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Lymphoplasmacytic lymphoma and Waldenström macroglobulinemia, a decade after the discovery of *MYD88*^{L265P}

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ABSTRACT

There has been remarkable progress over the past 80 years since Jan Waldenstrom first described patients with a hyperviscosity syndrome related to IgM paraprotein in 1944. The definition of Waldenstrom macroglobulinemia (WM) has evolved from a clinical syndrome to a distinct clinicopathologic entity with characteristic morphology, immunophenotype and molecular features. The landmark discovery of *MYD88* mutation among most WM cases in 2012 marked the dawning of an era of molecular genomic exploration that led to a paradigm shift in clinical practice. In the current World Health Organization (WHO) classification of hematologic neoplasms, WM is included in the category of lymphoplasmacytic lymphoma (LPL) of which WM represents over 90% of cases. LPL/WM is also better defined, resolving ambiguity in many cases that would have been classified as "low-grade B-cell lymphoma with plasmacytic differentiation" a decade before. Nevertheless, challenges still face pathologists because criteria for distinguishing LPL/WM from other types of low-grade B-cell lymphoma, particularly marginal zone lymphoma (MZL), remain imperfect. In this review, we highlight the current understanding of LPL and WM brought to light by new discoveries, which in turn are increasingly translated to improved diagnosis and personalized therapy. Key concepts in the diagnosis and their clinical implications are emphasized. Controversies and challenges are also discussed.

1. Introduction: a historical overview

In 1944, Jan G Waldenstrom described three patients, two of whom had a hyperviscosity syndrome manifested by oronasal bleeding, anemia, lymphadenopathy, hypofibrinogenemia and BM lymphocytosis associated with an abnormal high-molecular-weight serum protein known as macroglobulinemia [1]. Since then, there have been continuous efforts to better define the disease. As it became clear that the constellation of findings described above is in large part a clinical syndrome of IgM paraproteinemia associated with a variety of lymphoid malignancies [2], earlier efforts were directed at establishing WM as a distinct entity caused exclusively by LPL. Table 1 lists synonyms used in different classification schemes of lymphoma associated with WM [3–10]. These efforts lead to the ultimate designation of LPL/WM in the 4th edition of WHO classification where LPL and WM were officially tied, replacing the previous terms such as well-differentiated lymphocytic lymphoma with plasmacytoid differentiation, small lymphocytic lymphoma with plasmacytoid differentiation, immunocytoma, or lymphoplasmacytoid and/or lymphoplasmacytic lymphoma, used in the Rappaport, Kiel, Lukes-Collins and Revised European-American Lymphoma (REAL) classifications as well as the Working Formulation [5,6,11](Table 1). To distinguish LPL from other lymphomas or monoclonal gammopathy of uncertain significance (MGUS), emphasis was placed on appropriate morphologic findings, exclusion of other types of B-cell lymphoma and setting a minimum IgM threshold of 3 g/dL. However, lack of specific genetic hallmark and a non-specific immunophenotype of LPL, which overlaps with MZL, continued to pose diagnostic challenges for pathologists.

The concept of a minimal IgM cutoff was challenged by a consensus group at the 2nd International WM Workshop (IWWM-2) in 2002 [12]. Recognizing that symptomatic patients can have a relatively low level of serum IgM paraprotein, WM was defined as a BM disease with a typical immunophenotype and IgM monoclonal gammopathy. Cases of LPL associated with IgG or IgA were excluded [12]. Specific criteria for distinguishing LPL from MZL were not addressed. In the absence of tailored therapy, further discrimination between LPL and MZL at that

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Table 1
Terms used to designate lymphomas associated with WM in different classification systems.

Classifications	Terminology
Rappaport	Well-differentiated lymphocytic, plasmacytoid
Lukes-Collins	Plasmacytic-lymphocytic
Kiel	Immunocytoma, lymphoplasmacytic type
Working formulation	Small lymphocytic, plasmacytoid
REAL	Lymphoplasmacytoid lymphoma (immunocytoma)
WHO 4th	Lymphoplasmacytic lymphoma/Waldenström macroglobulinemia
WHO 4th R, 5th, ICC	Lymphoplasmacytic lymphoma

WHO: World Health Organization, R: revised, ICC: International Consensus Classification.

time did not seem to be a pressing clinical issue.

To our clinical colleagues, WM is only part of a spectrum of clinical manifestations related to IgM paraprotein. Patients with debilitating complications of IgM paraprotein, such as cold agglutinin anemia, neuropathy and or cryoglobulinemia, although overlapping with WM, may not have morphologically recognizable lymphomas in the BM and IgM paraprotein levels are usually below 3 g/dL. However, these conditions may still require medical intervention. Considered "IgM-related disorders", they are now officially recognized in the 5th Edition of WHO classification (WHO-HAEM5) as complications of IgM monoclonal gammopathy [9] (Table 2) and are designated as "monoclonal gammopathy of clinical significance" (MGCS) in the 2022 International Consensus Classification (ICC) [8].

While WM is generally considered a BM-based disease with 10–20% of patients also having lymphadenopathy and splenomegaly, LPL can be primarily a lymph node-based disease, producing IgM, IgG or IgA or no paraprotein. Furthermore, entities such as gamma heavy chain disease, although distinct from WM clinically, share underlying pathology that greatly overlaps with LPL. In essence, LPL and WM are closely related but independent concepts. Noting these variations, we proposed de-linking WM from LPL in a 2005 review [13].

Historically, the diagnostic reproducibility of LPL and MZL are similarly low in lymph node samples, merely 53–63%, even among expert hematopathologists [14]. A major breakthrough changed the landscape in 2012 when Treon and colleagues reported a recurrent point mutation in myeloid differentiation primary response 88 (*MYD88)* gene in 91% of WM cases [15]. The mutation (T→C) lead to an amino acid change of leucine to proline at position 265 (NM_002468.5, also referred to as position 273 in NM_001172567) of *MYD88* at 3p22.2 (known as $MYD88^{L265P}$ or $MYD88^{L273P}$). Subsequently, in 2014, mutations of C-X-C chemokine receptor type 4 (*CXCR4*) were identified in WM, with over 40 somatic variants in the coding region, affecting up to 40% of patients who almost always also carry *MYD88* mutation [16]. $MYD88^{L265}$ was later found in LPL with IgA or IgG paraproteins underscoring their biologic similarity to WM or IgM-LPL [17–20].

The discovery of $MYD88^{L265P}$, coupled with more sophisticated flow cytometry immunophenotypic profiling, refined diagnostic criteria for LPL of both IgM and non-IgM types. Expanding at least the morphologic spectrum permissible in the lymph nodes [21], the mutations have facilitated the differential diagnosis from other B-cell lymphomas with plasmacytic differentiation, thereby improving the diagnostic accuracy for appropriate clinical management. The discovery of $MYD88^{L265P}$, along with other findings revealed by studies at the genomic level, are also reshaping treatment strategies for LPL/WM patients.

Despite the advances in clinical understanding and improved technology available for testing mutations in the past decade, there are still remaining issues, both historical and emerging, awaiting to be addressed. Although the detection of $MYD88^{L265P}$ favors LPL over other low-grade B-cell lymphomas, it is not exclusively seen in LPL. Thus, "low-grade B-cell lymphoma with plasmacytic differentiation" remains the diagnostic default in clinical practice in some cases. The departure from the conventional criteria for WM also raises a question of where to draw a line between asymptomatic WM and MGUS. What is $MYD88^{WT}$ LPL/WM and how is it different from the $MYD88^{MUT}$ LPL/WM? Most studies in the literature regarding WM do not specify how MZL is excluded; they are presumed to be associated with LPL. When is "low-grade B-cell lymphoma with plasmacytic differentiation, $CD5^{-}$ and $CD10^{-}$", in the context of serum IgM paraprotein, considered as presumptive evidence of WM clinically, or is it relevant to distinguish MZL from LPL?

As for *MYD88* mutation testing, there is currently no standardized approach regarding sample source, assay design and residual disease monitoring. The utility of other somatic mutations such as *CXCR4* and *TP53* in diagnosis, prognosis, and treatment decisions remain to be clarified. Finally, as needle core biopsy specimens of tissue increasingly replace excisional biopsy specimens, the diminishing sample size makes histological assessment and molecular testing more challenging.

This review summarizes the clinical, pathologic and molecular genetic features of LPL/WM, emphasizing the impact of *MYD88* and *CXCR4* mutations on various aspects including the pathological evaluation and clinical management. Challenges in differential diagnosis and common pitfalls encountered in daily practice are elaborated.

Table 2
IgM-type lymphoplasmacytic lymphoma (LPL/WM), IgM monoclonal gammopathy of undetermined significance (IgM MGUS) and IgM-related disorders.

Entity	IgM monoclonal protein	BM infiltration	IgM-related symptoms	Tumor-related symptoms[a]
IgM MGUS	+	−/Minimal	–	–
LPL-WM (asymptomatic)	+	+	–	–
LPL-WM (symptomatic)	+	+	+/−	+
IgM-related disorders	+	+/−	+/−	–
Cold agglutinin disease	+	+	+	–
Cryoglobulinemia	+	–	+	–
IgM-associated immunoglobulin light chain (AL) amyloidosis	+	+/−	+/−	–

[a] Tumor related symptoms include 1) constitutional symptoms (fevers, drenching night sweats, fatigues, and/or unintentional weight loss), 2) cytopenias (anemia, and/or thrombocytopenia), 3) organomegaly, associated organ damage and extramedullary mass, such as lymphadenopathy, hepatosplenomegaly.

2. Clinical features

LPL is a rare form of non-Hodgkin lymphoma (NHL). It comprises 1–2% of NHL in the United States and Europe and has an incidence of 3–4 cases per million persons per year. Although rare overall, the incidence of LPL/WM increases markedly with age with a median age of 7th decade at diagnosis, and is more common in men than women, and in Caucasians than African American or other races [22,23]. Most cases are sporadic, but a familial predisposition is observed in approximately 20% of patients [24]. Hepatitis C and autoimmune diseases such as Sjogren syndrome and systemic lupus erythematosus also have been reported to be associated with LPL/WM [25].

The clinical manifestations are heterogeneous. Approximately 20–30% of patients can be asymptomatic at presentation. Symptomatic patients may have features attributable to one or more of the following disease features: (1) tumor infiltration of BM and extramedullary tissue sites (10–20%), causing constitutional symptoms (fever, night sweats, weight loss), cytopenias (anemia most common), central nervous system infiltration (Bing-Neel syndrome), organomegaly (10–20%, lymphadenopathy, hepatosplenomegaly), or an extramedullary mass.

Extramedullary masses are present in 10–20% of patients at time of diagnosis, but are more common at time of disease progression; 2) IgM paraprotein associated complications, in 10–15% of patients, resulting in hyperviscosity syndrome, cryoglobulinemia and/or cold agglutinin hemolytic anemia, and/or 3) tissue deposition by paraprotein and associated organ dysfunction, the least common manifestation occurring in approximately 3% of WM patients, including light or heavy chain deposition disease, amyloidosis, IgM demyelinating peripheral neuropathy and IgM deposition disease [26–28].

Patients with LPL/WM have a wide range of IgM paraprotein levels, ranging from 0.2 to 10.9 g/dL with a median of 2.2 g/dL in a study of 225 LPL/WM cases [13]. Whereas a paraprotein level >3 g/dL would strongly favor a diagnosis of WM, a lower level can been seen in up to 50% of WM patients [13,29]. Hyperviscosity occurs in 10–15% of WM patients and is usually associated with higher IgM paraprotein levels, particularly over 6.0 g/dL.

Clinical presentations also may be related to mutational status of *MYD88* and *CXCR4*. Patients with *MYD88*^{WT} WM have been reported to have less BM infiltration and more frequent extramedullary involvement compared to patients with *MYD88*^{MUT} WM [30]. Patients with *CXCR4*^{MUT} present with less lymphadenopathy, and those with *CXCR4* nonsense mutations have higher serum IgM paraprotein levels and frequency of symptomatic hyperviscosity, higher BM tumor burden, worse cytopenias and/or shorter time to initial treatment when compared to those with *CXCR4* frame shift mutation or *CXCR4*^{WT} [30–33].

LPL patients with a non-IgM paraprotein, such as IgG and/or IgA, are rare, and represent about 2–5% of cases. The IgG LPL type is more common than IgA type [17–20]. Non-IgM type LPL is less well understood, due to its rarity, heterogeneity, and limited studies with small case series. IgG/A can also coexist with IgM. Compared to patients with IgM-type LPL/WM, patients with non-IgM type LPL have more variable clinical manifestations, including older age, female predominance and more frequent lymphadenopathy, splenomegaly and/or extranodal involvement, although they share similar morphological and genetic features with IgM type LPL [17–20].

3. Diagnostic criteria of LPL/WM

LPL is defined in WHO-HAEM5 as a mature B-cell neoplasm composed of small B lymphocytes, plasmacytoid lymphocytes and plasma cells, usually involving BM and sometimes involving the lymph nodes and spleen, which does not fulfill the criteria for other small B-cell lymphomas [34]. There are two subtypes, IgM and non-IgM types, accounting for 95% and 5% cases, respectively. The latter includes cases of IgG or IgA monoclonal proteins, non-secretory LPL, and IgM LPL without BM involvement. WHO-HAEM5 also specifies the *MYD88*^{MUT} and *MYD88*^{WT} variants to emphasize their biological and clinical relevance.

The two essential diagnostic criteria outlined in the WHO-HAEM5 include both 1) morphologic evidence of significant BM infiltration by clonal small lymphocytes with plasmacytoid and/or plasma cell differentiation; and 2) an immunophenotype compatible with LPL cells expressing B cell antigens (CD19, CD20, CD22), CD25, with or without CD138. The WHO-HAEM5 has proposed three desirable criteria, two related to mutations in *MYD88* and *CXCR4*, and one related to monoclonal IgM (rarely IgG or IgA) demonstrated by serum electrophoresis and immunofixation [9]. For IgM-type LPL, monoclonal IgM remains a requisite but no minimal IgM level or 10% tumor in the BM is required [35]. Notably, clonal CD138-positive plasma cells are not absolutely required. Demonstration of clonal plasma cells or plasmacytoid cells can still be achieved either by flow cytometry or immunostaining using other markers to be detailed later.

Due to the overlap between WM and MGCS, application of these modified criteria requires knowledge of the clinical picture such as end organ damage or symptoms (described above). Distinguishing asymptomatic WM from MGUS-IgM type is discussed in more details in the section on differential diagnosis.

4. Morphologic features

4.1. Lymph node

A spectrum of histologic findings can be observed in lymph nodes depending on the extent of involvement [26,21]. There are well defined classical features of LPL and WM. The morphological spectrum of this neoplasm has expanded since the discovery of *MYD88*^{L265P}. In the classic cases, the lymphoid infiltrate involves the medullary, interfollicular or paracortical areas with preserved architecture, patent sinuses and scattered small residual lymphoid follicles with atretic germinal centers. In non-classic cases, non-specific features can be observed, such as complete architectural effacement, a vaguely nodular growth pattern with Castleman like changes or nodules mimicking follicular lymphoma (FL) Clusters of epithelioid histiocytes, hyperplastic germinal centers and areas of monocytoid cells, features commonly seen with MZL, can also be observed in some cases, but are usually focal. The infiltrate is characterized by proliferation of mostly monotonous small lymphocytes, with varying number of plasmacytoid lymphoid cells and plasma cells. Three morphologic variants were previously described by Bartl et al. [36]: lymphoplasmacytic (a mixture of small lymphocytes and mature plasma cells with Dutcher bodies), lymphoplasmacytoid (a predominance of small lymphocytes with minimal plasmacytic differentiation), and polymorphous (increased medium and large cells). The polymorphous variant tends to develop over the course of disease and is frequently associated with cytogenetic abnormalities, representing most likely evolution to a more aggressive disease. This variant is therefore considered an intermediate stage between the low-grade variants and diffuse large B-cell lymphoma (DLBCL). Dutcher bodies, increased mast cells and hemosiderin deposition within the sinuses are common. Rarely, involved lymph nodes may show amyloid deposition or crystal-storing histiocytosis. Fig. 1A–L.

4.2. Bone marrow

BM infiltration can be interstitial, paratrabecular, nodular, or diffuse. Fig. 2A A diffuse pattern is more common in progressive disease with a high tumor burden. A paratrabecular distribution can be seen, but usually does not elicit the extent of fibrosis as is seen in follicular lymphoma (FL) and is rarely the only pattern of infiltrate. Reactive germinal centers and a sinusoidal infiltrate are uncommon; if present, these features suggest MZL over LPL. The neoplastic cells are composed predominantly of monotonous small lymphocytes admixed with plasmacytoid lymphocytes and plasma cells in variable proportions, imparting a spectrum of cytologic features. The plasma cells are usually mature (Marchalko type) and may contain cytoplasmic globules (Russell bodies) and intranuclear pseudoinclusions (Dutcher bodies). Increased mast cells and hemosiderin-laden histiocytes are also commonly seen. The three morphological variants seen in lymph nodes can also be noted in the BM. Fig. 2B.

In peripheral blood, circulating lymphoma cells can be present and exhibit a similar morphologic spectrum. LPL/WM patients usually do not have marked leukocytosis and lymphocytosis at diagnosis and if present, MZL or chronic lymphocytic leukemia (CLL) should be considered. Rouleaux formation can be present secondary to elevated serum IgM paraprotein. Fig. 2C.

4.3. Extramedullary sites

Extramedullary involvement has been reported, including the lung, gut, skin, soft tissue, pleura or central nervous system [37]. The lymphoma often shows a diffuse growth pattern, composed of a mixture of lymphocytes and plasma cells. The growth pattern in the spleen is not well documented as splenectomy is rarely performed in the modern era of effective chemoimmunotherapy, but both red and white pulp can be involved [26,21,37]. Extramedullary involvement at presentation is

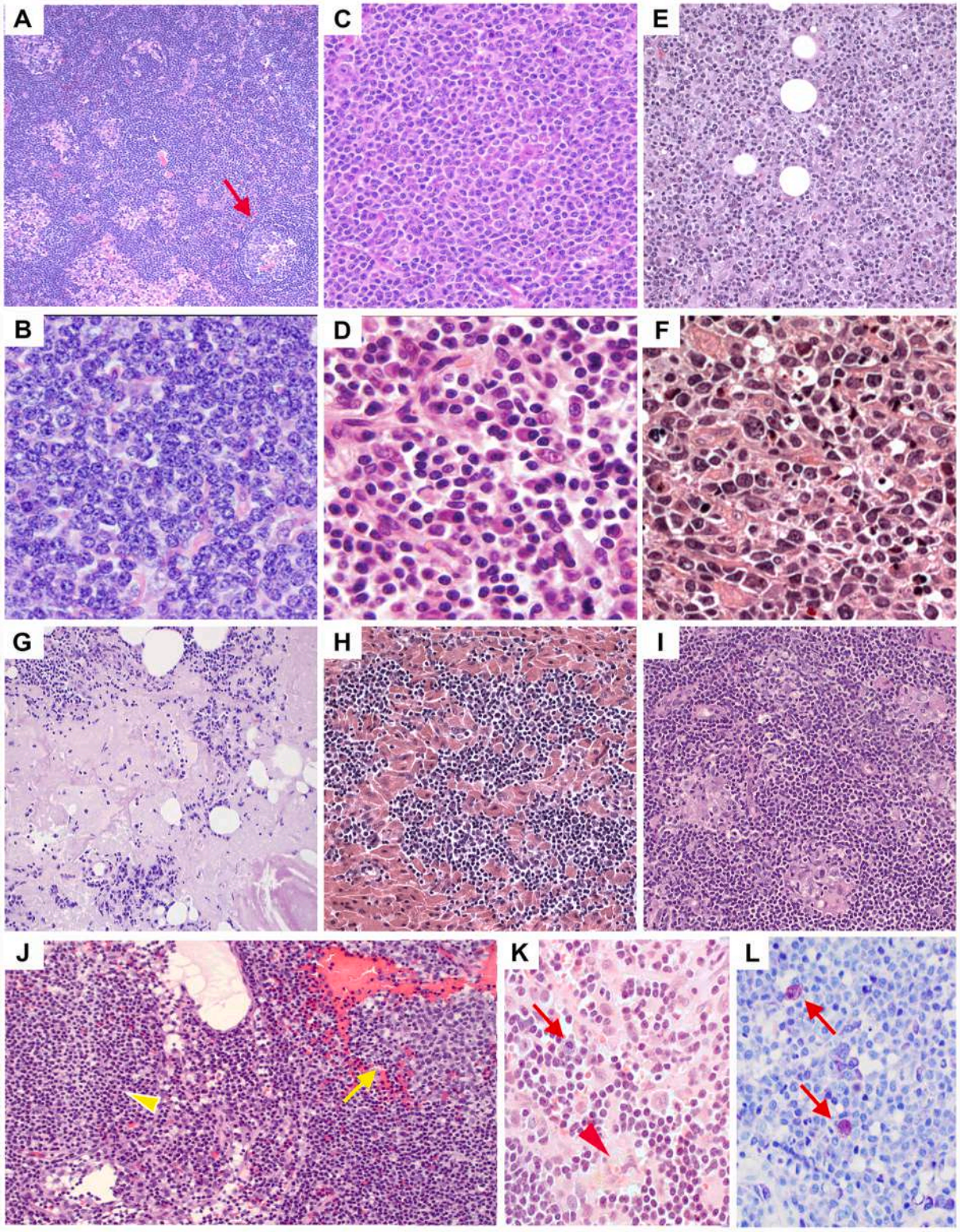

Fig. 1. A: LPL/WM, lymphoplasmacytoid variant, involving lymph node showing patent sinuses, a small atretic germinal center (arrow) and a monotonous infiltrate of small mature lymphoid cells, B: higher power view shows plasmacytoid features. Note in this type, plasma cells are rare and CD138 staining may not highlight sufficient monoclonal plasma cells. C–D: low and high-power view of LPL/WM, lymphoplasmacytic variant, in which the plasmacytic differentiation is prominent. E: LPL/WM-Polymorphous variant, a more heterogenous population of neoplastic cells ranging from small to large. F: Large cell transformation of LPL. G: LPL/WM involving lymph node associated with amyloid deposition. H: LPL/WM associated with Crystal-storing histiocytosis. I: LPL/WM with clusters of histiocytes, J: LPL/WM with residual reactive germinal centers (arrow) and foci of monocytoid cells (arrowhead). K–L: Mast cell (arrows, Giemsa stain in L), patent sinuses with histiocytes containing hemosiderin (arrowhead, K). A-K, H&E; B, D, F, K–L: 400×; A: 100×;C, E, G–J: 200×.

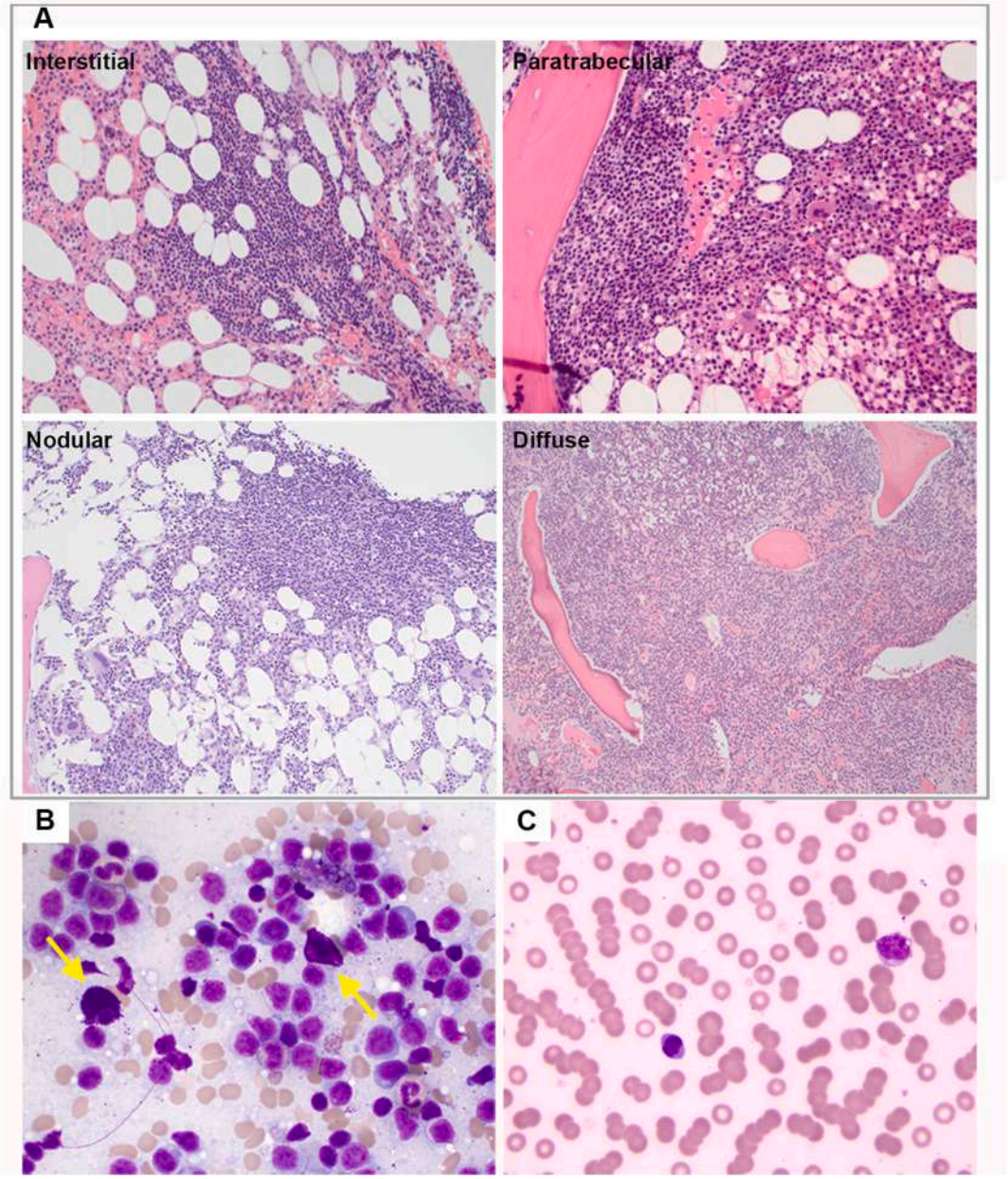

Fig. 2. A: Patterns of BM involvement include interstitial, paratrabecular, nodular and diffuse. Note the lack of significant fibrosis seen often in follicular lymphoma in the case with a paratrabecular pattern. H&E, 200×. B: Aspirate smear showing small lymphocytes, plasmacytoid lymphocytes, and plasma cells. Two mast cells are admixed (arrows). C: Peripheral blood shows Rouleaux formation, B–C: Wright-Giemsa, 500×.

uncommon and can be a diagnostic challenge requiring testing for *MYD88*^{L265P} for confirmation. It is is often associated with disease progression and/or relapse after therapy. Fig. 3A–B.

4.4. *Unusual patterns and cytologic features*

Besides classic morphology, some unusual growth patterns and cytologic variations can be found in LPL/WM cases, especially at advanced stage of disease. These features include extensive plasmacytic/plasmacytoid differentiation, absence of a lymphoid component, and a pure monotypic plasma cell infiltrate post therapy. The IgG/IgA type of LPL tends to show more a plasmacytic component, to the extent where plasma cells can outnumber B cells, mimicking plasma cell neoplasms (PCNs). Similar to Richter transformation in CLL/SLL, large cell transformation in LPL/WM, clonally related or unrelated, may be preceded by a stage of more heterogeneous tumor infiltrate, the so-called polymorphous morphology described above. Classic Hodgkin lymphoma may also complicate LPL/WM patients [13,38]. The large cell transformation is usually accompanied by new onset or increasing size of lymphadenopathy, organomegaly, worsening cytopenia, rarely hypercalcemia and even lytic bone lesion with acquisition of *MYC* rearrangement [13,39]. Paradoxically, the serum IgM level may drop as the tumor become less differentiated.

5. Immunophenotypic features

Immunophenotypic and genetic evidence suggests that the neoplastic cells in LPL/WM are memory B-cells that have undergone somatic hypermutation in *IGH* variable (V_H) region but devoid of isotype switching for the IgM subtype [40]. There is preferential usage of the

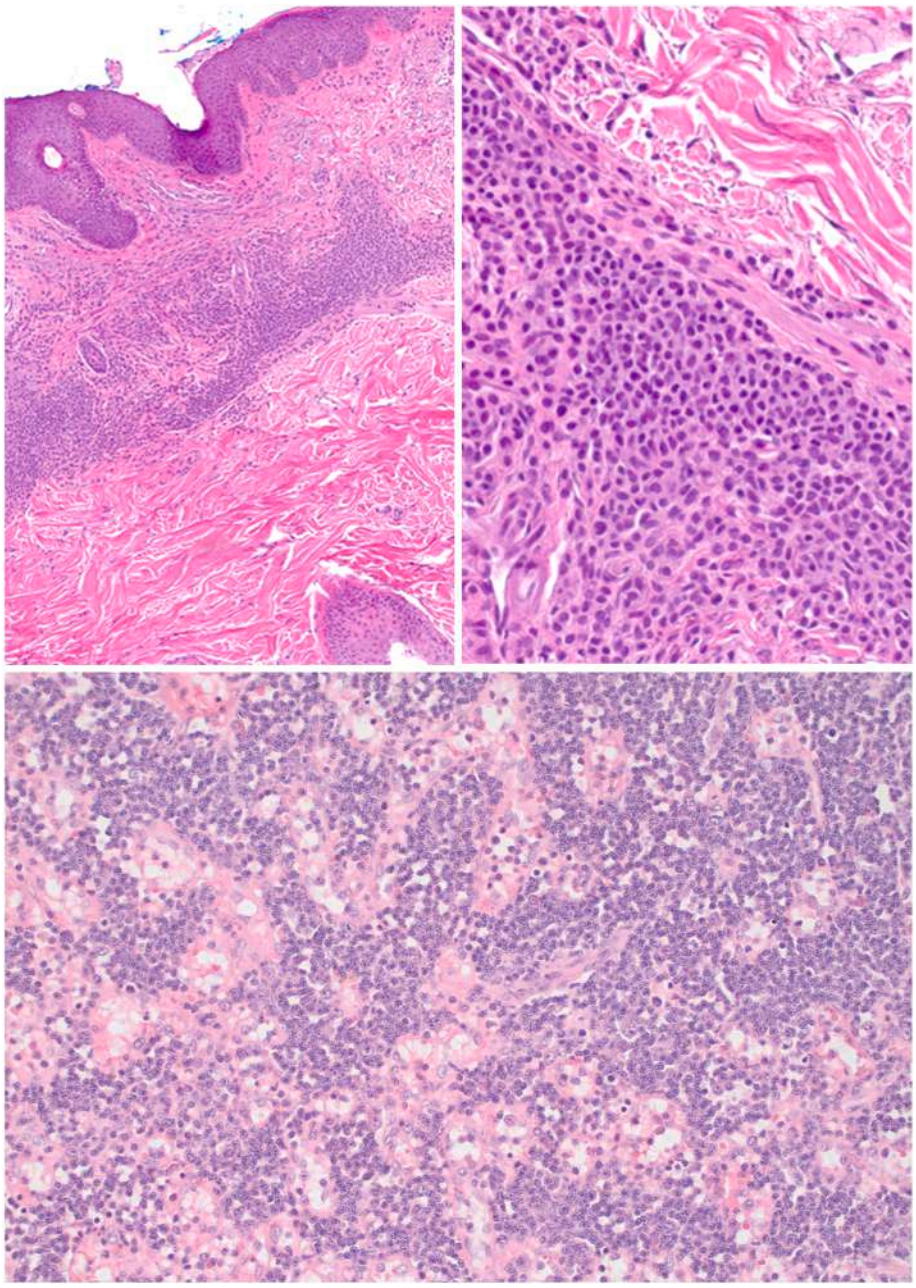

Fig. 3. Skin involvement by LPL/WM (top panel) showing a monotonous infiltrate of plasmacytoid cells in the dermis, sparing the epidermis. H&E. 400×. A case of spleen involved by LPL/WM (bottom panel). The neoplastic cells are monotonous and fill the red pulp. H&E: 400×.

V_H3 family in >90% of the LPL/WM cases. In the IgA/IgG type of LPL, isotype switch has occurred, but the cells fail to fully develop into plasma cells.

The neoplastic B cells express monoclonal Ig light chain, pan-B-cell markers (CD19, CD20, CD22, moderate to bright CD79a, PAX5), and have a memory B cell immunophenotype featured by CD22dim, $CD25^+$, $CD27^+$ and IgM^+ (so-called "WM phenotype") [40]. The neoplastic cells are also positive for CD44 bright, FMC-7 and BCL2 [41–44]. CD38 expression is variable and can be negative. CD27 and CD200 though usually positive, can be decreased or partial. The LPL/WM are typically negative for CD5, CD10, CD23 and CD103. However, CD5, CD10 and CD23 can be observed in a subset or the entire neoplastic population, in 5%, 3%, and 25–70% cases, respectively [41]. CD23 expression, when present, is usually dim and partial. In line with their paraprotein types, most cases show IgM expression, and rarely IgG or IgA. Cases of non-IgM type show similar immunophenotypic features [17].

The plasmacytic component in LPL/WM is usually positive for monoclonal cytoplasmic Ig light chain and sometimes even surface Ig light chain, although polyclonal plasma cells may be present in the background. The clonal plasma cells express bright CD38 and variable CD138, and show retention of lymphoid antigen markers including CD19, CD27, CD45, and CD81 in most cases. The plasma cells consistently lack CD56 and CD117, although weak CD56 expression can be observed in occasional cases of LPL of non-IgM type [17]. CD19 or CD27 can be also absent or decreased in a subset of LPL/WM cases, usually not simultaneously. Absence or decreased CD19 and CD45 are more common in non-IgM type LPL cases [17]. Fig. 4A.

WHO-HAEM5 requires no mandatory demonstration of CD138+ clonal plasma cells for a diagnosis of LPL/WM. However, demonstration of clonal plasma cells or plasmacytoid cells can still be achieved by flow cytometric detecting cells with a profile of plasma cells: CD38 bright, $CD19^+$ $CD20^-$ $CD22^-$ $CD43^+$ CD45dim; or immunohistochemical

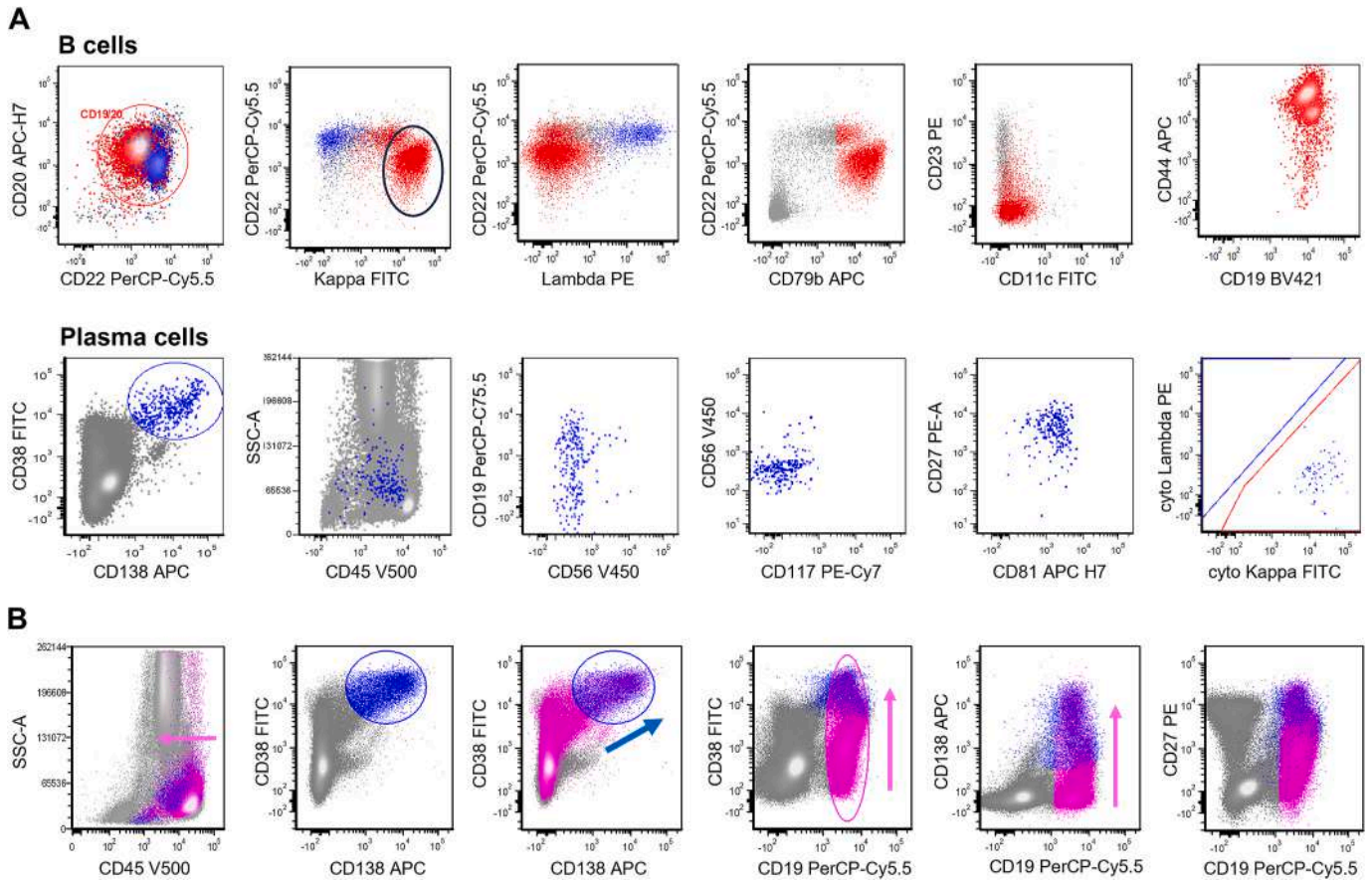

Fig: 4. A-4B. **4A:** Flow cytometric immunophenotyping plots of LPL-IgM type. Top panel: κ: red, λ: blue. The neoplastic B cells (red, within the black circle) show dimmer CD22 compared with normal polyclonal B cells in the background, but brighter CD79b and CD44. The neoplastic cells are κ monoclonal and negative for CD23, CD11c and ROR1 (not shown). Middle panel: plasma cells show expression of CD19 (partial), CD27, CD45 dim, CD81 and monoclonal κ. CD56 and CD117 are absent. **4B** (bottom panel)**:** Spectrum of plasmacytic differentiation in a case of LPL/WM: $CD19^{+}$ B cells (red purple) and CD38bright/CD138+ plasma cells (blue) form a continuum along the CD38 axis as marked by the arrows. As B cells transition to plasma cells which retain CD19, CD38 and CD138 levels progressively increase. Note the gradually ascending intensity of CD138 (blue arrow) on the CD38/CD138 gating plot (blue circle) and descending intensity of CD45 on the CD45 vs SSC plot (red purple arrow). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

staining for MUM1/IRF4, XBP1, or BLIMP1 with Ig light chain restriction. Failure to demonstrate clonal CD138+ plasma cells could be due to insufficient number of such cells in a biopsy (too few or too dim CD138). The relaxed requirement of CD138+ clonal plasma cells follows the concept that "plasmacytic differentiation" in LPL/WM falls into a spectrum, as shown in Fig. 4B. In essence, the immunophenotype mirrors morphology.

6. Impact of *MYD88* mutation

Initially identified in 91% of WM cases by whole genome sequencing followed by Sanger sequencing confirmation, $MYD88^{L265P}$ mutation is detectable in 93–97% of LPL/WM patients with more sensitive allele specific polymerase chain reaction (AS-PCR) or digital droplet PCR (ddPCR); the mutation is present in both sorted B cells and plasma cells [15,45,46]. As a highly recurrent somatic mutation in LPL/WM patients, $MYD88^{L265P}$ helps to discriminate these neoplasms from other IgM-secreting hematolymphoid malignancies. $MYD88^{L265P}$ is essentially absent in patients with multiple myeloma (including IgM type), and rare in other B-cell lymphomas with plasmacytic differentiation, such as MZL (nodal, extranodal, splenic), CLL/SLL, and a subset of large B cell lymphoma (DLBCL) of non-germinal center B-cell type, particularly those of leg type and those at immune privileged sites (central nervous system and testis) [15,47–51]. Detection of $MYD88^{L265P}$ is particularly helpful in challenging cases involving extramedullary sites such as a pleural effusion or Bing–Neel syndrome (lymphoma involving leptomeningeal tissue and/or the central nervous system). Conversely, absence of $MYD88^{MUT}$ in suspected LPL/WM cases may suggest alterative diagnosis, with the most common diagnosis being IgM PCN followed by MZL [52].

$MYD88^{L265P}$ is an oncogenic, non-synonymous, gain-of-function mutation, and plays an important role in lymphomagenesis by constitutively activating NF-κB and its associated signaling pathways to promote B-cell proliferation and survival. As a signaling adaptor in the canonical NF-κB pathway, mutant MYD88 preferentially and constitutively recruits IL-1R associated kinase 4 (IRAK4), a serine-threonine kinase, to preferentially phosphorylate IRAK1. Phosphorylated IRAK1 then constitutively activates the MAPK and NF-κB pathways through interaction with TRAF6 and TAK1, resulting in proliferation and survival of B cells. In addition, Bruton tyrosine kinase (BTK), an integral protein in the B-cell receptor (BCR) signaling cascade, is preferentially complexed with $MYD88^{L265P}$ but not $MYD88^{WT}$, which subsequently activates the NF-κB, MAPK, PI3K, NFAT pathways through cascade responses. The constitutively active NF-κB increases autocrine signaling of IL-6 and other cytokines, which further enhances proliferation and survival of neoplastic cells through the alternative JAK/STAT signaling cascade [53]. Fig. 5.

$MYD88^{L265P}$ is also detectable in patients with IgM MGUS. Mutations in *MYD88* and *CXCR4* are less frequent in the IgA/IgG type of LPL, reported to be 43–80% for *MYD88*, and 25% for *CXCR4* by Sanger sequencing [19,20]. The frequency of $MYD88^{L265P}$ is 60–90% in IgM MGUS detected by using sensitive ddPCR methods [30,54] suggesting an

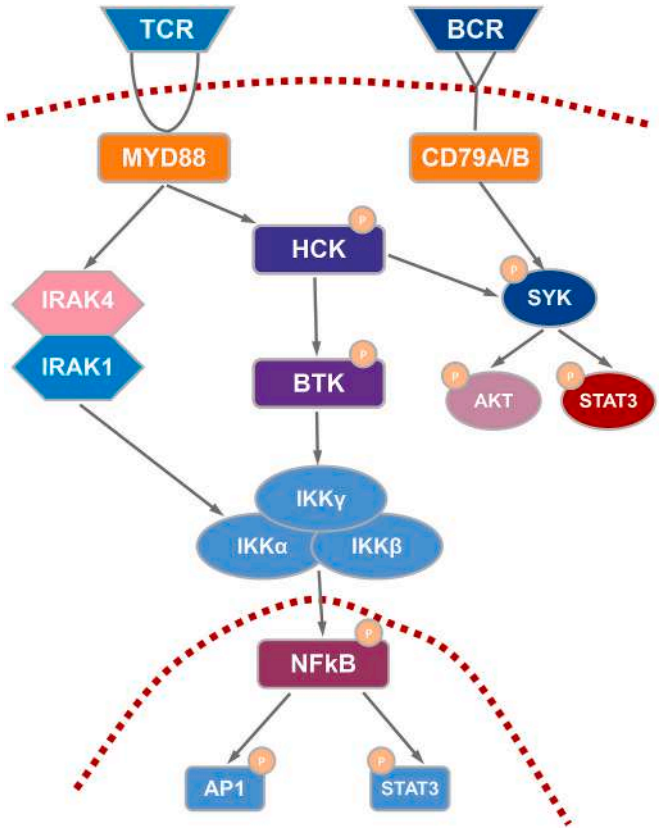

Fig. 5. *MYD88* and *BCR* components (CD79A&B) activate the NF-kB Pathway. Mutations of *MYD88* activate hematopoietic cell kinase (HCK), a member of SRC family, or directly activates IRAK4 and IRAK1, which in turn activates NF-κB pathway way via AP1 and STAT3 to regulates cell proliferation, apoptosis. In parallel, Immunoglobulin B-cell receptor and a heterodimeric signaling unit CD79A and CD79B can be activated as a result of increased cytokine activity and activation of HCK, with downstream activation of SYK which in turn activates AKT and STAT3.

early oncogenic role in WM pathogenesis. However, the mutant allelic fraction does not usually correlate with BM tumor burden [55]. Furthermore, $MYD88^{MUT}$ is not limited to malignant clones; it is also detectable in immunophenotypically normal B-cells suggesting that $MYD88^{MUT}$ alone may not be sufficient to drive oncogenesis [35,40,56].

The non-L265P variants (M232T, S243N, V217F, S219C, R209C) of *MYD88* mutation have been identified in 4% of WM patients and their clinicopathological features are less well defined [30,57]. Variant mutations have been more commonly reported in other B-cell lymphomas such as CLL/SLL or MZL [51].

$MYD88^{WT}$ WM have a distinct transcriptional profile but overlap with the $MYD88^{MUT}$ LPL/WM [58]. $MYD88^{WT}$ WM carry somatic mutations which point to activation of NF-κB pathway (*TBL1XR1*), epigenetic dysregulation (*KMT2D, KMT2C,* and *KDM6A*), or impaired DNA damage repair (*TP53, ATM* and *TRRAP*). These mutations overlap with those found in DLBCL [59], underscoring their shared pathogenesis [59].

7. Impact of *CXCR4* mutation

CXCR4 mutation is the second most common mutation in LPL/WM, occurring in 30–40% of cases and almost always associated with *MYD88* mutations [50]. *CXCR4* mutations have been identified exclusively in the C-terminal domain, leading to truncated receptor protein associated with higher expression of CXCR4. The mutational landscape of *CXCR4* is complex, with >40 nonsense and frameshift mutations described. Interestingly, the observed somatic *CXCR4* mutations in LPL/WM neoplastic cells are similar to a germline mutation found in a congenital immune deficiency disorder characterized by warts, hypogammaglobulinemia, infections and myelokathexis (WHIM syndrome) and therefore are also termed $CXCR4^{WHIM}$ [60]. The most common *CXCR4* mutation (50%) is nonsense mutation, at S338 ($CXCR4^{S338X}$) with substitution of C→G (54%), or C→A (25%), or a frameshift mutation (21%) [61,62]. CXCR4 is a chemokine receptor that binds to its ligand CXCR12 to regulate cell proliferation, migration, chemotaxis and cell cycle. The $CXCR4^{S338X}$ results in a premature stop codon, causing a truncated and non-functional CXCR4 protein. $CXCR4^{WHIM}$ impairs its desensitization and internalization causing perpetual activation of its downstream targets through PI3K- AKT–NF–κB, as well as JAK-STAT3 and MAPK signaling cascades [61].

$CXCR4^{WHIM}$ may be seen in 16-20% IgM-MGUS and is rare in other lymphoproliferative disorders, with sporadic cases reported in patients with MZL, CLL/SLL and the activated B-cell subtype of DLBCL [8,30,55,63]. Therefore, detection of $CXCR4^{WHIM}$ can help to confirm diagnosis in challenging cases. However, *CXCR4* mutation is usually subclonal in nature and a patient can harbor multiple mutations within separate clones. Though not required for diagnosis, mutation analysis of *CXCR4* is useful to guide treatment and prognostication.

8. Other molecular and cytogenetic features

8.1. Other recurring mutations

The heterogeneous clinical presentation, variable morphologic patterns and diverse immunophenotypic features in LPL/WM cases strongly suggest a multifactorial pathogenesis [25]. In addition to the highly recurrent $MYD88^{L265P}$ and $CXCR4^{WHIM}$, about 50% of cases have mutations in other genes. Hunter and colleagues identified mutation of genes involving *KTM2D* (18%), *TP53* (~8%), *CD79*A/B (7–12%), *ARID1A* (7–17%), *NOTCH2* (~4%) and *PRDM1* (~4%) [16,30,44,63,64]. We found about 30% of cases carry *IGLL5* mutations (unpublished data) by next generation sequencing (NGS). *ARID1A* and its frequently deleted homolog *ARID1B* on chromosome 6q are among those most frequently mutated genes. As switch/sucrose nonfermentable (SWI/SNF) family members, both serve as chromatin remodeling genes, modulating gene regulation such as *TP53* [63]. Mutations in *CD79A* and *CD79B,* which are part of a BCR complex, result in chronic activation of BCR signaling. In the study by Poulain and colleagues, mutations in *CD79*A/B were mutually exclusive with *CXCR4* mutations, suggesting these mutant BCR components may have independent roles in facilitating $MYD88^{MUT}$-mediated disease progression [60].

Alterations in *TP53*, including mutation and 17p deletions, occur in 5–10% of treatment-naïve LPL/WM patients, but are more frequent, up to 25–30%, in patients exposed to alkylator agents and/or nucleoside analogues [65,66]. Mutations in *TP53* are associated with poorer survival.

8.2. Copy number alterations

Unlike myeloma and other low-grade B-cell lymphomas, there are no disease-defining cytogenetic abnormalities associated with LPL/WM, including a lack of highly recurrent translocations. The karyotype is usually simple with a median of 2–3 chromosomal abnormalities per patient. Deletion of 6q is the most frequent chromosomal abnormality, occurring in 30–50% of patients, and is associated with increased risk and a shorter time of progression to symptomatic disease [40,63,67]. Del(6q) is more commonly detected by karyotyping in patients with polymorphous type of histology [68]. Loss of regulatory genes on chromosome 6q could affect modulation of NF-kB activity (*TNFAIP3* and *HIVEP2*), BCL2 and apoptosis (*FOXO3*), BTK (*IBTK*), plasma cell differentiation (*PRDM1*) and *ARID1B*.

Other abnormalities observed in LPL/WM include trisomies of 4, 12 and/or 18 and deletion of 13q and 17p, at much lower frequencies

(mostly <15%) [40]. Notably, the frequency of specific copy number abnormalities progressively increases from IgM MGUS, through smoldering WM to symptomatic WM (18% vs 20% and 73%, respectively), suggesting a multistep transformation of clonal B cells from benign to malignant, and specific copy number abnormalities may be acquired during transformation [40]. Deletion of 17p/*TP53* is present in 8–15% of WM patients and has been associated with an adverse prognosis [65,69].

8.3. Familial predisposition/evidence of genetic susceptibility

The genetic basis for predisposition to WM was first investigated in the familial setting, and familial aggregation of WM has been observed for >60 years. Relatives of WM patients were found to have an increased risk for developing WM, related B-cell malignancies and/or IgM MGUS in epidemiological studies and population-based registry studies [70,71]. The subsequent linkage analysis in multi-generational pedigrees supports a role for genetic factors in predisposition, and the diversity of reported inheritance patterns suggests variable penetrance and/or genetic heterogeneity. Efforts have been directed at searching for common genetic variations in WM etiology. McMaster et al. identified two high-risk susceptibility chromosomal loci, at 6p25.3 and 14q32.13, in a genome-wide association study in leveraged familial cases as independent single nucleotide polymorphisms (SNPs). These results were replicated in a nonfamilial sample, and together suggest 4% of the familial risk [72]. In a more recent study using whole exome sequencing in 64 WM/LPL pedigrees, several pathogenic (P) or likely pathogenic (LP) variants were identified, including *TREX1* and *SAMHD1*, genes responsible for innate immune response, genotoxic surveillance and DNA repair, and enrichment in genes that play a role in telomere maintenance and DNA repair [73]. However, P/LP variants in highly penetrant genes constitute only a modest proportion of the deleterious variant load in familiar WM cases, and each pedigree is largely unique in its genetic architecture. These studies point to genetic susceptibility and support the notion that multiple genes and pathways are likely involved in the etiology of LPL/WM.

9. Diagnostic challenges and differential diagnosis

9.1. Differential diagnosis

LPL remains an entity diagnosed by exclusion of other neoplasms producing monoclonal proteins. IgM paraprotein has been reported in B cell lymphoproliferative disorders including primary cold agglutinin disease (CAD), CLL/SLL (20.2%), MZL (7.1%), FL (4.7%), mantle cell lymphoma (2.9%), DLBCL with or without concurrent low-grade B-cell lymphoma (3.1%), and rarely angioimmunoblastic T-cell lymphoma (1.0%) [13]. In one study of 64 patients with an original diagnosis of $MYD88^{WT}$ WM, 7 were reclassified as IgM MM and 6 MZL [52].

9.1.1. Marginal zone lymphoma

The differential diagnosis between LPL and MZL, especially splenic MZL which often involves BM, can be challenging (see Table 3). An elevated IgM paraprotein >3 g/dL can be seen in MZL, especially MZL of mucosa-associated lymphoid tissue (MALT lymphoma). In contrast to patients with SMZL, LPL patients less often have lymphocytosis and splenomegaly [74]. Splenomegaly is usually a late stage finding in LPL/WM when patients present with high tumor burden and widespread disease.

Morphologically, LPL and MZL share overlapping morphological features. However, the lymphoid infiltrate in LPL has a relatively monotonous cytomorphology, whereas MZL is characterized by a more heterogenous population consisting of small lymphocytes, monocytoid or plasmacytoid lymphoid cells and occasionally mixed large, transformed cells. Exceptions are sometimes seen in SMZL where cellular heterogeneity is not as noticeable as the nodal or extranodal MZL. Mast cells are more often seen in LPL than MZL [75]. A sinusoidal pattern in the BM is more in line with splenic MZL [26]. MZL cases can also show significantly increased plasma cells and therefore this feature is non-specific, Fig. 6A. Some investigators have suggested that a paratrabecular distribution is more common in LPL/WM than in MZL 74,76, others have contradicted that [75]. The definition of paratrabecular may in part explain this discrepancy. A paratrabecular pattern was described as the contact surface of the infiltrate with the trabecular bone is larger than the maximum diameter perpendicular to the bone [74,76] and minimal or no intervening adipocytes between the lymphoma cells and trabecular bone [77]. When defined as such, a paratrabecular pattern is more common than previously recognized in LPL— owing to the aid of $MYD88^{L265P}$, but the prevalence of this pattern in LPL does not appear to surpass that of MZL in our experience, and is usually associated with other patterns, with minimal or no visible stromal response seen in FL and some cases of MZL. On the other hand, a well-defined nodular pattern is indeed more frequent in MZL. Other features typical of MZL include recognizable germinal centers, well defined or expanded follicular dendritic cell (FDC) meshwork highlighted by CD21 and CD23 stains, in contrast to absent or ill-defined FDC meshwork in LPL (Fig. 7) [77]. In spleen, SMZL usually involves white pulp whereas LPL could involve both but more often the red pulp.

LPL/WM can involve the so-called mucosa associated lymphoid tissue (MALT) sites such as orbit, gut, lung or skin. The distinction between extramedullary LPL/WM from MALT lymphoma can be challenging. LPL/WM involving extramedullary sites is rare in the modern era and the diagnosis is usually made in the context of evidence of medullary disease already established before and by excluding MALT lymphoma. Extramedullary LPL/WM also recapitulates the medullary disease in that the neoplastic cells are monotonous. In contrast, MALT lymphoma typically shows germinal center hyperplasia, follicular colonization and lymphoepithelial lesions. MALT lymphoma infrequently involves bone marrow (2–5%) and is associated with unique microorganism infection at specific anatomic sites, such as *Helicobacter pylori* in gastric MALT lymphoma, and, importantly, disease-specific translocations involving *MALT1, BCL10, TNFAIP3,* or *BIRC3* detectable by FISH and other ancillary studies.

Immunophenotypically, LPL/WM is often indistinguishable from various types of MZL. However, LPL/WM cases usually have homogeneous expression of CD25 and weak expression of CD22 (~90% of cases), whereas MZL is usually CD25 negative with moderate CD22 (~80% cases) [42]. Other distinguishing markers include surface membrane IgM and CD79b, which are often upregulated in LPL/WM, whereas CD305 (LAIR1) and IRTA1 is commonly expressed in MZL but not in LPL/WM [78,79]. CD11c is expressed in 20–30% of MZL but is usually negative in LPL/WM.

Table 3
Comparison of bone marrow findings in lymphoplasmacytic lymphoma (LPL) versus marginal zone lymphoma (MZL).

	LPL	MZL
Morphology		
Lymphoid infiltrate	Homogeneous	Heterogeneous
Pattern		
Para-trabecular	++	++
Nodular	++	+++
Diffuse	++	++
Intra-sinusoidal	–	++
Interstitial	+++	++
Germinal center	–	+
Immunophenotype		
CD22	Weak	Strong
CD25	Majority cases	A small subset of cases
CD21/CD23	Absent, ill-defined	Well-defined or expanded
Common mutations		
$MYD88^{L265P}$	>95%	6–10%
CXCR4	30–40%	Rare
KLF2	None	20% (nodal)
PTPRD	None	20% (nodal)

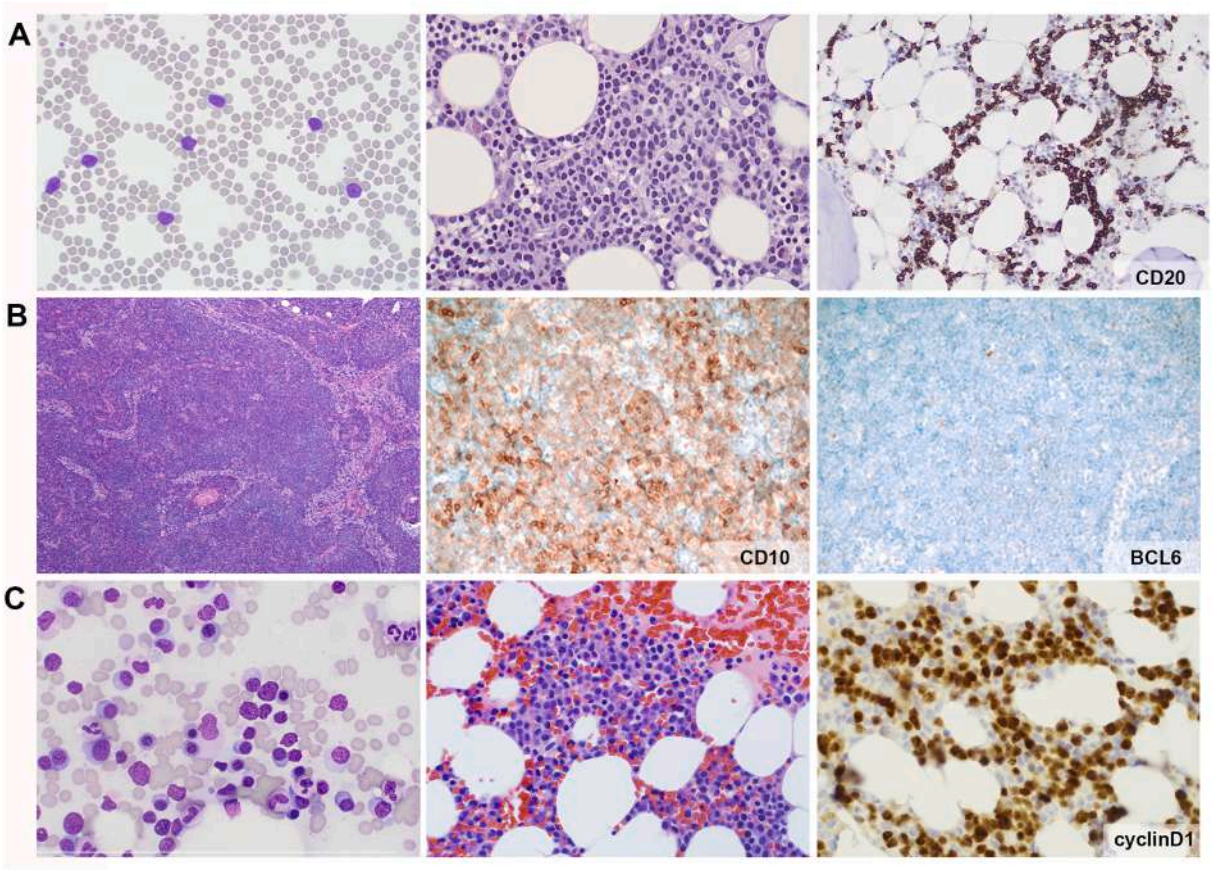

Fig. 6. A: Typical SMZL showing peripheral blood lymphocytosis (left, Giemsa, 500×), BM infiltrate (middle, H&E, 200×), and a sinusoidal infiltrate highlighted by CD20 stain (right, immunostaining, 200×). B: CD10-positive LPL. Hematoxylin-eosin section shows nodal involvement by monotonous neoplastic cells (left) that are positive for CD10 (middle), but negative for BCL6 (right) by immunostains. C: A case of IgM myeloma showing lymphoplasmacytoid morphology (left: Wright-Giemsa, 500×), clot shows interstitial infiltrate (middle, HE, 200×) and cyclinD1 expression by immunostaining (right, 200×).

In our opinion, there are well-recognized morphological patterns of classic MZL and classic LPL. Between the two ends lies a spectrum of gray zone cases regarding the extent of monocytoid cells, cellular heterogeneity and reactive germinal centers or follicular colonization in lymph node samples. *MYD88*^{L265P} can help to resolve equivocal cases, especially when the sample size is limited, but does not totally overrule morphologic findings if classic. *KLF2* and *PTPRD* mutations are more commonly seen in nodal MZL (20% each) and are usually absent in LPL/WM [80]. Of interest, 7q31-q32 deletions is reported in about 40% and chromosome 3/3q gains in approximately 25% of SMZL cases whereas deletion of 6q is more common in LPL/WM. Of note, 7q22-q32 deletion is not entirely specific to SMZL. It has been previously reported in rare cases of LPL/WM and hairy cell leukemia variant [81,82].

9.1.2. Other low-grade small B-cell lymphomas

LPL cases with a diffuse growth pattern and CD5 expression may simulate CLL/SLL or MCL. A serum IgM level can even reach 3 g/dL in CLL/SLL. However, in LPL/WM cases, proliferation centers are not evident, and plasmacytoid differentiation is usually detectable and can be focally prominent, especially in the subcapsular and perivascular regions. On the other hand, the presence of proliferation centers and/or LEF1 expression, a marker with a high specificity for CLL/SLL, would support a diagnosis of CLL/SLL. ROR1 is another marker often expressed by CLL/SLL cells and can be tested by flow cytometry. In LPL/WM cases with CD10 expression mimicking FL, additional germinal center markers such as BCL6 and LMO2 can be helpful (Fig. 6B). *MYD88*^{MUT} and *CXCR4*^{MUT} can be found in CLL/SLL, but only a subset of those cases producing IgM paraprotein carry these mutations [51]. Demonstration of clonal plasma cells (either CD138+ or CD38 bright only without significant CD138) is more commonly seen in LPL/WM and rare in other low-grade B-cell lymphoma.

9.1.3. Primary cold agglutinin disease (CAD)

Primary CAD is a newly introduced category in WHO-HAEM5. By definition, CAD represents a very indolent BM clonal B-cell lymphoproliferative disorder that induces cold agglutinin-mediated autoimmune hemolytic anemia without any obvious underlying lymphoma or specific infections. Cold agglutinins are the autoantibodies in CAD, most often of the IgM κ class, whereas monoclonal IgG, IgA, "biclonal" IgA + IgG, or a λ phenotype is rare [83]. Although similarly affecting the elderly population at onset, most patients with CAD present with circulatory symptoms and hemolytic anemia to various extent [84]. BM may show nodular aggregates of B-cells (74% in a 54-case series), typically interstitial, without plasmacytoid features, and plasma cells tend to be singly scattered throughout the marrow if present. Mast cells are not part of the morphology [85] CAD harbors somatic mutations in immunoglobulin heavy and light chain genes with a preferential usage of *IGHV*4-34 gene in >85% cases and *IGKV*3-20 in 59% cases, whereas LPL/WM has a usage of *IGHV*3-23 in 24% of cases [86–88]. like MZL, trisomies of 3, 12 and 18 are common, and mutation of *KMT2D* is most common (~70%) followed by mutation of *CARD11* (~30%), but *MYD88*^{L265P} is absent [89]. Of note, cold agglutinin mediated hemolytic anemia can be secondary complication of LPL/WM.

9.1.4. MGUS

Since both WHO-HAEM5 and ICC have adopted the recommendation by IWWM that LPL/WM can be diagnosed even when monoclonal B cells and plasma cells are <10%, the distinction of LPL/WM or LPL of IgA/G

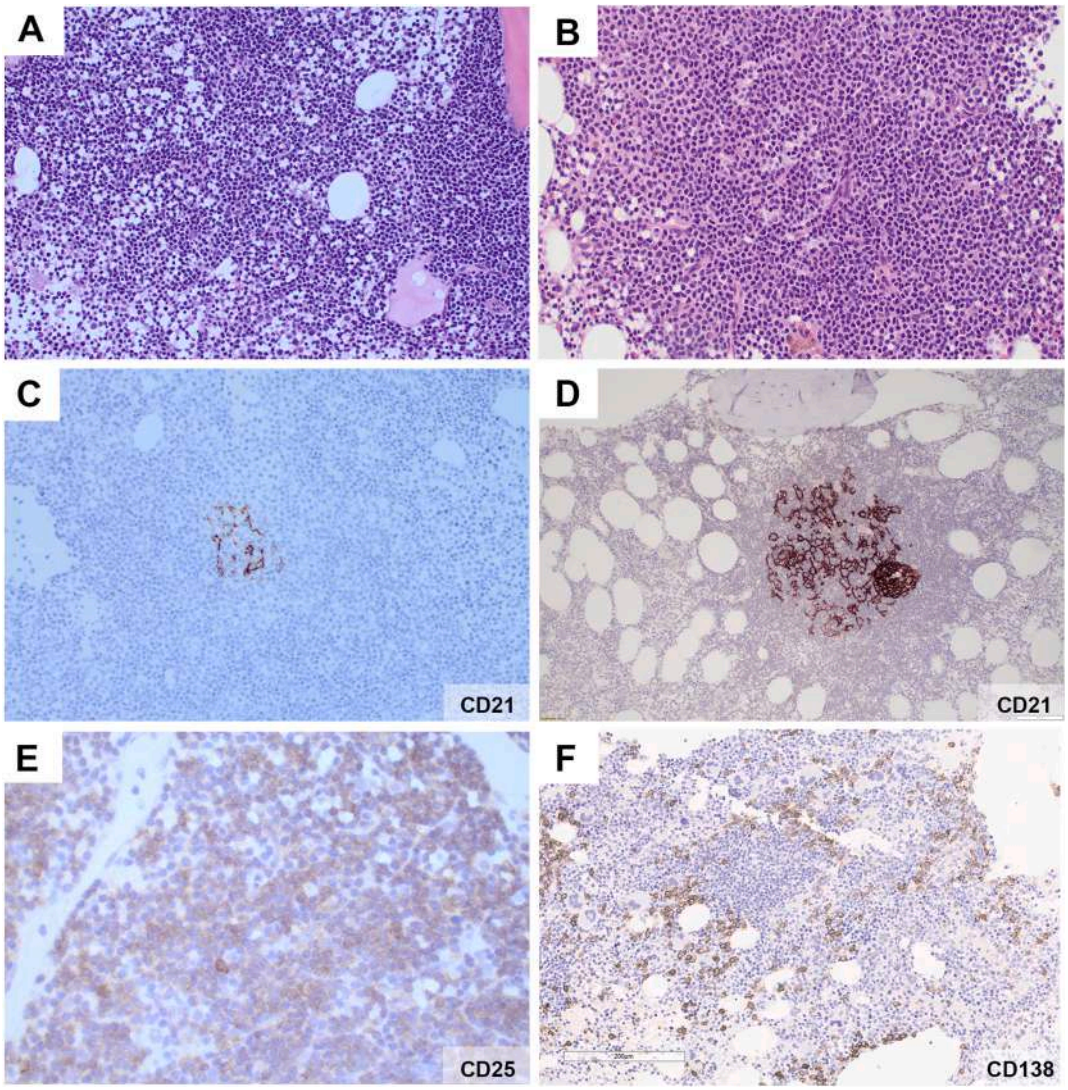

Fig. 7. A–B: Comparison of LPL/WM(A) and splenic MZL (B): both show mainly small lymphoid cells, but the MZL shows more heterogenous population with scattered larger cells. C–D: Follicular dendritic meshwork highlighted by CD21 are absent or ill-defined and hardly noticeable on routine H&E in LPL/WM (C) but more defined and expanded in MZL (D). Note also CD25 is usually expressed in LPL/WM (E). CD138+ plasma cells usually surround the lymphoid aggregate in both LPL and SMZL but are more common in LPL/WM (F). A and B: 200×, C–F: immunostaining, 200×.

type from MGUS becomes more nuanced.

Two types of MGUS are recognized in WHO-HAEM5 and ICC [8,90,91]: 1) IgM MGUS, not otherwise specified (NOS), for cases with clonal B cells that may harbor *MYD88*^{MUT} in ~90% cases; and 2) IgM MGUS-PC, defined as detectable clonal plasma cells without a clonal B-cell component and with a wild-type *MYD88*. These two subclasses are considered precursors to WM and other B-cell neoplasms, or PCNs, respectively. Therefore, IgM MGUS-PC also includes patients with t(11;14)(q13;q32) or other cytogenetic abnormalities typical of myeloma.

According to the current IWWM definition, IgM type of MGUS-NOS is defined as lack of morphological evidence of lymphoma (lymphoplasmacytic aggregates) in the presence of serum IgM paraprotein [92] even if monoclonal B cells are detected by flow cytometry. This definition is slightly different from the WHO-HAEM5 definition which allows a diagnosis of MGUS as long as paraprotein level is below 3 g/dL, and neoplastic infiltrate (B cells and plasma cellss combined) <10%, and absence of related end-organ damage and organomegaly [9]. As *MYD88*^{L265P} (60–90%) and *CXCR4*^{MUT} (16-20%%) can both be detected in MGUS, their presence does not distinguish MGUS from WM.

9.1.5. Plasma cell neoplasms

LPL cases with monoclonal proteins of IgG or IgA type can be misdiagnosed as myeloma [17]. These variants often show more plasmacytic differentiation, to the extent that the plasma cells may occasionally even outnumber B cells. Morphologically, plasma cells in LPL are small and mature, whereas plasma cells in myeloma show a spectrum of histological grades, from small mature to large and pleomorphic with distinct nucleoli [17]. Marked cytological atypia and immaturity is generally not a feature of plasma cells in LPL/WM as in myeloma. IgM myeloma may show lymphoplasmacytoid morphology that mimics LPL/WM. These tumors usually carry t(11;14)(*IGH*::*CCND1*) and positive for cyclin D1 by immunostaining (Fig. 6C) [93]. These tumors can even express CD20 or PAX5 sometimes further confounding interpretation.

Plasma cells in LPL and PCN also differ immunophenotypically. The "lymphoma type of plasma cells" in LPL are often positive for CD19, CD27, CD45, and CD81, and are usually negative for CD56 and CD117; dim CD56 expression has been noted in rare cases of non-IgM LPL cases [17]. In contrast, plasma cells from PCNs are more commonly negative or show reduced or absent expression of CD19, CD27, CD45, and CD81, and more often are positive for CD56 and/or CD117. Of note, CD19,

CD27 and CD45 are sometimes decreased in LPL, especially the IgG/A type. But total absence of these markers simultaneously is extremely rare. Lytic bone lesions are common in patients but not in WM patients unless large cell transformation has occurred. *MYD88*^{L265} has not been described in PCNs, but PCNs may show a complex karyotype and aberrations of t(11;14), t(4;14), t(6;14), t(14;16), t(14;20), and/or 1q+/1p-. Deletion of 13q can be seen in both myeloma and LPL/WM.

9.1.6. *Heavy chain diseases*

Heavy chain diseases (HCD) include gamma or mu heavy chain type and may morphologically be consistent with LPL, but serum protein studies show only monoclonal heavy chain. *MYD88*^{L265P} has been reported in a subset of mu HCD, but not in gamma HCD [94,95]. The distinction is based primarily on the results of serum protein studies. The plasma cells usually lack light chain expression by immunostaining or flow cytometry.

9.1.7. *Monoclonal immunoglobulin deposition diseases*

Light chain, light and heavy chain or heavy chain deposition disease are usually caused by plasma cell neoplasms, however, rare cases of lymphoma may present similar clinical features. LPL can be the underling pathology and CLL/SLL or MZL are others. Serum protein studies detect only monotypic light chain or heavy chain or both. Renal involvement and dysfunction are the most common clinical presentations, followed by hepatic, cardiac and neural deposits in a minority of cases [96–98]. The diagnosis is established by demonstration of the involved Ig in the involved organs.

9.2. *LPL in unusual scenarios*

Rare LPL cases in unusual scenarios have been occasionally reported in the literation that are diagnostically very challenging. One scenario is related to unusual immunoglobulin features, such as non-secretory, non-IgM type (IgG or IgA), and biclonal (IgM and IgG or IgA), in occasional LPL cases [74]. The bi-clonal LPL cases can be either of different immunoglobulin heavy chains, commonly IgM and IgG, or have different light chains, or a combination of both [99,100]. We have described a case of LPL with κ monoclonal B cells in BM and λ monoclonal B cells in lymph node [26,99]. Though LPL is considered a monoclonal process arising from a single transformed progenitor cell, the presence of two unrelated clonal populations in occasional LPL cases support the notion of clonal heterogeneity and clonal evolution in the pathogenesis of LPL.

Composite neoplasms are another diagnostic challenge. These cases are extremely rare, and have been presented in case reports, including monoclonal B-cell lymphocytosis (MBL), CLL/SLL, MALT lymphoma, MCL and myeloma [101]. When discordant κ and λ between the plasma cell and B cell components is present, a composite PCN and LPL should be considered (Fig. 8). Multiple immunophenotypically distinct subsets identified by flow cytometry analysis provide some clues. The clonal relationship between these composite neoplasms, whether related or independent, often requires molecular studies to discern. For example, clonal B cells can be present as precursors of neoplastic plasma cells in myeloma patients and are not necessarily an indication of composite PCN and MBL [102].

9.3. *Molecular testing*

MYD88 or *CXCR4* testing is recommended in all patients suspected of LPL/WM [103], especially those who are potential candidates for BTK inhibitor therapy. Testing for deletion of 6q and 17p *TP53* are also recommended in certain patient subgroups depending on clinical treatment need.

There are currently no standardized protocols for mutational analysis of *MYD88* or *CXCR4* in patients with LPL/WM. CD19 enriched or unselected BM or tissue samples can all be used. Unselected BM sample may yield a false negative result when the infiltrate is patchy and focal. Involved lymph node samples usually yield better results. In general, use of peripheral blood is not recommended in routine diagnosis. The utility of cell-free DNA from plasma has been shown to be promising in several small studies, which potentially offers a non-invasive, convenient and cost-effective approach for screening [104,105].

Available detection modalities include Sanger or next-generation sequencing (NGS), allele-specific PCR (AS-PCR) and ddPCR. While NGS and Sanger sequencing can detect other *MYD88* variants, Sanger sequencing has a relatively lower sensitivity [44]. NGS may miss *MYD88*^{L265P} and *CXCR4* mutations in up to 35% and 66% of patients, respectively, particularly in those with low BM disease burden (<10%), hypocellular or hemodiluted samples [106,107].

At a minimum, allele specific PCR for *MYD88*^{L265P} can be used on unselected BM samples, to achieve a detection limit of at least 1×10^{-3}. The ddPCR offers a far superior sensitivity at 5×10^{-5}, optimal for screening and MRD monitoring on unselected BM samples [108]. Sanger sequencing or NGS could achieve a similar level of sensitivity when CD19 enriched BM samples are used [15]. The newly introduced Competitive Allele-Specific TaqMan® PCR (Cast-PCR) is also another

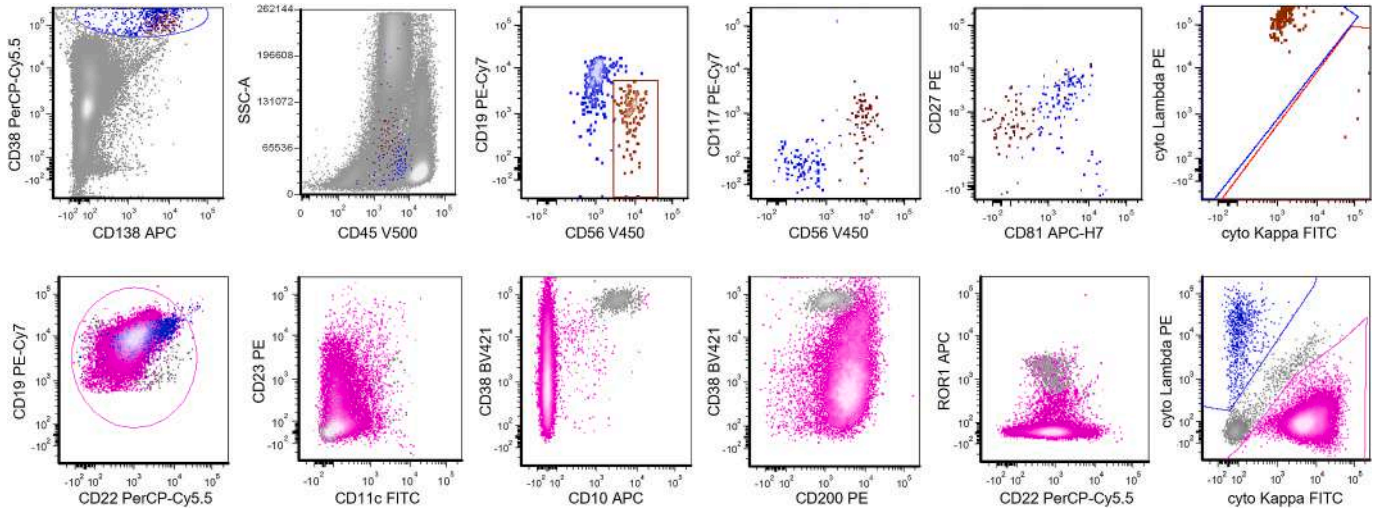

Fig. 8. A case of LPL/WM composite with a plasma cell neoplasm. Top: A distinct subset of λ monoclonal plasma cells (Brown) expressing CD56, CD117dim, with decreased CD19, CD27 and CD81. Cyclin D1 immunostaining is positive among the plasma cells (not shown). Bottom: Purple red: κ, blue: λ: The κ monoclonal B cells are positive for CD19, CD22, CD23 partial/dim, CD38 variable and CD200; negative for CD10, CD11c and ROR1.

effective technique when DNA content is low and can be used in whole BM samples [109]. Ideally, a case should not be considered *MYD88*^{WT} until after having been tested with a sensitive technique such as ddPCR. If a diagnosis of LPL/WM is still strongly suspected after a negative result from ddPCR, sequencing of entire *MYD88* genes is recommended to identify non-*MYD88*^{L265P} variants or other mutations [110]. To achieve this end, the IWWM-11 consensus recommends including probes that cover the entire *MYD88* gene in all NGS-targeted panels.

BM samples enriched for CD19^{+} cells by magnetic beads or flow cytometry sorting also allow detection of subclonal mutations (e.g. *CXCR4* and *TP53*) and non-*MYD88*^{L265P} variants [111]. Similarly, fluorescence in situ hybridization (FISH) analysis of chromosomes 6q and 17p should ideally be performed on enriched samples. Optical genomic mapping (OGM) is an emerging new technology that may replace or supplement FISH or karyotyping [112].

To cover the diverse *CXCR4* mutation spots, testing with Sanger and NGS using CD19 enriched B cells are recommended by IWWM-11 consensus [103]. Alternatively, since only nonsense mutations, but not frameshift mutations have been shown to be prognostically relevant and the vast majority of nonsense mutations reside in *CXCR4*^{S338X} [61,113], an AS-PCR or ddPCR assay targeting *CXCR4*^{S338X} is considered adequate when NGS is unavailable.

Testing of *TP53* alterations is recommended prior to each new line of therapy. Using CD19 enriched samples may help to avoid contamination of somatic variants associated with clonal hematopoiesis of indeterminate potential (CHIP) from myeloid cells that are common in an older patient population. Testing *TP53* abnormalities is particularly relevant in relapsing or refractory patients, and is generally useful for prognostication and adjusting therapy.

9.4. Post-therapy monitoring of disease

Post-therapeutic monitoring of LPL/WM can be challenging, as monotypic plasma cells can predominate in the absence of monotypic B cells in 25% of LPL/WM patients after therapy, with levels ranging from minimal (<1%) to 46% of cellularity. These monotypic plasma cells may persistent 1–50 months after the last course of chemotherapy [114].

Traditionally, treatment response relies on assessment of serum paraprotein levels after therapy. A drop in serum paraprotein levels is indicative of a good response to therapy, but a maximal response can be delayed for months after various therapeutic regimens. Current guidelines for assessment of response, IgM flare (rising IgM after treatment) and IgM rebound (rising IgM after discontinuation of treatment), or extramedullary disease have been updated recently and detailed in the IWWM-11 recommendation reports [92,115,116].

At the molecular level, the *IGH*-based PCR remains the classical, standard method. Although *MYD88*^{L265P} mutation detection by ddPCR has been shown to correlate with *IGH*-based assay in post-therapeutic monitoring, [108] its utility in minimal residual disease (MRD) monitoring has not been validated. MRD monitoring may provide a more detailed evaluation of response and survival outcome and could potentially be used to determine the duration of therapy and whether maintenance therapy is required.

10. Treatment and prognosis

The discovery of *MYD88* and *CXCR4* mutations in LPL/WM has facilitated rational drug development, including the development of BTK and CXCR4 inhibitors, and promoted genomic-based personalized therapy. For the management of symptomatic, treatment-naïve patients, the traditional chemoimmunotherapy (CIT) regimens remain central to the first-line therapy which are rituximab-based, either in conjunction with bendamustine (BR) or with Bortezomib and dexamethasone (BDR) or with cyclophosphamide (DRC) [117]. The class of covalent BTK inhibitors (cBTKi) (ibrutinib, acalabrutinib, zanubrutinib) can produce major responses in 70–80% of patients, when used either as single agents or in combination with rituximab [110,118].

The mutation status of *MYD88* and *CXCR4* can affect treatment response and clinical outcomes in patients receiving cBTKi. The response rate to ibrutinib is most superior among *MYD88*^{L265P}/*CXCR4*^{WT} genotype followed by *MYD88*^{L265P}/*CXCR4*^{WHIM} and least among *MYD88*^{WT}/*CXCR4*^{WT} genotype [33,119]. However, inferior response among the *MYD88*^{WT} genotype can be improved with more potent and selective second generation of BTK inhibitors such as acalabrutinib and zanubrutinib [63,111]. Mutations in *TP53* and *TERT* show a trend toward lower deep response rates as well as a less favorable progression-free survival (PFS) [61,65,103,113].

Clinical trials have shown that second-generation cBTKi are more potent and less toxic than ibrutinib, particularly in patients with *CXCR4*^{WHIM} or *MYD88*^{WT} or altered *TP53* [35]. As such, Treon and colleagues have recently proposed genomic-based treatment algorithms based on mutation status, disease characteristics and comorbidities in treatment-naïve and previously treated patients [110]. In the setting of relapsed or refractory disease, newer cBTKi or non-covalent BTKi (pirtobrutinib and nemtabrutinib), proteasome inhibitors and BCL-2 inhibitors as well as stem cell transplant are alternatives [110,116].

As an indolent B-cell lymphoma, the overall survival (OS) of LPL/WM patients is estimated to be around 40–50% at 10 years. The causes of death are mainly disease progression, transformation to aggressive lymphoma or therapy-related complications [120,121]. A revised International Prognostic Scoring System for WM (WM IPSS) has been proposed in the era of rituximab-based CIT to improve risk stratification, which is based on 4 identified risk factors including advanced age, LDH (>250 IU/L), albumin (<3.5 g/dL) and β2-microglobulin (B2M) (>4 mg/L) [122]. The previously recognized risk factors such as serum monoclonal protein levels, anemia and thrombocytopenia in the original WM IPSS are no longer valid after rituximab-based CIT became the first line treatment regimen [123]. For the asymptomatic WM patients, the BM tumor burden, IgM level, B2M and serum albumin levels are still used to calculate a score of risk for progression to symptomatic disease [124]. The median time to progression is 1.8, 4.8, and 9.2 years, respectively, for the high, intermediate and low risk groups [125].

MYD88^{WT} is considered an independent risk factor for progression in asymptomatic WM patients, and is associated with a higher risk of transformation to large B-cell lymphoma, and thereby a shorter OS than LPL/WM patients carrying *MYD88*^{L265P} [52,59,126,127]. However, the data are controversial [128]. The impact of *CXCR4*^{WHIM} on OS has not been proven among patients treated with proteosome inhibitors [52]. Although patients with *TP53* alterations have shorter OS and/or progression free survival (PFS) treated with BTK inhibitors in some studies, its impact in the primary treatment setting is still under active investigation [110,115]. Survival data in the era of genomic testing and cBTKi therapy are emerging from clinical trials and may help better outline the survival outlook of patients in various risk groups stratified based on mutation profile in the future.

11. Summary

The discovery of *MYD88* and *CXCR4* mutations marked the dawning of genomic medicine which has greatly advanced our understanding of the biology of LPL and WM, and in turn, reshaped our practice in the past decade. The progress has not only improved diagnostic accuracy but also informed therapeutic decision making. Still in current practice, morphologic evaluation remains the pivotal starting point, with incorporation of sensitive ancillary studies necessary to fully characterize the neoplastic cells. While *MYD88*^{L265P} resolved much ambiguity, it does not totally trump morphology. The tentative diagnosis of "B-cell lymphoma with plasmacytic differentiation, CD5 and CD10 negative" ideally should only be reserved for cases truly difficult to subclassify. Modified diagnostic criteria with removal of a minimal threshold in IgM and tumor burden in the latest classification schemes are most applicable to symptomatic WM patients. Distinction from nodal MZL producing a

paraprotein, with or without *MYD88*^{MUT} needs further exploration. Unusual scenarios such as an atypical immunophenotype with CD5 and/or CD10 expression, non-IgM type LPL, biclonal LPL and composite neoplasms are pitfalls to be kept in mind during the diagnostic workup. Molecular testing using sensitive PCR-based techniques or CD19^{+} enriched BM samples is recommended for detecting *MYD88*^{L265P} whereas genomic profiling provides a global glimpse of the tumor genetics and detection of other *MYD88* variants. Questions remain to be answered as to the pathogenesis of *MYD88*^{WT} variant, the collaboration of *MYD88*^{L265P} and other genetic alterations, and the interplay of tumor and their environment. In the era of multi-omics, the future holds greater promise for deeper insights into the biology and transformative clinical practice.

CRediT authorship contribution statement

Lianqun Qiu: Writing – review & editing, Writing – original draft. **Pei Lin:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

All the authors declare no conflict of Interest.

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