Lymphoplasmacytic Lymphoma With a Non-IgM Paraprotein Shows Clinical and Pathologic Heterogeneity and May Harbor *MYD88* L265P Mutations

Rebecca L. King, MD,[1] Wilson I. Gonsalves, MD,[2] Stephen M. Ansell, MD, PhD,[2] Patricia T. Greipp, DO,[3] Lori A. Frederick, MS,[1] David S. Viswanatha, MD,[1] Rong He, MD,[1] Robert A. Kyle, MD,[2] Morie A. Gertz, MD,[2] Prashant Kapoor, MD,[2] William G. Morice, MD, PhD,[1] and Matthew T. Howard, MD[1]

From the [1]Division of Hematopathology, [2]Department of Hematology, and [3]Division of Laboratory Genetics, Mayo Clinic, Rochester, MN.

Key Words: Lymphoplasmacytic lymphoma; IgG; IgA; Non-IgM; Waldenström macroglobulinemia; *MYD88*; Bone marrow; Paraprotein

Am J Clin Pathol June 2016;145:843-851

DOI: 10.1093/AJCP/AQW072

ABSTRACT

Objectives: *Lymphoplasmacytic lymphoma (LPL) with non–immunoglobulin M (IgM) paraproteinemia remains poorly understood. The goal of this study was to investigate the clinicopathologic features of LPL in the bone marrow in patients with immunoglobulin G (IgG) or immunoglobulin A (IgA) paraproteins and evaluate* MYD88 *L265P mutation status to determine the relationship of these cases to Waldenström macroglobulinemia (WM).*

Methods: *Bone marrows from LPL cases with IgG or IgA paraproteins diagnosed between January 1, 2007, and June 30, 2014, were retrieved from the clinical archive. Clinicopathologic features were retrospectively reviewed.* MYD88 *L265P mutation status was assessed by allele-specific polymerase chain reaction prospectively on all cases.*

Results: *Of 27 cases, four were reclassified as multiple myeloma, all* MYD88 *mutation negative.* MYD88 *L265P mutations were present in 10 (43%) of 23 remaining cases. No association between* MYD88 *status and bone marrow morphologic or phenotypic features, including the presence of Dutcher bodies, mast cells, expression of CD19 by plasma cells, or hemosiderin, was identified, although these features were present in a subset of cases, similar to WM. Clinical features of WM such as hyperviscosity were uncommon in this group and did not correlate with* MYD88 *status.*

Conclusions: *Non-IgM LPLs are a clinically and pathologically heterogeneous group and often harbor* MYD88 *L265P mutation, albeit at a lower rate than classic WM.* MYD88 *status does not correlate with any specific pathologic or clinical manifestations.*

Lymphoplasmacytic lymphoma (LPL) is a low-grade B-cell neoplasm exhibiting a spectrum of B-cell differentiation ranging from small lymphocytes to plasma cells. LPL typically involves the bone marrow (BM) and spleen but may also involve lymph nodes and other extranodal sites.[1] Most patients have the clinical syndrome of Waldenström macroglobulinemia (WM), which is defined as LPL with an associated immunoglobulin M (IgM) serum monoclonal protein of any size.[1] WM often presents with clinical symptoms related to the IgM paraproteinemia, including hyperviscosity, cryoglobulinemia, demyelinating neuropathy, and occasionally amyloid light chain (AL) amyloidosis.[2]

LPL is most often diagnosed in the BM, although the diagnosis can be challenging due to morphologic and immunophenotypic overlap with other low-grade B-cell lymphomas and multiple myeloma. Immunophenotypically, LPL is characterized by a clonal B-cell population with expression of pan–B-cell markers, including CD19, CD20, CD79a, and Pax5, with some cases expressing CD38.[3-6] CD138 is not expressed on the B cells, although there is typically also a clonal plasma cell population with expression of CD38 and CD138. Reported expression of CD5 by the B cells varies from 10% to 40% and of CD10 approximately 7% to 10%. In contrast to multiple myeloma, the plasma cells most often retain expression of CD19 and CD45, albeit not universally.[5] Overall, however, the phenotype of the B cells and plasma cells in LPL is relatively nonspecific and does not help in distinguishing it from other low-grade B-cell disorders that may exhibit plasmacytic differentiation.

Recently, whole-genome sequencing studies have identified mutations in the *MYD88* gene (*MYD88* L265P) in 90% to 95% of WM cases, and testing for this abnormality

offers an appealing diagnostic aid in these difficult-to-classify cases.[7-10] Follow-up studies have confirmed this finding and have shown that the mutation is much less frequently present in marginal zone lymphoma (MZL), plasma cell myeloma, and other low-grade B-cell neoplasms.[9,11,12]

Due to their frequent co-occurrence, the terms *LPL* and *WM* are often used interchangeably in clinical practice and the literature. However, although the clinical diagnosis of WM is restricted to cases with an IgM monoclonal protein, the World Health Organization (WHO) recognizes that LPL can rarely present with paraproteins of immunoglobulin G (IgG) or immunoglobulin A (IgA).[1] Due to the paucity of literature describing the clinicopathologic features of LPL associated with non-IgM paraproteinemia, as well as the challenging nature of the diagnosis, the relationship of such cases to WM remains unclear.

In this study, we investigated the clinicopathologic features of a cohort of patients with low-grade B-cell lymphomas showing lymphoplasmacytic differentiation in patients with IgG or IgA monoclonal proteins and evaluated *MYD88* L265P mutation status in an effort to determine the relationship of these cases to "typical" WM/LPL cases. Here we highlight the challenge that these cases pose to both the pathologist and the clinician.

Materials and Methods

Case Selection and Clinicopathologic Review

Following institutional review board approval, the laboratory information system at the Mayo Clinic in Rochester, Minnesota, was queried for BM cases diagnosed as either LPL or low-grade B-cell lymphoma with plasmacytic differentiation between January 2007 and June 2014. Initial diagnoses of LPL were made based on WHO criteria. The concurrent serum electrophoresis and immunofixation results were reviewed for each patient, and only those who had a non-IgM paraprotein were included in the study cohort. The electronic medical record for each patient in the study cohort was reviewed. For all cases except two in which slides could not be located, Wright-Giemsa–stained BM aspirate slides and H&E-stained BM core biopsy specimens were reviewed by two hematopathologists (R.L.K. and M.T.H.).

If available, flow cytometric immunophenotyping reports and/or histograms were retrospectively reviewed and the immunophenotype of the clonal B-cell and plasma cell populations was recorded. Flow cytometric immunophenotyping, when performed as part of the diagnostic evaluation, was done according to standard, previously published methods.[4,13,14] Corollary cytogenetic information, including chromosome analysis and fluorescence in situ hybridization (FISH), was also reviewed, as available.

MYD88 L265P Mutation Analysis

DNA was extracted using the Qiagen DNeasy kit (Qiagen, Valencia, CA) from archived BM aspirate sample pellets fixed in methanol–acetic acid. A single-tube multiplex allele-specific polymerase chain reaction (PCR) was performed using primers situated in exon 5 of *MYD88* (NM_002468.4), including one primer specifically targeting the c.794T > C; Leu265Pro (L256P) alteration. Reaction products were analyzed using capillary electrophoresis (QIAxcel; Qiagen); *MYD88* control amplification yields a PCR product of 141 base pairs (bp), and if present, an additional specific 72-bp product denotes the L265P mutation.

Statistical Analysis

Statistical analysis was performed using the biostatistical software JMP 10.0.1 (SAS Institute, Cary, NC). The Fisher exact test was used to compare differences between nominal variables, and the Kruskal-Wallis test was used for continuous variables.

Results

The initial study group included 27 patients diagnosed with either a WHO diagnosis of LPL or a more general diagnosis of low-grade B-cell lymphoma with plasmacytic differentiation involving the BM. Upon review of clinical, morphologic, and laboratory data, four patients were reclassified as having multiple myeloma **Table 1**. The initial diagnosis was lymphoplasmacytic neoplasm in two cases and low-grade B cell lymphoma with plasmacytic differentiation in the other two cases. None of the four patients had lytic bone lesions. In the first case, there was a t(11;14) *CCND1/IGH* identified in plasma cells, supporting a diagnosis of multiple myeloma.[15] This case showed CD20 positivity by immunohistochemistry in plasma cells, a feature that is commonly seen in CCND1-positive plasma cell neoplasms.[16] This patient was subsequently lost to follow-up at our institution. In the second case, although there were distinct lymphoid aggregates in the BM, no clonal B-cell population was identified by flow cytometry, and the lymphoid aggregates were composed of predominantly T cells. She responded well to bortezomib, cyclophosphamide, dexamethasone, and rituximab followed by autologous stem cell transplant (SCT). The third patient had amyloidosis, and although clonal B cells and plasma cells were identified by flow cytometry, the BM showed predominantly atypical plasma cells. FISH studies revealed a deletion of chromosome 13q, supporting

DOI: 10.1093/ajcp/aqw072

Table 1
Features of Cases Initially Diagnosed as B-Cell Lymphoma With Plasmacytic Differentiation and Reclassified as Multiple Myeloma[a]

Case No.	Initial Diagnosis	Genetics	Paraprotein	Amyloid	Pathology
1	Lymphoplasmacytic neoplasm	t(11;14)	IgA κ	No	Clonal B cells by flow; CD20 positivity in plasma cells
2	Low-grade B-cell lymphoma with plasmacytic differentiation	46(XX)	IgA κ	Yes	No clonal B cells by flow; lymphoid aggregates in BM are T cells
3	Low-grade B-cell lymphoma with plasmacytic differentiation	del13q	IgG κ	No	Clonal B cells by flow; morphology shows only atypical plasma cells; responded to MM treatment
4	Lymphoplasmacytic neoplasm	IgH translocation to unknown partner	IgG λ	Yes	Clonal B cells by flow; morphology shows predominantly atypical plasma cells; responded to MM treatment

BM, bone marrow; IgA, immunoglobulin A; IgG, immunoglobulin G; IgH, immunoglobulin H; MM, multiple myeloma.
[a]All four cases are negative for a *MYD88* mutation.

Table 2
Summary of Clinical Features of *MYD88*-Mutated and Unmutated Cases

Clinical Features	*MYD88* Positive (n = 10)	*MYD88* Negative (n = 13)	*P* Value
Age, median (range), y	74 (54-80)	79 (46-86)	.385
Male sex, No.	8	9	.660
M spike (range), g/dL	2.7 (1-7.8)	1.6 (0-5.6)	.475
Paraprotein, No.			
IgG κ	6	9	.414
IgG λ	2	4	1.000
IgA κ	1	0	.435
IgA λ	1	0	.435
Organomegaly, No.	1	5	.179
Lymphadenopathy, No.	3	7	.402

IgA, immunoglobulin A; IgG, immunoglobulin G.

Table 3
Summary of Pathologic Features of *MYD88*-Mutated and Unmutated Cases[a]

Pathologic Characteristic	*MYD88* Positive (n = 10)	*MYD88* Negative (n = 11)	*P* Value
BM involvement, median (range), %	55 (5-90)	20 (10-70)	.119
Dutcher bodies[b]	1	1	1.000
Hemosiderin[b]	2	4	.608
Mast cells[b]	5	3	.619
Interstitial only	5	2	.417
Aggregates only	3	7	.213
Both aggregates and interstitial	2	2	1.000
Nonparatrabecular aggregates	2	4	.405
Paratrabecular aggregates or both	3	5	1.000
Distribution			
Lymphocytes > plasma cells	8	5	.197
Plasma cells > lymphocytes	2	4	.660
Approximately equal	0	2	.486
Immunophenotype			
Plasma cells CD19 + (at least partial)[c]	10	6	.087
Plasma cells CD45 + (at least partial)[c]	9	8	1.000
B cells CD10–/CD5–[d]	8	6	.577

BM, bone marrow.
[a]Values are presented as numbers unless otherwise indicated.
[b]Cases with less than 10% involvement were not scored for these features; n = 8 for each mutation status.
[c]Data only available on a subset of cases; n = 10 for each mutation status.
[d]Data only available on a subset of cases; n = 9 for *MYD88* positive and n = 8 for *MYD88* negative.

multiple myeloma. The patient's disease was refractory to rituximab, cyclophosphamide, vincristine, and prednisone but was subsequently successfully treated with bortezomib and dexamethasone followed by autologous SCT. The fourth patient had a small clonal B-cell population detected by flow cytometry, but the morphology demonstrated predominantly atypical plasma cells. Overall, this patient clinically was believed to have myeloma and AL amyloidosis. This patient was successfully treated with lenalidomide and dexamethasone followed by autologous SCT. All four of these cases were negative for the *MYD88* L265P mutation.

Of the remaining 23 patients, 17 (74%) were diagnosed by the signing pathologist as having low-grade B-cell lymphoma with plasmacytic differentiation, five (22%) as having LPL (WHO diagnosis), and one (4%) as having a lymphoplasmacytic neoplasm. In this group, seven (30%) received some form of therapy prior to evaluation at our institution. *MYD88* L265P mutations were present in 10 (43%) of 23 cases. No statistically significant association with WM symptoms, paraprotein level, or specific BM morphologic or phenotypic features was identified **Table 2** and **Table 3**. Only two IgA cases were identified, both with *MYD88* mutations; however, the small number of cases precludes drawing conclusions with regard to an association. Clinical features are summarized in **Table 4**.

No patients had a definitive extramedullary tissue diagnosis of another type of lymphoma either prior to or following the BM diagnosis during the follow-up period of the study. One patient had a breast biopsy performed concurrent to the BM diagnosis, which showed a lymphoplasmacytic infiltrate similar to that seen in the BM. A second patient had a lymph node biopsy prior to being seen at our institution that reportedly showed a low-grade B-cell lymphoma

DOI: 10.1093/ajcp/aqw072

Table 4
Clinical Characteristics of All Patients Included in the Study

Mutation Status	Patient No.	Age, y	Sex	Paraprotein	Paraprotein Level, g/dL	Progressive Anemia	Lymphadenopathy
MYD88 positive	1	54	M	IgA κ	1.0	Yes	No
	2	70	M	IgA λ	2.5	Yes	No
	3	80	M	IgG κ	3.0	Yes	No
	4	62	M	IgG κ	3.0	No	No
	5	74	M	IgG κ	2.9	No	Yes
	6	80	M	IgG λ	1.8	No	No
	7	66	F	IgG κ	4.3	No	No
	8	72	F	IgG κ	1.4	Yes	Yes
	9	80	M	IgG λ	2.0	No	No
	10	76	M	IgG κ, κ	7.8	Yes	Yes
MYD88 negative	11	78	M	IgG κ	0.7	No	No
	12	67	M	IgG κ	3.0	Yes	Yes
	13	68	M	IgG κ	0.7	No	Yes
	14	73	F	IgG κ	7.0	Yes	Yes
	15	60	F	IgG κ	0[a]	Yes	No
	16	46	M	IgG κ	0.6	No	Yes
	17	64	M	IgG κ	1.6	Yes	Yes
	18	61	M	IgG κ, κ	3.7	Yes	No
	19	55	F	IgG λ	1.4	No	No
	20	86	M	IgG λ	1.1	Yes	Yes
	21	81	M	IgG λ	3.4	No	Yes
	22	55	M	IgG λ, λ	5.6	Yes	No
	23	79	F	IgG κ	4.2	Yes	No

ASCT, autologous stem cell transplantation; BR, bendamustine and rituximab; CyBorD, cytoxan, bortezomib, and dexamethasone; IgA, immunoglobulin A; IgG, immunoglobulin G; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; R-CVP, rituximab, cyclophosphamide, vincristine, and prednisone.
[a]IgG κ identified by immunofixation.

with plasmacytic differentiation, not further classifiable; this material was not available for review by our pathologists. Both of these patients were negative for *MYD88* mutations.

Reports of clinical features typically associated with WM were extracted from the medical record (Tables 2 and 4). Serum viscosity was measured in 10 patients and ranged from 0 to 2.7. Generally, clinical hyperviscosity syndrome is unlikely unless the serum viscosity is greater than 4.[17] Interestingly, two patients (both negative for *MYD88*) reported visual changes at diagnosis, although their serum viscosity was only 1.5 and 2.0, respectively. The relationship of these findings to the patients' lymphoma is uncertain given the lack of hyperviscosity in either case. Neuropathy was reported in three (13%) patients: one patient had coexisting light chain amyloidosis that was presumed to be the cause of the peripheral neuropathy, the second patient did not have amyloidosis but electromyography revealed an axonal length-dependent sensorimotor peripheral neuropathy, and the third patient experienced burning sensation in her feet at the time of diagnosis, but no confirmatory testing was performed. One patient had a low-grade hemolytic anemia, whereas cryoglobulinemia (type II and type III) was identified in two (9%) patients. No other autoimmune phenomena were noted in any patients. Coagulopathy was not reported in any patient. Acute kidney injury was present in four (17%) cases, attributable to amyloid deposition in one, light chain deposition disease in one, membranous glomerulonephritis in one, and unknown causes in the fourth. None of these features showed significant correlation with *MYD88* mutation status. Management in this group of patients was heterogeneous, as shown in Table 4.

The degree of BM involvement by LPL ranged from 5% to 90%. Slides were available for review in 21 of 23 cases **Image 1**. In 13 (62%) cases, the infiltrate was noted to be predominantly lymphoid, with a minor plasma cell component. In six (29%), plasma cells predominated, and in two (10%), approximately equal numbers of both were seen. The infiltrates were prominent aggregates in 10 (48%) cases, purely interstitial in seven (33%) cases, and a combination of interstitial and aggregates in four (19%) cases. Among cases with aggregates, six (43%) of 14 had nonparatrabecular aggregates, and the remaining eight (57%) had some degree of paratrabecular aggregates with or without a nonparatrabecular component. The breakdown of these features by *MYD88* status is shown in Table 3.

DOI: 10.1093/ajcp/aqw072

Organomegaly	Visual Changes	Neuropathy	Amyloid	Regimens Used for Treatment
No	No	No	No	None (observation)
No	No	No	Yes	R-CHOP followed by consolidative ASCT
Yes	No	No	Yes	R-CVP
No	No	No		Lenalidomide-cytoxan-dexamethasone; BR followed by maintenance rituximab
No	No	No	No	R-CVP; BR
No	No	No		None (observation)
No	No	No		BR
No	No	No		BR
No	No	No	Yes	Melphalan-dexamethasone
No	No	No	No	BR
No	No	Yes	No	None (observation)
Yes	Yes	No		R-CVP
No	No	No	No	R-CVP; R-CHOP
No	No	No	No	Lenalidomide-rituximab-cytoxan-dexamethasone
Yes	No	No	No	R-CVP
Yes	No	No		Lenalidomide-rituximab-cytoxan-dexamethasone
No	No	No	Yes	CyBorD followed by consolidative ASCT
No	No	No		Bortezomib-dexamethasone
No	No	No		Rituximab; bortezomib
No	No	No		Rituximab-prednisone; chlorambucil
No	No	No		None
Yes	Yes	No		Pentostatin-cytoxan-rituximab; rituximab-bendamustine followed by rituximab maintenance
Yes	No	Yes		Rituximab

Review of the core biopsy specimen for Dutcher bodies and hemosiderin was performed in cases with slides available and with greater than 10% lymphomatous involvement. Plasma cells had identifiable Dutcher bodies in two (13%) of 16 evaluable cases. Of note, one additional case with less than 10% involvement did have easily identifiable Dutcher bodies despite the low level of involvement. Histiocytes containing hemosiderin in close association with the lymphomatous infiltrate were seen in six (38%) of 16 cases. Similarly, aspirate smears were evaluated for increased numbers of mast cells when there was greater than 10% involvement by lymphoma. Pathologist reviewers noted whether mast cells were easily identifiable and present in greater than three consecutive high-power fields (×600). Aspirate smears showed increased numbers of cytologically normal mast cells in eight (50%) of 16 (50%). *MYD88* status did not correlate significantly with the presence of these features (Table 3). Amyloid was present in four (17%) of 23 cases, identified as κ or λ light chain in two cases each by mass spectrometry.[18] *MYD88* mutations were found in three (75%) of four patients with amyloid.

B-cell and plasma cell flow cytometry was performed in 20 (87%) of 23 cases. One additional case had only B-cell flow cytometry performed. All 20 patients had both a clonal B-cell and a clonal plasma cell population with the same light chain. In all cases, plasma cells expressed both CD38 and CD138. CD19 was expressed in at least a subset of plasma cells in 16 (80%) of 20, and CD45 was expressed in 17 (85%) of 20. B cells were positive for CD19 and showed surface light chain expression in all cases. CD5 was expressed on B cells in three (18%) of 17 cases in which it was evaluated, and one with CD5 expression also showed expression of CD10. The remaining 14 (82%) showed a CD5–, CD10– B-cell phenotype. The breakdown of these phenotypic features by *MYD88* status is shown in Table 3.

Conventional karyotype was performed on 18 (78%) of 23 lymphoma cases and revealed a normal karyotype in 12, with three additional cases showing only loss of the Y chromosome. The remaining three cases showed 20q deletion (3/20), trisomy 15 (4/20), and t(9;12) (q22;q22) (3/20), respectively.

Discussion

The diagnosis of low-grade B-cell lymphoma with plasmacytic differentiation in the BM is challenging due to

DOI: 10.1093/ajcp/aqw072

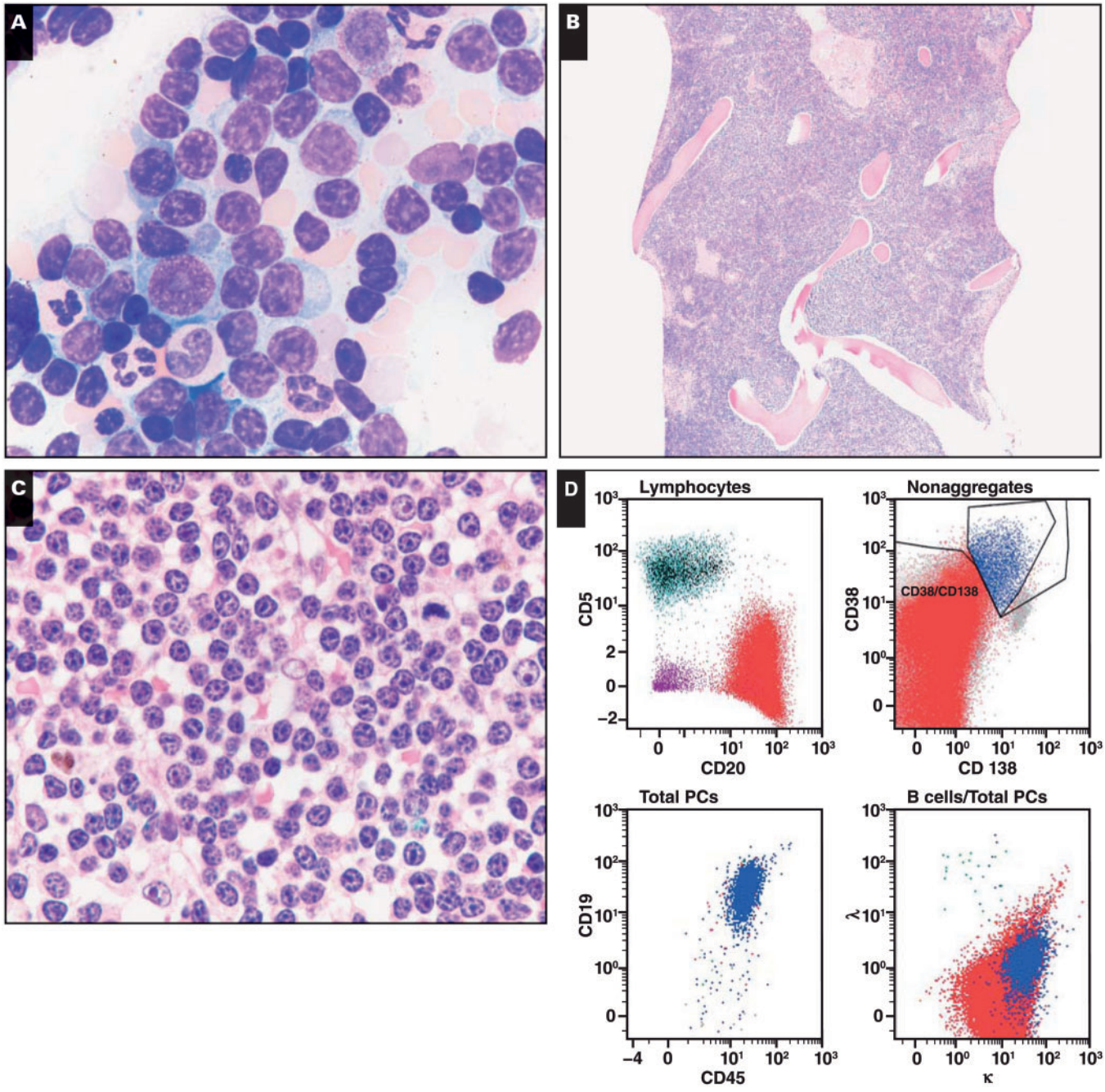

Image 1 Bone marrow features of a representative case from this series. The patient has an immunoglobulin G κ serum monoclonal protein. **A**, Bone marrow aspirate shows extensive involvement by an infiltrate with a spectrum of cytology from small lymphocytes to lymphoplasmacytoid cells to true plasma cells (Wright-Giemsa, ×2,000). **B**, **C**, Bone marrow core biopsy specimen shows marrow replacement by a diffuse lymphoplasmacytic infiltrate (H&E; **B**, ×80; **C**, ×800). **D**, Flow cytometry reveals a CD20-positive, κ light chain–restricted B-lymphocyte population in red (gating strategy not shown). Variable CD38 is present on lymphocytes. Gating on CD138/CD38-positive plasma cells (PCs) (blue) shows a population of κ-restricted PCs that coexpress CD19 and CD45.

overlapping histopathologic and immunophenotypic features of various entities. Typically, the differential diagnosis rests between LPL and MZL, which are the most common low-grade lymphomas to exhibit plasmacytic differentiation. In addition, multiple myeloma can exhibit lymphoplasmacytoid features and may mimic a low-grade lymphoma pathologically. Finally, flow cytometry may reveal a possibly unrelated monoclonal B-cell lymphocytosis (MBL), which can further complicate the picture. Often a definitive diagnosis cannot be made at the time of BM biopsy. In our series, only five (18%)

DOI: 10.1093/ajcp/aqw072

of 27 were initially given a WHO diagnosis of LPL. Since pathologic features alone are often insufficient to distinguish between LPL and MZL, we may rely on the presence or absence of the clinical findings typically associated with WM, especially an IgM paraprotein, to aid in the diagnosis of LPL. However, MZL may be associated with an IgM paraprotein, and infrequently, patients with LPL can have non-IgM paraproteins.[1] Although the WHO acknowledges their existence, these patients with non-IgM LPL are rare and therefore are poorly characterized. Given the difficulties in distinguishing LPL from other B-cell and plasma cell disorders, cases with non-IgM paraproteins present a diagnostic dilemma. Their clinical relationship to WM is unclear due to the challenges associated with identifying them.

The recent discovery of *MYD88* L265P mutations in more than 90% of LPL/WM has aided our understanding of this disease entity and heralds a possible new era in lymphoma classification. However, it is clear that approximately 10% of MZLs also carry this mutation,[9,12,19] which raises the question of whether the histopathologic or molecular genetic features should take precedence in the classification of these diseases. Although a few series evaluating *MYD88* mutation status in LPLs have included small numbers of non-IgM cases,[9,19,20] most have evaluated well-established LPL/WM, usually with an IgM paraprotein. By comparison, our study is unique in that we began with a series of LPL cases associated with non-IgM paraproteinemia and attempted to refine our understanding of this disease based on *MYD88* status and clinicopathologic review.

Here we have demonstrated that non-IgM, BM-based low-grade B-cell lymphomas with plasmacytic differentiation are pathologically heterogeneous and remain difficult to classify even with the addition of modern molecular tools. In retrospect, we were able to identify four cases from our original series that were reclassified as multiple myeloma, some with a concurrent MBL. This distinction is critical for therapeutic decision making and may be even more challenging when faced with a posttherapy BM.

The *MYD88* mutation was identified in 43% of our cases, suggesting that many of these non-IgM cases may in fact be biologically related to WM. These results are supported by a small number of cases from other studies, in which non-IgM LPL cases were evaluated for *MYD88*. Treon et al[9] initially reported *MYD88* mutations in three of three non-IgM LPL cases, and Bassarova et al[19] report two of two IgG LPLs positive for the mutation. Manasanch et al[20] presented a case of IgG LPL that was negative for the mutation. Selection bias is probable in these reports; in the current diagnostic era, it is more likely that a non-IgM secreting neoplasm will be given a formal diagnosis of LPL if it harbors a *MYD88* mutation.

Dissecting out which cases in our series represent "non-LPL" low-grade B cell lymphomas remains challenging, even with current technologies. Although some of the cases in our series may represent BM involvement by MZL, all of the included cases had predominantly BM-based infiltrates, and none had a definitive extramedullary diagnosis of MZL. The *MYD88*-positive cases, many of which were initially diagnosed as low-grade B cell lymphoma with plasmacytic differentiation, are more easily now classified as LPL in our practice. Exact classification of the *MYD88*-negative cases remains challenging.

In addition to providing a potential molecular marker for disease classification, the discovery of the *MYD88* mutation has focused the spotlight on the nuclear factor κB (NF-κB) pathway in LPL.[9] The somatic *MYD88* L265P mutation triggers activation of NF-κB via two distinct pathways involving Bruton's tyrosine kinase (BTK) and interleukin 1 receptor–associated kinases, IRAK1 and IRAK4.[21] The presence of this mutation in such a high proportion of LPL/WM, as well as in up to 80% of IgM monoclonal gammopathy of uncertain significance, suggests that it is likely an early oncogenic event.[9,12,22-24]

In addition, recent data suggest that those with *MYD88* mutation-positive LPL/WM have greater disease burden, higher serum IgM levels, more symptomatic disease, and higher risk of death than their mutation-negative counterparts.[25,26] Treon et al[26] also highlighted the significance of C-terminus mutations in the *CXCR4* gene, showing that cases with *MYD88* L265P mutations and concomitant *CXCR4* nonsense mutations have more aggressive features and a higher disease burden than *MYD88*-mutated cases with either wild-type or frameshift mutations in *CXCR4*; however, overall survival was not affected by the presence of the *CXCR4* mutation.

Interestingly, the presence or absence of *MYD88* in our series did not correlate significantly with any specific pathologic or clinical features that are typically associated with LPL. Although historically, the presence of mast cells, hemosiderin-laden macrophages, and paratrabecular aggregates was often associated with LPL BM infiltrates, these features are generally thought to be nonspecific.[6,27,28] In a series of pathologically and clinically well-established cases, paratrabecular lymphoid aggregates, lymphoplasmacytoid cells, Dutcher bodies, and mast cells were recently shown to be significantly associated with LPL compared with MZL in the bone marrow.[19] In contrast, our study does not support the specificity of these features in ambiguous non-IgM cases where the diagnosis of LPL is not well established. We do, nevertheless, find that non-IgM LPL cases may have "classic" LPL features such as mast cells, hemosiderin, and paratrabecular infiltrates similar to IgM LPLs. Notably, the lack of correlation of these features with *MYD88* mutation status suggests the heterogeneity within this disease is incompletely understood.

DOI: 10.1093/ajcp/aqw072

This study reveals that WM-associated symptoms are not often seen in the non-IgM cases, an unsurprising finding given that most such symptoms are directly attributable to the large IgM protein itself.[29] However, many of the clinical features of WM are nonspecific, and often one or two of these features may be seen in a given patient with non-IgM disease.

From a therapeutic perspective, the presence or absence of mutations in *MYD88* (and possibly *CXCR4*) may prove more significant than the presence of a specific paraprotein or histopathologic features. Ibrutinib, a small-molecule inhibitor of BTK, has shown efficacy in LPL/WM and demonstrates improved response rates in patients with *MYD88* mutations compared with their mutation-negative counterparts, with the response rate being slightly abrogated in those with the *MYD88* mutant/*CXCR4* mutant genotype.[30] This again demonstrates the need to more accurately identify and subclassify these non-IgM LPL cases to ensure appropriate molecular evaluation.

With only two cases in our series having biopsy-proven extramedullary disease, conclusions about nodal-based non-IgM LPL without marrow involvement are not able to be drawn here. This interesting cohort most assuredly exists, although distinction from nodal MZLs clearly will remain challenging. Future studies in this area are warranted to continue to understand non-IgM LPL.

In conclusion, this study confirms the clinical, pathologic, and genetic heterogeneity inherent to low-grade B-cell lymphomas with plasmacytic differentiation and illustrates that many non-IgM LPL cases share characteristics with IgM LPL. The addition of *MYD88* L265P testing to the diagnostic armamentarium has enhanced our understanding of the biology of these unusual lymphomas. Whether *MYD88* should be routinely used to render a WHO diagnosis of LPL in otherwise ambiguous cases is a subject for continued debate. Regardless, it is clear that many non-IgM LPL cases do show pathologic similarity to IgM cases, harbor *MYD88* mutations, and likely warrant *MYD88* testing for potential therapeutic reasons.

Corresponding author: Rebecca L. King, Mayo Clinic, Hilton 8-17, 200 First St SW, Rochester, MN 55905; rebecca.king98@gmail.com.

References

1. Swerdlow S, Harris NL, Jaffe ES, et al, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France; IARC; 2008.
2. Dispenzieri A, Buadi F, Kumar SK, et al. Treatment of immunoglobulin light chain amyloidosis: mayo stratification of myeloma and risk-adapted therapy (mSMART) consensus statement. *Mayo Clin Proc*. 2015;90:1054-1081.
3. Howard MT, Hodnefield J, Morice WG. Immunohistochemical phenotyping of plasma cells in lymphoplasmacytic lymphoma/Waldenström's macroglobulinemia is comparable to flow cytometric techniques. *Clin Lymphoma Myeloma Leuk*. 2011;11:96-98.
4. Morice WG, Chen D, Kurtin PJ, et al. Novel immunophenotypic features of marrow lymphoplasmacytic lymphoma and correlation with Waldenström's macroglobulinemia. *Mod Pathol*. 2009;22:807-816.
5. Rosado FG, Morice WG, He R, et al. Immunophenotypic features by multiparameter flow cytometry can help distinguish low grade B-cell lymphomas with plasmacytic differentiation from plasma cell proliferative disorders with an unrelated clonal B-cell process. *Br J Haematol*. 2015;169:368-376.
6. Remstein ED, Hanson CA, Kyle RA, et al. Despite apparent morphologic and immunophenotypic heterogeneity, Waldenström's macroglobulinemia is consistently composed of cells along a morphologic continuum of small lymphocytes, plasmacytoid lymphocytes, and plasma cells. *Semin Oncol*. 2003;30:182-186.
7. Treon SP, Hunter ZR. A new era for Waldenström macroglobulinemia: MYD88 L265P. *Blood*. 2013;121:4434-4436.
8. Treon SP, Hunter ZR, Castillo JJ, et al. Waldenström macroglobulinemia. *Hematol Oncol Clin North Am*. 2014;28:945-970.
9. Treon SP, Xu L, Yang G, et al. MYD88 L265P somatic mutation in Waldenström's macroglobulinemia. *N Engl J Med*. 2012;367:826-833.
10. Poulain S, Roumier C, Decambron A, et al. MYD88 L265P mutation in Waldenström macroglobulinemia. *Blood*. 2013;121:4504-4511.
11. Ondrejka SL, Lin JJ, Warden DW, et al. MYD88 L265P somatic mutation: its usefulness in the differential diagnosis of bone marrow involvement by B-cell lymphoproliferative disorders. *Am J Clin Pathol*. 2013;140:387-394.
12. Jimenez C, Sebastian E, Chillon MC, et al. MYD88 L265P is a marker highly characteristic of, but not restricted to, Waldenström's macroglobulinemia. *Leukemia*. 2013;27:1722-1728.
13. Morice WG, Kurtin PJ, Hodnefield JM, et al. Predictive value of blood and bone marrow flow cytometry in B-cell lymphoma classification: comparative analysis of flow cytometry and tissue biopsy in 252 patients. *Mayo Clin Proc*. 2008;83:776-785.
14. Morice WG, Hanson CA, Kumar S, et al. Novel multi-parameter flow cytometry sensitively detects phenotypically distinct plasma cell subsets in plasma cell proliferative disorders. *Leukemia*. 2007;21:2043-2046.
15. King RL, Howard MT, Hodnefield JM, et al. IgM multiple myeloma: pathologic evaluation of a rare entity. *Am J Clin Pathol*. 2013;140:519-524.
16. Owen RG, Kyle RA, Stone MJ, et al. Response assessment in Waldenström macroglobulinaemia: update from the VIth International Workshop. *Br J Haematol*. 2013;160:171-176.
17. Roccaro AM, Sacco A, Jimenez C, et al. C1013G/CXCR4 acts as a driver mutation of tumor progression and modulator of drug resistance in lymphoplasmacytic lymphoma. *Blood*. 2014;123:4120-4131.
18. Ghobrial IM. Are you sure this is Waldenström macroglobulinemia? *Hematology Am Soc Hematol Educ Program*. 2012; 2012:586-594.

DOI: 10.1093/ajcp/aqw072

19. Bassarova A, Troen G, Spetalen S, et al. Lymphoplasmacytic lymphoma and marginal zone lymphoma in the bone marrow: paratrabecular involvement as an important distinguishing feature. *Am J Clin Pathol*. 2015;143:797-806.
20. Manasanch EE, Braylan R, Stetler-Stevenson M, et al. Lack of MYD88 L265P in non-immunoglobulin M lymphoplasmacytic lymphoma. *Leuk Lymphoma*. 2014;55:1402-1403.
21. Yang G, Zhou Y, Liu X, et al. A mutation in MYD88 (L265P) supports the survival of lymphoplasmacytic cells by activation of Bruton tyrosine kinase in Waldenstrom macroglobulinemia. *Blood*. 2013;122:1222-1232.
22. Xu L, Hunter ZR, Yang G, et al. MYD88 L265P in Waldenström macroglobulinemia, immunoglobulin M monoclonal gammopathy, and other B-cell lymphoproliferative disorders using conventional and quantitative allele-specific polymerase chain reaction. *Blood*. 2013;121:2051-2058.
23. Landgren O, Staudt LM. MYD88 L265P somatic mutation in IgM MGUS. *N Engl J Med*. 2012;367:2255-2257.
24. Varettoni M, Arcaini L, Zibellini S, et al. Prevalence and clinical significance of the MYD88 (L265P) somatic mutation in Waldenström's macroglobulinemia and related lymphoid neoplasms. *Blood*. 2013;121:2522-2528.
25. Patkar N, Subramanian PG, Deshpande P, et al. MYD88 mutant lymphoplasmacytic lymphoma/Waldenström macroglobulinemia has distinct clinical and pathological features as compared to its mutation negative counterpart. *Leuk Lymphoma*. 2015;56:420-425.
26. Treon SP, Cao Y, Xu L, et al. Somatic mutations in MYD88 and CXCR4 are determinants of clinical presentation and overall survival in Waldenström macroglobulinemia. *Blood*. 2014;123:2791-2796.
27. Lin P, Molina TJ, Cook JR, et al. Lymphoplasmacytic lymphoma and other non-marginal zone lymphomas with plasmacytic differentiation. *Am J Clin Pathol*. 2011; 136:195-210.
28. Sovani V, Harvey C, Haynes AP, et al. Bone marrow trephine biopsy involvement by lymphoma: review of histopathological features in 511 specimens and correlation with diagnostic biopsy, aspirate and peripheral blood findings. *J Clin Pathol*. 2014;67:389-395.
29. Treon SP. How I treat Waldenström macroglobulinemia. *Blood*. 2009;114:2375-2385.
30. Treon SP, Tripsas CK, Meid K, et al. Ibrutinib in previously treated Waldenström's macroglobulinemia. *N Engl J Med*. 2015;372:1430-1440.

DOI: 10.1093/ajcp/aqw072