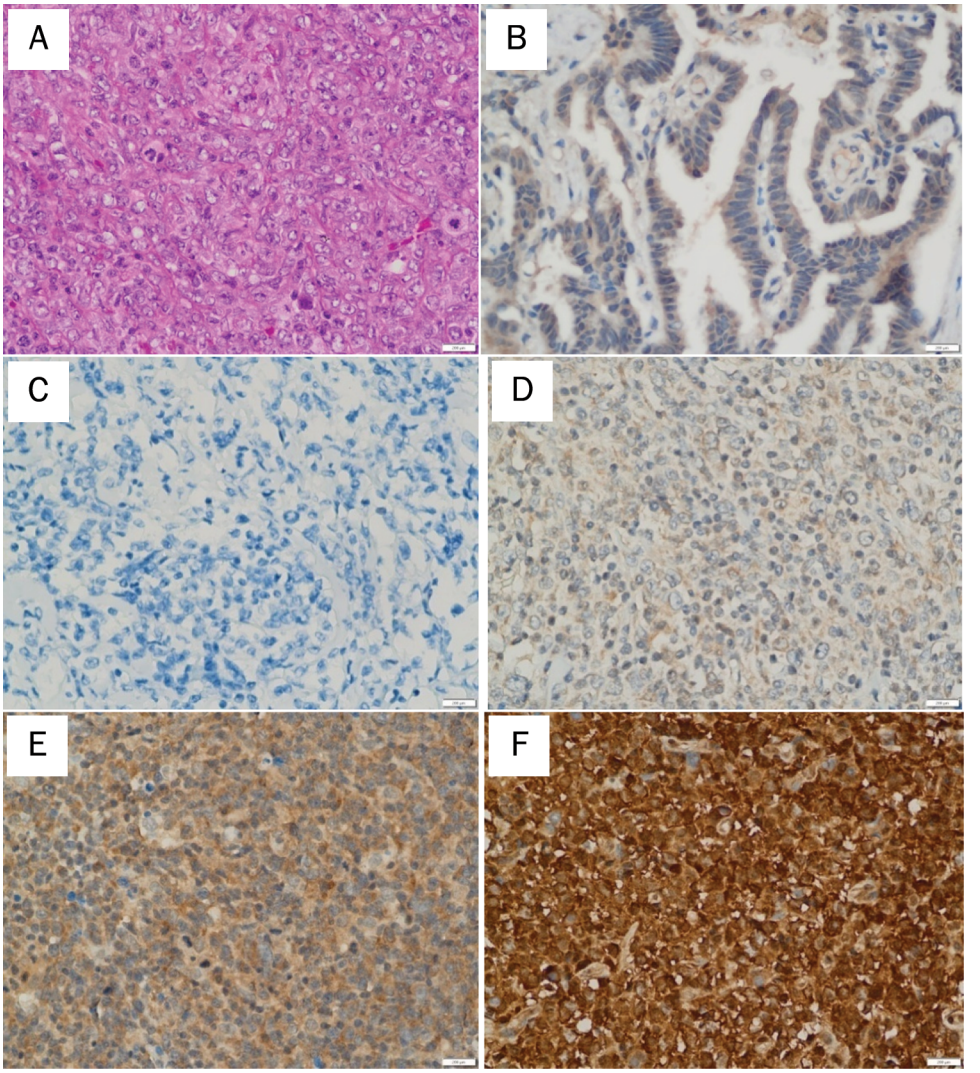

Figure 1. IHC staining of MYD88 protein expression and scoring examples. (A) H&E stain of a DLBCL section. (B) Positive control tissue (human papillary lung adenocarcinoma). (C-F) Examples of MYD88 staining scores: (C) Score 0 (no staining), (D) Score 1+ (weak staining), (E) Score 2+ (appreciable cytoplasmic staining), and (F) Score 3+ (intense cytoplasmic staining). All images were captured at 40x magnification.

(77.8%). By NCCN-IPI score, most patients were classified as high-intermediate (49.4%) or high risk (29.1%), while only 21.5% were low risk, indicating a population with significant baseline prognostic risk. Laboratory findings revealed elevated LDH in 88.5% and high Ki-67 proliferation index (≥70%) in 86.7% of cases. Additionally, MYD88 protein expression was positive in 87.2% of patients. Clinico-pathological features of DLBCL patients are shown in Table 1.

Although patients aged >63 years showed a higher proportion of MYD88 negativity compared with those ≤63 years (72.7% vs. 27.3%), this difference was marginally significant (p=0.051). Although MYD88 expression was found more frequently in males, presence of B symptoms, extranodal site, non-GCB subtype based on Hans algorithm, advanced Ann Arbor stage, NCCN-IPI score high-intermediate and high-risk groups, or high Ki-67 proliferation index, the differences were not statistically significant. Additionally, elevated LDH levels, which were more frequent among MYD88-positive patients compared with negative cases (84.0% vs. 54.6%), showed a trend for a significant association (p=0.064). Table 2 shows the association between MYD88 protein expression and clinicopathological variables. In Table 2, distribution of almost variables was not

Table 1. The demographic, clinical, laboratory, and immunohistochemical characteristics of the DLBCL patients

Characteristics	Number (n=86) n (%)
Age (years)	
Min, Max	22, 87
Median (Q1, Q3)	63 (54, 72)
≤63	47 (54.7)
>63	39 (45.3)
Sex	
Male	51 (59.3)
Female	35 (40.7)
Location (n=85)	
Nodal	23 (27.1)
Extranodal	62 (72.9)
Extranodal involvement (n=62)	
CNS	6 (9.7)
Others	56 (90.3)
Hans algorithm	
GCB	34 (39.5)
Non-GCB	52 (60.5)
Ann Arbor stage (n=81)	
Limited (I-II)	18 (22.2)
Advanced (III-IV)	63 (77.8)
B symptom (n=77)	
Absent	27 (35.1)
Present	50 (64.9)
ECOG performance status score (n=79)	
<2	40 (50.6)
≥2	39 (49.4)
LDH level (n=78)	
Normal	9 (11.5)
Above normal	69 (88.5)
NCCN-IPI score (n=79)	
Low risk (0 to 3)	17 (21.5)
High intermediate risk (4 to 5)	39 (49.4)
High risk (≥6)	23 (29.1)
Ki-67 (n=75)	
≥70%	65 (86.7)
<70%	10 (13.3)
CD10	
Positive	34 (39.5)
Negative	52 (60.5)
MYD88 protein expression	
Positive	75 (87.2)
Negative	11 (12.8)
Treatment (n=63)	
R-CHOP	35 (55.5)
CHOP	25 (39.7)
Other	3 (4.8)
Survival status	
Died	48 (55.8)
Alive	38 (44.2)

CNS=central nervous system; GCB=germinal center B-cell; ECOG=Eastern Cooperative Oncology Group; LDH=lactate dehydrogenase; NCCN-IPI=National Comprehensive Cancer Network International Prognostic

Table 2. The association between MYD88 protein expression and clinicopathological variables

Clinicopathological data	Total (n=86) n (%)	MYD88 protein expression; n (%)		p-value
		Negative (n=11)	Positive (n=75)	
Age (years)				
≤63	47 (54.7)	3 (27.3)	44 (58.7)	0.051
>63	39 (45.3)	8 (72.7)	31 (41.3)	
Sex				
Male	51 (59.3)	7 (63.6)	44 (58.7)	0.754
Female	35 (40.7)	4 (36.4)	31 (41.3)	
Location				
Nodal	23 (26.7)	1 (9.1)	22 (29.3)	0.327
Extranodal	62 (72.1)	10 (90.9)	52 (69.3)	
NA	1 (1.2)	0 (0.0)	1 (1.4)	
Extranodal involvement[a] (n=62)				
CNS	6 (9.7)	1 (10.0)	5 (9.6)	0.970
Other	56 (90.3)	9 (90.0)	47 (90.4)	
Hans algorithm				
GCB	34 (39.5)	5 (45.5)	29 (38.7)	0.667
Non-GCB	52 (60.5)	6 (54.5)	46 (61.3)	
NA	0 (0.0)	0 (0.0)	0 (0.0)	
Ann Arbor stage				
Limited (I-II)	18 (20.9)	3 (27.3)	15 (20.0)	0.728
Advanced (III-IV)	63 (73.3)	7 (63.6)	56 (74.7)	
NA	5 (5.8)	1 (9.1)	4 (5.3)	
B symptom				
Absent	27 (31.4)	4 (36.4)	23 (30.7)	0.558
Present	50 (58.1)	5 (45.5)	45 (60.0)	
NA	9 (10.5)	2 (18.1)	7 (9.3)	
ECOG performance status score				
<2	40 (46.5)	5 (45.5)	35 (46.7)	0.409
≥2	39 (45.4)	4 (36.4)	35 (46.7)	
NA	7 (8.1)	2 (18.1)	5 (6.6)	
LDH level				
Normal	9 (10.5)	3 (27.3)	6 (8.0)	0.064
High	69 (80.2)	6 (54.6)	63 (84.0)	
NA	8 (9.3)	2 (18.1)	6 (8.0)	
NCCN-IPI score				
Low risk (0 to 3)	17 (19.8)	3 (27.3)	14 (18.7)	0.22
High intermediate risk (4 to 5)	39 (45.4)	2 (18.2)	37 (49.3)	
High risk (≥6)	23 (26.7)	4 (36.4)	19 (25.3)	
NA	7 (8.1)	2 (18.1)	5 (6.7)	
Ki-67				
≥70.0%	65 (75.6)	9 (81.8)	56 (74.7)	0.342
<70.0%	10 (11.6)	2 (18.2)	8 (10.7)	
NA	11 (12.8)	0 (0.00)	11 (14.6)	
CD10				
Positive	34 (39.5)	5 (45.5)	29 (38.7)	0.667
Negative	52 (60.5)	6 (54.5)	46 (61.3)	
NA	0 (0.0)	0 (0.0)	0 (0.0)	
Treatment				
R-CHOP	35 (40.7)	3 (27.3)	32 (42.7)	0.588
CHOP	25 (29.1)	4 (36.4)	21 (28.0)	
Other	3 (3.5)	1 (9.1)	2 (2.7)	
NA	23 (26.7)	3 (27.2)	20 (26.6)	
Survival status				
Died	48 (55.8)	6 (54.6)	42 (56.0)	0.928
Alive	38 (44.2)	5 (45.5)	33 (44.0)	

NA=not assessment case; CNS=central nervous system; GCB=germinal center B-cell; ECOG=Eastern Cooperative Oncology Group; LDH=lactate dehydrogenase; NCCN-IPI=National Comprehensive Cancer Network International Prognostic

(a) Extranodal involvement

statically significant difference.

The association between MYD88 expression and clinicopathological data, as well as IHC study results, was examined

By univariate analysis, no significant associations were observed between MYD88 protein expression and clinicopathological characteristics (Table 2). Nevertheless, certain trends were noted. MYD88 positivity tended to be higher among younger patients (≤63 years, p=0.051) and in those with elevated serum LDH levels (p=0.064). Most MYD88-positive cases demonstrated extranodal involvement, advanced Ann Arbor stage (III-IV), non-GCB subtype by Hans algorithm, and high Ki-67 proliferation index (≥70%), though these associations were not statistically significant.

Treatment for our patients was 35 R-CHOP (55.5%), 25 CHOP (39.7%), and 3 others (4.8%). After the median follow-up of 14.77 months (3 days to 106.9 months), 48 patients died (55.8%). The median OS was 20.7 months (95% confidence interval [CI] 9.93 to not reach). The 3-year survival rate was 44.4% (95% CI 32.68 to 54.42).

Although a longer median OS for the negative MYD88 protein expression group (31.0 months) compared to the positive group (19.0 months) was observed, the difference was not statistically significant (HR 1.075, p=0.869) (Figure 2). Kaplan-Meier curves for OS in DLBCL according to Ann Arbor stage, ECOG status, NCCN-IPI score, and treatment regimens are presented in Figure 3-6, respectively. In the univariate analysis, advanced Ann Arbor stage (hazard ratio [HR] 3.07, p=0.018), high ECOG performance status (HR 2.60, p=0.003), and high-risk NCCN-IPI score ≥6 (HR 5.11, p=0.001) were significant poor prognostic factors for survival. Multivariate analysis found no independent prognostic factors. Although higher NCCN-IPI scores (≥6) and positive MYD88 protein expression showed trends toward poorer survival (adjusted HR 5.416, p=0.102; and adjusted HR 2.915, p=0.210, respectively), these associations did not reach statistical significance (Table 3).

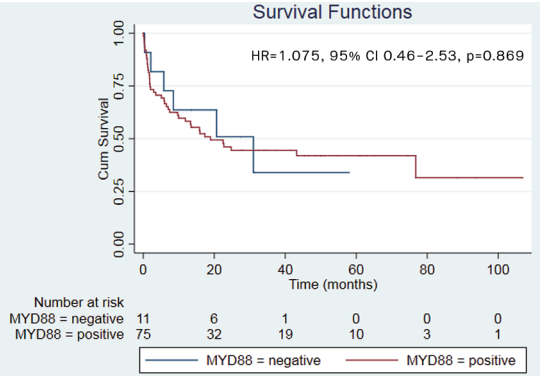

Figure 2. Kaplan-Meier curves for overall survival in diffuse large B-cell lymphoma. Survival analysis is shown according to MYD88 expression status.

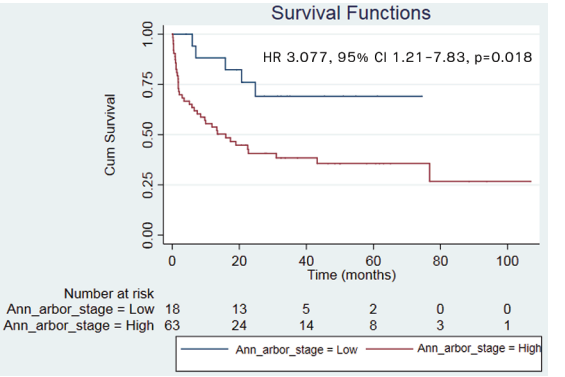

Figure 3. Kaplan-Meier curves for overall survival in diffuse large B-cell lymphoma. Survival analysis is shown according to Ann Arbor stage.

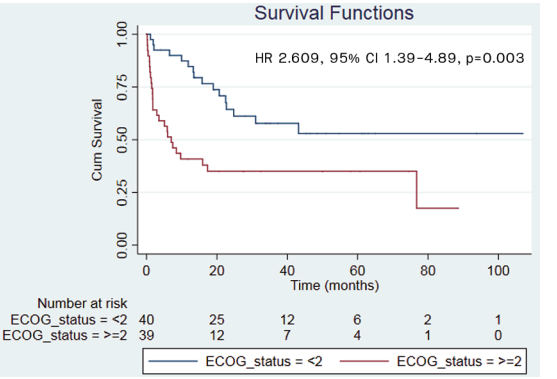

Figure 4. Kaplan-Meier curves for overall survival in diffuse large B-cell lymphoma. Survival analysis is shown according to ECOG performance status.

Discussion

The prevalence of MYD88 protein expression in DLBCL in our study was 87.2%, which was quite high compared to previous studies from China and Korea, which reported only 38% or 38.7%, respectively[11,12]. The discrepancy of prevalence across studies may lie in various factors, which may have affected the rates of expression: genetic, clinical features of the population studied, and techniques being used. We reviewed and summarized some features in each study (Table 4). Ethnic or geographic genetic backgrounds

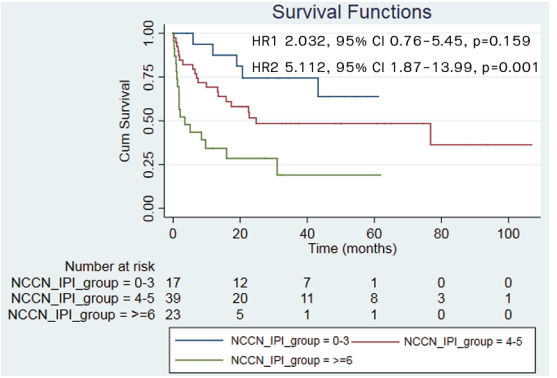

Figure 5. Kaplan-Meier curves for overall survival in diffuse large B-cell lymphoma. Survival analysis is shown according to the NCCN-IPI risk category.

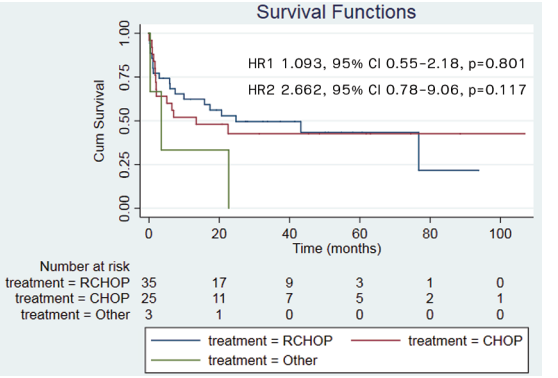

Figure 6. Kaplan-Meier curves for overall survival in diffuse large B-cell lymphoma. Survival analysis is shown according to the treatment regimen.

Table 3. Univariate and multivariate analyses of overall survival by Cox regression

Variables	Univariate model			Multivariate model		
	Crude HR	95% CI	p-value	Adjusted HR	95% CI	p-value
Age (years)						
≤63	Reference	-	-	Reference	-	-
>63	1.537	0.9 to 2.7	0.139	1.135	0.43 to 2.96	0.795
Sex						
Male	Reference	-	-	Reference	-	-
Female	0.671	0.4 to 1.2	0.194	0.451	0.18 to 1.13	0.090
Location						
Nodal	Reference	-	-	Reference	-	-
Extranodal	1.078	0.6 to 2.1	0.820	1.196	0.36 to 4.03	0.772
Ann Arbor stage						
Limited (I-II)	Reference	-	-	Reference	-	-
Advanced (III-IV)	3.077	1.2 to 7.8	0.018*	1.385	0.43 to 4.46	0.585
Hans algorithm						
GCB	Reference	-	-	Reference	-	-
Non-GCB	1.317	0.7 to 2.4	0.370	1.740	0.67 to 4.50	0.253
ECOG performance status score						
<2	Reference	-	-	Reference	-	-
≥2	2.609	1.4 to 4.9	0.003*	2.052	0.68 to 6.21	0.203
NCCN-IPI score						
0-3	Reference	-	-	Reference	-	-
4-5	2.032	0.8 to 5.4	0.159	1.551	0.33 to 7.24	0.577
≥6	5.112	1.9 to 14.0	0.001*	5.416	0.71 to 41.10	0.102
MYD88 protein expression						
Negative	Reference	-	-	Reference	-	-
Positive	1.075	0.5 to 2.5	0.869	2.915	0.55 to 15.54	0.210
LDH level						
Normal	Reference	-	-	Reference	-	-
Above normal	1.303	0.5 to 3.7	0.616	0.789	0.13 to 4.64	0.793
Treatment						
R-CHOP	Reference	-	-	Reference	-	-
CHOP	1.093	0.5 to 2.2	0.801	1.009	0.41 to 2.47	0.984
Other	2.662	0.8 to 9.1	0.117	1.509	0.24 to 9.52	0.662

HR=hazard ratio; CI=confidence interval; GCB=germinal center B-cell; ECOG=Eastern Cooperative Oncology Group; NCCN-IPI=National Comprehensive Cancer Network International Prognostic; LDH=lactate dehydrogenase

* Statistically significant at p<0.05

Table 4. Clinico-pathological characteristics of MYD88 protein expression in DLBCL

Characteristic	Authors/year/country		
	Niu et al. 2020 China	Choi et al. 2012 Korea	Present study 2025 Thailand
Sample size of DLBCL (n)	100	124	86
Prevalence of MYD88 expression (%)	38	38.7	87.2
Age (years) (%)			
<60	55.2	39.6	51.2
≥60	44.7	60.4	36
Sex (%)			
Male	44.7	58.7	51.2
Female	55.3	41.7	36.0
Location (%)			
Nodal	42.8	41.7	25.6
Extranodal	51.2	58.3	60.5
NA	-	-	1.2
Extranodal involvement (%)			
CNS	36.8	-	5.8
Other	51.2	-	54.7
Hans algorithm (%)			
GCB	26.3	20.8	33.7
Non-GCB	73.7	79.2	53.5
Ann Arbor stage (%)			
Limited (I-II)	26.3	62.5	17.4
Advanced (III-IV)	73.7	37.5	65.1
NA	-	-	4.7
B symptom (%)			
Absent	81.6	68.8	26.7
Present	18.4	31.2	52.3
NA	-	-	8.1
ECOG performance status score (%)			
<2	50	89.6	40.7
≥2	50	10.4	40.7
NA	-	-	5.8
LDH level (%)			
Normal	65.8	52.1	7.0
Above normal	31.5	47.9	73.3
NA	2.7	-	7.0
Ki-67 (%)			
Positive	71	-	65.1
Negative	29	-	9.3
NA	-	-	12.8
NCCN-IPI score (%)			
Low risk	63.1*2	79.2*3	16.3*2
High intermediate risk	26.3	-	43.0
High risk	10.6	20.8	22.1
NA	-	-	5.8
Survival status (%)			
Died	57.9	-	48.8
Alive	34.2	-	38.4
Unknown	7.9	-	-

DLBCL=diffuse large B-cell lymphoma; NA=not assessment case; CNS=central nervous system; GCB=germinal center B-cell; ECOG=Eastern Cooperative Oncology Group; LDH=lactate dehydrogenase; NCCN-IPI=National Comprehensive Cancer Network International Prognostic
Median age of the present study 63 years; *2 NCCN-IPI score: low risk 0-3, high intermediate risk 4-5, high risk ≥6; *3 NCCN-IPI score: low risk 0-2, high risk 3-5

could influence MYD88-related pathways. Studies in different Asian populations have demonstrated diverse frequencies of MYD88 L265P mutations, which may translate to different expression patterns[16-25]. Our cohort included a higher proportion of patients aged 63 years (58.7%), the recognized association between MYD88 activation. The proportion of extranodal DLBCL was also greatest in our study (69.3%), compared with approximately 50% in prior Asian reports[11,12]. Extranodal involvement, especially CNS, is known to correlate with MYD88 mutation or expression. In addition, our patients presented with more advanced stage disease (Ann Arbor III-IV 74.7%) and higher LDH level (84%), both consistent with aggressive molecular subtypes such as non-GCB. Although a lower percentage of non-GCB subtype in our study (61.3%) compared to 79% in Korea and 74% in China, other more aggressive features may take preponderance. Technical differences may also play an important role. Variability in immunohistochemical staining (antibody clones, cut-off thresholds, tissue fixation, or detection platforms) could markedly alter apparent prevalence. For instance, using more sensitive antibodies or process of interpretation. The IHC expression in our study was interpreted by two pathologists with a high correlation of the process. This may have added to the high reliability of our results. The discrepancy of high MYD88 IHC positivity shown in this study (87.2%) differs from previous studies conducted in China and Korea. This may be explained by several factors, including genetic, variations in clinical characteristics of the studied populations, and laboratory techniques used. In the study from China, a tissue microarray technique was used with a mouse anti-MYD88 antibody (1:700, cat. no. ab133739, Abcam). Similarly, the Korean study also used a tissue microarray technique with a mouse anti-MYD88 antibody (1:300 dilution, clone 2B2,

Origene, Rockville, MD). Rabbit antibodies generally provided stronger, sharper, and more sensitive signals compared to mouse antibodies, with cleaner staining and less background[26]. Therefore, our study used a rabbit polyclonal anti-MYD88 antibody (1:500; Gene Tex, Lot 445527) that could play a role in the higher MYD88 protein expression rate compared to the studies from China and Korea.

In terms of OS, although MYD88 protein expression was highly prevalent among patients with DLBCL, its association with OS was not statistically significant. Although the multivariate analysis showed a trend toward poorer outcomes in patients with positive MYD88 protein expression, this did not reach significance. Poor ECOG performance, advanced Ann Arbor stage, and high NCCN-IPI scores (≥6), which are known as adverse clinical parameters, were associated with inferior survival in univariate analysis, in line with established prognostic models. However, these factors lost statistical significance in the multivariate analysis. Other features, indicative of tumor burden and proliferation, frequently found in our study, which may have contributed to poor outcomes of our patients, such as elevated LDH (84%) and high Ki-67 positivity (76.7%) were not found as significant unfavorable factors for survival in our study. These likely reflected overlapping prognostic influences and the relatively limited sample size, different managements among patients (R-CHOP, CHOP, and other treatments), and the small number of survival events. Previous studies also reported inconsistent prognostic implications of MYD88 protein expression. The authors reported that only age ≥60 years (HR 1.982), high neutrophil lymphocyte ratio (HR 0.518), or high LDH levels were independent prognostic factors for OS of DLBCL[11,12].

Findings of previous studies and our study emphasized that survival in DLBCL was influenced not only by molecular subtype but also by the interplay of demographic, clinical, and pathological factors, as well as regional and methodological variations. These findings underscore the importance of considering population-specific characteristics when interpreting MYD88 protein expression and its prognostic implications in DLBCL.

The present study had some strengths. First, it represented the initial investigation of MYD88 protein expression by an IHC study in DLBCL conducted in the Thai population. Second, the results from the IHC study of tumor tissue were reliable due to an independent interpretation by two pathologists with a high correlation index. Our data should be useful in clinical practice because the test is widely accessible in most laboratories and at a low cost compared to PCR.

We also acknowledged several limitations in our study. First, our study did not perform a PCR or molecular genetic study due to financial constraints. So, the performance of the IHC study could not be confirmed. Second, all patients in the present study were Thai, who may not represent genetic aberrations similar to those in other populations. Third, the study included 86 patients, with only 11 MYD88-negative cases. This imbalance could limit statistical power, particularly in survival analysis and multivariate modeling (increasing the risk of unstable hazard ratios, wide confidence intervals, and type II error). Another limitation of this study is the follow-up period used for the OS analysis. In this study, the follow-up time was limited to one year after diagnosis until the last follow-up or death. Therefore, this relatively short follow-up period may affect the ability to fully evaluate long-term survival outcomes. MYD88 protein expression IHC is often used as a representative to detect the mutated protein's over-expression[11], nevertheless, it is not as specific as PCR or next-generation sequencing (NGS). Future research should be conducted in other populations, with a larger cohort, longer follow-up time, and with more comprehensive genetic studies for comparison.

Regarding the clinical implications, the Kaplan-Meier OS analysis of MYD88 protein expression in DLBCL showed that the median survival time of patients with MYD88-positive tumors was shorter than that of MYD88-negative patients (average 20 months vs. 35 months). Although the difference was not statistically significant, the result suggests a trend toward poorer outcomes in the MYD88-positive group. These findings may help improve clinical practice by supporting usual MYD88 IHC staining in DLBCL tissue to identify patients who may have an unfavorable prognosis. This information could be beneficial for clinicians in guiding patient counseling, as well as planning closer clinical follow-up and imaging surveillance for patients with MYD88-positive tumor staining.

Conclusion

The present study found a higher prevalence of MYD88 protein expression in DLBCL compared with previous reports. MYD88 protein expression was mainly observed in the non-GCB subtype. High serum LDH was independently associated with MYD88 protein expression. Univariate analysis showed that

advanced Ann Arbor stage (III-IV), ECOG ≥2, and high-risk NCCN-IPI score predicted poor OS, though MYD88 protein expression itself was not independently linked to OS. No statistically significant independent prognostic factors were identified, possibly due to limited sample size. Overall, these findings suggest that clinical parameters remain important indicators of prognosis; the independent prognostic value of MYD88 protein expression in DLBCL requires confirmation in larger, multi-institutional studies with molecular correlation.

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Authors Contributions

WR (Principal investigator) was accountable for literature review, writing a proposal for approval, collecting data and analysis, and manuscript arrangement of research for publication. WB and SY (assistant researchers) took responsibility for supervising the IHC staining quality carried out by the laboratory scientist and interpreted MYD 88 expression in tissue specimens.

Clinical Trial Registration

As this study did not involve a clinical trial, these documents were not applicable.

Conflicts of Interest

The authors declared no conflicts of interest.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article. Raw data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

This trial was approved by the Bangkok Metropolitan Administration Human Research and Ethics Committee (No. S006hc/67_EXP). Informed consent was waived by the ethical policy.

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Use of Artificial Intelligence

No artificial intelligence tools were used in any part of the research process.

References

1. Imsamran W, Chaiwerawattana A, Wiangnon S, Pongnikorn D, Suwanrungrung K, Sangrajrang S, et al., editors. Cancer in Thailand. Vol. viii, 2010-2012. Bangkok: New Thammada Press (Thailand); 2015.
2. World Health Organization. WHO classification of tumours: Haematolymphoid tumours. 5th ed. Lyon: IARC; 2024.
3. Sukpanichnant S. Analysis of 1983 cases of malignant lymphoma in Thailand according to the World Health Organization classification. Hum Pathol 2004;35:224-30.
4. Wright G, Tan B, Rosenwald A, Hurt EH, Wiestner A, Staudt LM. A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B cell lymphoma. Proc Natl Acad Sci USA 2003;100:9991-6.
5. 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;403:503-11.
6. Kawasaki T, Kawai T. Toll-like receptor signaling pathways. Front Immunol 2014;5:461. doi: 10.3389/fimmu.2014.00461.
7. Bekeredjian-Ding I, Jego G. Toll-like receptors--sentries in the B-cell response. Immunology 2009;128:311-23. doi: 10.1111/j.1365-2567.2009.03173.x.
8. Xia Y, Shen S, Verma IM. NF-κB, an active player in human cancers. Cancer Immunol Res 2014;2:823-30.
9. Gay NJ, Symmons MF, Gangloff M, Bryant CE. Assembly and localization of Toll-like receptor signalling complexes. Nat Rev Immunol 2014;14:546-58.
10. Jardin F. Next generation sequencing and the management of diffuse large B-cell lymphoma: From whole exome analysis to targeted therapy. Discov Med 2014;18:51-65.
11. Niu J, Ma Z, Nuerlan A, Li S, Cui W, Gao H, et al. Prognostic value of MYD88 L265P mutation in diffuse large B cell lymphoma via droplet digital PCR. Mol Med Rep 2020;22:1243-56.
12. Choi JW, Kim Y, Lee JH, Kim YS. MYD88 expression and L265P mutation in diffuse large B-cell lymphoma. Hum Pathol 2013;44:1375-81.
13. Lee JH, Jeong H, Choi JW, Oh H, Kim YS. Clinicopathologic significance of MYD88 L265P mutation in diffuse large B-cell lymphoma: A meta-analysis. Sci Rep 2017;7:1785. doi: 10.1038/s41598-017-01998-5.
14. Vermaat JS, Somers SF, de Wreede LC, Kraan W, de Groen RAL, Schrader AMR, et al. MYD88 mutations identify a molecular subgroup of diffuse large B-cell lymphoma with an unfavorable prognosis.

Haematologica 2020;105:424-34.
15. Takano S, Hattori K, Ishikawa E, Narita Y, Iwadate Y, Yamaguchi F, et al. MyD88 mutation in elderly predicts poor prognosis in primary central nervous system lymphoma: Multi-institutional analysis. World Neurosurg 2018;112:e69-73.
16. Xu Y, Li J, Ouyang J, Li J, Xu J, Zhang Q, et al. Prognostic relevance of protein expression, clinical factors, and MYD88 mutation in primary bone lymphoma. Oncotarget 2017;8:65609-19.
17. Fukumura K, Kawazu M, Kojima S, Ueno T, Sai E, Soda M, et al. Genomic characterization of primary central nervous system lymphoma. Acta Neuropathol 2016;131:865-75.
18. Hattori K, Sakata-Yanagimoto M, Okoshi Y, Goshima Y, Yanagimoto S, Nakamoto-Matsubara R, et al. MYD88 (L265P) mutation is associated with an unfavourable outcome of primary central nervous system lymphoma. Br J Haematol 2017;177:492-4.
19. Kim Y, Ju H, Kim DH, Yoo HY, Kim SJ, Kim WS, et al. CD79B and MYD88 mutations in diffuse large B-cell lymphoma. Hum Pathol 2014;45:556-64.
20. Nagakita K, Takata K, Taniguchi K, Miyata-Takata T, Sato Y, Tari A, et al. Clinicopathological features of 49 primary gastrointestinal diffuse large B-cell lymphoma cases; comparison with location, cell-of-origin, and frequency of MYD88 L265P. Pathol Int 2016;66:444-52.
21. Nakamura T, Tateishi K, Niwa T, Matsushita Y, Tamura K, Kinoshita M, et al. Recurrent mutations of CD79B and MYD88 are the hallmark of primary central nervous system lymphomas. Neuropathol Appl Neurobiol 2016;42:279-90.
22. Ogura G, Kikuti YY, Kikuchi T, Carreras J, Sato T, Nakamura N. MYD88 (L265P) mutation in malignant lymphoma using formalin-fixed paraffin-embedded section. J Clin Exp Hematop 2013;53:175-7.
23. Oishi N, Kondo T, Nakazawa T, Mochizuki K, Tanioka F, Oyama T, et al. High prevalence of the MYD88 mutation in testicular lymphoma: Immunohistochemical and genetic analyses. Pathol Int 2015;65:528-35.
24. Taniguchi K, Takata K, Chuang SS, Miyata-Takata T, Sato Y, Satou A, et al. Frequent MYD88 L265P and CD79B mutations in primary breast diffuse large B-cell lymphoma. Am J Surg Pathol 2016;40:324-34.
25. Wang CZ, Lin J, Qian J, Shao R, Xue D, Qian W, et al. Development of high-resolution melting analysis for the detection of the MYD88 L265P mutation. Clin Biochem 2013;46:385-7.
26. Rocha R, Nunes C, Rocha G, Oliveira F, Sanches F, Gobbi H. Rabbit monoclonal antibodies show higher sensitivity than mouse monoclonals for estrogen and progesterone receptor evaluation in breast cancer by immunohistochemistry. Pathol Res Pract 2008;204:655-62.