INTRODUCTION

Small B cell lymphomas are mature B cell lymphoid neoplasms arising from the various differentiation stages of B cell development. Although in the World Health Organization (WHO) classification of lymphomas, they are described based on their morphology, phenotype and genetics, the differential diagnosis can be difficult because of some overlapping characteristics. Differences observed in the clinical outcome of cases representing the same entity have also made it difficult to understand fully the biology of the small B cell lymphomas, but interpretation of the results of molecular profiling studies has helped pathologists and clinicians in this regard. Some specific genetic changes defined on the neoplastic B cells have revealed the route of lymphomagenesis. But there are still debates on description, biology and differential diagnosis of some entities. Focusing on the interactions between the neoplastic cells and the cells or structures of the microenvironment is the new concept which holds promise for explaining some of the unresolved questions. Herein, we provide an overview of the recent advances in the diagnosis, prognosis and biology of small B cell lymphoma entities together with their description in the WHO lymphoma classification.

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL)

Chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) is a hematopoietic neoplasm of B-lymphocytes found in the peripheral blood, bone marrow, and/or lymph nodes. The term SLL is used for non-leukemic cases having the morphology and immunophenotype of CLL. Many cases of CLL/SLL are thought to correspond to the recirculating naïve B cells. Cases that show Ig gene variable region mutations may correspond to a subset of memory B cells [1].

The nodal presentation of CLL/SLL is characterized by the diffuse infiltration of small lymphocytes admixed with prolymphocytes and para-immunoblasts, giving rise to proliferation centers or pseudofollicles. CLL/SLL cells are small lymphocytes with round nucleus, clumped chromatin and scant cytoplasm. Nucleoli are generally absent. Prolymphocytes are medium-sized cells with dispersed chromatin and small nucleoli, whereas para-immunoblasts are medium to large cells with round nuclei, dispersed chromatin, central eosinophilic nucleoli and basophilic cytoplasm. The number of para-immunoblasts and size of pseudofollicles vary from case to case. Some cases show plasmacytoid differentiation.

In the spleen, white pulp involvement is usually prominent, but the red pulp may be involved. Bone marrow involvement may be nodular, interstitial, diffuse or a combination of the three; pseudofollicles are rarely seen in the marrow [1].

Immunophenotypically, CLL/SLL cells are positive for IgM, IgM and IgD, CD5, CD19, CD20...
tyrosine kinase the T- and natural killer cell-associated ZAP-70 expression of the activation marker CD38 and the more aggressive type of CLL/SLL is also recognized by Hodgkin’s disease variant fuse large B cell lymphoma and 0.5-2% being a more indolent whereas unmutated cases have a more aggressive clinical course. The mutated form of CLL/SLL is more indolent whereas unmutated cases have a more aggressive clinical course. A more aggressive type of CLL/SLL is also recognized by expression of the activation marker CD38 and the T- and natural killer cell-associated ZAP-70 tyrosine kinase. Gene expression profiles demonstrate that unmutated CLL cells express more ZAP-70 mRNA than mutated CLL cells.

In cases of CLL/SLL, chromosomal translocations are rare, and no specific mutations have been identified. Although cytogenetic lesions are rare in the early course of the disease, some appear as the disease progresses. The most common is a deletion at 13q14.3, which occurs in more than 50% of cases. This deleted region contains two micro-RNA genes, miR-15a and miR-16-1. miR-15a and miR-16-1, which negatively regulate bcl-2, are frequently deleted and/or downregulated in patients with CLL. Mutations in miRNA transcripts may have functional importance and may predispose to CLL. Other cytogenetic abnormalities in CLL/SLL are trisomy 12 and deletions at 11q22-23, 6q21 or 17p13.

Richter syndrome, transformation of CLL/SLL into aggressive B cell lymphoma, occurs in up to 10% of the cases, most representing a diffuse large B cell lymphoma and 0.5-2% being a Hodgkin’s disease variant. It has been shown that in some cases, Epstein-Barr virus (EBV) infection and fludarabine treatment may lead to Hodgkin transformation of CLL/SLL. It has been speculated that unmutated cases can show diffuse large B cell lymphoma variant of Richter transformation, whereas mutated cases can transform into Hodgkin’s disease or diffuse large B cell lymphoma.

**Mantle cell lymphoma (MCL)**

Mantle cell lymphoma (MCL) is a B-cell neoplasm associated with a very poor prognosis. The neoplastic cells of MCL are thought to correspond to pre-germinal center B lymphocytes in primary lymphoid follicles and the mantle zones of secondary follicles. Approximately 25% of MCL cases bear mutated IgH genes, with rates of mutations much lower than those observed, for example, in follicular lymphomas, which clearly derive from germinal center B cells.

Lymph nodes, spleen, and bone marrow (with or without blood involvement) are the most commonly involved sites. The gastrointestinal tract and Waldeyer’s ring are the most often involved extranodal sites.

Nodal involvement generally adopts mantle zone, nodular or diffuse growth patterns. MCL is usually composed of monomorphic, uniform, small-medium sized B lymphocytes with scant cytoplasm, irregular nuclei and inconspicuous nucleoli. Although transformation into a diffuse large B cell lymphoma is rarely seen, transformation into a higher grade tumor may occur and these cases are described as ‘blastoid variant’ of MCL. Several MCL cytologic variants have been described, but the classic and pleomorphic blastoid variants are the most relevant because they often show genetic abnormalities and potentially correlate with a worse prognosis.

Rare cases of ‘in situ-like MCL’ without associating the localized disease have been described recently.

Several types of B cell lymphomas show plasmacytic differentiation. Although this phenomenon is not usual, a few cases of MCL exhibiting clonal plasma cell differentiation have also been reported. The tumor cells are positive for CD20, CD22, CD79a, CD5, CD43, surface IgM and IgD, FMC7, and bcl-2 and usually negative for CD10, CD23, and bcl-6. Virtually all cases show nuclear expression of Cyclin D1. Cyclin D1 is overexpressed as a consequence of t(11;14), which juxtaposes the Cyclin D1 gene next to the immunoglobulin heavy chain gene. Phenotypic variants may in-
include negativity for CD5 or Cyclin D1, and rare expression of CD10, CD23, bcl-6 or the T-cell markers CD8 or CD7 \cite{17,18}.

The t(11;14)(q13;q32) is the genetic hallmark of MCL \cite{1}. This abnormality can be demonstrated by cytogenetics and/or fluorescent in situ hybridization (FISH). Many cases also have point mutations and/or deletion of the ATM (ataxia telangiectasia mutated) gene. The blastoid variant of MCL frequently shows additional chromosomal abnormalities, such as 13q14 deletions, trisomy 12, as well as the deletion or inactivation of p53, p16 and p18 loci \cite{19}.

Variable region genes are unmutated in the majority of the cases, but a subset of the cases show somatic mutation. However, unlike the situation in CLL, the hypermutational status of the Ig genes in MCL is not of prognostic significance and is not associated with ZAP-70 expression \cite{20}.

**Follicular lymphoma (FL)**

Follicular lymphoma (FL) is the most common type of low-grade B cell lymphoma, characterized by a clinically indolent course. It is thought to originate from follicular center B lymphocytes \cite{1}.

FL predominantly involves the lymph nodes, but spleen, bone marrow, peripheral blood, and Waldeyer’s ring involvement have also been reported. The gastrointestinal tract, soft tissue and skin are the most often involved extranodal sites.

Histologically, FL is composed of centrocytes and centroblasts, and usually has a follicular growth pattern. Neoplastic follicles are often ill-defined, and lack mantle zones, polarization and starry-sky pattern. Interfollicular infiltration of the neoplastic cells can be a good diagnostic criterion for FL. Diffuse pattern may be seen and it is thought to be of clinical significance. In the WHO classification, FL is graded as 1, 2, 3a, and 3b according to the number of centroblasts per high-power field \cite{1}. Histological grade correlates with prognosis in FL, with grades 1 and 2 being indolent and grade 3 being more aggressive. In grade 3 FL, the presence of a diffuse component is commonly seen and some studies have demonstrated that this finding is correlated with a worse outcome \cite{21}.

Cases of ‘in situ localization of FL’ have been reported in the literature \cite{22}. It appears to represent early microscopic involvement of FL within the lymph nodes. The clinical significance of these cases without other evidence of lymphoma is not known yet.

The tumor cells are positive for CD19, CD20, CD22, CD79a, surface Ig (IgM+/IgG-, IgG or rarely IgA), bcl-2, CD10, and bcl-6 and negative for CD5, CD43, CD23 and Cyclin D1 \cite{1}.

The genetic hallmark of FL, t(14;18)(q32;q21), which juxtaposes the bcl-2 gene with the IgH gene, is seen in 80-90% of FLs \cite{23}. It is not associated with the prognosis. Bcl-2 protein is expressed in the majority of the cases, and its expression reduces as histological grade increases. Although FL is rarely seen in pediatric patients, it should be noted that bcl-2 expression in pediatric FL is relatively infrequent in contrast to its adult counterpart \cite{24,25}. Primary cutaneous follicle center cell lymphoma is a variant of FL and is often bcl-2-negative as well \cite{26}.

The proliferation index, as determined by Ki-67 immunohistochemistry on tissue sections, has been found to have prognostic value in FL \cite{27}. High Ki-67 staining in the reactive lymphoid follicles is useful for the differentiation of reactive follicular hyperplasia and FL. The proliferation index of the neoplastic follicles in low grade FL (grades 1 and 2) is lower than in reactive follicular hyperplasia and grade 3 FL. But in a recent study of Wang et al., high proliferation index in low grade FL was determined in nearly 20% of their cases. The clinical behavior of these low grade FL cases showing high proliferation index was correlated with inferior disease-specific survival but higher five-year disease free rate similar to grade 3 FL \cite{28}.

A number of cytogenetic abnormalities have been described in FL, including p53 mutations, loss of p16, upregulated MYC expression resulting from translocation or other mechanisms, gains of chromosome arms 7p or 7q, Xp, 12q and 18q, as well as losses on 6q and possibly mutations of bcl-2 and/or bcl-6 genes. The presence of additional genomic aberrations, in particular 17p and 6q deletions, is more frequent in grade 2 and 3 FL patients and correlated with shorter survival and a higher rate of transformation into diffuse large B cell lymphoma \cite{23,29}. 
Marginal zone lymphomas (MZL)

There are three entities described in WHO lymphoma classification in the category of MZL: extranodal MZL (EN-MZL) [i.e. MZL of the mucosa associated lymphoid tissue (MALT)], splenic MZL (S-MZL) and nodal MZL (N-MZL). Although the cellular origin of these lymphoma entities are B cells of marginal zone area of the lymphoid follicle, the clinical presentation, outcome and molecular characteristics of each type are different. For diagnostic and therapeutic purposes, these tumors must be clearly distinguished from each other. EN-MZL is the most commonly seen type of MZL and has been described with its characteristic clinical presentation, biology and molecular pathogenesis. However, S-MZL and N-MZL still contain unknowns that need to be clarified. We thus, in this section of the article, focus on the debates and recent data about the characterization of these two less-understood MZL entities.

Nodal marginal zone lymphoma (N-MZL)

N-MZL has been defined in WHO classification as the primary nodal B cell lymphoma histologically characterized by perifollicular marginal zone involvement of the lymph nodes by polymorphous small lymphocytes or centrocyte-like cells with clear cytoplasm, which can be described as monocytoid in appearance B cells. They may also show plasmacytic differentiation and transformation into large cell lymphoma. At the time of presentation, there should be no evidence of extranodal or splenic involvement or of an autoimmune disease in the background presenting the risk of misdiagnosis as EN-MZL. Since the neoplastic cells have polymorphous morphologic features with plasma cell differentiation and no specific characteristic immunophenotype or cytogenetic abnormality, diagnosis is dependent on the morphology, excluding the other indolent B cell entities by immunophenotyping and clinical picture. Morphological differentiation from FL could be difficult if follicular colonization is prominent. In that situation, immunohistochemistry will be useful for better clarifying marginal zone nature of the cells. As it is a rarely seen (1-2%) entity lacking characteristic phenotypic and molecular findings, the diagnosis could be missed if it is not remembered. Because of the low incidence, there are only a few studies on a limited number of patients present in the literature describing the phenotypic and molecular characteristics of N-MZL. The higher amount of large cells and mitosis has been found to be correlated with an aggressive clinical course but did not affect the clinical outcome of the patients. This may be because of the multi-agent therapy protocols. The bone marrow infiltration pattern has been described most frequently as nodular and paratrabecular, like several other types of indolent B cell lymphomas. Increased proliferation index and amount of large cells have been observed in the recurrent cases. The amount of plasmacytic differentiation could create a problem in the differential diagnosis from lymphoplasmacytic lymphoma (LPL), but presence of nodular pattern has been described as helpful for the diagnosis of N-MZL.

The phenotypes of the neoplastic cells are positive for CD20 and Bcl-2 but negative for CD5, CD23, Bcl-1 (Cyclin D1), CD10, and immunoglobulin superfamily receptor translocation-associated 1 (IRTA1). IgD is mostly negative, but the cases which show similarity to the lymph node involvement of S-MZL have been described as IgD-positive.

IRTA1 is a membrane receptor with Ig related extracellular and thyrosine based intracytoplasmic regions, expressed on marginal zone B cells of MALT, reactive marginal zone hyperplastic areas of mesenteric lymph nodes and also on neoplastic cells of EN-MZL. The value of its expression in diagnosis or in explanation of the biology of MZL entities has not been revealed in the recently published series, which may in part be due to the inconvenience of its commercial availability.

The molecular and cytogenetic findings are neither helpful for explaining the pathogenesis nor for defining the cellular origin of N-MZL. It is still an incompletely understood entity. The heterogeneity of the morphology and biology may be related to the micro-environmental differences.

In the recent series, most cases at presentation show disseminated disease and high recurrence rate with large cell transformation. The data about the clinical presentation is totally different than the definition in WHO classification.
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From the clinician’s point of view, these findings could be enough to warrant changing their approaches while treating N-MZL.

**Splenic marginal zone lymphoma (S-MZL)**

S-MZL is defined in WHO lymphoma classification as the primary B cell neoplasm of the spleen arising from the B lymphocytes of marginal zone cells of the white pulp. The neoplastic cells either replace the follicle centers or infiltrate red pulp. They are accepted as representing the post-germinal center B cells of unknown state of differentiation \[1\]. The incidence is low (1%), but it has more clear clinical and phenotypic characteristics that could make diagnosis straightforward compared to N-MZL. The clinical presentation can be characterized by splenomegaly, hilar lymphadenopathy, and bone marrow and peripheral blood involvement as stage IV disease in most of the patients, but the clinical course is indolent. Transformation into large cell lymphoma rate is higher than in CLL but lower than in FL \[37,38\].

Morphology of the neoplastic cells may contain plasmacytic differentiation, and small lymphocytes and plasmacytoid cells with villous projections can be seen in the peripheral blood.

The diagnostic material may be mostly bone marrow biopsies, and the infiltration pattern of the bone marrow biopsies may be variable, such as nodular, interstitial or, most characteristically, intra-sinusoidal.

The characteristic immunophenotype of neoplastic cells in S-MZL is CD20, IgM and IgD positivity. CD5, Bcl-6, CD10, Bcl-1, CD23, and CD43 are negative. Proliferation index is low. CD103, DBA44, and FCM-7 are the markers helpful in differential diagnosis from hairy cell leukemia (HCL) \[39\]. The phenotypic and morphological overlapping entity could be LPL, with the examples of the cases showing plasmacytic differentiation and increase in serum monoclonal Ig heavy chain. CD22 and CD25 expressions are proposed to be useful for differentiation of the two entities with overlapping morphological and clinical features.

Molecular studies revealed that similar to CLL, in half of the S-MZL cases, the neoplastic cells could contain somatic hypermutation on Ig genes and have more favorable prognosis when compared to the unmutated cases. These findings support the speculation that these mutated type lymphomas could be generated by longer antigenic stimulations including autoimmunity or chronic infections such as hepatitis C \[40\].

Several numerical chromosomal abnormalities with allelic losses have been described in S-MZL. Among them, losses of chromosome 7q are demonstrated to be related to the aggressive behavior of the disease \[41-43\].

Gene profiling studies have also provided information about the molecules that could be responsible for the pathogenesis or progression of the disease. BCR- and TNF-related signals and the expression of related molecules SYK, BTK, BIRC3, TRAF3, TRAF5, CD40, and LBT are increased \[42\]. In addition to these BCR-related genes, the increase in the T cell related genes may predict the importance of splenic microenvironment on disease pathogenesis or progression. Agents blocking the activation of these pathways or inducing apoptotic cell death through lymphomagenesis could be used as new therapeutic approaches.

**Hairy cell leukemia (HCL)**

HCL is a small B cell neoplasm composed of cells with characteristic oval or bean- shaped small nuclei and abundant cytoplasm with hairy projections. Diffuse infiltration of bone marrow, peripheral blood and splenic white pulp is a typical finding at presentation \[41\].

CD103, CD22 and CD11c are the diagnostic markers expressed on the neoplastic cells. Tartaric acid resistant acid phosphatase enzyme (TRAP) positivity can be demonstrated in a variable number of cells either histochemically or by monoclonal antibodies. But TRAP is not a specific marker for HCL. The characteristic bone marrow infiltration with increased reticulin fibrosis may make it difficult to obtain an adequate amount of neoplastic cells for flow cytometry. In this situation, DBA44 could be used as a diagnostic marker. Another marker that could be helpful for differentiation of HCL from non-MCL entities is Bcl-1 (Cyclin D1) in combination with DBA44. Bcl-1 expression is not related to a chromosomal abnormality, and its clinical relevance has not been clarified yet \[39\]. Other markers such as TIA-1, CD123, and CD52 could have either diagnostic or even therapeutic value \[44\]. TIA 1 expression is found only in HCL cells when compared to several different B cell lymphomas and could
be used as a diagnostic marker. Although its cellular localization has been defined, its function in the neoplastic cells of HCL is not yet understood [45]. The strong expression of CD52 in HCL is not very helpful in diagnosis but could be more useful for choosing anti-CD52 antibody as an alternative immunotherapy agent with other chemotherapeutics in resistant cases [46].

Hairy-cell leukemia-variant (HCL-V) accounts for 10% of HCL cases. The morphology of the neoplastic cells is between that of HCL and prolymphocytic lymphoma (PLL). CD11c and CD103 are positive on HCL-V cells, but unlike typical HCL, the cells are CD25- and CD123-negative. HCL-V is more aggressive and resistant to therapy compared to HCL [47].

If splenectomy is performed, diagnosis of HCL or HCL-V is straightforward by diffuse involvement of red pulp. Otherwise, examination of the expression of characteristic markers CD103 and CD123 is very helpful in the differential diagnosis between HCL / HCL-V and S-MZL or B-PLL. The increased blood T cell large granular lymphocytic leukemia (T-LGL) in HCL patients without peripheral blood involvement could cause misdiagnosis of T-LGL. Diagnosis could be made by bone marrow biopsy or splenectomy. Because two of the diseases involve red pulp, immunohistochemistry is essential for the diagnosis [39].

The molecular and cytogenetic studies have revealed the hypermutated nature of VH genes in HCL. But no specific cytogenetic abnormality has been defined for explanation of the oncogenic events. The hypermutation in Ig genes reveals the mature memory cell origin of HCL [48].

**Lymphoplasmacytic lymphoma/ waldenstrom macroglobulinemia (LPL/WM)**

LPL/WM is the most debatable entity of the small B cell lymphomas. Without knowing the clinical presentation, immunophenotype and some laboratory findings, the differential diagnosis of LPL and other small B cell lymphomas with plasmacytic differentiation may be impossible for the pathologists.

In the WHO classification, LPL/WM is described as the B cell neoplasm with plasmacytoid lymphocytes or plasma cells usually involving bone marrow, lymph nodes and spleen. The neoplastic cells lack CD5 and CD23. The patients often have serum paraproteinemia, hyperviscosity or cryoglobulinemia. Excluding the characteristic features of the other lymphomas with plasmacytes is recommended for differential diagnosis [1].

Following the WHO definition, two consensus panels were held on LPL/WM in 2002 and 2004 [49,50]. The first meeting panel accepted WM as a distinct clinicopathological entity limited to the cases of LPL and advised not using this terminology for the other type of lymphomas. But the differentiation of LPL and MZL with plasmacytes was not defined in this meeting [49]. The second consensus meeting included discussion of differentiation criteria of LPL and MZL [50]. WHO classification does accept WM as a clinical syndrome and this term was also used for the lymphomas with paraproteinemia such as CLL or MZL. The consensus panel of the second workshop recommended that the diagnosis of WM should be restricted to LPL. Berger et al. recommended accepting WM as a clinical entity as WHO suggested because of the frequent overlapping characteristics of LPL and S-MZL cases with plasmacytic differentiation [1, 51, 52]. The differential diagnosis of LPL and CLL/SLL includes CD5 and CD23 positivity with low intense CD20 expression and ZAP-70 expression in mutated cases. 13q deletion could be demonstrated in 50% of the CLL cases. Serum IgM paraprotein is usually present. Very rare cases of MCL have been demonstrated with plasmacytoid differentiation. The characteristic CD5 and CD20 expression with negative CD23 and overexpression of bcl-1 (Cyclin D1) are the phenotypic characteristics of MCL. Demonstration of t(11:14)(q13:q32) is the chromosomal abnormality that could support the diagnosis of MCL with plasmacytoid differentiation. Distinguishing multiple myeloma (MM) form LPL/WM may be easy in the typical cases which are composed of atypical plasma cells infiltrating bone marrow. But in a small number of cases, the neoplastic cells of MM could have lymphoid morphology and CD20 expression. In daily practice, we do see these types of difficult cases. In our study on MM, we saw three cases of CD20-positive MM and one was characterized with diffuse strong staining. All of these cases have typical clinical features of MM (unpublished data). These findings reveal that immunophenotype and morphology of the neoplastic cells may not be enough for differentiation of MM and LPL/WM. CD138 and CD19 can be helpful alternative markers for differentiation of the two entities. CD138 positivity supports MM and CD19 positivity supports LPL/WM [52, 53]. Some cytogenetic
abnormalities, if present, such as deletion 13q and 14q32, could be supportive for diagnosis of MM \cite{55, 56}. The patients with clinical parameters consistent with MM should be treated as MM. Bone lesions are one of the key parameters for differentiation \cite{54}.

The overlapping phenotypic and clinical features of LPL/WM and the other small B cell lymphoid neoplasias are the main reasons for the debates on the entity. The argument on the terminology and classification of LPL/WM will likely continue until the key abnormalities specific to these overlapping disease entities are defined.

CONCLUSION

In the REAL and WHO classification of lymphomas, the lymphoma entities are defined based on their morphological, phenotypic, genetic, and clinical characteristics and their biology. Since 2001, several studies have been done on small B cell lymphomas. The gene expression profile data was especially helpful for scientists in explaining some unknowns. In this review, we attempted to present the new data on small B cell lymphomas. The effect of micro-RNA genes on the biology of CLL/SLL, Ig gene mutated and Cyclin D1(Bcl-1) negative forms of MCL, and biology of childhood FL are some of the new findings that will take their place in the updated WHO blue book. N-MZL, S-MZL and LPL/WM are the entities which seem more prone to future changes in their definitions in the new WHO blue book. Following the last meeting of the European Association for Hematopathology on small B cell lymphomas, held in Vienna in September 2006, lymphoma research will be focusing on the cells of the microenvironment. This new route may help to definitively resolve some arguments regarding these debatable entities and to explain their biology.

References

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