Brief Communication
Diagnostic Hematology

Ann Lab Med
https://doi.org/10.3343/alm.2024.0644
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

MYD88 L265P Variant Detection with Droplet Digital PCR in Waldenström Macroglobulinemia: Clinical Implications as a Tumor Burden and Prognostic Marker

Woo Jin Shin, M.D.[1,2], Yoo Jin Kang, B.S.[2], Aram Kim, M.T.[2], Jeong-Ok Lee, M.D., Ph. D[3], and Sang Mee Hwang, M.D., Ph. D. [1,2]

[1]Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul, Korea; [2]Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seongnam, Korea; [3]Department of Internal Medicine, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam, Korea

Waldenström macroglobulinemia (WM) is a B-cell lymphoproliferative disease characterized by IgM monoclonal gammopathy and bone marrow (BM) infiltration caused by lymphoplasmacytic lymphoma. The *MYD88* L265P variant is present in >90% of WM cases. We used droplet digital PCR (ddPCR) to detect *MYD88* L265P in initial BM samples from 15 patients with WM and assessed the implication of variant burden as a tumor load and prognostic marker. *MYD88* L265P burden correlated with clinical indicators, including peripheral blood and BM lymphocyte percentages ($P<0.001$ and $P=0.003$, respectively), serum lactate dehydrogenase level ($P=0.045$), and platelet count ($P=0.003$). Patients classified into intermediate and high groups according to the Revised International Prognostic Score System for WM had higher *MYD88* L265P copies/μL than patients in very low and low groups ($P=0.017$), as had patients with minor response or stable disease after primary treatment than those with complete, partial, or very good partial response ($P=0.034$). *MYD88* L265P burden correlates well with multiple clinical indicators and has prognostic relevance, making it a potential marker for assessing tumor burden and predicting prognosis in WM.

Key Words: Droplet digital PCR, *MYD88* L265P variant, Waldenström macroglobulinemia

Received: December 4, 2024
Revision received: February 16, 2025
Accepted: May 6, 2025
Published online: June 27, 2025

Corresponding author:
Sang Mee Hwang, M.D., Ph. D.
Department of Laboratory Medicine, Seoul National University Bundang Hospital, 173-82 Gumi-ro, Bundang-gu, Seongnam 13620, Korea
E-mail: sangmee1@snu.ac.kr

Waldenström macroglobulinemia (WM) is a B-cell lymphoproliferative disease characterized by lymphoplasmacytic lymphoma in the bone marrow (BM) and IgM monoclonal components in the blood [1, 2]. Whole-genome sequencing has revealed that *MYD88* L265P is a frequent somatic alteration in WM, detected in >90% of patients [3]. Various assays and techniques, including Sanger sequencing, allele-specific quantitative PCR (qPCR), droplet digital PCR (ddPCR), and high-throughput sequencing, have been employed to detect *MYD88* L265P in WM; however, these methods have not yet been standardized [1, 4]. Recently, ddPCR has been recognized as a reliable and highly sensitive diagnostic method that addresses certain limitations of qPCR and applies to various hematologic malignancies [5]. Here, we present a ddPCR assay for detecting the *MYD88* L265P variant in patients with WM. Using the assay, we assessed the implication of *MYD88* L265P burden as a tumor load and prognostic marker.

We retrospectively reviewed the medical records of 15 patients with pathologically confirmed WM at Bundang Seoul National University Hospital, Seoul, Korea, between 2020 and 2023. The Institutional Review Board approved the study (SNUBH-2403-886-104). Informed consent was waived because of the retrospective nature of the study.

Peripheral blood (PB) (N=15) for flow cytometry and BM aspirates (N=15) for *MYD88* L265P molecular testing were collected upon diagnosis. The *MYD88* L265P variant was evaluated using Sanger sequencing and ddPCR. DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). ddPCRs were run using a QX200 Droplet Digital PCR System (Bio-Rad, Hercules, CA, USA) per the manufacturer's instructions. The thermal cycling conditions were as follows: enzyme activation at 95°C for 10 mins, 40 cycles of denaturation at 94°C for 30 secs and annealing and extension at 55°C for 1 min, enzyme inactivation at 98°C for 10 mins, and final hold at 4°C. Gating was conducted using positive and negative controls, and the data were analyzed using QuantaSoft v1.7.4 (Bio-Rad).

The presence of WM cells in PB was assessed using multiparametric flow cytometry (MFC) using a BD FACSCanto flow cytometer and BD FACSDiva software (BD Biosciences San Jose, CA, USA). The eight-color MFC panel used for the initial diagnostic evaluation of suspected B-cell lymphoproliferative disease included the following monoclonal antibodies: Kappa, Lambda, CD5, CD10, CD19, CD20, CD23, CD43, CD45, CD79b, CD81, and CD200 [6]. A B-cell population exhibiting light chain restriction was interpreted as suggestive of a B-cell lymphoproliferative disease.

Associations between categorical variables were analyzed using Fisher's exact test, whereas the Mann–Whitney U test was used for the inference of continuous variables. Univariate and multivariate logistic regression analyses were conducted to identify risk factors of refractory WM among clinical indicators and ddPCR fractional abundance and copies/μL. Odds ratios (ORs) were calculated with 95% confidence intervals (CIs). Data were analyzed using R4.2.2 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was set at $P<0.05$.

Among the 15 patients, six (40.0%) tested positive for *MYD88* L265P by Sanger sequencing, and 12 (80.0%) by ddPCR. Three patients with ddPCR-positive results, with fractional abundances of 1.1%, 2%, and 63.6%, had variant allele frequencies of 3.5%,

Table 1. Clinical characteristics and laboratory findings of 15 patients with WM

Clinical characteristics	Patients with WM (N=15)	*MYD88*^{L265P} (N=12)	*MYD88*^{WT} (N=3)
Median age (yrs), (range)	61.0 (45.0–89.0)	70.0 (51.0–89.0)	52.0 (45.0–58.0)
Sex, N (%)			
Male	12 (80)	11 (91.7)	1 (33.3)
Female	3 (20)	1 (8.3)	2 (66.7)
Hb (g/L), median, (range)	74 (50–127)	76 (50–127)	74 (62–83)
Leukocytes ($\times 10^9$/L), median, (range)	6.5 (1.0–43.7)	6.6 (1.0–43.7)	3.5 (1.1–9.0)
Platelets ($\times 10^9$/L), median, (range)	112.0 (24.0–318.0)	210.5 (24.0–318.0)	78.0 (76.0–112.0)
β2-Microglobulin (mg/L), median, (range)	4.3 (1.7–7.3)	4.6 (1.7–7.3)	2.9 (1.9–4.6)
LDH (IU/L), median, (range)	172.0 (74.0–274.0)	143.5 (74.0–274.0)	173.0 (172.0–183.0)
IgM (g/L), median, (range)	24.05 (4.56–58.50)	25.65 (4.56–46.05)	22.25 (9.50–58.50)
BM lymphocytes (%), median, (range)*	37.4 (6.0–96.7)	31.7 (6.4–96.7)	53.2 (6.0–90.4)
Revised IPSSWM, N (%)			
Very low	3 (20.0)	2 (16.7)	1 (33.3)
Low	2 (13.3)	2 (16.7)	0 (0.0)
Intermediate	6 (40.0)	4 (33.3)	2 (66.7)
High	4 (26.7)	4 (33.3)	0 (0.0)
Initial treatment regimen, N (%)			
Bendamustine and rituximab	10 (66.7)	9 (75.0)	1 (33.3)
Rituximab, cyclophosphamide, and dexamethasone	3 (20.0)	2 (16.7)	1 (33.3)
Bortezomib, thalidomide, and dexamethasone	1 (6.7)	1 (8.3)	0 (0.0)
Fludarabine, mitoxantrone, and dexamethasone	1 (6.7)	0 (0.0)	1 (33.3)

*% among BM nucleated cells on BM aspirate smears.
Abbreviations: WM, Waldenström macroglobulinemia; LDH, lactate dehydrogenase; BM, bone marrow; IPSSWM, International Prognostic Score System for WM.

https://doi.org/10.3343/alm.2024.0644

38%, and 68.06%, respectively, as indicated by next-generation sequencing. Table 1 summarizes the patients' clinical characteristics and laboratory findings.

We performed Spearman's correlation analysis between *MYD88* L265P fractional abundance and multiple clinical indicators. Fractional abundance was positively correlated with PB lymphocyte percentage ($P<0.001$), MFC-gated PB lymphocyte count ($P=0.019$), BM aspirate lymphocyte percentage ($P=0.003$), serum lactate dehydrogenase (LDH) level ($P=0.045$), and negatively correlated with platelet count ($P=0.003$) (Fig. 1). The PB and BM lymphocyte percentages included normal and abnormal lymphoid cells. dFLC is calculated as the absolute value of the difference between κ and λ FLC concentrations.

We categorized 12 patients harboring *MYD88* L265P into four groups according to the Revised International Prognostic Score System for WM (rIPSSWM): very low (N=2), low (N=2), intermediate (N=4), and high (N=4) [7]. Patients in the intermediate and high groups had higher *MYD88* L265P copies/μL ($P=0.017$)

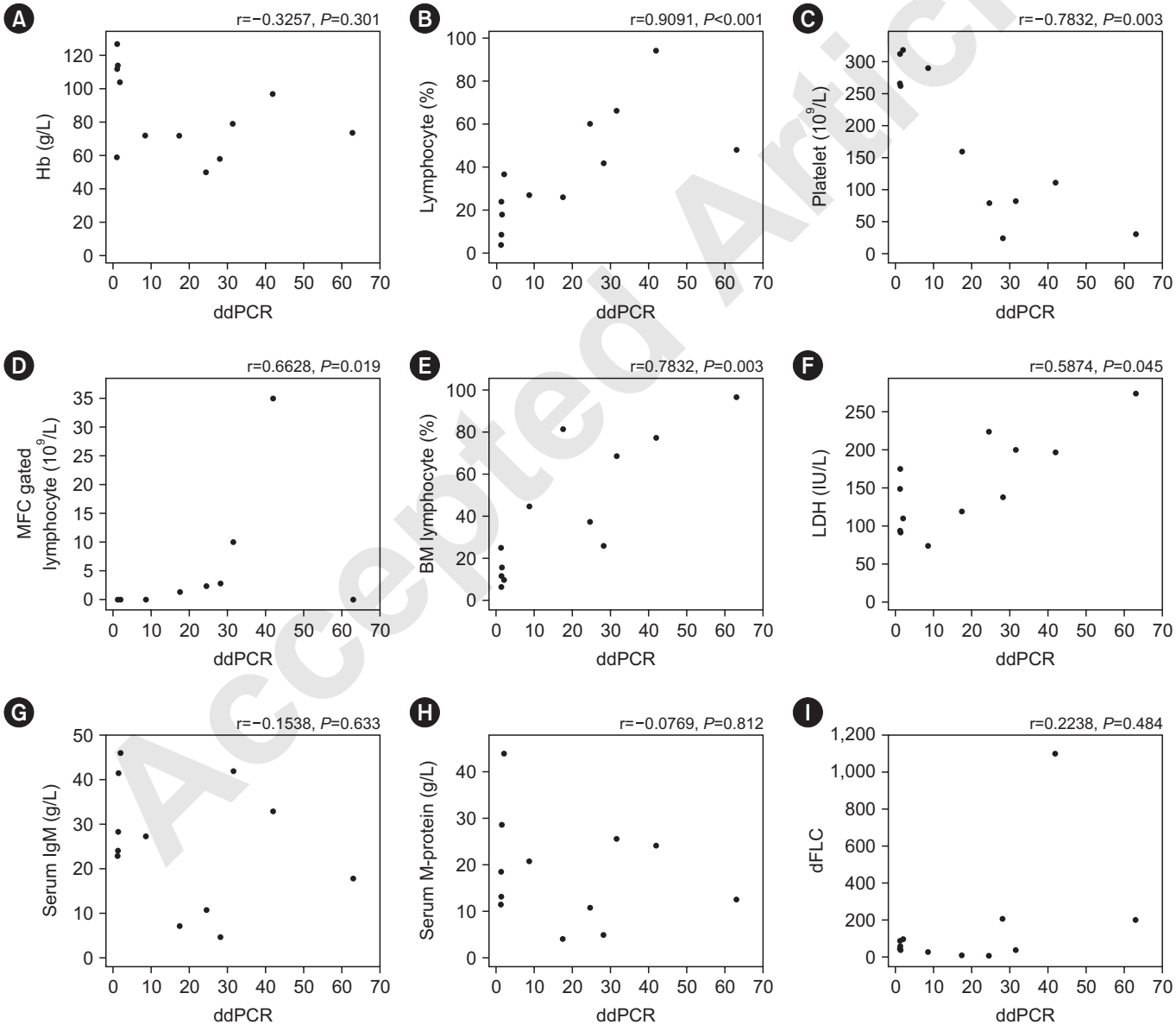

Fig. 1. Correlation of different clinical indicators with ddPCR fractional abundance in 12 *MYD88*^{L265P} patients. Fractional abundance is correlated positively with PB lymphocyte percentage ($P<0.001$) (B), MFC-gated PB lymphocyte count ($P=0.019$) (D), BM lymphocyte percentage ($P=0.003$) (E), serum LDH level ($P=0.045$) (F), and negatively with platelet count ($P=0.003$) (C).
Abbreviations: ddPCR, droplet digital PCR; PB, peripheral blood; MFC, multiparametric flow cytometry; BM, bone marrow; LDH, lactate dehydrogenase.

and fractional abundance ($P=0.027$) than those in the very low and low groups (Fig. 2A). Patients in the poor prognostic group had a higher BM lymphocyte percentage ($P=0.017$) than those with good prognosis. The median follow-up duration was 16.5 months. Treatment responses were available for 11 patients [8]: complete response (CR) (N=1), very good partial response (VGPR) (N=3), partial response (PR) (N=5), minor response (MR) (N=1), and stable disease (SD) (N=1). Patients with MR or SD had higher *MYD88* L265P copies/μL than those with CR, VGPR, or PR ($P=0.034$) (Fig. 2B).

Clinical indicators and ddPCR fractional abundance were assessed using univariate logistic regression analysis. Multivariate analysis included clinical indicators reflecting tumor burden in WM, along with ddPCR fractional abundance (Supplemental Data Table S1). Besides PB lymphocyte count (OR, 1.024, 95% CI, 1.008–1.041), we found no significant independent factor predictive of refractory response, including MR and SD.

MYD88 L265P identification can aid in the differential diagnosis of WM from other lymphoproliferative neoplasms with similar clinical features, such as multiple myeloma [9, 10]. *MYD88* variant status affects overall survival and may influence the efficacy of Bruton's tyrosine kinase inhibitors [1, 11]. Additionally, a high *MYD88* variant burden is associated with progression from IgM monoclonal gammopathy of undetermined significance (MGUS) or from smoldering WM to WM [12]. ddPCR was recently demonstrated to be effective in identifying *MYD88* L265P in WM, resolving certain challenges associated with qPCR [10, 13].

Given that ddPCR allows for the accurate quantification of variant burden, we conducted Spearman's correlation analysis between *MYD88* L265P fractional abundance and various clinical parameters. *MYD88* variant burden determined using ddPCR correlated with well-known disease status markers, such as PB and BM involvement, as evaluated either based on morphology or using MFC and dFLC. *MYD88* variant burden is positively correlated with known prognostic markers of hematologic malignancies, LDH, and platelet count [7, 14, 15]. Therefore, ddPCR-based quantitative analysis of *MYD88* L265P, along with various clinical parameters, has the potential for assessing tumor burden and predicting prognosis in WM.

We found no correlation between *MYD88* L265P burden and serum IgM or M protein levels. WM has considerable interpatient clinical heterogeneity, with significant variation in serum IgM levels and BM cellular contents. Studies have failed to demonstrate a correlation between IgM concentration and overall BM disease burden [16]. While the percentages of CD20- and CD138-positive cells in the BM weakly correlated with serum M protein levels, M protein levels were not correlated with any particular cell type, including small lymphocytes, plasmacytoid lymphocytes, or plasma cells [4, 17]. These findings highlight the need for further research to identify specific WM cells responsible for M protein secretion. Using unsorted BM aspirate samples for the *MYD88* L265P ddPCR assay, we did not find a correlation

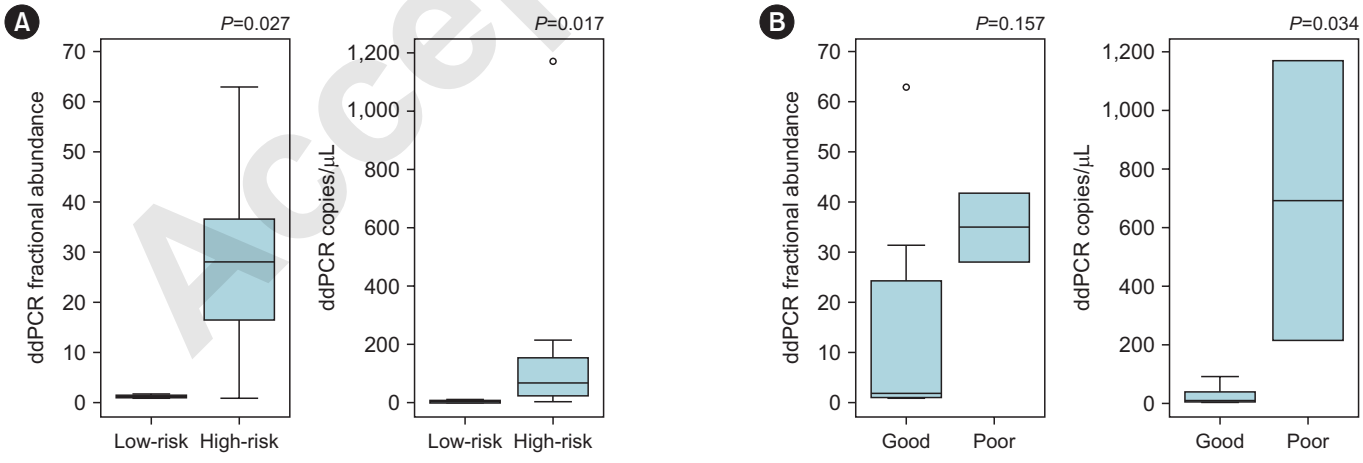

Fig. 2. Difference in ddPCR quantification in *MYD88*^{L265P} patients according to prognosis and treatment response. (A) Patients were classified into low-risk (very low and low) and high-risk (intermediate and high) groups based on the rIPSSWM. *MYD88*^{L265P} fractional abundance ($P=0.027$) and copies/μL ($P=0.017$) were higher in the high-risk group than in the low-risk group. (B) Patients achieving complete response, very good partial response, or partial response to treatment were categorized as having a good prognosis, whereas those with minor response or stable disease were classified as having a poor prognosis. Patients in the poor prognostic group had higher *MYD88*^{L265P} fractional abundance ($P=0.157$) and copies/μL ($P=0.034$) than those in the good prognostic group.
Abbreviations: ddPCR, droplet digital PCR; rIPSSWM, Revised International Prognostic Score System for WM.

https://doi.org/10.3343/alm.2024.0644

with serum IgM or M protein levels.

Somatic variants in *MYD88* and *CXCR4* are important in shaping the clinical presentation of WM and significantly influence overall survival [18]. In our study, the variant burden was significantly higher in patients with a higher rIPSSWM score. In patients with MR or SD, absolute copies/μL at initial diagnosis were significantly higher than those in patients who achieved CR, VGPR, or PR. These findings show that *MYD88* L265P quantification can be used for risk assessment and treatment response prediction. Previous study findings revealed that the cumulative incidence of progression to symptomatic WM increased with increasing *MYD88* and *CXCR4* BM variant burden in patients with IgM MGUS and smoldering WM [12].

Our study had some limitations. First, this was a single-center study with a limited sample size. Furthermore, WM has an indolent progression, and the median follow-up duration was relatively short compared with that in previous studies. Univariate and multivariate logistic regression analyses were performed to identify independent predictors of treatment response, and *MYD88* variant burden was not a significant independent factor for refractory response. Further studies in larger populations and with longer follow-up periods and survival analyses are needed to determine whether *MYD88* L265P burden quantified using ddPCR can serve as an independent prognostic factor. Assessing the treatment response in WM is challenging because of the variability in the rate of serum IgM reduction across treatments and the inconsistency between IgM and BM responses [8]. Further evaluation using follow-up samples from a larger prospective series of patients is required to assess the use of *MYD88* L265P quantification for disease monitoring and early relapse identification. Despite these challenges, the strong correlation between *MYD88* L265P burden and clinical indicators of tumor burden suggests that it may serve as a more reliable biomarker than IgM for disease monitoring.

In conclusion, quantitative analysis of *MYD88* L265P using ddPCR correlated well with several clinical indicators of tumor burden and treatment response in WM. ddPCR facilitates the monitoring of tumor burden and disease progression through quantitative analysis while providing sensitive *MYD88* L265P detection.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.3343/alm.2024.0644.

ACKNOWLEDGEMENTS

None.

AUTHOR CONTRIBUTIONS

Shin WJ analyzed and interpreted the data, performed statistical analysis, and wrote the manuscript; Kang YJ and Kim A performed the experiments; Lee JO and Hwang SM conceived and designed the study and revised the manuscript; Hwang SM analyzed the data and reviewed and revised the manuscript.

CONFLICTS OF INTEREST

None declared.

RESEARCH FUNDING

This work was supported by the SNUBH research fund (grant No. 02-2021-0051).

REFERENCES

1. Pratt G, El-Sharkawi D, Kothari J, D'Sa S, Auer R, McCarthy H, et al. Diagnosis and management of Waldenström macroglobulinaemia—A British Society for Haematology guideline. Br J Haematol 2022;197:171–87.
2. Dogliotti I, Jiménez C, Varettoni M, Talaulikar D, Bagratuni T, Ferrante M, et al. Diagnostics in Waldenström's macroglobulinemia: a consensus statement of the European Consortium for Waldenström's Macroglobulinemia. Leukemia 2023;37:388–95.
3. Treon SP, Xu L, Yang G, Zhou Y, Liu X, Cao Y, et al. MYD88 L265P somatic mutation in Waldenström's macroglobulinemia. N Engl J Med 2012;367:826–33.
4. Ahn A, Park CJ, Cho YU, Jang S, Seo EJ, Lee JH, et al. Clinical, laboratory, and bone marrow findings of 31 patients with Waldenström macroglobulinemia. Ann Lab Med 2020;40:193–200.
5. Galimberti S, Balducci S, Guerrini F, Del Re M, Cacciola R. Digital droplet PCR in hematologic malignancies: a new useful molecular tool. Diagnostics (Basel) 2022;12:1305.
6. Park M, Lim J, Ahn A, Oh EJ, Song J, Kim KH, et al. Current status of flow cytometric immunophenotyping of hematolymphoid neoplasmsin Korea. Ann Lab Med 2024;44:222–34.
7. Kastritis E, Morel P, Duhamel A, Gavriatopoulou M, Kyrtsonis MC, Durot E, et al. A revised international prognostic score system for Waldenström's macroglobulinemia. Leukemia 2019;33:2654–61.
8. Owen RG, Kyle RA, Stone MJ, Rawstron AC, Leblond V, Merlini G, et al. Response assessment in Waldenström macroglobulinaemia: update from the VIth International Workshop. Br J Haematol 2013;160:171–6.
9. Ondrejka SL, Lin JJ, Warden DW, Durkin L, Cook JR, Hsi ED. MYD88 L265P somatic mutation: its usefulness in the differential diagnosis of

bone marrow involvement by B-cell lymphoproliferative disorders. Am J Clin Pathol 2013;140:387–94.
10. Drandi D, Genuardi E, Dogliotti I, Ferrante M, Jiménez C, Guerrini F, et al. Highly sensitive *MYD88*^{L265P} mutation detection by droplet digital polymerase chain reaction in Waldenström macroglobulinemia. Haematologica 2018;103:1029–37.
11. Treon SP, Cao Y, Xu L, Yang G, Liu X, Hunter ZR. Somatic mutations in MYD88 and CXCR4 are determinants of clinical presentation and overall survival in Waldenström macroglobulinemia. Blood 2014;123:2791–6.
12. Moreno DF, López-Guerra M, Paz S, Oliver-Caldés A, Mena MP, Correa JG, et al. Prognostic impact of MYD88 and CXCR4 mutations assessed by droplet digital polymerase chain reaction in IgM monoclonal gammopathy of undetermined significance and smouldering Waldenström macroglobulinaemia. Br J Haematol 2023;200:187–96.
13. Ferrante M, Furlan D, Zibellini S, Borriero M, Candido C, Sahnane N, et al. MYD88L265P detection in IgM monoclonal gammopathies: methodological considerations for routine implementation. Diagnostics (Basel) 2021;11:779.
14. Durot E, Kanagaratnam L, Zanwar S, Kastritis E, D'Sa S, Garcia-Sanz R, et al. A prognostic index predicting survival in transformed Waldenström macroglobulinemia. Haematologica 2021;106:2940–6.
15. Morel P, Duhamel A, Gobbi P, Dimopoulos MA, Dhodapkar MV, McCoy J, et al. International prognostic scoring system for Waldenström macroglobulinemia. Blood 2009;113:4163–70.
16. De Tute RM, Rawstron AC, Owen RG. Immunoglobulin M concentration in Waldenström macroglobulinemia: correlation with bone marrow B cells and plasma cells. Clin Lymphoma Myeloma Leuk 2013;13:211–3.
17. Kim HJ, Hur M, Kim H, Moon H-W, Yun Y-M, Kim SY, et al. A rare case of Waldenström macroglobulinemia/lymphoplasmacytic lymphoma with light chain discrepancy between B lymphocyte population and serum paraprotein. Ann Clin Lab Sci 2015;45:593–7.
18. Varettoni M, Arcaini L, Zibellini S, Boveri E, Rattotti S, Riboni R, et al. Prevalence and clinical significance of the MYD88 (L265P) somatic mutation in Waldenström's macroglobulinemia and related lymphoid neoplasms. Blood 2013;121:2522–8.

https://doi.org/10.3343/alm.2024.0644