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Characteristics of splenic marginal zone lymphoma: Clinical, hematologic, and flow cytometry findings of 34 cases

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Abstract:

BACKGROUND: Splenic marginal zone lymphoma (SMZL) is a low-grade disorder that regularly presents with peripheral blood (PB) involvement. A precise description of clinical, laboratory features and immunophenotypic characterization of SMZL are still lacking. Here, we reviewed 34 patients presenting with SMZL to describe the clinical, hematologic features, and flow cytometry immunophenotypic findings of this type of lymphoma at diagnosis.

OBJECTIVES: The aim of this study is to confirm that SMZL has a specific immunologic profile which enables the hematopathologist and clinician to differentiate this low-grade B-cell lymphoma from other B-cell lymphoproliferative disorder, especially chronic lymphocytic leukemia and hairy cell leukemia, which could sometimes stimulate SMZL morphologically and to emphasize that a correlation of immunophenotypic findings, clinical, and hematologic features of patients plus careful morphological examination of PB and/or bone marrow (BM) aspirate can lead confidently to the correct diagnosis.

MATERIALS AND METHODS: Flow cytometry immunophenotypic findings of 34 cases of SMZL were reviewed. The analysis was performed by BD FACS Calibur™ and FACSCanto II flow cytometers. B lymphocytes were identified according to their Side-Scattered (SSC)/CD19 distribution. A marker was considered positive when expressed in more than 20% of cells above the control.

RESULTS: Median age was 60 years, range (35–84 years), both sexes were affected equally. All patients presented with splenomegaly, 71% of patients had absolute lymphocytosis and 88% of patients showed PB involvement. Seventy-four percent of patients had anemia and (53%) of them had thrombocytopenia. Cells from all cases expressed pan B-cell antigens (CD19, CD20), 74% of cases expressed CD79b and Human Leukocyte Antigen – antigen D Related (HLA-DR) expressed in nearly almost all cases (97%). Half of the patients expressed CD11c and SIgD, 41% expressed CD5 and FMC7 while CD25 and CD103 showed positivity in less than 5% of cases. Preferential expression of Kappa light chain was demonstrated, CD10 and CD38, SIgG were negative.

CONCLUSION: SMZL has a distinct immunologic profile which if correlated with morphologic findings of PB or BM aspirates, clinical and hematologic features can help to make the accurate diagnosis and lessens the need of further invasive diagnostic procedure.

Keywords:

Clinical, flow cytometer, hematologic, immunophenotyping, multiparameter, splenic marginal zone lymphoma

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Introduction

Splenic marginal zone lymphoma (SMZL) is a rare indolent lymphoma subtype which accounts for <1% of all

non-Hodgkin lymphomas.^[1] In the revised European-American Classification of Lymphoid Neoplasms, classification, SMZL, was considered a provisional entity and included with marginal zone lymphoma of mucosa-associated lymphoid

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tissue type and nodal marginal zone lymphoma in the category of marginal zone lymphomas.^[2] In the WHO classification, a Clinical Advisory Committee agreed that SMZLs could be considered a distinct disease and could be recognized and defined in the WHO classification without provisional specification.^[3]

Typically, the disease affects middle-aged patients. Characteristic features are splenomegaly, moderate lymphocytosis with villous morphology, intrasinusoidal pattern of involvement of various organs, especially bone marrow (BM).^[4] Cytopenia is often related to hypersplenism and less frequently to autoantibodies or BM infiltration. Lymphadenopathy and/or other organ involvement are infrequent, but they may develop during the disease. B symptoms and an increase of lactate dehydrogenase are rare at presentation.^[5] Autoimmune phenomena are present in 20% of patients.^[6] The clinical course is usually chronic and the median survival is around 10 years.^[7] A proportion (10%) of cases undergo transformation to diffuse large B-cell lymphoma.^[8] The lymphomatous cells express surface immunoglobulin of IgM and IgD classes and positive for CD19, CD22, CD20, and CD79a, and negative for CD5, CD10, CD23, CD43, and cyclin D1.^[4,7] SMZL shows heterogeneous and frequently complex cytogenetic findings. Trisomy 3 is a frequent cytogenetic abnormality, it has been reported in nearly half cases of SMZL.^[9] Other cytogenetic alterations include abnormalities in chromosomes 1, 7, and 8.^[10]

Despite the advances provided by the WHO lymphoma classification and the application of immunophenotyping as a powerful tool in the classification of lymphoid malignancies, the rarity of SMZL, morphological and clinical heterogeneity, and the lack of specific immunophenotypic markers create a diagnostic dilemma in distinguishing of SMZL from other small B-cell neoplasm such as chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), and mantle cell lymphoma (MCL), for this reason; we performed a retrospective review of clinical findings, hematologic data and immunophenotypic pattern of 34 cases of SMZL at presentation to determine whether SMZL has a characteristic clinical, hematological, and immunophenotypic findings that allow differentiation from other B-cell neoplasm.

Materials and Methods

Patients

Thirty-four patients with the diagnosis of SMZL were identified from the records of the Flow Cytometry Laboratory at the BM Transplant Center, from January 2014 to June 2016. The results of flow cytometry analysis of 28 peripheral blood (PB) samples and six BM samples were retrospectively reviewed. The diagnosis of SMZL

was based on clinical features, peripheral and/or BM lymphocytes morphology and immunophenotypic analysis. Clinical information, such as age, gender, clinical presentation (B symptoms, autoimmune complications, and absolute lymphocytosis), and physical examination (presence of peripheral lymphadenopathy, splenomegaly), was abstracted from clinical records on all patients. Hematological data collected included total white blood cell (WBC) count, absolute lymphocyte count (ALC), the percentage of atypical lymphocytes in the PB, hemoglobin level, and platelet count.

Morphologic examination

The BM aspirate smears and PB specimens were obtained and prepared by using air-dried with Leishman's stain and then examined under light microscopy for detecting the presence of atypical lymphocytes.

Immunophenotyping by flow cytometry

PB or BM samples were collected in K3-ethylenediaminetetraacetic acid (EDTA) tube as anticoagulant. BM samples were immediately diluted 1/1 (vol/vol) in phosphate-buffered saline (PBS). All specimens were processed within 24 h of collection. Four-color immunophenotyping was performed using combinations of antibodies labeled with fluorescein isothiocyanate, phycoerythrin, or peridinin chlorophyll protein, and allophycocyanin.

All antibodies were purchased from BD Biosciences. Antibodies used in this study included, CD3, CD4, CD5, CD7, CD8, CD10, CD11c, CD19, CD20, CD23, CD25, CD38, CD45, CD79b, CD103, FMC7, anti HLA-DR, anti-IgG, anti-IgD, anti- κ , anti- λ light chains, 100 μ l of well-mixed, EDTA-anticoagulated whole blood or BM sample, were stained with 6 μ l of 4-color direct fluorescent-labeled antibodies, which had to be fit together according to the detection protocol and the different fluorescence (according to standard operating procedure of flow cytometry laboratory and the manufacturer's instructions). Samples were incubated for 15 min at room temperature in a dark place, then 2 ml of BD FACS lysing solution was added and incubated for 10 min at dark place, then the tubes were centrifuged at 1500 rpm for 5 min, supernatant aspirated, and 2 ml of BD CellWash solution was added to wash the cells two times. After the last wash, the cell buttons in each tube resuspended with 0.5 ml of BD CellFIX solution and subjected to data acquisition and analysis. Four-color flow cytometric analysis was performed using a BD FACS Calibur™ flow cytometer (Becton-Dickinson, Bio) and FACSCanto II flow cytometer (Becton Dickinson Immunocytometry Systems, San José, CA, USA). Ten thousand events were acquired per tube to ensure the best definition of each cell population. The acquired data were analyzed using CellQuest software (Becton

Dickinson, San Jose, CA) and FACSDiva software. Lymphocytes were identified by a combination of Side-Scattered (SSC) properties and intensity of staining for CD45 and B lymphocytes were identified according to their SSC/CD19 distribution. A marker was considered positive when expressed in more than 20% of cells above the control. The proportion of T-lymphocytes was estimated by the expression of CD3.

Statistical analysis

The analysis of data was carried out using the available statistical package of SPSS-24 (Statistical Packages for Social Sciences-Version 24.0. Armonk, NY: IBM Corp). Data were presented in simple measures of frequency, percentage, median, and range (minimum-maximum values).

Results

Clinical features

The relevant clinical presentations for 34 patients at the time of diagnosis are shown in Figure 1. Among the 34 patients, there were 17 men and 17 women (male: female ratio 1:1) with a median age of 60 years (range 35–84 years). All patients had splenomegaly, pallor observed (in 35% of patients) and palpable lymphadenopathy (in 32% of patients). B symptoms were present in 8 patients (24%). Eighteen patients (53%) showed thrombocytopenia. One out of 34 patients (3%) developed autoimmune manifestation (autoimmune hemolytic anemia). Jaundice was observed in one patient, (88%) of patients had PB involvement. Anemia was recorded in 74% of patients. Absolute lymphocytosis was seen in 71% of patients.

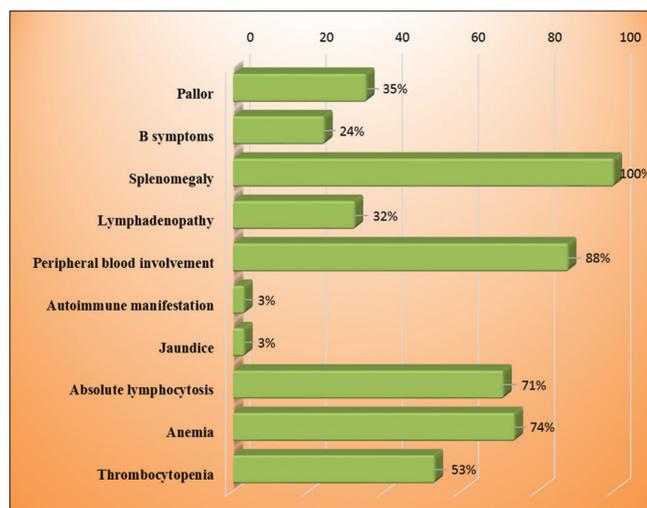


Figure 1: Clinical features of 34 splenic marginal zone lymphoma patients at diagnosis. Clinical features are listed on the Y-axis. Percentage of positive cases for each clinical presentation is indicated on the X-axis. In addition, percentage of positive cases is indicated adjacent to the bars

Table 1 summarized hematologic characteristics of patients at presentation. The presenting hemoglobin ranged from 3.2 to 14 g/dl (median 10.3 g/dl), with a half of patients having a hemoglobin <11 g/dl. The median WBC count was $17.9 \times 10^9/L$ (range: $4-100.7 \times 10^9/L$). The median of absolute lymphocytes count was ($12 \times 10^9/L$); 12 patients (35%) had absolute lymphocytes count above $16 \times 10^9/L$. The platelet count ranged from 22 to $265 \times 10^9/L$ (median $110 \times 10^9/L$) with 32% of patients having platelets count $<100 \times 10^9/L$. Morphologically, the percentage of atypical lymphocytes which was observed in the PB ranged from 19%–95%.

Immunophenotypic analysis by flow cytometry

The immunologic markers of the neoplastic cells in the PB or BM of 34 patients with SMZL are shown in Figure 2. All cases of SMZL (34/34) expressed CD19, CD20, and CD45, and 97% (33/34) of cases expressed HLA-DR. Seventy-four percent (25/34) of the cases expressed CD79b, 59% (20/34) of cases expressed CD23. CD11c

Table 1: Hematologic characteristics of splenic marginal zone lymphoma patients at diagnosis

Hematological parameters	n=34
Median WBC ($\times 10^9/l$) (range)	17.9 (4-100.7)
WBC >30 ($\times 10^9/l$)	11 (32)
Median ALC ($\times 10^9/l$) (range)	12 (2.9-86.7)
Absolute lymphocytes count >16 ($\times 10^9/l$)	12 (35)
Median Hb (g/dl) (range)	10.3 (3.2-14)
Hb <11 (g/dl)	17 (50)
Median platelet ($\times 10^9/l$) (range)	110 (22-265)
Platelet <100 ($\times 10^9/l$)	11 (32)
Median percentage of atypical lymphocytes in the peripheral blood (range)	61 (19-95)

All values are n (%) unless otherwise indicated. WBC = White blood cell, Hb = Hemoglobin, ALC = Absolute lymphocytes count

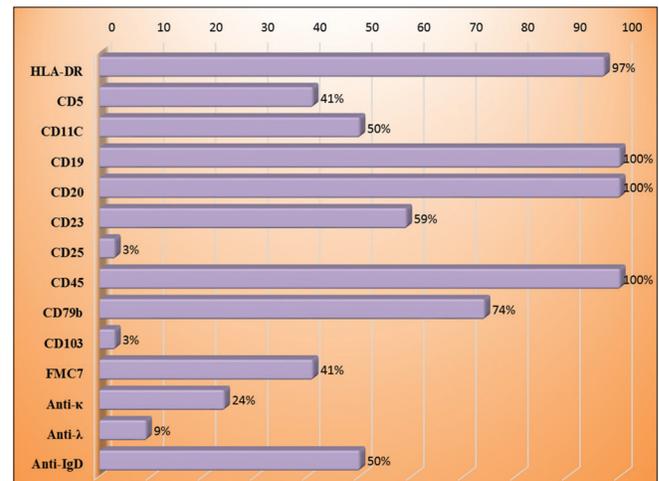


Figure 2: Antigen expression profile of splenic marginal zone lymphoma.

Immunophenotyping results from the 34 splenic marginal zone lymphoma cases are summarized as percentage of cases that are positive for each antigen. Antigens are listed on the Y-axis. Percentage of positive cases is indicated on the X-axis. In addition, percentage of positive cases is indicated adjacent to the bars

and SmIgD were positive in half of cases (17/34). CD5 and FMC7 were expressed in 41% (14/34) of cases. The light chains of surface immunoglobulin were expressed in 32% (11/34) of cases, and there was preferential expression of Kappa light chain. CD25 and CD103 were positive in only 3% (1/34) of cases. All 34 cases of SMZL were negative for CD10, CD38, and SmIgG.

Table 2 compared findings of CD5 positive SMZL cases with those markers that are usually positive (CD23), weakly expressed (SmIg, CD20), or usually negative (FMC 7, CD79b) in CLL. Most of them had bright CD20, positive FMC7, and expressed SIgD strongly, and half of them were positive for CD79b. None of these CD5 positive SMZL had the phenotype characteristics of CLL (CD5+, CD23+, weak SmIgD, and FMC7-)

Table 3 summarizes the findings with two HCL markers (CD25 and CD103) in the 17 CD11c+ SMZL cases. CD25 and CD103 were positive in 6% (1/17). No single case had the typical marker profile of HCL (CD11c+, CD25+ and CD103+).

Discussion

SMZL is a well-defined rare neoplasm that commonly follows an indolent course. Most patients display a stable clinical course and do not require treatment for years.^[7] Due to the rarity of SMZL, only a few series dealing with such disorder are available.^[5-8,11-16] SMZL is an indolent disorder that probably takes years for full clinical manifestation, the extension of disease at clinical presentation probably depends on the duration of the prediagnostic phase. This may explain the ample clinical variability of the disease at diagnosis. The present study integrates clinical, hematologic, and immunophenotypic observations in a relatively large number of patients with SMZL. Our data suggest that the immunophenotypic findings by flow cytometry are a valuable diagnostic hallmark, which is if coupled with clinical, hematologic features of patients plus careful morphological examination of PB and/BM aspirate can lead confidently to the correct diagnosis.

The results of this study confirm that SMZL is a disease of middle-aged patients with a median age of 60 years (range: 35–84 years) in agreement with many other studies.^[7,15,16] In contrast to previous series,^[7,14] in agreement with Thieblemont *et al.*,^[6] SMZL seems to affect both sexes equally. The most frequent presenting features were splenomegaly (100%) and anemia (74%), in keeping with other series.^[11,15,16] B symptoms and lymphadenopathy were rare at presentation and were noted in 24% and 32% of patients, respectively, as reported by Berger *et al.* and Arcaini *et al.*^[11,16] Absolute

Table 2: Comparison with chronic lymphocytic leukemia markers in 14 CD5+ splenic marginal zone lymphoma cases

Marker	CD5+ SMZL (n=14)	
	Number of positive cases	Percentage of positive cases
Bright CD20	13	93
CD23	10	71
FMC7	12	86
CD79b	7	50
SIgD		
Weak	2	14
Moderate to bright	6	43

SMZL=Splenic marginal zone lymphoma

Table 3: Comparison with hairy cell leukemia markers in 17 CD11c+ splenic marginal zone lymphoma cases

Marker	CD11c+ SMZL (n=17)	
	Number of positive cases	Percentage of positive cases
CD25	1	6
CD103	1	6

SMZL=Splenic marginal zone lymphoma

lymphocytosis observed in 71% of patients which is very close to what reported by Catovsky and Matutes^[5] (75%) and Troussard *et al.*^[13] (76%). Thirty-two percent of cases had WBC count higher than $30 \times 10^9/L$ and platelets count lower than $100 \times 10^9/L$ while 50% of patients had Hb level lower than 11 g/dl which is very close to the figures obtained by Iannitto *et al.*,^[15] in contrast to lower results reported by Parry-Jones *et al.*^[12] A large number of patients (30/34) (88%) showed PB involvement by circulating neoplastic cells in contrast to lower figures reported by other series, Berger *et al.*^[11] (43%), Arcaini *et al.*^[16] (59%), and Chacón *et al.*^[7] (65%). The percentage of these atypical lymphoid cells in the PB ranged from 19% to 95%. Traverse-Glehen *et al.*^[17] reported a range relatively close to our range (29%–75%).

Immunophenotyping by flow cytometry plays a major diagnostic role in the diagnosis of B-cell lymphoma and by defining the immunologic profile characteristics of some diseases such as CLL and HCL. We have analyzed here the immunophenotype of the neoplastic cells in the PB or BM aspirates of 34 patients with SMZL to see whether immunophenotyping helps to distinguish SMZL from other B-cell lymphoproliferative diseases, especially CLL and HCL. Almost all patients were positive for the pan B-cell antigens, CD19 and CD 20 (34/34), in addition to HLA-DR (33/34) which is consistent with prior publication by Kost *et al.*^[18] CD79b was positive in 74% of cases which is very close to what reported by Zomas *et al.*^[19] (75%). In the current study, we observe a higher percentage of CD5 and CD23 expression (41% and 59%, respectively) compared to what was previously reported by Isaacson *et al.*^[20] (26%

and 29%, respectively) and Matutes *et al.*^[21] (19% and 31%, respectively). Kojima *et al.*^[22] reported a positivity for CD23 marker in CD5 positive SMZL in 50% of cases (5/10). This higher percentage of positivity for CD23 in our series is not surprising because CD23 expressed in 56% of cases of lymphoplasmacytic lymphomas^[23] and 30% of non-CLL conditions.^[24] CD5 was observed in 12%–50% of patients with SMZL.^[25] Interestingly, all those CD5 positive SMZL (14/34) patients in this study had heterogeneous or dim to moderate CD5 expression.

Fifty percent of our patient (17/34) expressed CD11c in concordance with Kost *et al.*^[18] (57%) and Matutes *et al.*^[21] (47%); on the other hand, Isaacson *et al.*^[20] observed a lower figure (19%). CD103, an HCL marker, expressed in only one patient (3%); similar to what was reported by Isaacson *et al.*^[20] (1/16); unlike a previous study which has demonstrated a higher frequency (15%) while Kost *et al.*^[18] did not find any positivity for CD103 in their series.

There was preferential expression of Kappa light chain in agreement with other investigators.^[20-22,26,27] Fifty percent of patients expressed surface IgD compared to lower figures observed by Isaacson *et al.*^[20] (20%) and Matutes *et al.*^[21] (7%); in contrary, Wu *et al.*^[26] demonstrated a positivity for SIgD in 4 out of 5 patients (80%) and Dierlamm *et al.*^[27] found a positivity for this surface immunoglobulin in most of their cases by immunohistochemistry.

Regarding CD25 expression, we noted a positivity for this HCL marker in (1/34) 3% of cases in contrast to higher figures reported by other authors.^[20-22] On the other hand, Wu *et al.*,^[26] and Sánchez *et al.*^[28] demonstrated a negativity for this marker in their series. We observed a negative expression of CD10 and CD38 in our study which confirms previous observation by Wu *et al.*,^[26] who reported a negativity of these two markers by immunohistochemistry; on the contrary, Isaacson *et al.*^[20] and Matutes *et al.*^[21] reported expressions for CD10 and CD38; (28%, 38%) and (30%, 30%), respectively.

A recent review by Matutes *et al.*^[29] of data on 400 CLL patients shows that in contrast to SMZL, cells from more than 75% of the cases express weak SmIg and are CD5⁺ and CD23⁺ in more than 90% of cases, whereas the expression of FMC7 was rare. Although CD5 and CD23 were positive in 41% (14/34) and 59% (20/34) of SMZL cases in our series, the immunologic profile of such cases was clearly different from that of CLL when considering other B-cell markers [Table 3], such as bright expression of CD20 and a positivity for FMC7 and CD79b which are rarely seen in CLL.^[30]

When a comparison between the phenotypes of SMZL and HCL was made, we found major differences. Some

of the markers characteristic of HCL such as CD25 and CD103 were rarely positive in SMZL although a small proportion of cases were CD25 positive or CD103 positive, none of them coexpressed these markers. There was no coexpression of CD103, CD11c, and CD25 which is considered unique for HCL and is often used as an absolute criterion for establishing the diagnosis of this B-cell lymphoproliferative disorder.^[31,32] Therefore, CD25 and CD103 are the two HCL markers more useful to distinguish SMZL from HCL. Those discrepancies sometimes observed in the percentage of expression of the previously mentioned CD markers in our study compared with previous publications is unclear, it may be related to technical issues or could be due to differences in individual laboratory protocols, or secondary to relatively smaller number of cases studied compared with other series or may be due to ethnical variation.

Conclusion

Our findings indicate that SMZL is a disease of middle-aged patients with various clinical presentations. Immunophenotyping by flow cytometry is a highly applicable and sensitive tool for the detection of neoplastic cells in the PB and/or BM aspirate. Despite the expression of CD5, CD23 in some cases and CD25, CD103 expression in a minority of cases, SMZL has an immunologic profile distinct from that of CLL and HCL that could be useful for the differential diagnosis between these B-cell disorders if the results of four-color staining monoclonal antibodies are interpreted in combination by an expert hematopathologist. In addition, correlation of immunophenotypic findings by flow cytometry with morphologic findings of the PB or BM aspirates and clinical, hematologic features can lead confidently to the correct diagnosis.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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