

Marginal zone-related neoplasms of splenic and nodal origin

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Background. The marginal zone is an anatomically distinct B-cell compartment of lymphoid tissue with an abundant antigenic influx. Among marginal zone-derived lymphomas the WHO classification listed, in addition to extranodal marginal zone B-cell lymphoma of MALT type, two other marginal zone B-cell neoplasms: splenic marginal zone B-cell lymphoma (+/- villous lymphocytes) and nodal marginal zone B-cell lymphoma (+/- monocytoid B cells). These two entities are well characterized histologically, but specific biological markers are lacking. Treatment options are heterogeneous, including a watch-and-wait policy, surgery with or without chemotherapy, purine analogs, and interferon. No prospective studies have been conducted so far.

Information sources. Clinical and pathologic data were reviewed by searches of the published medical literature, including searches in PubMed®, important printed publications, and abstracts presented at recent hematology and pathology meetings.

State of the art. Splenic and nodal marginal zone lymphomas are typical low-grade lymphomas with an indolent course. A subset of patients, however, presents with more aggressive disease and have a shorter survival. Clinical and biological prognostic factors identified in reported series are heterogeneous. The role played by hepatitis C virus (HCV) in marginal zone lymphomas is not fully elucidated, but there is demonstration that eradication of HCV infection in splenic lymphoma with villous lymphocytes causes regression of the lymphoma. The optimal treatment has not yet been identified. Retrospective series, however, show that splenectomy is a good option if symptoms from the presence of spleen enlargement or cytopenias need to be treated. The utility of purine analogs and of anti-CD20 immunotherapy needs to be clarified in prospective trials.

Perspectives. Clinicians and pathologists should cooperate to define stringent diagnostic criteria for these indolent disorders. The optimal therapeutic approach and the role of new treatments need to be assessed in prospective clinical trials.

Key words: marginal zone, splenic marginal zone lymphoma, nodal marginal zone lymphoma, low-grade non-Hodgkin's lymphoma

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The marginal zone

Definition, anatomic distribution and morphology

The marginal zone (MZ) is an anatomically distinct B-cell compartment, well developed in those lymphoid organs in which an abundant influx of antigens is known to occur, such as spleen white pulp (Figure 1), Peyer's patches of the small bowel (Figure 2) and tonsils, but less evident in lymph nodes, other than mesenteric ones.¹

The MZ surrounds the lymphocytic corona of the mantle zone and consists of medium-sized lymphoid elements with often pale staining nuclei with one or two nucleoli and a variable amount of clear cytoplasm. On electron microscopy, marginal zone B-cells (MZBCs) contain numerous small mitochondria, a few cisternae of rough endoplasmic reticulum and a well developed Golgi apparatus.² Marginal zone B-cells are admixed with a variable number of larger blast elements, small lymphocytes, macrophages, granulocytes and plasma cells.

In normal peripheral lymph nodes, the marginal zone is poorly developed, whereas in various reactive lymphadenopathies, mostly of infectious origin (e.g., *Toxoplasma gondii* and human immunodeficiency virus infections),³ proliferation of B-cells with monocytoid-like features may frequently occur close to the subcapsular sinuses (so called *immature sinus histiocytosis*) (Figure 3).^{4,5} Such proliferation may also be observed in lymph nodes removed from patients with carcinoma (e.g., breast and gastric carcinoma).⁶

Immunophenotypic and genotypic characteristics

The typical splenic MZBCs express the B-cell antigens CD20 and CD79a, but they lack CD5, CD10, CD23 and CD43. MZBCs are strongly sIgM⁺, and negative or weakly positive for IgD, also bearing the alkaline phosphatase, CD21/CD35 (CR1/2) and C3.⁷

To date, however, no specific marker is available for MZBCs; promising results are expected from a novel monoclonal antibody raised against the human IRTA1 (immune receptor translocation associated-1 protein) antigen.⁸ Preliminary data revealed that IRTA1 selectively reacts with a subset of B-cells homing to the marginal zone or their anatomically equivalent areas of tonsils, lymph nodes and spleen.⁹

Although the term *marginal zone B-cells* is related *strictu sensu* to a well defined microanatomical area of the spleen,¹⁰ subsequent immunohistochemical studies have indicated that the monocytoid B-cells (MBCs) expanded in reactive lymph nodes are the nodal counterpart of spleen marginal zone B-cells.^{2,11} The antigenic

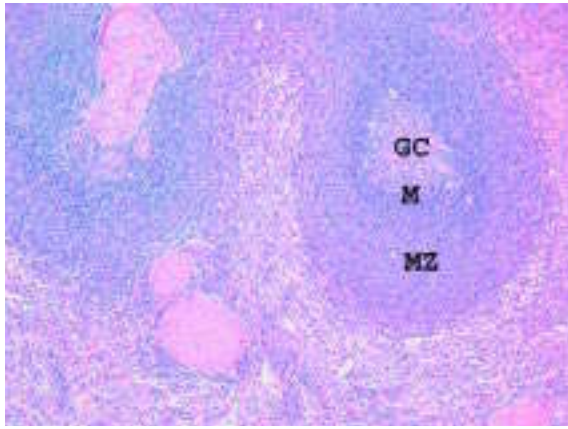


Figure 1. Normal spleen, white pulp; the germinal centers (GC) are surrounded by a lymphocytic corona of mantle cells (M) and a marginal zone area (MZ) (hematoxylin-eosin).

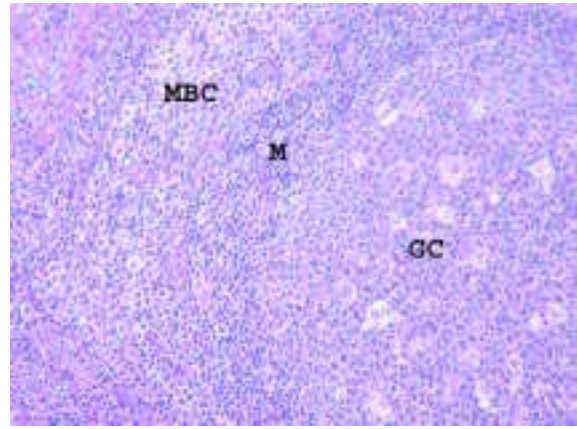


Figure 3. Toxoplasmic lymphadenitis; an expanded marginal zone area composed of monocytoid B-cells (MBC) surrounds the lymphoid follicle (hematoxylin-eosin).

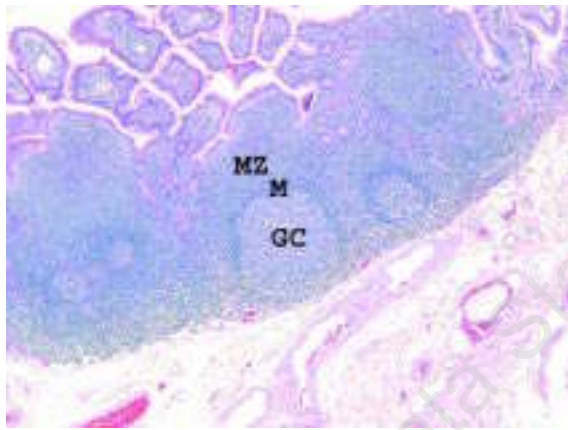


Figure 2. Small bowel; the lymphoid Peyer's patches consist of GC surrounded by M and MZ, prominent at the mucosal side (hematoxylin-eosin).

profile of the monocytoid B-cells (nodal counterparts of MZBCs) largely overlaps with that of splenic MZBCs, other than for IgM, bcl-2 (which have been reported to be, respectively, variable and lacking in MBCs), KiB3 epitope of the CD45RA molecule (positive in MBCs, negative in splenic MZBCs) and DBA44 antigen (negative in splenic MZBCs, expressed in about 20% of MBCs).¹²

The exact relationship, however, between splenic MZBCs and nodal MBCs is still a matter of debate. A recent immunobiological study by Stein *et al.*¹² suggested that monocytoid B-cells represent a B-cell subpopulation distinct from splenic MZBCs. By analysis of mutated IgV_H genes, both MZBCs of the spleen and Peyer's patches have been demonstrated to be mostly memory B-cells, with a minor component of naive elements, these latter probably being responsible for thymus-independent type 2 (TI-2) antigen response.¹⁰ In contrast, nodal MBCs in toxoplasmic lymphadenitis were revealed to be composed mainly of naive B-cells and to contain only a small subset (25%) of non-antigen-selected post-germinal center B-cells.¹²

Functions

The origin and functions of marginal zone B-cells are still debated.^{13,14} Studies in T-cell-deprived rabbits¹⁵ have indicated that MZ cells serve as precursors of plasma cells of Marschalko type. Within 24 hours after antigenic challenge, marginal zone lymphocytes demonstrate an increase in nuclear and cellular size and a marked increase in polyribosomes; their transformation into immunoblasts, plasma blasts and plasma cells occurs independently from regulatory T-cells. In the splenic white pulp, MZ cells have been considered to serve as antigen- and immune-complex binding and transporting cells. This transport results in antigen localization at the

Table 1. Marginal zone B-cell lymphomas.

Updated Kiel classification
Monocytoid, including marginal zone cell
REAL classification
Marginal zone B-cell lymphoma
Extranodal marginal zone B-cell lymphoma (low-grade B-cell lymphoma of MALT type)
Nodal marginal zone B-cell lymphoma (<i>provisional entity</i>)
Splenic marginal zone B-cell lymphoma (<i>provisional entity</i>)
WHO classification
Extranodal marginal zone B-cell lymphoma of MALT type
Nodal marginal zone B-cell lymphoma (± monocytoid B cells)
Splenic marginal zone B-cell lymphoma (± villous lymphocytes)

surface of dendritic reticulum cells in splenic follicles. Procedures that depleted the number of MZBCs inhibited the localization of antigen in follicular centers. More recent studies in rodents demonstrated that MZBCs have a part in immunologic responses to thymus-independent type 2 (TI-2) antigens, such as bacterial capsular polysaccharide, indicating that they are a first line of the immune reaction against blood-borne antigens.¹⁶ This immune activity of MZBCs seems to need the presence of complement.¹⁷ In humans, MZ maturation is completed at 2 years of age and, as a consequence, human TI-2 responses are impaired in infancy.¹⁸ Interestingly, in Wiskott-Aldrich syndrome, the MZ is significantly reduced and this may explain the inability of patients with this syndrome to mount a response to thymus-independent antigens and their tendency to infection with encapsulated bacteria.¹⁹

Marginal zone-related neoplasms

In the past, a number of cases of lymphoma considered to be derived from monocytoid B-cells have been described,²⁰ including cases primarily located in extranodal sites (e.g., stomach, salivary gland, thyroid). In 1992 the updated Kiel classification²¹ included monocytoid B-cell lymphoma (and the lymphoma of mucosa-associated lymphoid tissue – MALT) among low grade neoplasms (Table 1).

In 1994, the REAL and, more recently, the WHO lymphoma classification established a new combined clinico-pathologic approach to lymphoma diagnosis and subtyping. Within each lymphoma category, distinct diseases are defined according to a combination of morphology, immunophenotype, genetic features and clinical syndromes.

On such bases, the REAL classification²² indicates three types of lymphoma originating from the marginal zone (Table 1): marginal zone B-cell lymphoma comprising extranodal marginal zone B-cell lymphoma (low-grade B-cell lymphoma of MALT type) and nodal marginal zone B-cell lymphoma and, separately, splenic marginal zone B-cell lymphoma. Nodal and splenic marginal zone lymphomas (MZL) were listed as provisional entities.

In the WHO classification three marginal zone B-cell lymphomas were listed^{23,24} (Table 1): extranodal marginal zone B-cell lymphoma of MALT type, nodal marginal zone B-cell lymphoma (+/- monocytoid B cells), and splenic marginal zone B-cell lymphoma (+/- villous lymphocytes). A Clinical Advisory Committee agreed that nodal and splenic marginal zone lymphomas could be considered distinct diseases and could be recognized and defined in the WHO classification, without provisional specification.²⁵

Current problems

Despite the advances provided by the WHO lymphoma classification, pathologists and clinicians are

still facing many problems regarding splenic and nodal marginal zone lymphomas. In particular, the biological relationship between the nodal and extranodal subtypes of MZL needs to be further clarified.

The rarity of these disorders and some difficulties in the differential diagnosis from other low-grade lymphoma subtypes are obstacles in conducting epidemiological surveys and in properly describing clinical features and outcomes. MZLs represent a therapeutic dilemma in every day clinical practice and no prospective studies on large series have been published so far. From a clinical point of view, it seems necessary to obtain and validate clinical and biological prognostic parameters in order to recognize, from among these indolent disorders, in which patients the disease will have a more aggressive behavior.

Open issues in MZLs include: a) both in nodal and splenic MZL, the role played by the immune function, especially autoimmunity, as already established in some MALT lymphomas; b) the possible pathogenetic role of hepatitis C virus (HCV) infection, which, in some reports,^{26,27} has been associated with non-Hodgkin's lymphomas, often with MZ features.

For these reasons, clinicians and pathologists should co-operate in retrospective analyses, as well as in biological studies and prospective clinical trials in this field.

Splenic marginal zone B-cell lymphoma with or without villous lymphocytes

Splenic MZL is a rare indolent lymphoma subtype which accounts for less than 1% of all non-Hodgkin's lymphomas.²⁸ The term *splenic marginal zone cell lymphoma* was coined by Schmid in 1992²⁹ in a study on four female patients with primary splenic low-grade non-Hodgkin's B-cell lymphoma with morphologic and immunophenotypic features resembling splenic marginal zone cells.

Splenic MZL, which is characterized by the presence in the peripheral blood of lymphocytes with villous projections (Figure 4), has been termed *splenic lymphoma with villous lymphocytes* (SLVL).^{30,31} However, it is widely accepted that SLVL represents a histologically homogeneous entity identical to the condition characterized by histopathologists as splenic MZL.³² In nearly two thirds of cases, the diagnosis of SLVL is made by cytological and flow cytometry analysis of circulating lymphoma cells, and/or on bone marrow biopsies, without spleen histology. There are no stringent criteria about the percentage of villous lymphocytes necessary for diagnosis.³³ In general, two thirds of cases have more than 25% peripheral leukocytes with typical features of SLVL, but diagnosis is still possible, although more difficult, with a lower percentage of circulating lymphoma cells. On cytological smears, SLVL cells are slightly larger than chronic lympho-

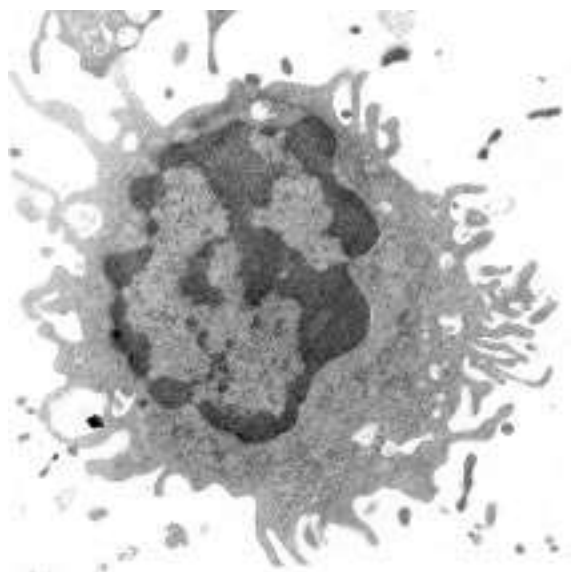


Figure 4. Electron microscopy in a case of SLVL: the neoplastic circulating lymphoid cell exhibits prominent villous projections.

cytic leukemia (CLL) cells, have a round nucleus with condensed chromatin and a single nucleolus in the majority of cases. The cytoplasm of villous lymphocytes is usually abundant, with irregularities in the border resulting in short, thin villi distributed around the cell or concentrated at one pole. Cells always express B-lineage antigens (CD19 and CD20) and have light chain restriction with surface immunoglobulin (Ig) of moderate to strong intensity. The heavy chain is usually IgM with or without co-expression of IgD. Most cases are positive for CD22 (strongly expressed), CD24, and FMC7, while CD10, CD23, CD38 are positive in only one-third of cases. CD11c is positive in nearly half of cases and CD25 in 25%, but CD25⁺ cases usually do not show the typical hairy cell leukemia profile (CD25⁺, CD11c⁺, HC2⁺, B-ly-7⁺).³⁴ In the Matutes' score for CLL,³⁵ which employs a panel of five antibodies (CD5, CD23, FMC7, CD22 and surface immunoglobulin) to distinguish CLL from other B-cell chronic leukemias, most cases of SLVL have a low score (< 3). Interestingly, a similarity between SLVL and hyperreactive malarial splenomegaly (HMS) has been proposed.³⁶ In fact, in West Africa these conditions have overlapping demographic and clinical features.³⁷ Polymerase chain reaction (PCR) evaluation of the Ig gene seems a good tool to discriminate true HMS from splenic lymphoma³⁸ and gives information about the differentiation status of the cell of origin. In African SLVL cases, V_H genes are relatively unmutated, indicating the possibility that this neoplasm

Table 2. Series of splenic marginal zone B-cell lymphoma ± villous lymphocytes.

	N° pts	Diagnosis	Diagnostic criteria	M/F ratio	Median age (yrs)	Survival
Mulligan <i>et al.</i> ⁵⁹	50	SLVL	Cytology Immunophenotype	1.77	68	5-yrs OS: 78%
Troussard <i>et al.</i> ⁶⁰	100	SLVL	Cytology Immunophenotype	0.7	70	5-yrs OS: 78%
Thieblemont <i>et al.</i> ⁶¹	81	SMZL/SLVL	Histology Cytology Immunophenotype	0.95	66 (all) 63 SMZL 75 SLVL	Median OS: 10.5 yrs. (all)
Chacón <i>et al.</i> ⁶²	60	SMZL	Histology (spleen)	1.7	63	5-yrs OS: 65%
Iannitto <i>et al.</i> ⁶⁴	57	SMZL/SLVL	Histology (spleen and bone marrow) Cytology Immunophenotype	1.59	61	5-yrs OS: 78%

SMZL: splenic marginal zone B-cell lymphoma; SLVL: splenic lymphoma with villous lymphocytes.

derives from a splenic T-independent B-cell, with malarial infection as a potential co-factor.³⁹

Recently, Thieblemont *et al.*⁴⁰ reported on a seven gene pattern by cDNA macroarrays analysis, to be used in the differential diagnosis of non-follicular small B-cell lymphomas, including splenic MZL. Genes expressed in all samples tested resulted to be *bcl2*, *mmp17*, *α3 IV collagen*, *notch2*, *bcl1*, *upar* and *timp1*. A validation by means of quantitative real-time PCR showed that in splenic MZL there is a significantly increased expression of *notch2*, a gene encoding for a transmembrane receptor influencing cell fate. Interestingly, in knock-out mice lacking B-cell expression of RBP-J, a key mediator of Notch signaling, marginal zone B-cells are absent leading the animals to have increased mortality from blood-borne bacterial infections.⁴¹

Pathology

Morphology. In the splenic white pulp (Figure 5), a central zone of small lymphoma (*lymphocyte-like*) cells surrounds or replaces reactive germinal centers. This zone merges with a peripheral area of medium-sized (monocytoid-like) cells and scattered larger blasts. Subsequently, the lymphoma progresses to the red pulp in the form of a patchy or micronodular infiltrate, which often spreads to the sinuses. Scattered histiocytes may be observed in the lymphoid aggregates. Lymphoma cells may show a variable degree of plasmacytic differentiation, which is often prominent in cases associated with autoimmune disorders (e.g., autoimmune hemolytic anemia)⁴² (Figure 5). Involvement of splenic hilar lymph nodes may occur and this consists of a vaguely

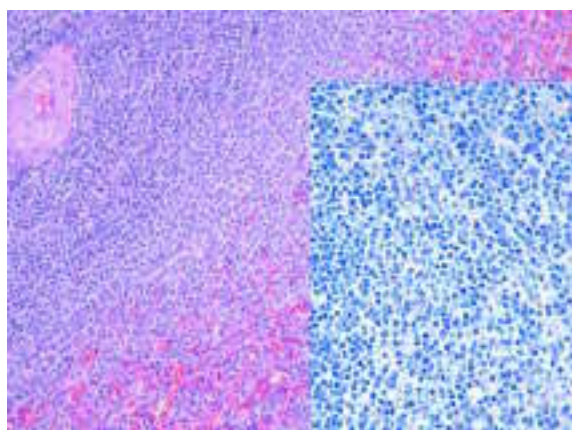


Figure 5. SMZL; the architecture of the white pulp is effaced by a nodular lymphoma proliferation consisting of small (*lymphocyte-like*) and medium-sized (monocytoïd like) cells (hematoxylin-eosin). Inset: Giemsa staining and higher magnification more clearly illustrate the cytologic features of the SMZL cells, some of which also show a variable degree of plasmacytic differentiation.

nodular infiltrate that is based on pre-existing follicles. Lymphoma cells are represented by small lymphocytes, similar to those seen in the center of splenic follicles, whereas the number of blasts is usually low. The neoplastic infiltrate surrounds germinal centers, with a marginal zone pattern. Alternatively, the germinal centers may be partially colonized or completely effaced by the lymphoma cells.⁴³ The intensity of follicular replacement varies in different areas in each given case. Transformation into high-grade lymphoma may occur, similarly to other indolent B-cell neoplasms.⁴⁴

Immunophenotype. Splenic marginal zone lymphoma (SMZL) cells express surface IgM and IgD and are CD20⁺, CD79a⁺, CD5⁻, CD10⁻, CD23⁻, CD43⁻, and CD103⁻; DBA44 is usually negative, except few cases reported to be weakly positive.⁴⁵ In addition, SMZL has been observed to variably react with the novel monoclonal antibody IRTA 1 (Figure 6). Cyclin D1/*bcl1* is negative,⁴⁶ whereas *bcl-2* protein is intensely expressed. Negativity for CD5, CD23, *bcl-1/cyclin D1*, CD10, CD103 is useful to exclude the other low-grade lymphoproliferative disorders (chronic lymphocytic leukemia, mantle cell lymphoma, hairy cell leukemia and follicular lymphoma).

Cytogenetic and molecular findings. Immunoglobulin heavy and light chains are clonally rearranged,⁴⁷ and most cases show somatic mutations of Ig heavy chain variable region genes.⁴⁸ SMZL shows heterogeneous and frequently complex cytogenetic findings. Trisomy 3 is a frequent cytogenetic abnormality in marginal zone lymphomas. It has been reported in nearly half cases of SMZL⁴⁹ and in 17% of cases of SLVL.⁵⁰ A study found this abnormality more frequently in extranodal (85%) and nodal MZL (50%) than in SMZL

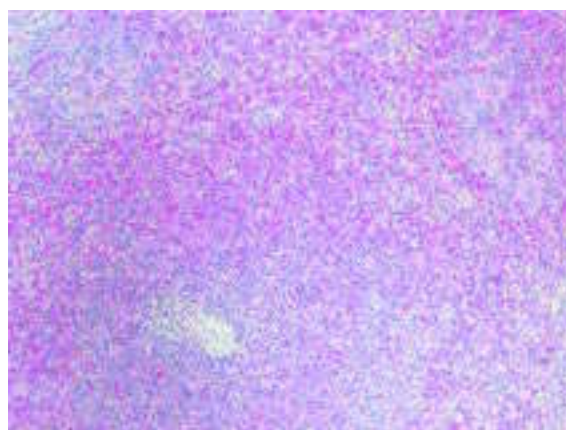


Figure 6. SMZL; the IRTA1 MoAb (kindly provided by Prof. B. Falini, Perugia, Italy) stains most of the lymphoma cells (alkaline-phosphatase anti-alkaline phosphatase, APAAP technique).

(18%).⁵¹ It has been postulated that the alterations of chromosome 3 at 3q27 may involve the proto-oncogene *bcl-6*.⁵² Other cytogenetic alterations include abnormalities in chromosomes 1, 7, and 8. Translocations involving 7q have been shown to be involved in dysregulation of cyclin-dependent kinase 6 expression.⁵³

Postulated cell of origin. Limited information is available to date regarding the postulated cell of origin of SMZL. A recent study, however, suggests that there are two types of SMZL, one originating from naive marginal zone B-cells, and another from memory marginal zone B-cells.⁵⁴

Bone marrow pathology

Bone marrow involvement by SMZL usually occurs in the form of a micronodular, interstitial and sometimes paratrabeular infiltrate. Moreover, a peculiar intrasinusoidal pattern of involvement (Figure 7) has been observed by Franco *et al.*,⁵⁵ who also documented changes of bone marrow infiltration pattern (from intrasinusoidal to nodular) and increase of the tumor burden following splenectomy.^{56,57}

Clinical features and outcome

Retrospective analyses of the natural history and the clinical outcome of patients with SMZL with or without villous lymphocytes are very useful in the comprehension of this rare disorder. However, clarification of the clinical characteristics of this lymphoma has met many obstacles: the rarity of the disease; the absence of reliable diagnostic marker(s); the indefinite overlapping with similar disorders such as extranodal marginal zone B-cell lymphoma of MALT type and nodal marginal zone B-

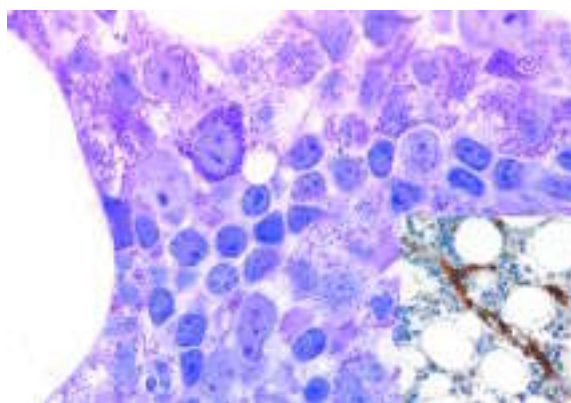


Figure 7. SMZL, bone marrow biopsy: the lymphoma cells show a characteristic sinusoidal pattern of involvement (Giemsa) and express the CD20 B-cell related antigen (inset: streptavidin-biotin-peroxidase complex, SABC method).

cell lymphoma; the heterogeneity in the diagnostic criteria (cytology, cytofluorimetry, histology); and the variability of therapeutic approaches. Despite these limits, retrospective reports represent a fundamental step for better understanding of the presenting clinical features and clinical outcome, and for evaluating prognostic parameters. These analyses are a useful basis for future biological and therapeutic studies (Table 2). In almost all patients symptomatic splenomegaly is the presenting feature. Anemia-related symptoms or B symptoms are rare. Autoimmune phenomena are often reported in association with SMZL.⁵⁸

Many series of patients with SLVL carry interesting notations but have limited numbers of cases. The first relatively large series of SLVL patients was described more than ten years ago by Mulligan *et al.*⁵⁹ from the Royal Marsden Hospital of London. They reported on 50 patients (32 men and 18 women) with SLVL followed for a median period of 3.7 years. Diagnosis was based on cytologic and immunophenotypic criteria.^{20,21} The median age of the patients was 68.4 years. Fourteen had received no therapy and experienced quite a long survival. Eighteen patients received chemotherapy as the first-line approach but only eight (36%), treated with chlorambucil, were good responders. In twenty patients splenectomy was performed either at onset or during the course of disease: one patient died post-splenectomy, but the remaining 19 subjects obtained a good response with a return of hemoglobin and/or platelet count to the normal and a decrease of leukocyte count by $\geq 50\%$. This indicates that splenectomy may be a good therapeutic option if symptomatic splenomegaly or cytopenias due to hypersplenism are present. Twelve patients died. One-third of deaths were caused by disease progression while one-half were

due to other malignancies or vascular disorders. The outcome appeared generally favorable with an indolent clinical course and a 5-year survival rate of 78%. These results were substantially confirmed in 1996 on 100 patients with SLVL by Troussard *et al.*⁶⁰ from the *Groupe Français d'Hématologie Cellulaire*. This series comprised 40 males and 60 females with a median age of 70 years. No patient had adenopathy. Hemoglobin level was < 10 g/dL in 16% and platelet count was $< 100 \times 10^9/L$ in 15%. In 28% of patients a monoclonal component was detected. No case was CD10⁺ and CD25⁺, while 20% resulted CD5⁺, 61% CD11c⁺, and 71% DBA44⁺. Thirty-two patients received no treatment with a 5-year survival of 88%; 19 patients received chemotherapy and 10 achieved a good partial response with a mean duration of response of 6 months. Splenectomy was performed in 28 patients as first-line treatment and in 6 at relapse. Of 28 patients splenectomized as first-line therapy, 21 had a good partial remission but 30% relapsed. Splenic irradiation was chosen as first-line treatment in 7 patients, with a clinical response in all cases. The 5-year overall survival was 78%. Of the 15 deaths registered, 9 were due to lymphoid disease or treatment toxicity. Recently, Thieblemont *et al.*⁶¹ reviewed 81 cases of splenic MZL with or without villous lymphocytes observed at the Department of Hematology of Lyon between 1987 and 2001. The median age of the entire series was 66 years. Of these patients, 11 had SLVL and 70 splenic MZL without villous lymphocytes with a statistically different median age (75 years for SLVL and 63 years for splenic MZL). Ninety-five percent of patients had stage IV disease and 16, all with splenic MZL without villous lymphocytes, had autoimmune events (hemolytic anemia, immune thrombocytopenia, acquired coagulation disorders, positive Coombs' test). A monoclonal component was detected in the serum of 46% of the patients. In 20 patients a *wait-and-see* policy was chosen at diagnosis, with half of these remaining without progression. Splenectomy was performed in 79% of patients, followed by adjuvant chemotherapy in 47%. In splenectomized patients, performance status ameliorated and cytopenias often resolved. However, in 70% of cases involvement of bone marrow and/or peripheral blood persisted. Partial responders were more prone to disease progression than complete responders, but the overall survival, the risk of histologic transformation, and the risk of death from lymphoma were not different. The median time to progression was 3.7 years, and the median survival was 10.5 years, without difference between the two types and with respect to treatment. Among splenic MZL patients, the presence or absence of circulating villous lymphocytes did not correlate with differences in the clinical presentation: SLVL cases had only an older median age and

absence of immune alterations. Despite this and other convincing evidence that SLVL is the leukemic counterpart of splenic marginal zone lymphoma, some perplexity remains about the diagnostic homogeneity of retrospective studies based on cytologic and immunophenotypic diagnoses on peripheral blood. A recent paper by Chacón *et al.*⁶² gives an important clarification on this point. In Chacón's study 60 splenic MZL patients, all diagnosed with splenectomy, were analyzed for clinical features at presentation and prognostic factors. The series was homogeneous for both the diagnostic criteria and first-line treatment (i.e. splenectomy). There was a male predominance and the median age was 63 years. Only a quarter had an ECOG performance status of 2. The most frequent presenting symptoms were symptomatic splenomegaly (73%) and systemic symptoms (58%). Bone marrow was involved in 83% of cases and 65% were leukemic. Lymphadenopathy was present in 25% at the abdominal level and in 17% superficially. After splenectomy, 29 patients received chemotherapy, obtaining a response in more than 90% of cases. The median overall survival has not been reached. The median survival of 16 patients who died from splenic lymphoma was 17.5 months.

Therapy

Randomized, prospective trials for splenic MZL are lacking. Most patients are without symptoms at diagnosis and many of them do not need specific treatment.⁵⁷ As for other indolent lymphomas the therapeutic approach is a balance between a conservative policy and a more aggressive approach to control the disease. This latter is reserved to younger patients or to those with a poor prognosis. Some data available from reported series may be useful for future investigation.

Splenectomy. Splenomegaly is a common feature of non-Hodgkin's lymphoma, but isolated spleen involvement (primary splenic lymphoma) is quite rare. Some years ago Gobbi *et al.*,⁶³ wondering whether primary splenic lymphoma really exists, extensively reviewed the literature on this topic and opted for a restrictive definition (exclusive involvement of spleen and of splenic hilar lymph nodes). In this setting, splenectomy is the most effective treatment for primary splenic lymphoma patients. This recommendation remains particularly valid for splenic MZL.

Since the study by Mulligan *et al.*⁵⁹ splenectomy appeared the treatment of choice for SLVL. The procedure should be performed in the presence of cytopenias and/or symptoms. The good response to splenectomy has been confirmed by two French studies.^{60,61} Obviously, an additional advantage of splenectomy is a more precise and homogeneous diagnostic approach.⁶² However, cytology and immunophenotyping studies remain a valid

Table 3. Prognostic factors in splenic marginal zone B-cell lymphoma with or without villous lymphocytes.

Reference	Adverse prognostic factors
Troussard <i>et al.</i> ⁶⁰ (SLVL)	Leukocytes > 30×10 ⁹ /L Lymphocytes < 4×10 ⁹ /L Chemotherapy
Thieblemont <i>et al.</i> ⁶¹ (SMZL/SLVL)	Monoclonal component β2-microglobulin level Leukocytes > 20×10 ⁹ /L Lymphocytes > 9×10 ⁹ /L
Chacón <i>et al.</i> ⁶² (SMZL)	ECOG performance status ≥ 2 Incomplete response Non-hematopoietic site involvement
Baldini <i>et al.</i> ⁶⁵ (SMZL) Gruszka-Westwood <i>et al.</i> ⁶⁶ (SLVL) Chacón <i>et al.</i> ⁶² (SMZL)	p53 alteration
Algara <i>et al.</i> ⁸⁷ (SMZL)	IgV _H unmutated + 7q deletion

SMZL: splenic marginal zone B-cell lymphoma; SLVL: splenic lymphoma with villous lymphocytes.

approach in evaluating advanced-age patients not suitable for splenectomy. The typical intrasinusoidal bone marrow involvement on bone marrow biopsies is an important diagnostic feature in splenic MZL.^{55,64} It has been intriguingly noted that splenectomy may be followed by a reduction or disappearance of peripheral lymphoma cells and/or monoclonal component.⁶⁵ As previously mentioned, however, it has also been shown that splenectomy may cause a modification of bone marrow involvement (from intrasinusoidal to nodular) and, more clinically relevant, an increase of tumor burden.⁵⁶

Splenic irradiation. Radiation therapy seems a rational approach in the treatment of splenic MZL if surgery is contraindicated (advanced age and/or co-morbidity). In the series of Mulligan⁵⁹ three of seven patients had a response to splenic irradiation. In Troussard's study⁶⁰ splenic irradiation was the first-line treatment in 7 patients (6-8 Gy over 2 weeks): all patients responded and none of these died; three relapsed at 8, 35 and 98 months. Low-dose radiotherapy (4 Gy) can also produce a hematologic response with reduction of splenomegaly and circulating lymphoma cells.⁶⁶

Purine analogs. Purine analogs play an important role in the treatment of indolent lymphoproliferative disorders⁶⁷ but have rarely been used in splenic MZL.⁶⁸ Bolam *et al.*⁶⁹ reported the cases of 4 patients with resistant and relapsed SLVL treated with fludarabine: two of them reached a long-lasting good response and two died from an unrelated cause. Prophylactic ciprofloxacin was adminis-

tered during the periods of severe neutropenia and no significant toxicity was observed. Lefrère *et al.*⁷⁰ retrospectively reviewed ten cases of SLVL treated with fludarabine, two as first-line therapy and the others at relapse/progression. Treatment was started a median of 17 months after diagnosis. Fludarabine was given at a dose of 25 mg/m²/day i.v. in 5-day courses for a median of four courses. Five patients achieved a good and durable response (median follow-up 14 months). Oral sulfamide was used as prophylaxis and no opportunistic infections occurred; one case of transient facial paralysis caused therapy discontinuation and one episode of autoimmune hemolytic anemia resolved after steroid therapy. Recently, an Epstein-Barr virus-positive B-cell lymphoproliferative disorder has been reported by Abruzzo *et al.*⁷¹ in a patient with splenic MZL treated with fludarabine.

Data on the role of cladribine in splenic MZL are even rarer. One case of SLVL has been reported by Virchis: the patient, resistant to alkylating agents and splenectomized, obtained a good partial remission lasting more than 2 years.⁷² Lefrère *et al.*⁷³ evaluated the efficacy and toxicity of cladribine in 7 SLVL patients (in one as first-line and in six as salvage treatment). The schedule was 0.1 mg/kg/day i.v. for 7 days. Responses were short-lived and many infections occurred.

There are some encouraging data about the efficacy of pentostatin in SLVL.⁷⁴ The drug was administered at a dose of 4 mg/m² i.v. every other week for 8 courses. In ten patients given the drug as first-line or as salvage treatment the overall response rate was 80% (4 complete and 4 partial responses) with acceptable toxicity.

Monoclonal antibodies. As other B-cell malignancies, splenic MZL lymphoma expresses CD20 antigen on the cell surface and consequently is a rational indication for the use of anti-CD20 monoclonal antibodies, either unconjugated or conjugated with radioisotopes.

Thomas *et al.*⁷⁵ reported the results of treatment with rituximab in 8 patients with SLVL and 6 with nodal MZL. Rituximab was administered at a dose of 375 mg/m² weekly for 8 or more weeks and 71% of the patients obtained a response. Rituximab benefited in particular those with SLVL. Toxicity was acceptable (grade 1-2 fever, chills, hypotension, at the first infusion). No relapse occurred in complete responders after a median follow-up of 10 months.

Interferon- α . An innovative therapeutic achievement is represented by the beneficial effects of interferon- α in HCV-positive SLVL cases,⁷⁶ in analogy with *Helicobacter pylori* eradication in gastric lymphoma of MALT type.⁷⁷

The background for this experience is ample. In addition to type II cryoglobulinemia,⁷⁸ HCV infection has been reported to be associated with some

types of non-Hodgkin's B-cell lymphoma (lymphoplasmacytoid lymphoma and diffuse primary hepatosplenic large B-cell lymphoma).⁷⁹ Other epidemiological studies did not find a statistically significant association between HCV and non-Hodgkin's lymphoma.⁸⁰ Another study, however, reported that monocytoid B-cell lymphoma is the non-Hodgkin's lymphoma most frequently associated with HCV.⁸¹ It has been demonstrated that both HCV-positive and HCV-negative cases of nodal MZL harbor somatic mutations in the immunoglobulin heavy chain variable genes but with usage of different segments between the two groups, indicating different antigenic stimulation in lymphoma B-cell precursor selection.⁸²

In the study by Hermine *et al.*,⁷⁶ 9 patients with SLVL and HCV infection were treated with interferon- α 2b (3 MU subcutaneously three times a week for six months); six patients had clinical features of cryoglobulinemia (purpura, arthralgia, neuropathy, renal disease). After interferon treatment, 7 patients obtained a complete hematologic remission and HCV-RNA negative status. The two non-responders were treated with interferon plus ribavirin (1000 to 1200 mg per day) and obtained clearance of HCV-RNA as well as the hematologic response (one complete remission and one partial remission). These data were compared with those of six cases of SLVL without HCV infection treated with interferon; none of them had a response of any grade to interferon.

Prognostic factors

The prognostic stratification of patients with non-Hodgkin's lymphoma is a strategic instrument to decide appropriate treatments. The parameters of the International Prognostic Index, initially conceived for aggressive lymphoma⁸³ and also validated for low-grade lymphoma,⁸⁴ have no prognostic impact in splenic MZL.⁶¹ New prognostic indicators seem necessary for these patients who have a generally indolent disease that sometimes, however, appears relatively more aggressive with shorter survival (Table 3).

Clinical factors. In the series by Troussard,⁶⁰ leukocyte count > 30 \times 10⁹/L, lymphocyte count <4 \times 10⁹/L and initial treatment with chemotherapy were adverse prognostic factors in multivariate analysis. The authors argued that chemotherapy had a negative impact because of its toxicity in a disease more frequently affecting elderly patients. In the series of Chacón *et al.*⁶² chemotherapy was not confirmed as an adverse prognostic factor. These authors interpreted Troussard's finding arguing that patients treated with chemotherapy may have a more aggressive disease *per se*, with respect to patients treated with splenectomy alone. In their series, at univariate analysis failure to obtain a complete response, involvement of a non-hematopoietic site,

and high ECOG score were correlated to a poorer overall survival, while liver involvement and incomplete response to therapy were correlated to a shorter failure-free survival. In multivariate analysis only failure to obtain a complete response and involvement of a non-hematopoietic site remained negatively associated with failure-free and overall survival. Thieblemont⁶¹ reported that survival was significantly shorter in the presence of a monoclonal component, elevation of β_2 -microglobulin, leukocyte count $> 20 \times 10^9/L$, and lymphocytes $> 9 \times 10^9/L$. An immunologic event or a monoclonal component was correlated with a shorter time to progression.

Biological parameters. In the series of Chacón *et al.*⁶² of five patients with p53 alterations, four died of their disease with a median survival of 17 months. p53 alteration was a poor prognostic factor in univariate analysis. In a series of 15 patients with non-villous splenic MZL reported by Baldini *et al.*,⁸⁵ five patients had p53 mutations and in 4 of them the disease evolved. The median survival of these five patients was shorter than that of remaining cases. Similar results were obtained by Gruszka-Westwood *et al.*,⁸⁶ who investigated p53 changes in 59 cases of SLVL. They found that p53 abnormalities were correlated with a significantly worse survival. With analogy to CLL, the presence of somatic mutations in the immunoglobulin V_H gene was also a favorable prognostic factor in splenic MZL.⁸⁷ Unmutated status is frequently associated with 7q deletion and identifies a subgroup of patients with an adverse clinical course.

Nodal marginal zone B-cell lymphoma ± monocytoid B-cells

The term *monocytoid B-cell lymphoma* (MBCL) was introduced in 1986 by Sheibani²⁰ to define a peculiar lymphoma type composed of neoplastic lymphoid cells with morphologic and immunophenotypic resemblances to monocytoid B-cells.

In the first reports on MBCLs,^{88,89} both primary nodal and primary extranodal (particularly gastric) cases were included in a single category. Today, primary nodal marginal zone B-cell lymphoma is retained by the WHO lymphoma classification as a rare (1.8% of all non-Hodgkin's lymphoma)²⁸ but distinct clinico-pathologic subtype within the wide spectrum of marginal zone-derived lymphoma. *Conditio sine qua non* for such a diagnosis is a primary lymph node localization in the absence of a prior or concurrent extranodal site of involvement.

Pathology

Morphology. Several studies^{89,90} have described in detail the patterns of lymph node involvement and cytological features of primary nodal marginal zone B-cell lymphoma (Figure 8). Lymph node patterns of infiltration include *marginal zone*



Figure 8. Nodal MBCL: lymphoma cells surround and partially colonize the germinal center (Giemsa).

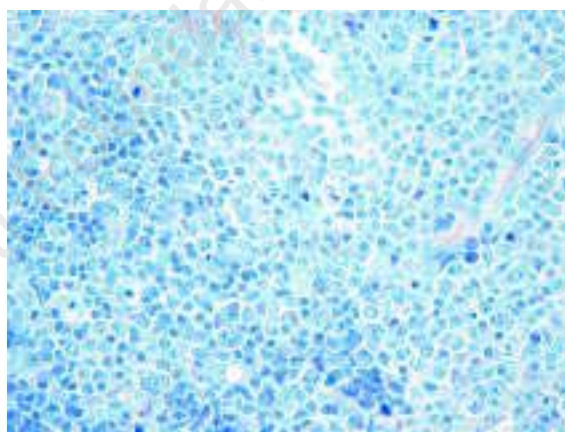


Figure 9. Nodal MBCL (the same case pictured in Figure 8): higher magnification detailing the cytologic features of the lymphoma population, including cells medium in size with a quite irregular nucleus and a fairly broad rim of gray cytoplasm, scattered larger blast cells, and elements with features of plasma cell differentiation.

like/perifollicular, sinusoidal, nodular and diffuse. A combination of different patterns in a single case is common. In the early stage, the typical *marginal zone-like* pattern consists of an enlargement of the marginal zone, due to a perifollicular proliferation of neoplastic MBCs, which surround residual reactive germinal centers and a thin layer of small lymphocytes of the mantle zone. Continued perifollicular proliferation results in progressive expansion of interfollicular areas, with formation of large, often nodular-appearing masses in the paracortical areas. In advanced cases of MBCLs the lymph node architecture is effaced with diffuse proliferation of neoplastic cells, but resid-

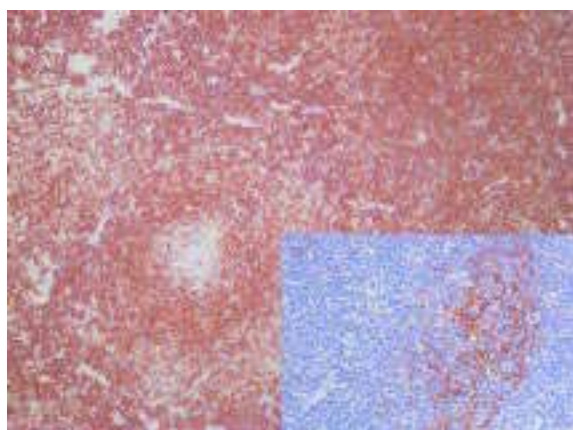


Figure 10. Nodal MBCL: lymphoma cells intensely express the B-cell related antigen CD79a but lack the CD23 antigen (inset); in contrast, the dendritic meshwork within the residual GC stains for the CD23 (SABC method).

ual follicles may still be detectable, at least in some areas.⁹⁰

Cytologically (Figure 9), in most cases, the lymphoma cells are medium-sized with a slightly or markedly irregular, roundish to reniform or indented nucleus, with multiple, inconspicuous nucleoli and a fairly broad rim of clear (in hematoxylin-eosin) or gray (in Giemsa) cytoplasm; a variable number of large size blast cells may also be present, but usually account for less than 10% of neoplastic elements. In some cases, the lymphoma cells may show some degree of differentiation towards plasma cells, sometimes resembling an almost pure plasmacytic infiltrate.⁸⁸

In a minority of cases, the neoplastic population consists of small *centrocyte-like* cells in the absence of blast cells and without signs of plasmacytic differentiation.

Campo *et al.*⁹¹ have proposed differentiation of nodal marginal zone lymphomas into two different subtypes, *MALT-type* and *splenic-type*, on the basis of the nodal pattern of involvement. Immunophenotypically, the *MALT-type* nodal marginal zone B-cell lymphoma lacks surface IgD (positive in the *splenic-type*) and is frequently associated with an extranodal component (often detected only after extensive clinical staging and follow-up). However, this subtyping is still matter of debate, and above mentioned morpho-functional divergences might be ascribed to the complex histologic and immunophenotypic spectrum which characterizes marginal zone-derived lymphomas.⁷ Sporadically, a predominant cytologically low grade, nodal, marginal zone B-cell lymphoma may show areas mainly composed of large cells;⁸⁹ these peculiar morphologic features have been defined by Berger,⁹² who reported MZBCL as a *large cell rich variant*, giving rise to the possibility of lymphoma progression.

Immunophenotype. The antigenic profile of nodal monocytoid B cell lymphoma largely overlaps with similar but extranodal forms (CD20⁺, CD79a⁺, CD5⁻, CD10⁻, CD23⁻, cyclin D1/ bcl1⁻) (Figure 10); in some cases, lymphoma cells show a IgD⁺, CD43⁻ profile, similar to that of their splenic counterpart.

Cytogenetic and molecular findings. Few data are available on nodal MBCL genetics. However, the t(11;18)(q21;q21) and trisomy 3 associated with extranodal (MALT) forms are not frequent.

Postulated cell of origin. A recent study,⁹³ based on the pattern of immunoglobulin heavy-chain variable region mutations, suggests that nodal MBCL, previously considered a memory B-cell derived malignancy, is biologically heterogeneous and may arise from different subsets of marginal zone B-cells: a) naive B-cells with unmutated V_H genes, b) memory B-cells with somatic mutations without intraclonal variation and c) germinal center B-cells defined by their capacity to undergo the somatic hypermutation process.

Clinical features and outcome

On the basis of the pathologic findings and clinical features, nodal MZL clearly appears to be a classic indolent lymphoma. One fundamental issue is the relationship between primary nodal MZL and secondary nodal localization during the course of advanced MALT-type MZL and another is to define whether these two forms are distinct entities or aspects of the same disease. To answer this question, Nathwani *et al.*⁹⁴ compared the clinical features of 20 cases of nodal MZL and of 73 cases of MALT-type MZL lymphoma with nodal localization. In this series, nodal MZL was more frequently in advanced stage with peripheral and para-aortic lymphadenopathy, as in other primary nodal indolent B-cell lymphomas (follicular, small lymphocytic, lymphoplasmacytic).²⁸ In contrast, there was no difference with respect to age, sex distribution, B symptoms, elevated lactate dehydrogenase, performance status, IPI score, or histologic transformation. This would indicate that nodal MZL is not the advanced stage of MALT-type MZL. The 5-year overall survival rate for patients with nodal MZL was significantly lower than that of MALT-type MZL (56% vs 81%), as was the 5-year failure-free survival (nodal MZL 28%; MALT-type MZL 65%). This difference remained significant also after stratification according to the IPI score. These data indicate that nodal MZL is a distinct entity.

Other studies on non-MALT marginal zone B-cell lymphomas

Berger *et al.*⁹² addressed the problem of non-MALT marginal zone lymphomas clinically. They reviewed 124 cases of non-MALT marginal zone lymphomas treated in the Hospital of Lyon. Fifty-

nine cases of *splenic subtype* and 37 of *nodal subtype* were included in the study. The remaining 28 cases did not fit the two WHO categories. For the cases with both nodal and splenic involvement, the authors created a new category of *disseminated subtype*, and denominated the cases without splenic or nodal localization but with marrow and peripheral blood involvement as *leukemic subtype*. This new clinical classification resulted in a more rational approach to the patient with non-MALT marginal zone lymphomas. The male-female ratio was 1 and the median age was 60. Patients with the *nodal subtype* were younger, while those with the *leukemic subtype* were older. Bone marrow involvement was found in 72% of cases, but with a lower frequency in the *nodal subtype*. Only 21% of cases had B symptoms and 18% had a poor performance status. A hemoglobin level < 12 g/dL was present in half of the cases, especially in the *splenic* and *disseminated subtypes*. Fifteen percent of cases had a monoclonal component in serum and/or urine at diagnosis. The median overall survival and time to progression (TTP) for all subtypes were 9.1 years and 3.8 years, respectively. The median TTP for *splenic* and *leukemic subtypes* were 6.9 and 5.6 years, respectively, while the median TTP for nodal and *disseminated subtypes* were 1.3 and 1.1 years, respectively. The TTP was not different in the presence of complete remission or not. The authors speculated that *disseminated* disease may be the terminal stage of *splenic* and *nodal subtypes*, while the *leukemic subtype* might be the preliminary phase of the *splenic subtype*.

We studied a series of 30 patients with non-MALT MZL.⁹⁵ All histologies were reviewed according to the REAL and WHO classifications. Twelve patients had a *nodal subtype* and 18 a *splenic subtype*. Fifteen were male and 15 were female; their median age was 58 years (range 25–74). Five of the 12 nodal cases (42%) and 17 of the 18 splenic subtypes (94%) had stage IV disease; 30% had B symptoms. Twenty patients (30% of nodal and 90% of splenic) had bone marrow involvement and 10 (1 nodal and 9 splenic) had peripheral blood involvement. Liver involvement was present in 10% of nodal and in 40% of splenic subtypes. Five cases (20%) had bulky disease. Only 2 (with nodal lymphoma) had a poor performance status (ECOG \geq 2). Two autoimmune events occurred in 2 nodal cases (1 hemolytic anemia and 1 Sjögren's disease). Four of 18 patients with splenic lymphoma (24%) had a monoclonal component (3 IgM, 1 IgG). Anemia and thrombocytopenia were present in 60% of splenic cases but in no patient with nodal lymphoma. Two nodal and 6 splenic cases had HCV-positive serology. The median follow-up was 3 years (range 1–15). Of the patients with splenic MZL, 61% had splenectomy. Chemotherapy (chlorambucil in 7, anthracycline-based chemotherapy in 9) was

administered to 16 patients (all those with nodal disease and 4 with splenic subtype): 30% obtained a CR and 35% a PR, with a median duration of 3 and 1.1 years. Median progression-free survival (PFS) was 1 year for the nodal type and not reached for the splenic type; median overall survival was not reached for either. Our analysis indicates that in the context of non-MALT marginal zone lymphomas, nodal and splenic subtypes have peculiar clinical features and outcomes: splenic subtype, although presenting with stage IV disease has an indolent outcome, while the nodal subtype, less disseminated at onset, shows a more pronounced tendency to progression.

Conclusions

Although the WHO lymphoma classification has provided significant advances in terminology and clinico-pathologic subtyping, several issues remain to be addressed. In particular, pathologists are still searching for specific biological markers, useful for identifying marginal zone cells and derived lymphomas. This would allow better definition of the relationship between the various marginal zone lymphoma entities.

Clinicians' interest in these disorders has been increasing over the last few years. Some series recently reported provide important contributions to understand the clinical course of these lymphomas and to identify clinical and biological prognostic parameters. Co-operation of clinicians and pathologists in defining stringent diagnostic criteria seems most relevant. The optimal therapeutic approach and the role of new treatment modalities in these indolent disorders requires the design of prospective clinical trials.

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