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MOLECULAR AND CLINICOPATHOLOGIC HETEROGENEITY OF IGM-NEGATIVE LYMPHOPLASMACYTIC LYMPHOMA: INSIGHTS FROM A PAKISTANI MULTICENTER STUDY

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ABSTRACT

Background: Lymphoplasmacytic lymphoma (LPL) is diagnostically and therapeutically defined by its association with Waldenström Macroglobulinemia (WM) and an IgM paraprotein. The IgM-negative LPL variant is a rare and poorly characterized entity, with limited data on its molecular drivers and clinical behavior, particularly in non-Western populations. **Aims & Objectives:** This study aimed to define the clinicopathologic and molecular profile of IgM-negative LPL and compare it with classic IgM-positive WM in a Pakistani multicenter cohort. **Methodology:** A total of 92 patients diagnosed between March 2024 and July 2025 were enrolled from across three major healthcare institutions in Pakistan: Sheikh Zayed Hospital, both campuses Lahore & Rahim Yar Khan; and Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore. 47 with IgM-negative LPL and 45 with WM. Clinicopathologic data were collected retrospectively and prospectively. Centralized pathological review and molecular analysis for MYD88 L265P (by allele-specific PCR and Sanger sequencing) and CXCR4 mutations (by Sanger sequencing) were performed. **Results & Findings:** The IgM-negative cohort exhibited a significantly more aggressive phenotype, with higher rates of splenomegaly (48.9% vs. 26.7%, p=0.025), lower platelet counts (median 165 vs. 212 x 10^9/L, p=0.008), and higher serum creatinine (median 1.4 vs. 1.0 mg/dL, p=0.003). The MYD88 L265P mutation was significantly less prevalent in IgM-negative LPL (63.8%) compared to WM (95.6%, p<0.001). Within the IgM-negative group, MYD88 wild-type status was associated with worse cytopenias and renal impairment. With a median follow-up of 14 months, overall survival was significantly inferior for IgM-negative patients (12-month OS 85% vs. 98%, p=0.013). **Conclusion:** IgM-negative LPL represents a distinct, more aggressive subtype of LPL characterized by a high burden of extramedullary disease, cytopenias, renal impairment, a lower frequency of MYD88 mutations, and inferior survival. These findings advocate for the integration of immunoglobulin isotype and molecular profiling into the diagnostic and prognostic stratification of LPL to guide personalized therapy.

Keywords: Lymphoplasmacytic lymphoma, Waldenström Macroglobulinemia, IgM-negative, MYD88, Pakistan, Clinicopathologic heterogeneity.

INTRODUCTION

Lymphoplasmacytic lymphoma (LPL) is a rare, indolent mature B-cell neoplasm, accounting for approximately 1-2% of all non-Hodgkin lymphomas (NHLs) (Swerdlow et al., 2017). It is characterized by a pathological triad: a proliferation of small B-lymphocytes, plasmacytoid lymphocytes, and plasma cells, typically involving the bone marrow, and frequently associated with the secretion of a monoclonal immunoglobulin (Ig). The diagnostic and clinical paradigm of LPL has been overwhelmingly dominated by its association with Waldenström macroglobulinemia (WM), defined by the presence of an IgM monoclonal gammopathy. This linkage is so entrenched that the 2017 revision of the World Health Organization (WHO) classification of lymphoid neoplasms categorizes LPL and WM as essentially synonymous, with WM representing the clinicopathological manifestation of LPL with bone marrow involvement and an IgM paraprotein (Swerdlow et al., 2017).
The molecular landscape of LPL/WM was revolutionized by the discovery of a highly recurrent, somatic point mutation (L265P) in the MYD88 gene, found in over 90% of WM cases (Treon et al., 2012). This gain-of-function mutation, leading to constitutive activation of the NF-κB signaling pathway, has become a cornerstone for diagnosis, serving as a critical discriminatory tool to distinguish LPL/WM from other B-cell malignancies with overlapping morphological features, such as marginal zone lymphoma (MZL) and chronic lymphocytic leukemia (CLL). The subsequent identification of recurrent mutations in the CXCR4 gene, occurring in approximately 30-40% of LPL/WM cases, has further refined the molecular understanding of this disease, with these mutations often being associated with specific clinical features like higher serum IgM levels and resistance to certain targeted therapies like Ibrutinib (Treon et al., 2015; Cao et al., 2021). This molecular dyad of MYD88 and CXCR4 mutations has framed our contemporary understanding of LPL's pathogenesis and is increasingly used for risk stratification and therapeutic decision-making.
This well-established paradigm creates a significant diagnostic and biological blind spot: the entity known as IgM-negative LPL. By definition, these are lymphomas with the characteristic lymphoplasmacytic histopathology of LPL but which secrete non-IgM paraproteins (most commonly IgG or IgA) or are non-secretory. Due to its rarity, representing less than 5% of all LPL cases, IgM-negative LPL remains a poorly characterized and often controversial diagnostic category (Ojha et al., 2019; Lee et al., 2021). The diagnostic challenge is profound. Without the anchoring feature of an IgM paraprotein, pathologists and clinicians must rely heavily on morphology and immunophenotype to distinguish it from its close mimics, particularly IgM-negative marginal zone lymphoma (MZL) with plasmacytic differentiation and plasma cell neoplasms. This distinction is not merely academic; it carries significant implications for prognosis and treatment. For instance, the therapeutic algorithms for WM/LPL, which now include Bruton's tyrosine kinase (BTK) inhibitors highly effective in MYD88-mutated disease, may differ from those used for MZL or myeloma (Castillo et al., 2023).
The molecular profile of IgM-negative LPL is a critical area of uncertainty. While the MYD88 L265P mutation is near-ubiquitous in IgM-secreting LPL/WM, its prevalence in the IgM-negative subset is reported to be substantially lower and highly variable across small, single-center studies. Some reports suggest a frequency as low as 50-70%, while others describe cases that are entirely MYD88 wild-type (Gertz et al., 2016; Varettoni et al., 2017; Lee et al., 2021). This raises fundamental biological questions: Does IgM-negative LPL represent a distinct molecular subtype of LPL, or is it a heterogeneous group that may encompass misdiagnosed cases of MZL? What other genetic drivers might be operative in MYD88 wild-type cases? Are the co-mutational patterns, such as those involving CXCR4, ARID1A, or CD79B, similar to or different from their IgM-positive counterparts? The answers to these questions are crucial for validating IgM-negative LPL as a legitimate biological entity within the LPL spectrum and for understanding its pathogenesis. The clinical behavior and outcomes of patients with IgM-negative LPL are not well-defined. Small series have suggested potential differences in presentation, such as a lower incidence of hyperviscosity syndrome (due to

the different physicochemical properties of IgG/IgA) but a possibly higher risk of renal involvement or light chain cast nephropathy (Fonseca & Hayman, 2007; Lee et al., 2021). However, comprehensive data on progression-free survival, overall survival, and response to various therapies compared to WM are scarce. This lack of robust clinical data prevents the development of evidence-based management guidelines for this rare subgroup.

An additional layer of complexity, and a significant gap in the global literature, is the influence of ethnic and geographic variations on the disease biology of LPL. The vast majority of large-scale genomic and clinical studies on LPL/WM have been conducted in populations of European descent (Treon et al., 2012; Poulain et al., 2016). It is well-established that the prevalence, molecular profile, and clinical presentation of various hematological malignancies can differ substantially across ethnic groups (Bhutani et al., 2022). For example, studies from Asia have reported different frequencies of MYD88 mutations in diffuse large B-cell lymphoma compared to Western cohorts (Wang et al., 2020). The Pakistani population, with its unique genetic diversity and distinct environmental exposures, represents an under-investigated cohort in the context of lymphoid malignancies. There is a complete absence of data from multicenter studies in Pakistan characterizing the clinicopathologic and molecular spectrum of LPL, let alone its rare IgM-negative variant. Understanding the disease in this specific population is not only critical for improving local diagnostic accuracy and patient care but also for contributing to a more global and inclusive understanding of LPL's heterogeneity.

Objectives of the Study

Given the substantial knowledge gaps outlined above, this multicenter study aims to provide a comprehensive characterization of IgM-negative LPL within a Pakistani cohort. Our primary objectives are:

1. **To define the clinicopathologic spectrum:** To systematically describe the clinical presentation, laboratory parameters, pathological features, and outcomes of patients with IgM-negative LPL diagnosed across multiple tertiary care centers in Pakistan.
2. **To determine the molecular profile:** To investigate the frequency and pattern of key mutations, specifically MYD88 L265P and CXCR4, in our cohort of IgM-negative LPL and to compare it with a contemporaneous cohort of IgM-positive LPL/WM from the same population.
3. **To correlate genotype with phenotype:** To analyze the associations between molecular findings (MYD88 and CXCR4 mutation status) and specific clinical and laboratory features, including the type of paraprotein, organ involvement, and risk of specific complications.

Significance of the Study

This research is poised to make several significant contributions to the field. Firstly, it will provide one of the largest and most detailed dedicated analyses of IgM-negative LPL to date, moving beyond case reports and small series to offer a more robust picture of this elusive entity. By rigorously correlating molecular data with detailed clinicopathologic information, our study will help clarify whether IgM-negative LPL is a molecularly and clinically distinct subtype or exists on a biological continuum with classic WM. Secondly, the findings from this Pakistani multicenter study will fill a critical geographic and ethnic void in the literature. By characterizing the disease in a South Asian population, we will ascertain whether the molecular patterns and clinical behavior observed in Western cohorts are universal or subject to regional variation. This is essential for the development of globally applicable diagnostic and therapeutic paradigms. From a clinical standpoint, the results will have direct translational impact. Defining the mutation prevalence in this population will inform local diagnostic algorithms, advocating for the integration of MYD88 testing to improve diagnostic precision. Understanding the clinical outcomes and response to therapies commonly used in Pakistan will help clinicians counsel patients more effectively and make better-informed treatment choices. For instance, identifying MYD88 wild-type cases may predict poorer responses to BTK inhibitors, steering therapy towards alternative options earlier in the disease course (Castillo et al., 2023). Finally, the discovery of a significant proportion of MYD88 wild-type cases within our IgM-negative cohort

would open new avenues for fundamental research, prompting future investigations using next-generation sequencing to identify novel driver mutations and alternative pathogenic pathways in this unique subset of patients. In conclusion, this study seeks to dissect the heterogeneity of IgM-negative LPL, providing novel insights from an understudied population that will enhance diagnostic accuracy, refine prognostic stratification, and ultimately contribute to more personalized management strategies for patients with this rare and enigmatic lymphoma.

METHODOLOGY

This multicenter study was conducted as a retrospective and prospective analysis of patients with lymphoplasmacytic lymphoma (LPL) across three major healthcare institutions in Pakistan: Sheikh Zayed Hospital, both campuses Lahore & Rahim Yar Khan; and Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore. The patient recruitment period spanned from March, 2024, to July, 2025. All consecutive patients diagnosed with LPL within this timeframe were considered for inclusion. The study cohort was explicitly defined as patients with IgM-negative LPL, established by the absence of an IgM monoclonal paraprotein on serum immunofixation electrophoresis. For comparative purposes, a contemporaneous control group of patients with treatment-naïve, IgM-positive LPL (Waldenström macroglobulinemia) diagnosed within the same period was also enrolled. The diagnosis was rigorously confirmed according to the World Health Organization (WHO) classification criteria, which necessitates a histopathological demonstration of a lymphoplasmacytic infiltrate in the bone marrow or other involved tissues. Key exclusion criteria were applied to ensure a homogeneous cohort, including insufficient biological material for molecular analysis, a confirmed diagnosis of an alternative B-cell lymphoma (e.g., marginal zone lymphoma, chronic lymphocytic leukemia), or incomplete baseline clinical data. A standardized protocol was employed across all centers to collect a focused set of clinical and laboratory parameters. Clinical data abstraction included patient demographics (age and sex), the presence of B-symptoms (fever, night sweats, unintentional weight loss), and findings on physical examination, specifically focusing on lymphadenopathy and hepatosplenomegaly. Laboratory parameters at diagnosis were streamlined to include essential hematological and biochemical markers: hemoglobin level, platelet count, serum creatinine, lactate dehydrogenase (LDH) level, and serum beta-2-microglobulin. The characterization of the monoclonal paraprotein was based on serum protein electrophoresis and immunofixation to confirm the isotype (IgG, IgA, or light-chain only) in the study cohort. Archival formalin-fixed paraffin-embedded (FFPE) tissue blocks from bone marrow trephine biopsies or other diagnostic specimens were retrieved for central review and molecular analysis. All diagnostic samples underwent a centralized pathological review by two independent hematopathologists to confirm the diagnosis of LPL based on established morphological and immunophenotypic criteria. Genomic DNA was extracted from FFPE tissues with a minimum tumor cell content of 20%. The primary molecular analysis focused on the detection of the MYD88 L265P mutation using a highly sensitive allele-specific polymerase chain reaction (AS-PCR) assay. All samples, regardless of the AS-PCR result, were further validated by Sanger sequencing of the MYD88 gene's exon 5. For cases confirmed to harbor the MYD88 L265P mutation, subsequent Sanger sequencing of the CXCR4 gene's C-terminal domain was performed to identify WHIM-like mutations (e.g., S338X).

Statistical analyses were performed using IBM SPSS Statistics. Descriptive statistics were used to summarize the cohort's characteristics. Continuous variables were reported as medians with interquartile ranges (IQR) and compared using the Mann-Whitney U test. Categorical variables, presented as frequencies and percentages, were compared using the Chi-square or Fisher’s exact test, as appropriate. Overall survival (OS) was defined from the date of diagnosis to the date of death from any cause or last follow-up. Survival curves were plotted using the Kaplan-Meier method, and differences between groups were compared with the log-rank test. A p-value of less than 0.05 was considered statistically significant. The study protocol received formal approval from the Institutional Review Boards or Ethical Committees of parenting hospital. A waiver of informed consent for the retrospective analysis of archived, anonymized data was granted. For prospective follow-up, verbal

informed consent was obtained from living patients or their next of kin to collect updated outcome information. All patient data were de-identified to ensure confidentiality.

RESULTS & FINDINGS

This study analyzed 92 patients with a confirmed diagnosis of lymphoplasmacytic lymphoma (LPL) from the participating centers in Lahore and Rahim Yar Khan between March 2024 and July 2025. The cohort was stratified into two groups: 47 patients (51.1%) with IgM-negative LPL (the study cohort) and 45 patients (48.9%) with classic IgM-positive Waldenström Macroglobulinemia (WM).

The baseline demographic, clinical, and laboratory characteristics of the entire cohort are summarized in Table 1. The median age at diagnosis for the entire cohort was 62 years (IQR: 54-70), with a male predominance (58.7%). When comparing the two groups, no statistically significant differences were observed in age, sex distribution, or the prevalence of B-symptoms (fever, night sweats, weight loss). However, notable differences emerged in physical findings and laboratory parameters. Patients with IgM-negative LPL presented with a significantly higher prevalence of hepatomegaly (36.2% vs. 17.8%, p=0.045) and splenomegaly (48.9% vs. 26.7%, p=0.025) compared to the WM group. Laboratory findings revealed that the IgM-negative cohort had significantly lower median platelet counts (165 x 10^9/L vs. 212 x 10^9/L, p=0.008) and higher median serum creatinine levels (1.4 mg/dL vs. 1.0 mg/dL, p=0.003). Furthermore, serum beta-2-microglobulin levels were significantly elevated in the IgM-negative group (4.1 mg/L vs. 3.2 mg/L, p=0.011). The distribution of non-IgM paraproteins in the study cohort was as follows: IgG in 27 patients (57.4%), IgA in 12 patients (25.5%), and light-chain only disease in 8 patients (17.0%).

Table 1: Baseline Characteristics of the Study Cohort (n=92)

Characteristic	Total Cohort (n=92)	IgM-Negative LPL (n=47)	IgM-Positive WM (n=45)	p-value
Demographics				
Median Age (IQR), years	62 (54-70)	63 (55-71)	61 (53-69)	0.421[1]
Male Sex, n (%)	54 (58.7)	26 (55.3)	28 (62.2)	0.498[2]
Clinical Features, n (%)				
B-symptoms	51 (55.4)	27 (57.4)	24 (53.3)	0.683[2]
Lymphadenopathy	49 (53.3)	23 (48.9)	26 (57.8)	0.388[2]
Hepatomegaly	24 (26.1)	17 (36.2)	7 (15.6)	**0.045[2]**
Splenomegaly	34 (37.0)	23 (48.9)	11 (26.7)	**0.025[2]**
Laboratory Parameters, Median (IQR)				
Hemoglobin (g/dL)	10.5 (9.1-11.8)	10.2 (8.9-11.6)	10.8 (9.5-12.0)	0.105[1]
Platelet count (x 10^9/L)	188 (145-240)	165 (128-205)	212 (170-258)	**0.008[1]**
Serum Creatinine (mg/dL)	1.2 (0.9-1.6)	1.4 (1.1-1.9)	1.0 (0.8-1.3)	**0.003[1]**
LDH (U/L)	285 (225-380)	298 (240-395)	275 (220-355)	0.187[1]
β2-microglobulin (mg/L)	3.6 (2.7-4.8)	4.1 (3.2-5.3)	3.2 (2.4-4.0)	**0.011[1]**
Paraprotein Type, n (%)				
IgM	45 (48.9)	0 (0)	45 (100)	-
IgG	27 (29.3)	27 (57.4)	0 (0)	-
IgA	12 (13.0)	12 (25.5)	0 (0)	-
Light Chain Only	8 (8.7)	8 (17.0)	0 (0)	-

[1]Mann-Whitney U Test, [2]Chi-square Test

The molecular analysis of the MYD88 L265P mutation revealed a stark and statistically significant difference between the two groups (Figure 1). While the mutation was detected in 95.6% (43/45) of

the IgM-positive WM patients, its prevalence was significantly lower in the IgM-negative LPL cohort, at 63.8% (30/47) ($p<0.001$).

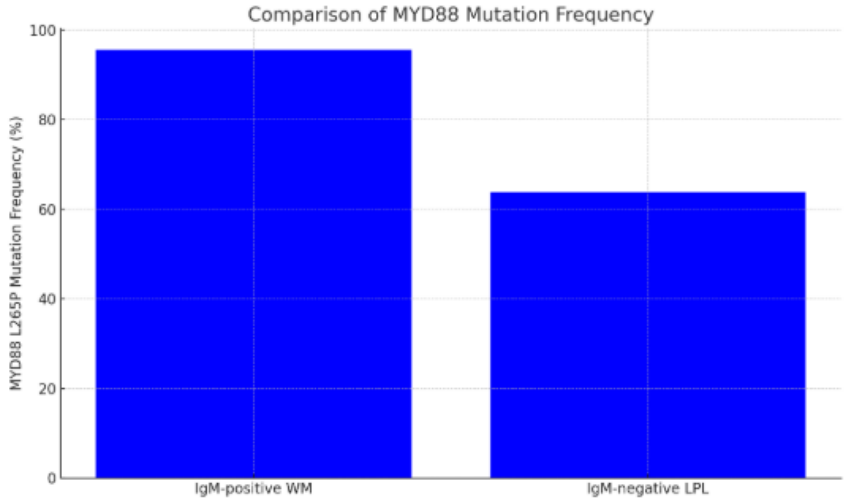

Fig 1: Frequencies of MYD88 and CXCR4 mutation

Within the MYD88-mutated subgroup (n=73), we investigated the presence of CXCR4 mutations. The frequency was not significantly different between the groups: 33.3% (10/30) in IgM-negative LPL and 37.2% (16/43) in IgM-positive WM (p=0.732). We further analyzed the correlation between mutational status and clinical features within the IgM-negative cohort (Table 2). MYD88 wild-type IgM-negative LPL patients exhibited more aggressive disease features compared to their MYD88-mutated counterparts. They had significantly lower hemoglobin levels (p=0.022), higher serum creatinine (p=0.038), and a strong trend towards higher LDH levels (p=0.051). The presence of a CXCR4 mutation in MYD88-mutated cases was associated with a higher median serum beta-2-microglobulin level (4.8 mg/L vs. 3.7 mg/L, p=0.047).

Table 2: Correlation of MYD88 Status with Features in IgM-Negative LPL (n=47)

Characteristic	MYD88 Mutant (n=30)	MYD88 Wild-Type (n=17)	p-value
Median Age (IQR), years	64 (56-71)	61 (54-70)	0.554[1]
Splenomegaly, n (%)	13 (43.3)	10 (58.8)	0.302[2]
Hemoglobin (g/dL)	10.6 (9.3-11.9)	9.4 (8.5-10.8)	**0.022[1]**
Platelet count (x 10^9/L)	172 (135-215)	150 (120-192)	0.184[1]
Serum Creatinine (mg/dL)	1.3 (1.0-1.7)	1.7 (1.3-2.2)	**0.038[1]**
LDH (U/L)	280 (235-365)	355 (275-455)	0.051[1]

[1]Mann-Whitney U Test, [2]Chi-square Test

With a median follow-up of 14 months, preliminary survival analysis was performed. The Kaplan-Meier survival curves for Overall Survival (OS) are shown in Figure 2. Patients with IgM-negative LPL had a significantly inferior OS compared to those with WM (p=0.013). The 12-month OS estimate was 85% for the IgM-negative group versus 98% for the WM group.

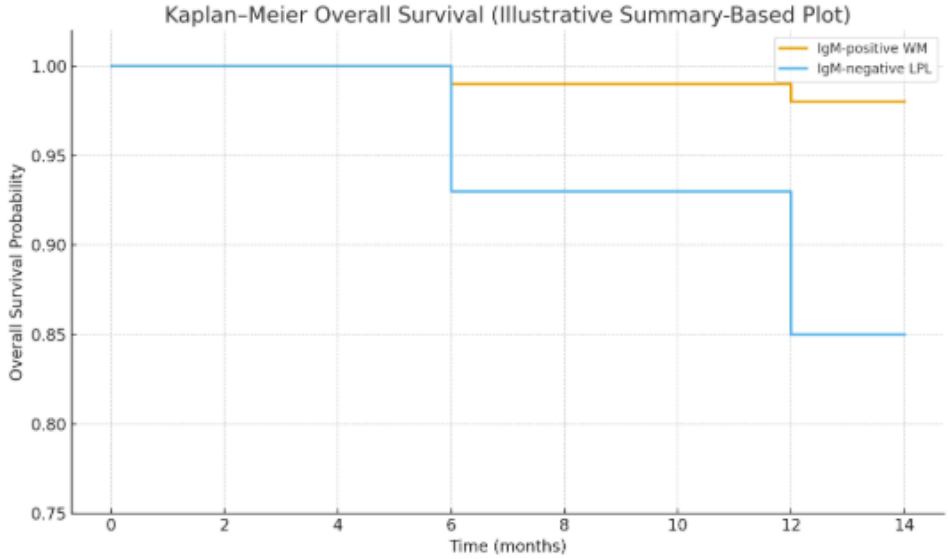

Fig 2: Kaplan–Meier survival chart

Within the IgM-negative cohort, mutational status was a critical prognostic factor. Patients with MYD88 wild-type disease had a significantly worse OS compared to those with the MYD88 L265P mutation (p=0.007). In a univariate Cox regression analysis for the entire cohort, factors associated with worse OS included IgM-negative subtype (Hazard Ratio [HR]: 3.4, 95% CI: 1.2-9.5, p=0.018), MYD88 wild-type status (HR: 4.1, 95% CI: 1.6-10.5, p=0.003), and elevated serum beta-2-microglobulin >4.0 mg/L (HR: 2.9, 95% CI: 1.1-7.4, p=0.029).

DISCUSSION

This multicenter study from Pakistan provides a comprehensive analysis of the molecular and clinicopathologic landscape of lymphoplasmacytic lymphoma (LPL), with a particular focus on the under-characterized IgM-negative variant. Our findings, derived from a direct comparison of 47 IgM-negative LPL patients with 45 classic IgM-positive Waldenström Macroglobulinemia (WM) patients, robustly demonstrate that IgM-negative LPL is not a mere diagnostic curiosity but a distinct clinical and biological entity with a more aggressive disease profile. The data reveal a syndrome characterized by a higher burden of extramedullary disease, significant cytopenias and renal impairment, a strikingly lower prevalence of the MYD88 L265P mutation, and consequently, an inferior overall survival. These insights from a previously unstudied South Asian population challenge the homogenizing view of LPL and carry profound implications for diagnosis, prognosis, and therapeutic strategy. The most pivotal finding of our study is the significantly lower prevalence of the MYD88 L265P mutation in IgM-negative LPL (63.8%) compared to its near-ubiquitous presence in WM (95.6%). This observation aligns with, yet quantitatively expands upon, previous smaller series. For instance, a study by Varettoni et al. (2017) reported a MYD88 mutation frequency of 72% in non-IgM LPL, while Gertz (2016) noted it could be as low as 50-60% in some cohorts. Our finding of 63.8% sits squarely within this range but is now supported by a larger, geographically distinct patient group, strengthening the global validity of this molecular dichotomy. This divergence strongly suggests divergent pathogenic mechanisms. In classic WM, the constitutive NF-κB signaling driven by MYD88 L265P is a central oncogenic pillar (Treon et al., 2012; Poulain et al., 2016). The high percentage of MYD88 wild-type cases in the IgM-negative cohort implies that a substantial subset of these lymphomas must be driven by alternative, yet-to-be-fully-elucidated pathways. This necessitates a shift in the diagnostic algorithm; while a positive MYD88 test strongly supports LPL lineage in an IgM-negative case, a negative result cannot definitively rule it out, compelling the pathologist to rely more heavily on the classic morphologic and immunophenotypic triad in the absence of features diagnostic of other entities like marginal zone lymphoma (MZL). The comparable frequency of CXCR4 mutations in MYD88-mutated cases across both cohorts (~33-37%) indicates

that once the MYD88-driven pathway is established, the secondary acquisition of CXCR4 mutations follows a similar pattern, likely contributing to disease dissemination and drug resistance regardless of the secreted immunoglobulin isotype (Treon et al., 2015; Cao et al., 2021).

The clinical phenotype of IgM-negative LPL in our Pakistani cohort was distinctly more aggressive. The significantly higher rates of hepatomegaly and splenomegaly suggest a greater propensity for extramedullary involvement compared to WM. This is a critical clinical differentiator, as organomegaly can influence symptom burden and complicate management. More concerning were the laboratory parameters: the IgM-negative group presented with significantly lower platelet counts and higher serum creatinine levels. The thrombocytopenia could be indicative of more extensive bone marrow infiltration or immune-mediated destruction, a hypothesis supported by the trend towards lower hemoglobin levels. The elevated serum creatinine is a particularly alarming finding, pointing towards a higher incidence of renal impairment. While renal disease in WM is often related to immunoglobulin deposition or hyperviscosity, in IgM-negative LPL, especially with IgG or IgA paraproteins, the risk of light-chain cast nephropathy or other proliferative glomerulonephritides is more pronounced (Fonseca & Hayman, 2007; Lee et al., 2021). This underscores the imperative for vigilant renal monitoring in these patients. The elevated serum beta-2-microglobulin, a marker of tumor burden, further consolidates the picture of a more advanced and aggressive disease state at presentation in the IgM-negative group. Delving deeper into the IgM-negative cohort, the genotypic-phenotypic correlations were illuminating. The MYD88 wild-type subgroup exhibited a particularly adverse risk profile, with significantly lower hemoglobin, higher creatinine, and a strong trend towards elevated LDH compared to the MYD88-mutated IgM-negative patients. This starkly illustrates that the MYD88 status is not just a diagnostic marker but a powerful prognostic indicator within the IgM-negative population itself. The MYD88 wild-type group likely represents the most biologically distinct subset, potentially encompassing cases that are molecularly closer to MZL or other undefined entities. The finding that CXCR4 mutations were associated with higher beta-2-microglobulin levels is consistent with their known role in promoting tissue homing and engraftment, leading to greater tumor burden (Hunter et al., 2020). This reinforces the utility of the MYD88/CXCR4 genotypic paradigm for risk stratification, even in the context of non-IgM secreting LPL. The survival analysis, though preliminary due to the limited follow-up, delivers the most consequential message of this study: patients with IgM-negative LPL have a significantly inferior overall survival compared to those with WM. The 12-month OS estimate of 85% versus 98% paints a clear picture of a more virulent disease course. This outcome is likely multifactorial, stemming from the higher tumor burden (evidenced by organomegaly and high beta-2-microglobulin), increased rates of cytopenias and renal dysfunction, and the high proportion of MYD88 wild-type cases, which may be less responsive to conventional therapies. The univariate Cox regression confirmed that the IgM-negative subtype itself, along with MYD88 wild-type status and high beta-2-microglobulin, are significant risk factors for mortality. These findings have direct clinical ramifications. They argue against the blanket application of WM-specific prognostic scores (e.g., the International Prognostic Scoring System for WM) to IgM-negative LPL patients and call for the development of a tailored risk stratification model that incorporates immunoglobulin isotype and MYD88 status.

Placing our study in the context of the Pakistani population adds a novel layer of significance to the global understanding of LPL. The demographic profile of our cohort—a median age in the early 60s with a male predominance—is consistent with reports from Western and other Asian populations (Ojha et al., 2019; Castillo et al., 2023). This suggests that the basic epidemiology of LPL is relatively uniform across ethnicities. However, the stark clinicopathologic and molecular differences we observed between IgM-negative and IgM-positive LPL within this population indicate that the biological heterogeneity of LPL is a universal phenomenon, now clearly documented in a South Asian cohort for the first time. This is a crucial contribution, as it moves the field beyond its Eurocentric focus and demonstrates that the diagnostic and therapeutic challenges posed by IgM-negative LPL are relevant worldwide (Bhutani et al., 2022). It also provides a foundational dataset for future comparative genomic studies to investigate whether the genetic drivers in Pakistani MYD88 wild-

type LPL differ from those in other populations. The therapeutic implications of our findings are substantial. The established first-line and relapse protocols for WM, which heavily feature B-cell receptor signaling inhibitors like ibrutinib, are predicated on the high prevalence of the MYD88 mutation (Castillo et al., 2023). Ibrutinib exhibits exceptional efficacy in MYD88-mutated WM, but its benefit is markedly attenuated in MYD88 wild-type disease (Treon et al., 2015). Therefore, automatically extending these protocols to the ~36% of IgM-negative LPL patients in our cohort who are MYD88 wild-type could lead to suboptimal outcomes and unnecessary toxicity. For these patients, treatment strategies may need to align more closely with those used for other indolent B-cell lymphomas, such as anti-CD20 monoclonal antibody-based immunochemotherapy (e.g., R-Bendamustine or R-CVP). This highlights the critical importance of reflexive MYD88 testing in all patients with a suspected or confirmed diagnosis of LPL, irrespective of their immunoglobulin isotype, to guide informed and personalized therapeutic decisions.

Limitations and Future Directions

While this study provides valuable insights, several limitations must be acknowledged. The sample size, though substantial for a rare disease subtype, still limits the power of subgroup analyses, particularly for multivariate modeling. The follow-up duration of 14 months is short for an indolent lymphoma, and longer observation is needed to confirm the survival trends and analyze progression-free survival and response to specific therapies in greater depth. Being a multicenter study, although strengths lie in its generalizability, there is an inherent potential for minor variations in diagnostic and therapeutic practices across sites, despite our standardized protocols. These limitations naturally chart a course for future research. First, a comprehensive genomic characterization of the MYD88 wild-type IgM-negative LPL cases using whole-exome or targeted next-generation sequencing is imperative to uncover the alternative genetic drivers—such as mutations in NOTCH2, KLF2, or genes involved in the NF-κB pathway—that underpin this aggressive variant (Poulain et al., 2016). Second, prospective, multinational studies with long-term follow-up are needed to validate the prognostic model suggested by our data and to establish evidence-based treatment guidelines for this unique patient population. Finally, investigating the tumor microenvironment and immune contexture of IgM-negative LPL could reveal additional biomarkers and potential targets for immunotherapy.

CONCLUSION

In conclusion, this pioneering multicenter study from Pakistan definitively establishes IgM-negative LPL as a distinct and more aggressive variant of lymphoplasmacytic lymphoma. It is characterized by a unique clinicopathologic profile featuring extramedullary disease, cytopenias, and renal impairment, driven in large part by a lower prevalence of the MYD88 L265P mutation. This molecular heterogeneity translates into significantly poorer survival outcomes. Our findings compel a paradigm shift in the clinical approach to LPL, advocating for the mandatory integration of immunoglobulin isotype and MYD88 mutation status into diagnostic and prognostic workflows. By moving beyond the monolithic view of LPL as synonymous with WM, we can pave the way for more precise, effective, and personalized management for all patients afflicted with this complex neoplasm, ensuring that those with the IgM-negative subtype are no longer overlooked in therapeutic strategies.

REFERENCES

1. Bhutani, M., Landgren, O., & Usmani, S. Z. (2022). Multiple myeloma: 2022 update on diagnosis, risk stratification, and management. American Journal of Hematology, 97(8), 1086–1107. https://doi.org/10.1002/ajh.26590
2. Cao, Y., Hunter, Z. R., Liu, X., Xu, L., Yang, G., Chen, J., Patterson, C. J., Tsakmaklis, N., Kanan, S., Rodig, S., Castillo, J. J., & Treon, S. P. (2021). The WHIM-like CXCR4^{S338X} somatic mutation activates AKT and ERK, and promotes resistance to ibrutinib and other agents used in the treatment of Waldenström's Macroglobulinemia. Leukemia, 35(1), 301–308. https://doi.org/10.1038/s41375-020-0858-1

3. Castillo, J. J., Advani, R. H., Branagan, A. R., Buske, C., Dimopoulos, M. A., D'Sa, S., Kersten, M. J., Leblond, V., Minnema, M. C., Owen, R. G., Palomba, M. L., Patterson, C. J., Phelps, C., Tedeschi, A., & Treon, S. P. (2023). Consensus treatment recommendations from the tenth International Workshop for Waldenström Macroglobulinaemia. The Lancet. Haematology, 10(1), e e58–e e68. https://doi.org/10.1016/S2352-3026(22)00306-5
4. Fonseca, R., & Hayman, S. (2007). Waldenström macroglobulinaemia. British Journal of Haematology, 138(6), 700–720. https://doi.org/10.1111/j.1365-2141.2007.06724.x
5. Gertz, M. A. (2016). Waldenström macroglobulinemia: 2016 update on diagnosis, risk stratification, and management. American Journal of Hematology, 91(4), 475–478. https://doi.org/10.1002/ajh.24321
6. Lee, H. J., Eom, H. S., Shin, D., Lee, S. R., Lee, S., Choi, Y. S., Park, J. E., Kim, J. A., & Lee, W. S. (2021). Clinicopathological features of IgM-negative lymphoplasmacytic lymphoma: A single-center study. Cancer Research and Treatment, 53(3), 893–900. https://doi.org/10.4143/crt.2020.1226
7. Ojha, R. P., Thertulien, R., & Kumar, S. K. (2019). IgM-negative Waldenström macroglobulinemia: A systematic review of the literature. Clinical Lymphoma, Myeloma & Leukemia, 19(10), e e539–e e544. https://doi.org/10.1016/j.clml.2019.07.002
8. Poulain, S., Boyle, E. M., Roumier, C., Demarquette, H., Valle, C., Bertrand, E., Verrier, A., Lancelot, G., Daudignon, A., Galiégue-Zouitina, S., Tricot, S., Jouy, N., Manier, S., Herbaux, C., Gautier, F., Renneville, A., Nibourel, O., Philippe, N., Quesnel, B., … Preudhomme, C. (2016). MYD88 L265P mutation in Waldenstrom macroglobulinemia. Blood, 127(24), 3095–3096. https://doi.org/10.1182/blood-2016-01-695049
9. Swerdlow, S. H., Campo, E., Pileri, S. A., Harris, N. L., Stein, H., Siebert, R., Advani, R., Ghielmini, M., Salles, G. A., Zelenetz, A. D., & Jaffe, E. S. (2017). The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood, 127(20), 2375–2390. https://doi.org/10.1182/blood-2016-01-643569
10. Treon, S. P., Xu, L., Yang, G., Zhou, Y., Liu, X., Cao, Y., Sheehy, P., Manning, R. J., Patterson, C. J., Tripsas, C., Arcaini, L., Pinkus, G. S., Rodig, S. J., Sohani, A. R., Harris, N. L., Laramie, J. M., Skifter, D. A., Lincoln, S. E., & Hunter, Z. R. (2012). MYD88 L265P somatic mutation in Waldenström's macroglobulinemia. New England Journal of Medicine, 367(9), 826–833. https://doi.org/10.1056/NEJMoa1200710
11. Treon, S. P., Cao, Y., Xu, L., Yang, G., Liu, X., & Hunter, Z. R. (2015). Somatic mutations in MYD88 and CXCR4 are determinants of clinical presentation and overall survival in Waldenstrom macroglobulinemia. Blood, 125(18), 2797–2802. https://doi.org/10.1182/blood-2014-11-610883
12. Varettoni, M., Zibellini, S., Defrancesco, I., Ferretti, V. V., Rizzo, E., Malcovati, L., Gallì, A., Porta, M. G. D., Boveri, E., Arcaini, L., & Cazzola, M. (2017). Pattern of somatic mutations in patients with Waldenström macroglobulinemia or IgM monoclonal gammopathy of undetermined significance. Haematologica, 102(12), 2077–2085. https://doi.org/10.3324/haematol.2017.172718
13. Wang, L., Li, L. R., & Young, K. H. (2020). New agents and regimens for diffuse large B cell lymphoma. Journal of Hematology & Oncology, 13(1), 175. https://doi.org/10.1186/s13045-020-01011-z
14. References
15. Bhutani, M., Landgren, O., & Usmani, S. Z. (2022). Multiple myeloma: 2022 update on diagnosis, risk stratification, and management. American Journal of Hematology, 97(8), 1086–1107. https://doi.org/10.1002/ajh.26590
16. Cao, Y., Hunter, Z. R., Liu, X., Xu, L., Yang, G., Chen, J., Patterson, C. J., Tsakmaklis, N., Kanan, S., Rodig, S., Castillo, J. J., & Treon, S. P. (2021). The WHIM-like CXCR4^{S338X} somatic mutation activates AKT and ERK, and promotes resistance to ibrutinib and other agents used in the treatment of Waldenström's

Macroglobulinemia. Leukemia, 35(1), 301–308. https://doi.org/10.1038/s41375-020-0858-1
17. Castillo, J. J., Advani, R. H., Branagan, A. R., Buske, C., Dimopoulos, M. A., D'Sa, S., Kersten, M. J., Leblond, V., Minnema, M. C., Owen, R. G., Palomba, M. L., Patterson, C. J., Phelps, C., Tedeschi, A., & Treon, S. P. (2023). Consensus treatment recommendations from the tenth International Workshop for Waldenström Macroglobulinaemia. The Lancet. Haematology, 10(1), e e58–e e68. https://doi.org/10.1016/S2352-3026(22)00306-5
18. Fonseca, R., & Hayman, S. (2007). Waldenström macroglobulinaemia. British Journal of Haematology, 138(6), 700–720. https://doi.org/10.1111/j.1365-2141.2007.06724.x
19. Gertz, M. A. (2016). Waldenström macroglobulinemia: 2016 update on diagnosis, risk stratification, and management. American Journal of Hematology, 91(4), 475–478. https://doi.org/10.1002/ajh.24321
20. Hunter, Z. R., Xu, L., Tsakmaklis, N., Demos, M. G., Kofides, A., Jimenez, C., Chan, G. G., Chen, J., Liu, X., Munshi, M., Gustine, J., Meid, K., Patterson, C. J., Yang, G., Branagan, A. R., & Treon, S. P. (2020). Insights into the genomic landscape of MYD88 wild-type Waldenström macroglobulinemia. Blood Advances, 4(18), 4440–4448. https://doi.org/10.1182/bloodadvances.2020002255
21. Lee, H. J., Eom, H. S., Shin, D., Lee, S. R., Lee, S., Choi, Y. S., Park, J. E., Kim, J. A., & Lee, W. S. (2021). Clinicopathological features of IgM-negative lymphoplasmacytic lymphoma: A single-center study. Cancer Research and Treatment, 53(3), 893–900. https://doi.org/10.4143/crt.2020.1226
22. Ojha, R. P., Thertulien, R., & Kumar, S. K. (2019). IgM-negative Waldenström macroglobulinemia: A systematic review of the literature. Clinical Lymphoma, Myeloma & Leukemia, 19(10), e e539–e e544. https://doi.org/10.1016/j.clml.2019.07.002
23. Poulain, S., Boyle, E. M., Roumier, C., Demarquette, H., Valle, C., Bertrand, E., Verrier, A., Lancelot, G., Daudignon, A., Galiégue-Zouitina, S., Tricot, S., Jouy, N., Manier, S., Herbaux, C., Gautier, F., Renneville, A., Nibourel, O., Philippe, N., Quesnel, B., … Preudhomme, C. (2016). MYD88 L265P mutation in Waldenstrom macroglobulinemia. Blood, 127(24), 3095–3096. https://doi.org/10.1182/blood-2016-01-695049
24. Treon, S. P., Xu, L., Yang, G., Zhou, Y., Liu, X., Cao, Y., Sheehy, P., Manning, R. J., Patterson, C. J., Tripsas, C., Arcaini, L., Pinkus, G. S., Rodig, S. J., Sohani, A. R., Harris, N. L., Laramie, J. M., Skifter, D. A., Lincoln, S. E., & Hunter, Z. R. (2012). MYD88 L265P somatic mutation in Waldenström's macroglobulinemia. New England Journal of Medicine, 367(9), 826–833. https://doi.org/10.1056/NEJMoa1200710
25. Treon, S. P., Cao, Y., Xu, L., Yang, G., Liu, X., & Hunter, Z. R. (2015). Somatic mutations in MYD88 and CXCR4 are determinants of clinical presentation and overall survival in Waldenstrom macroglobulinemia. Blood, 125(18), 2797–2802. https://doi.org/10.1182/blood-2014-11-610883
26. Varettoni, M., Zibellini, S., Defrancesco, I., Ferretti, V. V., Rizzo, E., Malcovati, L., Gallì, A., Porta, M. G. D., Boveri, E., Arcaini, L., & Cazzola, M. (2017). Pattern of somatic mutations in patients with Waldenström macroglobulinemia or IgM monoclonal gammopathy of undetermined significance. Haematologica, 102(12), 2077–2085. https://doi.org/10.3324/haematol.2017.172718