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Review Article

Primary Mediastinal Large B-cell Lymphoma: Diagnostic Challenges and Recent Advances

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Abstract

Primary mediastinal (thymic) large B-cell lymphoma (PMBL) is a subtype of uncommon aggressive large B-cell lymphomas primarily occurring in mediastinum although rare cases with non-thymic type of PMBL have been reported. Typical PMBL has characteristic clinical, morphological, and immunophenotypic features which the pathologists use as diagnostic paradigm in routine practice. However, the diagnosis can be occasionally challenging due to the overlapping clinicopathologic features with other lymphomas, among which are nodular sclerosis classic Hodgkin lymphoma, systemic diffuse large B-cell lymphoma (DLBCL) involving mediastinum, and B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic Hodgkin lymphoma (gray zone lymphoma). Recent depictions of the characteristic genetic/ molecular aberrations and unique gene expression profiling in PMBL have provided a robust tool to significantly improve the diagnostic accuracy. In addition, the progresses in understanding the pathogenesis of PMBL have paved the way discovering novel therapeutic agents for patients with refractory/relapsed disease.

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Introduction

Primary mediastinal (thymic) large B-cell lymphoma (PMBL) belongs to the group of aggressive large B-cell lymphomas. It was initially recognized as a rare subtype of diffuse large B-cell lymphoma (DLBCL) in the Revised European American Lymphoma classification in 1994. However, due to its unique clinical, pathologic, immunophenotypic and genomic features, PMBL has been recognized as a separate distinct lymphoma in the World Health Organization (WHO) classification since 2001.[1] The aims of this current review are to summarize the clinical, morphologic, and immunophenotypic features of PMBL; illustrate genetic and molecular characteristics of PMBL; and describe the main differential diagnosis of PMBL.

Epidemiology and clinical presentation of PMBL

PMBL constitutes approximately 2–3% of all non-Hodgkin lymphomas and predominantly affects young adults. The median age of patients is 35-years-old with a female:male ratio of ~2:1[1,2] although cases from other age groups have been occasionally reported. PMBL typically presents as a bulky (greater than 10 cm in the largest dimension) and fast-growing anterior mediastinal mass, often associated with localized supraclavicular lymph node involvement. Intrathoracic extension into the lungs, chest wall and pericardial and pleural spaces is common, whereas extrathoracic disease, including distant lymphadenopathy and bone marrow involvement, is very rare at initial presentation. Therefore, approximately 80% of patients initially present as stage I or II disease. However, nodal disease and extranodal dissemination to kidney, breast, adrenal cortex, ovary, liver, pancreas, and gastrointestinal organs may uncommonly occur at relapse.[3] In addition, rare cases of PMBL present as a non-mediastinal tumor without evidence of mediastinal involvement.[4] Due to the bulky disease, approximately 50% of patients have superior vena cava syndrome and present with facial swelling, dyspnea, headache, neck vein distention, and occasionally thrombosis. B symptoms are not uncommon.[2]

Cytomorphologic and immunophenotypic features of PMBL

Morphologically, the majority of PMBLs show a diffuse infiltrative pattern, although occasional cases with focal vague nodularity have been reported. The most characteristic morphologic feature of PMBL is sclerosis surrounding lymphoma cell nests, producing a so-called alveolar compartmentalization growth pattern (Fig. 1a).[1] Sclerosis varies from case to

Keywords: PMBL; Cytomorphology; Immunophenotype; GEP; Genetic abnormality.

Abbreviations: BLK, B-cell lymphocyte kinase; CGH, comparative genomic hybridization; cHL, classic Hodgkin lymphoma; CIITA, class II transactivator; COO, cell-of-origin; DLBCL, diffuse large B-cell lymphoma; GEP, gene expression profiling; GZL, gray zone lymphoma; HRS, Hodgkin and Reed-Sternberg; IHC, immunohistochemistry; IL, interleukin; IRF, interferon response factor; MHC, major histocompatibility class; NSCHL, nodular sclerosis classic Hodgkin lymphoma; PMBL, primary mediastinal large B-cell lymphoma; SHM, somatic hypermutation; TRAF1, TNF receptor associated factor 1; WHO, world health organization.

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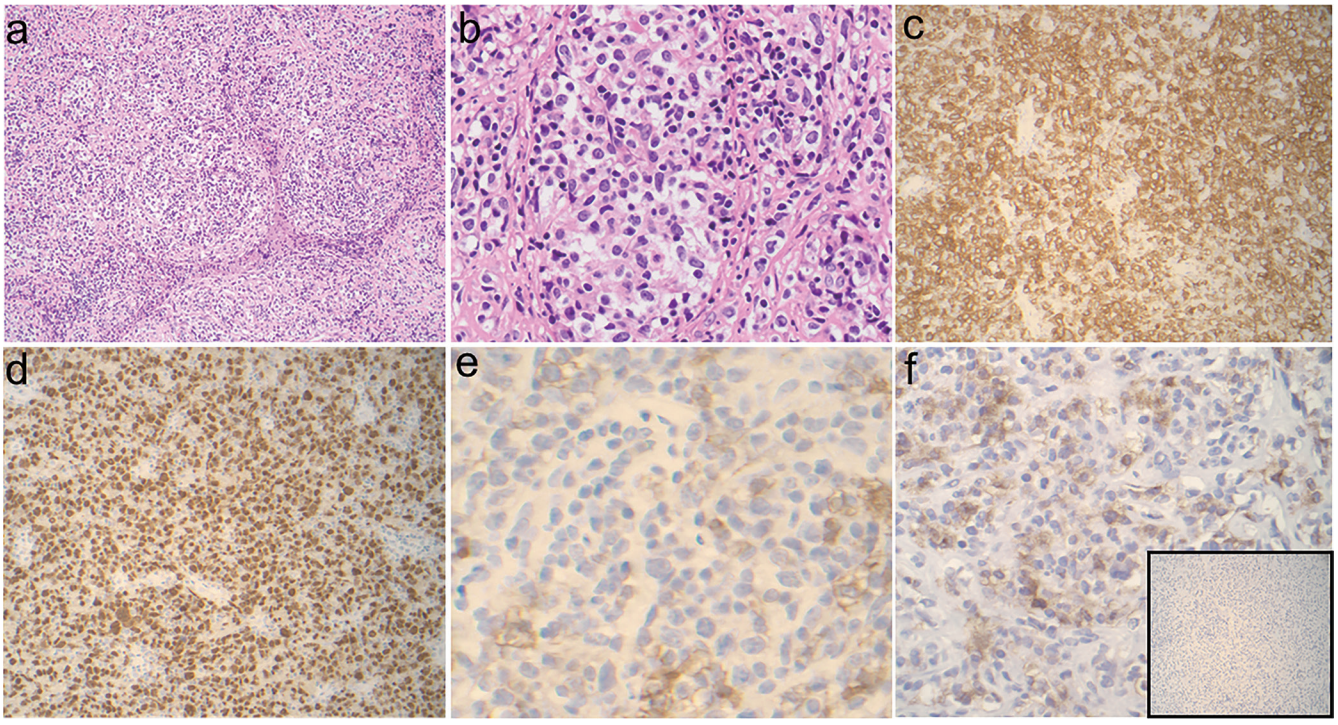

Fig. 1. Typical PMBL morphology and immunophenotype. (a) Low-power and (b) high-power magnifications show classical cytomorphologic features of PMBL in a 57-year-old female who had a mediastinal mass. (a) Atypical lymphoid cells are arranged in nests separated by delicate fibrosis, forming an alveolar compartmentalization pattern. (b) Atypical lymphoid cells are medium size with abundant clear cytoplasm (the original magnifications of A and B are 100× and 400×, respectively). The lymphoma cells are strongly and diffusely positive for (c) CD20 (200×) and (d) PAX-5 (200×). However, tumor cells are weakly and focally (~20%) positive for (e) CD30 (400×). While the lymphoma cells are partially positive for (f) CD23 (400×), both CD23 and CD21 (inset, 40×) show the absence of follicular dendritic cell meshwork despite a nest-like growth pattern.

case, ranging from typical delicate compartmentalizing sclerosis, intersecting bands of fibrosis (Fig. 2a), to occasional broad septa of dense collagen. Focal necrosis is sometimes seen. Cytologically, the lymphoma cells are usually medium to large size with irregular nuclear contours, small nucleoli, and abundant clear to eosinophilic cytoplasm (Fig. 1a, b). Not uncommonly, lymphoma cells can display centroblast-like (Fig. 2b), immunoblast-like, or anaplastic morphology (Fig. 2c). Occasionally, neoplastic cells are multinucleated, mimicking Hodgkin-Reed-Sternberg (HRS) cells (Fig. 2d). In addition, the mitotic rate is generally high.[5,6]

The normal counterparts of PMBL tumor cells are thought to be CD21(−)/CD23(+) medullary thymic B-cells with either a germinal center (GC) or post-GC origin.[7] Immunophenotypically, PMBL uniformly expresses leukocyte common antigen CD45 and is positive for pan B-cell membranous markers, including CD19, CD20 (Fig. 1c), and CD79a.[1] B-cell transcription factors, such as PAX5 (Fig. 1d), OCT2, PU.1 and BOB1, are expressed with strong nuclear staining.[8] CD30 (Fig. 1e) is expressed in ~70% of cases but is typically a heterogeneously dimmer in comparison to classic Hodgkin lymphoma (cHL) and anaplastic large cell lymphoma.[9] In addition, the CD30 expression is often focal, patchy, and only seen in a subset of tumor cells. Although rare EBER positive PMBL cases have been reported,[10,11] expression of EBER and/or CD15 in tumor cells opposes the diagnosis of PMBL. The PBML tumor cells are positive for CD23 (Fig. 1f) expression in ~70% of cases. Despite a common nest-like growth pattern, PMBL lacks follicular dendritic meshwork as demonstrated by negative CD21 staining (Fig. 1f inset). Variable expressions of CD10 (~20%), BCL6 (50–60%), MUM1 (40–70%), and BCL-2 (30–80%) have also been reported in PMBL.[8,12] The expression of human leukocyte antigen (HLA) class I and II molecules are diminished to absent in a majority of PMBL cases in contrast to other B cell lymphomas.[1] Surface light chain immunoglobulin expression is non-detectable in ~50% of cases by flow cytometry analysis.[13] The proliferation index determined by Ki-67 immunohistochemistry (IHC) is generally high, ranging from 40% to 90%. While variable MYC protein expression detected by IHC has been found in >90% of PMBLs, only approximately 33% PMBL cases showed high (> 30%) nuclear positivity.[14] Of interest, most MYC positive cases have no MYC translocation, and positive MYC expression does not confer a prognostic significance.[14] While PMBL is often positive for expression of CD274 (PD-L1) and CD273 (PD-L2, also known as PDCD1LG2), PD-L1 expression can be seen in tumor-associated macrophages, which may complicate the interpretation of PD-L1 expression in lymphoma cells.[15,16] Recently, Kim H *et al*. demonstrated that ~80% of PMBLs are positive for p63 expression but negative for GATA3 expression.[17] Other recent studies have reported that the expressions of PD-L1, PD-L2, MAL, CD200, TNFAIP2, TRAF1, and c-REL have high sensitivity and specificity (both ranging ~70–90%) in diagnosing PMBL.[18,19] As such, the use of some of these new markers, particularly MAL, c-REL, and CD200, is increasing in major academic laboratories.

Gene expression profiling of PMBL

Gene expression profiling (GEP) is a powerful tool to reveal lymphomagenesis. GEP studies[20,21] have shown that PMBL has the following features: (1) high expression of genes located at chromosome band 9p24, including *JAK2, PD-L1, PD-L2*, and *SMARCA2* (mainly due to copy number increases); (2) high expression of IL-13 and its downstream ef-

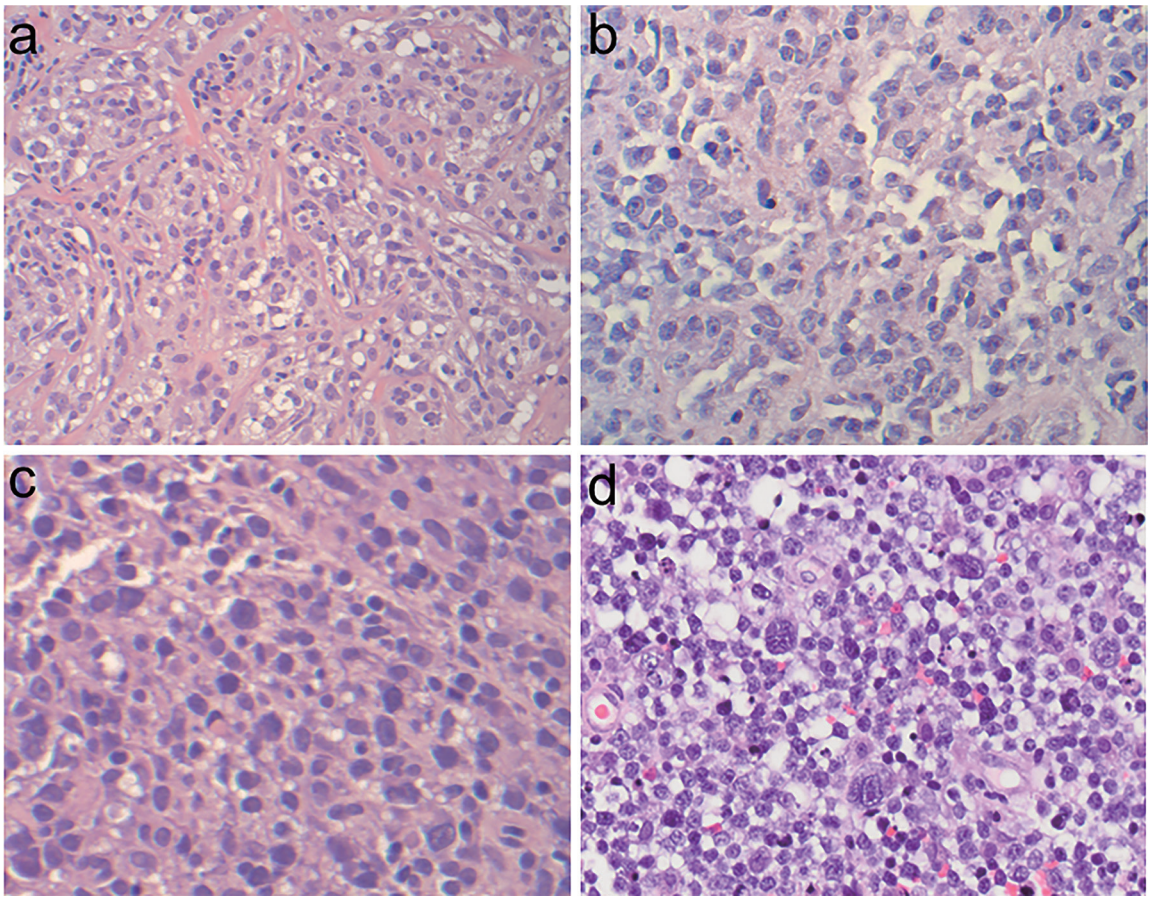

Fig. 2. Representative cases of PMBL with variable morphologic features (H&E, 400×). (a) A case of PMBL from a 16-year-old male shows intersecting fibrosis bands separating lymphoma cells. (b) A case of PMBL from a 26-year-old female presents as sheets of centroblast-like lymphoma cells with delicate fibrosis. (c) A case of PMBL from a 17-year-old female exhibits diffuse proliferation of lymphoma cells with anaplastic morphology. (d) A case of PMBL from a 38-year-old male demonstrates the majority of lymphoma cells with medium size without significant fibrosis. Scattered Hodgkin and Reed-Sternberg (HRS) cells are noted. Additional information regarding case (d): all lymphoma cells, including HRS cells, are positive for CD20; approximately ~20% of cells are positive for CD30 and CD23 and negative for CD15; no surface light chain expression was detected by flow cytometry. Fluorescence *in situ* hybridization (FISH) showed three copies of MYC, but no rearrangements of BCL2, BCL6, or MYC were detected. NGS showed amplifications of *PD-L1 (CD274), PD-L2 (PDCD1LG2*) *JAK2* and *KDM4C*, mutations of *IDH1* R132C, *ARID1A* P94fs*12, *B2M* L7*, L7fs*34, FBXO11 splice site, 1009-2A>G, and *TNFAIP3* F540fs*162, I629fs*71. The overall immunophenotypic and genomic profiles are most compatible with PMBL.

fector genes, including JAK2, STAT1, TNF family members, and TRAF1 (TNF receptor associated factor 1); (3) activation of NF-κB pathway manifested by nuclear shuttling of c-REL (2p16); and (4) low expression levels of multiple components of the B-cell receptor cascade, including AKT1, BLK (B-cell lymphocyte kinase), CD10, CD22, FOXP1, and the major histocompatibility complex (MHC) class II components. Interestingly, Rosenwald *et al*.[20] pointed out that PMBL and cHL share fascinating similarities between their GEPs, whereby over 33% of all PMBL signature genes are more highly expressed in cHL, including CD30, MAL, SNFT, TNFRSF6 and TARC. The striking overlapping GEP features between PMBL and cHL also manifest that both PMBL and cHL show amplifications/gains of genes located at 9p24[20] and both have low expression of genes involved in the B-cell receptor signaling cascade.[22]

Recent studies of B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and cHL, commonly referred as gray zone lymphoma (GZL) by Sarkozy *et al*.[23] provided additional information with regard to the GEP of PMBL in relationship between PMBL and thymic subtype of GZL in that thymic type of GZL resembled PMBL but the non-thymic type of GZL resembled DLBCL. These results added to the notion that the specificity of the thymic niche played an important role in the pathogenesis of PMBL, nodular sclerosis classic Hodgkin lymphoma (NSCHL), and thymic subtype of *bona fide* GZL.

Genetic/chromosomal aberrations

The detection rate of MYC alterations varies depending on the method used. For example, Scarpa *et al*.[24] reported that 25% of PMBL exhibited MYC alternations using Southern blotting and PCR-single strand conformation polymorphism (SSCP); however, only 6.25% (2/32) had rearrangement of the 2nd/3rd exon, which is characteristic of the translocations found in sporadic Burkitt lymphoma. BCL2 and BCL6 translocations were absent in the 16 PMBLs studied.[25] Chromosomal translocation of MHC class II transactivator (CIITA) on chromosome 16p13 has been reported in 38% of PMBL.[26] It has been shown that fusion of CIITA, which is the master transcriptional regulator of MHC class II expression, resulted in decreased surface HLA-DR expression. This decreased expression was correlated to the loss of HLA-DR expression, a phenomenon rarely seen in mature and im-

mature B-cell lymphoma/leukemia, that was identified in ~20% of PMBL.[26] Of pertinent note, identical CIITA fusion was also found in 15% of cHL,[26] providing additional evidence that mediastinal PMBL and cHL share overlapping genetic features. Rearrangement of chromosome 9p24.1 containing programmed death ligand (PDL) was found in 20% of 125 PMBL cases studied.[27] Moreover, 9p24.1 locus contains several important genes, including CD274 (PD-L1) and CD273 (PDCD1LG2, PD-L2).

Genomic landscape of PMBL

The genomic landscape used in this study refers to genomic changes other than chromosomal translocations, as aforementioned above, and includes gains/amplification due to copy number alterations (CNA), insertions, deletions, and mutations.

Immunoglobulin (Ig) genes

Despite that 50% PMBL cases did not express surface kappa or lambda light chains,[13] PMBL showed monoclonal Ig rearrangement. In addition, there is evidence of somatic hypermutation (SHM) and heavy chain class switch but without ongoing mutational activity.[28]

Gains/amplifications

Gains/amplifications of certain chromosomal regions, such as chromosome 9p24, are a frequent feature of PMBL. For instance, a gain of chromosome 9p24.1 with a 5.6-fold higher expression of CD273 (PD-L2) was found to be the overall best PMBL distinction compared to DLBCL.[20] In fact, copy number changes of PD-L1 (CD274) and PD-L2 (CD273) were reported in 71% of cases.[21] Other amplified genes from 9p24 locus included JAK2 and SMARCA2.[20] The expression of PD-L2 was easily assessed by IHC and was associated with gains of copy numbers.[16] Amplification of 2p16.1 locus, where the c-REL gene resides, was observed in 41% of PMBLs.[29] Comparative genomic hybridization (CGH) also revealed significant gains of chromosomes 9, 19, and X and a loss of 4 in PMBL compared to DLBCL.[30]

Genes mutations

Large-scale and high-throughput tools, such as next generation sequencing (NGS), have revolutionized our understanding of the mutational landscape of PMBL, which is described in following subsections.

JAK-STAT and NF-kB pathways: Recurrent somatic genomic abnormalities in JAK-STAT and NF-κB signaling pathways were traditionally regarded as the genetic hallmark of PMBL. JAK/STAT pathway transfers signals from cell-membrane receptors to the nucleus and is essential for a wide range of cytokines and growth factors leading to critical cellular events, such as hematopoiesis and immune system development. The most common molecular abnormalities identified in PMBL include JAK2 copy number gain on chromosome 9p24 band SOCS1 structure variant and somatic mutations in IL4R, STAT6, SOCS1, CSF2RB, PTPN1, and CISH. The end effect of these gene alterations is the constitutive activation of the JAK/STAT pathway, which in turn provides lymphoma cells with a proliferative advantage.[31] NF-κB signaling pathway activation is another important dysregulated pathway in the pathogenesis of PMBL. The most common genetic abnormalities in this pathway include chromosomal gains and amplifications of the *REL* gene locus on chromosome 2p16, *BCL10* (1p22), and *MALT1* (18p21); chromosome deletion and biallelic inactivating mutations of *TNFAIP3* on chromosome 6q23; and inactivating mutation of *NFKBIE*. Both TNFAIP3 and NFKBIE are negative regulators of the NF-κB pathway. In contrast, mutations of *NFKBIA*, a member of NF-κB pathway and often mutated in CHL, were absent in PMBL and cause constitutive NF-κB pathway activation.[32]

Tumor microenvironment: The tumor microenvironment changes involving immune evasion in PMBL lymphomagenesis have been increasingly studied particularly with the advent of therapy using immune checkpoint inhibitors. Copy number changes of PD-L1 (CD274) and PD-L2 (CD273) were already discussed above.[19] Genetic alterations discovered in other genes in this pathway include *B2M* (β_2-microglobulin), IL13RA, and *CD58*,[31] which in turn lead to decreased expression of MHC I and II molecules.

Interferon response elements pathway: Mottok *et al*.[33] recently performed whole-exome sequencing of a large cohort of PMBL cases and identified the interferon response factor (IRF) pathway as a potential oncogenic pathway in approximately 50% of PMBL cases. Recurrent oncogenic mutations have been seen in multiple IRF pathway members, including IRF2BP2, IRF4, IRF8, and IRF1, in a mutually exclusive pattern. IRF4 and IRF8 transcription factors are known to play an important role in B-cell development as well as in the germinal center response. The mutations of these genes dysregulate B-cells, facilitating tumorigenesis. In addition, this whole-exome sequencing study provides additional evidence that PMBL is distinct from DLBCL in terms of gene expression level, mutational landscape, and oncogenic driver genes. Conversely, PMBLs have significant overlapping driver gene mutations with classic Hodgkin lymphoma, further establishing the relation between PMBL and cHL.

Interleukin (IL-4)/IL-13 signaling pathway: Significantly increased expression of IL-4 induced gene 1 was observed in 94% (16/17) of PMBLs in contrast to low expression in 67% of DLBCL (12/18).[34] PMBL also expressed high levels of IL-13, IL-13 receptor α1 and its downstream effector genes.[22] Although STAT6 is a downstream effector gene of IL-13 and phosphorylated STAT6 (P-STAT6) is highly expressed in PMBL [72.7% (8/11) in comparison to 10% (1/10) in DLBCL, it was shown that upregulation of P-STAT6 was not via IL-13, but by high expression of JAK2.[35]

GEP and genomic analysis of PMBL have led to improved accuracy of PMBL diagnosis. For instance, Mottok A. from the Mayo Clinic recently developed a molecular assay to distinguish PMBL from DLBCL using routinely available formalin-fixed paraffin-embedded tissue biopsies.[36] The so-called Lymph3Cx assay is a quantitative gene RNA expression assay that analyzes 58 genes using the Nanostring platform. Of these 58 genes, 24 genes are overexpressed in PMBL and 6 genes are overexpressed in DLBCL. The rest of genes allow cell-of-origin (COO) determination in DLBCL. The assay not only distinguishes PMBL from DLBCL NOS, but also provides COO assignments to DLBCL NOS. Under the appropriate clinical circumstances, PMBL becomes a molecular diagnosis. Furthermore, the identification of recurrent genetic abnormalities in PMBL has provided novel targets in precision treatment. Agents, such as small molecule inhibitors of the JAK/STAT pathway and immune checkpoint inhibitors, have great potential in disease management.

Differential diagnosis of PMBL

The main differential diagnoses of PMBL are lymphomas involving mediastinum, namely NSCHL, GZL, and DLBCL,

NOS. Despite similar and overlapping GEPs and genomic landscapes between NSCHL and PMBL as mentioned above, NSCHL should be readily distinguished from PMBL. While PMBL contains HRS-like cells, the cellular components from these two entities are quite different. Specifically, PMBL contains mononucleated medium to large size lymphoma cells, while NSCHL consists of background inflammatory cells. CD30 and PAX5 are uniformly and weakly positive in NSCHL, but only variably positive and strongly positive in PMBL, respectively. Other B-cell specific transcription factors like BOB1 and Oct2 are strongly positive in PMBL, but only positive in a small fraction of NSCHL. PMBL has a strong expression of common B-cell antigens except dim CD22, whereas NSCHL does not express these antigens except variable and weak CD20. A recent report demonstrated that the majority of NSCHL is positive for GATA3 expression with no p63 expression, while PMBL is positive for p63 expression but negative for GATA3 expression.[17]

The differential diagnosis between PMBL and GZL is challenging, partially because GZL is an evolving entity, and the diagnostic criteria continue to emerge. Based on mutational analysis, GZL is divided into thymic GZL (involving the thymic niche, the traditional GZL) and non-thymic GZL.[37] Thymic GZL involves the mediastinum of young males and is associated with a more aggressive clinical course than NSCHL or PMBL. Non-thymic GZL is rarely reported and occurs more frequently in older patients. By definition, GZL demonstrates morphologic and immunophenotypic overlapping features with both PMBL and cHL but does not fulfill the diagnostic criteria of either entity.[1] GZL also commonly exhibits confluent sheets of tumor cells, with a background containing a paucity of inflammatory cells. Variable fibrosis can be present, including extensive coarse and fine compartmentalizing fibrosis. Cytomorphologically, neoplastic cells exhibit a broad range of size and shape from centroblast-like and immunoblast-like to Hodgkin-like cells in different areas of the same case. The immunophenotype of GZL is also variable with transitional and divergent patterns of both PMBL and cHL. Tumors morphologically resembling cHL usually show strong CD20, strong PAX-5, weak/absent CD30, and absent CD15 expression. Comparatively, tumors morphologically mimicking PMBL are frequently positive for CD30 and CD15 but negative for CD20 and CD79a. All GZLs should show expression of at least one B-cell marker, such as CD20, CD79a, and PAX-5. MUM1 and CD45 are invariably positive, and MAL expression is identified a significant subset of GZL.[1,38,39]

Distinguishing PMBL from systemic DLBCL, NOS involving mediastinum can be challenging in certain clinical settings. Systemic DLBCL, NOS can occasionally present as a mediastinal mass with or without extra-mediastinal involvement. The diagnosis of PMBL is relatively straightforward if the lymphoma exhibits the typical morphology and immunophenotypic features of PMBL, including medium to large-sized cells with occasional multilobated nuclei, abundant clear cytoplasm in the background of fibrosis, variable CD30, and CD23 expression.[1] However, if the typical morphology and immunophenotypic features are absent, the definitive diagnosis of PMBL versus systemic DLBCL with mediastinal involvement is extremely difficult to render. On the other hand, there are occasional reports describing large B-cell lymphoma with typical pathologic features of PMBL but without detectable mediastinal involvement. The differential diagnosis in such instances includes extra-mediastinal PMBL vs systemic DLBCL with PMBL features. Since the management and prognostics of PMBL and systemic DLBCL are different, a definitive diagnosis is clinically desirable. The most recent advent of the Lymph3Cx assay based on molecular profiling of tumors has proved to accurately distinguish PMBL from DLBCL, making PMBL a molecular diagnosis.[36] Moreover, appropriate applications of this assay could improve the diagnostic accuracy for patients with PMBL. The differential diagnoses of PMBL are summarized in Table 1.

Treatment of PMBL

Historically, PMBL was treated as a variant of DLBCL using the combination of rituximab with CHOP/CHOP-like regimens followed by mediastinal radiation therapy. Although this regimen has resulted in favorable outcomes for most patients, exposure to radiation may pose an adverse effect in young patients. Currently, many medical centers in the United States have considered using more intensive regimens, such as EPOCH-R or DA-EPOCH-R without mediastinal radiation, as the standard treatment for PMBL.[40] For refractory or relapsing disease, high-dose therapy followed by auto-SCT is regarded as the standard of care. More recently, immune checkpoint inhibitor pembrolizumab has demonstrated highly effective and safe antitumor activities in clinical trials[41] and has received FDA approval for treating patients with chemotherapy-refractory PMBL. JAK/STAT pathway inhibitors and CD19 CAR-T therapy are among other novel agents with great potential in PMBL management.

Conclusions

In summary, PMBL is an uncommon subtype of large B-cell lymphoma that primarily involves the mediastinum. PMBL displays a relatively unique GEP and mutational landscape that resembles NSCHL compared to DLBCL and has a better 5-year survival than DLBCL. While classical PMBL with typical diagnostic morphologic and immunophenotypic features poses minimal diagnostic challenges, atypical PMBL should be distinguished from cHL, GZL, and systemic DLBCL, NOS involving the mediastinum. It is expected that the appropriate application of available molecular tools will help to significantly increase the diagnostic accuracy of PMBL in the future.

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None.

Conflict of interest

Dr. Zhou has been an editorial board member, and Dr. Wang has been an editor-in-chief of *Journal of Clinical and Translational Pathology* since May 2021. The authors have no other conflicts of interest to declare.

Author contributions

Dr. Zhou and Dr. Wang contributed equally to initiating the study, writing the manuscript, and critical revision. They have approved the final manuscript.

Ethical statement

Written informed consents were obtained from all patients for publication of the images described in the figures.

Table 1. Comparison of PMBL, GZL, NSCHL, and DLBCL, NOS

	PMBL	GZL	NSCHL	DLBCL, NOS, with mediastinal involvement
Age (median)	35	20–40	15–35	70
Gender	M:F=~2:1		F>M	M>F
Location (mediastinum)	Anterior mediastinum	Mediastinum	Mediastinum (80% of cases)	Rare
Location (other than mediastinum)	Lung, pleura, pericardium, breast	Other locations	Other locations	GI, BM, liver, muscle
Growth pattern	D or N	D	N	D
Presence of fibrosis	Yes	Variable	Yes	No
Presence of HRS cells	Yes	Yes	Yes	No
Expression of common B-cell antigens CD19, CD20, CD79a	Yes	Variable	No (but dim CD20 can be seen ~20%)	Yes
Expression of B-cell transcription factors (BOB1 and OCT2)	Yes	Yes	Absent or partially and weakly	Yes
Expression of CD23	71–95%	67%	9%	12%
Expression of CD45	Yes	Yes	No	Yes
Expression of PAX-5 and its intensity	Yes	Yes	Yes, but dim	Yes
Expression of surface kappa or lambda by FCM	~50%	Variable	No	Vast majority
Rearrangement of Ig	Yes	Yes	Yes (among enriched HRS cells)	Yes
Somatic hypermutation of Ig	High load	Not known	Not known	Presence in ABC type
Ongoing somatic hypermutation of Ig	Absence	Not known	Not known	Presence in GC subtype
Gene expression profiling	Please see Text	Thymic GZL similar to PMBL and NSCHL	Similar to PMBL	Diverse
Translocation of MYC	Absent or rare	Absent	Absent	5–8%
Translocation of BCL2	Absent or rare	Absent	Absent	5–40%
Translocation of BCL6	Absent or rare	Absent	Absent	15–30%

PMBL, primary mediastinal large B-cell lymphoma; GZL, gray zone lymphoma; NSCHL, nodular sclerosis classic Hodgkin lymphoma; DLBCL, NOS, diffuse large B-cell lymphoma, not otherwise specified; M, male; F, female; GI, gastrointestinal tract; BM, bone marrow; D, diffuse; N, nodular; HRS, Hodgkin and Reed-Sternberg; FCM, flow cytometry; Ig, immunoglobulin; ABC, activated B-cell; GC, germinal center.

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