Splenic Marginal Zone Lymphoma With or Without Villous Lymphocytes

Hematologic Findings and Outcomes in a Series of 57 Patients

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BACKGROUND. Splenic marginal zone lymphoma (SMZL) is a well defined pathologic entity. However, questions regarding the bone marrow infiltration rate, the minimal diagnostic data set, and therapy remain unanswered.

METHODS. Clinical-pathologic features and outcomes of 57 consecutive patients who had splenomegaly with no clinically significant lymphadenomegaly and who were diagnosed with SMZL with or without (±) villous lymphocytes (VL) were reviewed.

RESULTS. SMVL ± VL occurred mostly in elderly males (median age, 62 years ± 10 years; male-to-female ratio, (1.85). Anemia was recorded in 49% of patients, and 30% of patients had moderate thrombocytopenia. Leukocytosis and leukopenia were found in 33% and 14% of patients, respectively, and typical VL were found in 84% of patients. Serology for hepatitis C virus infection was positive in 16% of patients, and a small monoclonal component was detected in 36% of patients. The bone marrow was infiltrated with an intrasinusoidal component in all patients. Thirteen patients were monitored using a watch-and-see policy, and they remained alive 1–5 years after diagnosis. Overall, 21 patients (36%) underwent splenectomy; and, in all patients, the diagnosis of SMZL was confirmed histologically in the surgical specimens. Twenty-five patients received single-agent therapy, which included either alkylators or pentostatine, and they achieved an overall response rate (ORR) of 65% and 87%, respectively: Polychemotherapy was administered to 6 patients (ORR, 83%). The median survival for all patients in the series was not reached, and it is expected that 70% of patients will be alive at 5 years.

CONCLUSIONS. Up to 20% of patients who had SMZL ± VL could be monitored using a watch-and-wait policy. The bone marrow intrasinusoidal infiltration pattern may be a valuable diagnostic hallmark, thus obviating diagnostic splenectomy. The issues regarding prognostic stratification and the best therapeutic strategy need to be addressed in properly designed, prospective trials. ***Cancer* 2004; 101:2050-7.** *© 2004 American Cancer Society.*

KEYWORDS: splenic marginal zone lymphoma, splenic lymphoma with villous lymphocytes, splenectomy, intrasinusoidal, bone marrow biopsy.

Splenic marginal zone lymphoma (SMZL) with or without (±) villous lymphocytes (VL) is a recently recognized, chronic B-cell lymphoproliferative disorder[1] that is listed as a distinct pathologic entity in the World Health Organization (WHO) classification system.[2] SMZL ± VL is infrequent, accounting for only ≈ 8% of all lymphomas, and it occurs primarily in the elderly (median age at onset, 68 years) with a male-to-female ratio of 1.0:1.8.[3]

When a leukemic component is present (≥ 10% of lymphocytes show a villous morphology), the diagnosis is splenic lymphoma with

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villous lymphocytes (SLVL).[4,5] These leukemic lymphoid cells display thin and unevenly distributed, short cytoplasmic projections. The immunophenotype of lymphomatous cells comprises surface immunoglobulin M (sIgM), and sIgD expression; CD19, CD20, CD79a positivity, and a lack of CD5, CD10, CD23, CD43, and nuclear cyclin D1.[2] In some patients, an atypical phenotype can be observed that expresses CD5, CD23, or cyclin D1.[6] In such cases, FMC7 expression can be useful in differentiating SMZL ± VL from chronic lymphocytic leukemia.

It has been reported that trisomy 3 is present in ≈ 20% of tumors,[7] and up to 40% show a deletion or translocation of the long arm of chromosome 7 (7q21–32).[8] This disease involves the spleen, the bone marrow,[9,10] often the blood, but rarely other tissues, without significant lymphadenomegaly or hepatomegaly.[4] SMZL and SLVL are indistinguishable histologically, in both the spleen and the bone marrow, and it is believed that they are different phases of the same process.[11]

The most common clinical findings on diagnosis, in addition to splenomegaly, are related to a mild degree of anemia, often secondary to hypersplenism.[3] Monoclonal gammopathy, mostly < 2 g/dL and of the IgM type, is detected in up to one-third of patients. Signs of hepatitis C virus (HCV) infection and/or of HCV-related chronic hepatic disease are present in ≈ 20% of patients.[12]

Diagnosis historically has been based on the histologic examination of surgically removed spleens or on the morphologic and immunophenotypic features of the leukemic cells. Splenectomy is regarded as the best therapeutic choice for patients who become symptomatic and/or develop cytopenia.[13] However, only a few series with > 50 patients have been published.[4,11,13–17]

Currently, the minimal diagnostic data set, the bone marrow infiltration rate, the factors that affect prognosis, and the best therapeutic approach remain matters of debate. Herein, we present our experience in 57 patients with SMZL ± VL who were diagnosed at 2 Italian centers.

MATERIALS AND METHODS

Patients

Between October, 1988 and July, 2001, 57 patients were diagnosed with SMZL ± VL at the Division of Hematology, University of Palermo, and at the Division of Hematology, University of Verona. The following data were gathered at the time of diagnosis: demographic data, clinical symptoms, blood counts at presentation, clinically detectable splenomegaly, hepatomegaly, lymphadenopathy, the presence of a serum monoclonal component, anti-HCV antibodies, patterns of bone marrow infiltration, treatment, type of response, response duration, and causes of death.

Diagnoses were made based on histologic examination of bone marrow and/or spleen integrated with immunophenotypic and cytologic data. Four diagnostic hallmarks were considered for diagnosis in patients with splenomegaly who were without clinically significant peripheral lymphoadenomegaly: 1) the presence of clonal (light chain-restricted), circulating CD19/CD20-positive (CD19+/CD20+) B lymphocytes that were negative for CD5, CD10, CD23, and CD25; 2) > 10% typical villous lymphocytes in the peripheral blood; 3) intrasinusoidal bone marrow infiltration pattern by B-mature lymphocytes; and 4) a diagnosis of SMZL on surgically removed spleen.

The diagnosis was considered definite when unambiguous spleen histology and/or a leukemic clone that showed typical immunophenotype and villous morphology was present. When these two major diagnostic criteria were lacking, but an intrasinusoidal bone marrow infiltration pattern and/or a peripheral B-cell clone that was negative for CD5, CD10, and CD23 and that did not show villous morphology was present, then the diagnosis was considered probable. Patients with histologically proven nodal marginal zone lymphoma, even if they showed some degree of splenomegaly, were not included.

Pathology

Paraffin sections from bone marrow trephine biopsies were available in all patients and also in spleen specimens from the 21 patients who underwent splenectomy. Histopathologic evaluation was performed using hematoxylin and eosin, periodic acid-Schiff, Giemsa, and Gomori stains. Immunostainings were performed with the streptavidin-biotin peroxidase complex, using the following antibodies: CD5, CD20, CD23, CD10, and CD43. Histologic data were reviewed by two of the authors (V.F. and F.M.) and were classified according to WHO criteria.

Immunophenotyping

Immunophenotyping and morphologic evaluations were performed at the referral centers on peripheral blood (PB) samples that were taken at the time of diagnosis and before any treatment started. Briefly, PB samples were collected in tubes containing K3 ethylenediamine tetraacetic acid as an anticoagulant. All samples were stained using a direct immunofluorescence stain-and-then-lyse technique with the following battery of phycoerythrin (PE) or fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies: CD19, CD20, CD22, CD23, CD5, CD10, CD103, CD11c,

TABLE 1
Immunophenotypical Analysis and Differentiation

Immunophenotype	IgM	IgD	CD5	CD19	CD20	CD23	CD43	CD79a	Cyclin D1
SMZL/SLVL	+	+	−	+	+	−	−	+	−
HCL	+	±	−	+	+	−	+	+	−
CLL/SLL	±	±	+	+	±	+	+	+	±
PLL	+	±	±	+	+	−	+	+	−
MCL	+	±	+	+	+	±	+	+	+

IgM: immunoglobulin M; SMZL/SLVL: splenic marginal zone lymphoma/splenic lymphoma with villous lymphocytes; +: positive; −: negative; ±: weakly positive; HCL: hairy cell leukemia; CLL/SLL: chronic lymphocytic leukemia/small lymphocytic lymphoma; PLL: prolymphocytic leukemia; MCL: mantle cell lymphoma.

CD25, sIgκ, and sIgλ (Becton Dickinson). Acquisition and analysis were performed using CellQuest software on a FACScan flow cytometer (Becton Dickinson).

B lymphocytes were identified according to their side scatter low intensity (SSClo/int)/CD19+ distribution, and their percentage was calculated after excluding cell debris and platelets according to conventional procedures. The common, albeit nonspecific, SMZL immunophenotypic pattern, according to the WHO criteria, is compared with that of other small B-cell lymphomas in Table 1.

Treatment

Treatment was administered according to local policy and was at the discretion of the physician in charge.

Response Criteria

Regrettably, clinical characteristics, the frequent use of splenectomy as a diagnostic tool, and the unsatisfactory response to the systemic therapy made it difficult to use classic response criteria. We used the following criteria to score and record clinical responses. A complete remission (CR) was defined as the disappearance of all signs and symptoms of the disease. A good hematologic response (GHR) was defined as a reduction of the bone marrow lymphomatous infiltration $> 50\%$ in the absence of any kind of cytopenia and splenomegaly. A minor hematologic response (MHR) was defined when at least 1 of the following criteria was fulfilled: 1) recovery from anemia or an increase > 2 g/dL in the hemoglobin level; 2) recovery from thrombocytopenia or an increase $> 50\%$ in the platelet count; 3) a reduction $> 50\%$ in lymphocytosis; 4) recovery from splenomegaly or a decrease $> 50\%$ in spleen size; and 5) a reduction $> 50\%$ in the bone marrow lymphoid infiltrate.

Statistics

All data were analyzed with the SPSS software package (Scientific Software for Professional Statistics, Chicago, IL). Overall survival was calculated as the time elapsing from diagnosis either to death or to last follow-up. Event-free survival was calculated as the time from the onset of therapy to the date of treatment after the institution of the initial watch-and-see policy, the development of recurrent or progressive disease, death from any causes, or loss of follow-up. Follow-up evaluation of patients who did not experience any of the above indicated events was recorded at the date of last contact. Survival curves were estimated by using the Kaplan–Meier method.

TABLE 2
Hematologic and Physical Features on Diagnosis in 57 Patients with Splenic Marginal Zone Lymphoma

Characteristic	No. of patients (%)
Male:female ratio	37:20
Age (yrs)	
Median	62 ± 10
Range	35–83
Hemoglobin < 11 g/dL	28 (49)
Leukocytes < 4000/μL	10 (17)
Platelets < 100.000/μL	17 (30)
Leukocytes > 30.000/μL	20 (33)
Lymphocytes > 5000/μL	25 (55)
Villous lymphocytes > 10%	48 (80)
HCV serology positive[a]	7 (17)
Monoclonal component[b]	18 (36)
Splenomegaly	52 (91)
Splenomegaly > 7 cm	40 (70)
Hepatomegaly	9 (16)
Abdominal lymph nodes	20 (35)
Superficial lymph nodes	9 (16)

HCV: hepatitis C virus.
[a]Serology was unavailable for 15 patients.
[b]Immunofixation was not performed for eight patients.

RESULTS

Presenting Features

Clinical and hematologic findings at the time of diagnosis are shown in Table 2. In this series, the disease

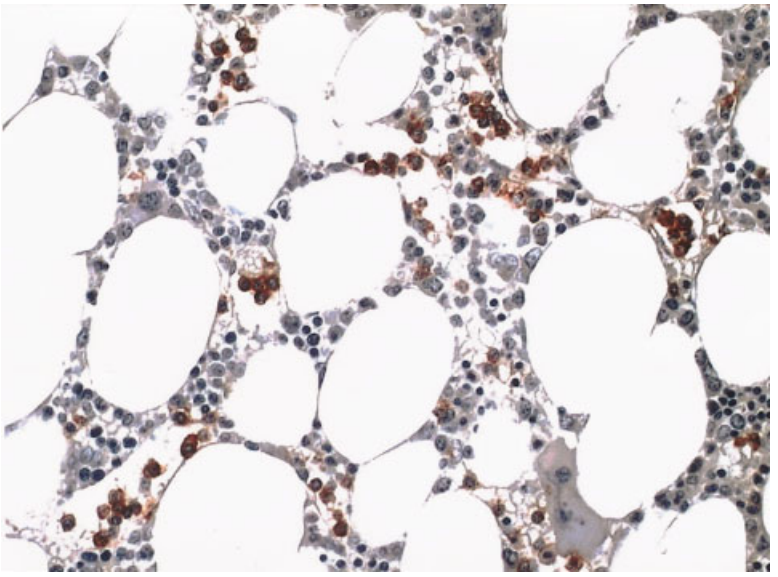

FIGURE 1. Bone marrow intrasinusoidal infiltration: Anti-CD20 immunostaining highlights the lymphomatous cells, which are lodged inside the bone marrow sinusoids (avidin-biotin complex method; CD20 immunostain; original magnification, × 250).

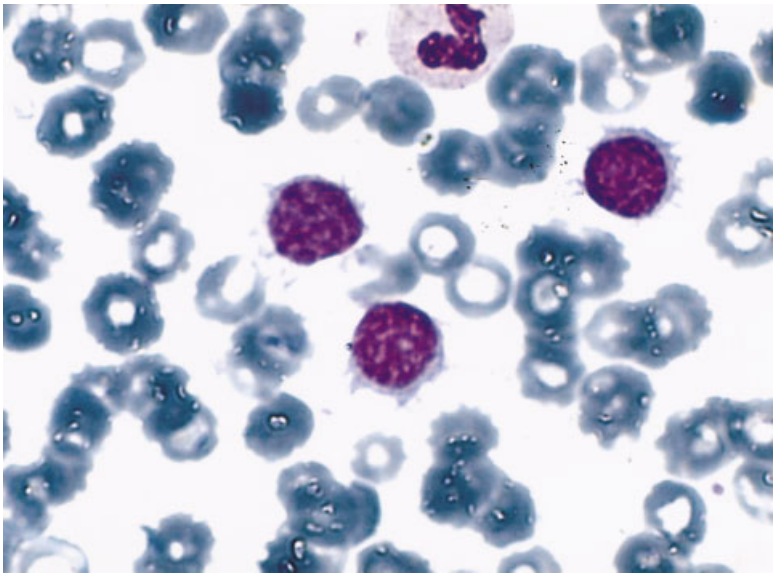

FIGURE 2. Circulating villous lymphocytes: Neoplastic lymphocytes are characterized by the presence of short polar projections (Giemsa stain; original magnification, × 1000).

occurred mostly in elderly males (median age, 62 years ± 10 years; male-to-female ratio, 1.85). All but 6 patients showed splenomegaly that was appreciated > 7 cm below the left costal margin in 40 patients (70%). Hepatomegaly was recorded in 14 patients (24%). Nine patients (16%) also had small (about 1 cm) superficial or abdominal lymph nodes.

Laboratory Characteristics

A slight-to-moderate anemia (hemoglobin < 11 g/dL) was recorded in 49% of patients. Seventeen patients (30%) showed moderate thrombocytopenia (≤ 100,000/μL), which was severe in only 1 patient (≤ 20,000/μL). Leukocytosis (leukocyte count ≥ 10,000/μL) and leukopenia (leukocyte count ≤ 4000/μL) were found in 33% and 17% of patients, respectively. Only 1 patient had < 500 neutrophils/mm^3. Morphologically typical villous lymphocytes were found in 84% of patients, irrespective of the leukocyte count (Fig. 1). A monoclonal component was detected in 36% of patients, and two-thirds were of the IgM type. Seventeen percent of the 42 patients screened were anti-HCV positive. A bone marrow trephine biopsy was performed in all patients. The bone marrow showed a slight-to-moderate lymphoid infiltration in all patients. An intrasinusoidal component was detected consistently, and the infiltrate was exclusively intrasinusoidal in 34% of patients (Fig. 2). In the remaining patients, the intrasinusoidal pattern

TABLE 3
Bone Marrow Infiltration Data

Pattern	No. of patients (%)
Intrasinusoidal	57 (100)
Intrasinusoidal and nodular	14 (25)
Intrasinusoidal, interstitial, and nodular	8 (14)
Intrasinusoidal and interstitial	3 (5)
Infiltration < 30%	22 (60)

was observed along with a prevalent nodular or interstitial component. Data regarding the bone marrow infiltration patterns are reported in Table 3.

Response to Therapy

Thirteen patients were monitored on a watch-and-see policy until the disease progressed and remain alive 1–5 years after diagnosis. The remaining patients received ≥ 1 lines of treatment (range, 1–4 treatments), including alkylating, purine analogues, splenic irradiation, or splenectomy. Overall, 21 patients (36%) underwent splenectomy; and, in all patients, the diagnosis of SMZL was confirmed histologically in the surgical specimens. Nine of 12 patients who underwent splenectomy at the time of diagnosis, all of whom showed a bone marrow intrasinusoidal infiltration pattern, were classified with SMZL, because they had no obvious leukemic component.

After splenectomy, nearly all the patients obtained a MHR or GHR. A clinical response (CR) lasting from 6 months to 7 years (median, 4 years) was observed irrespective of whether splenectomy was the first line of treatment or a salvage procedure. Eight out of the 13 patients who underwent bone marrow follow-up after splenectomy showed worsening of both the degree and the pattern of bone marrow infiltration.

Splenic irradiation was employed in two patients, who both improved clinically. The overall response rate (ORR) was 55%. Data on response to first-line, systemic therapy are reported on Table 4.

Six out of 8 patients (75%) who were treated with pentostatine obtained a clinical response (5 MHRs and 1 CR). In addition, with the inclusion of 6 patients who received pentostatine as salvage therapy, the ORR rate was 78% (8 MHRs and 3 CRs).

Overall, 17 patients received first-line therapy with alkylators, and 6 patients received combination chemotherapy. Only one patient obtained a CR, and the ORR for monochemotherapy and polychemotherapy was of 65% and 83%, respectively.

Outcomes

The median survival for the entire series was not reached, and 70% of patients are expected to be alive at 5 years (Fig. 3). Fourteen patients (24%) died, including 12 deaths from lymphoma progression and 2 deaths from a secondary malignancy. Seven of 10 lymphoma-related deaths occurred within 36 months of diagnosis.

TABLE 4
Response to Systemic Therapy

Therapy	No. of patients	Response			ORR (%)
		CR	GHR	MHR	
Watch and see	13	—	—	—	—
Splenectomy	12	—	8	3	91
Splenic irradiation	2	—	—	2	100
Monochemotherapy					
Chlorambucil	17	—	2	9	65
Pentostatine	8	1	—	6	87
Polychemotherapy[a]	6	1	2	2	83

CR: complete response; GHR: good hematologic response; MHR: minor hematologic response; ORR: overall response rate.
[a] Only one patient received combined chemotherapy without anthracyclines.

DISCUSSION

The results of our study, which was performed on 57 patients, confirm that SMZL ± VL is a disease of the elderly with a slight male prevalence. Patients mostly are asymptomatic, and the most frequent symptoms are related to mild anemia. We found that bone marrow also is involved constantly in patients who do not show villous lymphocytes in PB. The lymphoid infiltrate is often very fine and is confined to the bone marrow sinusoids in more than one-third of patients.

It is known that the intrasinusoidal infiltration pattern occurs in rare lymphomas of both T-cell lineage (large granular cell leukemia and hepatosplenic T-cell lymphoma) and B-cell lineage (intravascular large B-cell lymphoma).[10] However, in cases in which the infiltrate is made up of small B lymphocytes, it is characteristic of SMZL. In all 21 patients who were diagnosed with probable SMZL in the current study, the diagnosis was confirmed after examination of splenectomy specimens. Our data suggest that the bone marrow intrasinusoidal infiltration pattern may be a valuable diagnostic hallmark, which, coupled with careful morphologic and immunophenotypic examination of PB, can lead confidently to the correct diagnosis.

Another interesting finding in our series, which also was reported recently in the literature,[12] was the high HCV prevalence rate, and impressive results have been described with interferon therapy in HCV-positive patients who have SLVL.[18] Hermine et al. reported that antiviral therapy can result in a CR of HCV infec-

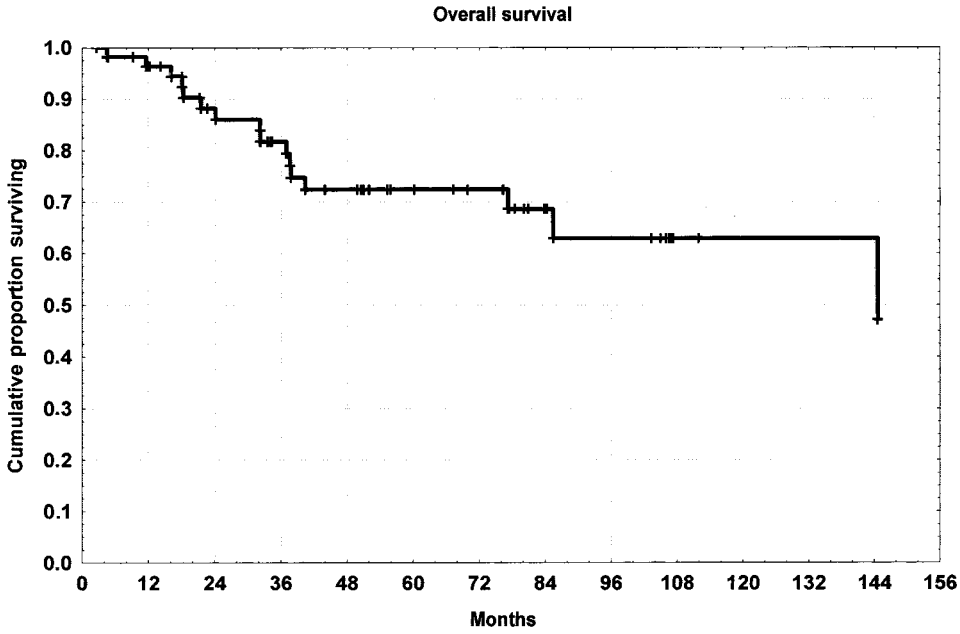

FIGURE 3. Overall survival for 57 patients who had splenic marginal zone lymphoma/splenic lymphoma with villous lymphocytes.

tion and SLVL in patients with coexisting cryoglobulinemia. Of the nine treated patients, seven patients achieved a CR after the loss of detectable HCV RNA. The other two patients obtained a CR and a partial remission after the addition of ribavirine and the loss of detectable HCV RNA. Thus, these results, if confirmed, stress the importance of prospective trials examining the response of HCV-positive patients with SMZL to the association of ribavirine and pegylated interferon, which allegedly have greater antiviral efficacy compared with interferon alone.[19] However, the question whether there is a correlation between HCV infection and lymphoma development should be addressed by a properly designed trial.[20]

Regarding treatment, it has been suggested that splenectomy should be the first line of therapy.[4,13–15] However, it must be noted that this assertion comes from retrospective analyses and is not supported by prospective, randomized, specifically designed trials. The presenting features, therapy, and outcomes of patients from the major retrospective series on SMZL are compared in Table 5. It should be stressed that at least 50% of the reported deaths were related to lymphoma progression or to a histology shift toward a high-grade, non-Hodgkin lymphoma.[14–17,21]

Purine analogues have been tested in a few small series of SMZL/SLVL. Fludarabine reportedly has produced a clinical response in 4 patients with recurrent disease, including 2 durable responses, and in 5 of 10 patients in another series. Some degree of activity also has been shown for cladribine: $\approx$ 50% of pretreated patients responded to the treatment, and 2 patients obtained a molecular complete response.[3]

Our experience with pentostatin, as reported in the current series, also was promising, with a 79% ORR and a long-lasting CR achieved in 14 patients. Rituximab has been employed both alone and in combination with chemotherapy consisting of fludarabine, cyclophosphamide, and vincristine.[22–24] It is worth noting that there were no episodes of disease recurrence in the complete responders after a median follow-up of 10 months.[22]

The current experience confirms that up to 20% of patients have only splenomegaly and/or minor modifications of the PB count and can be monitored safely with a watch-and-see policy. Splenectomy easily leads to the recovery of cytopenias both if it is undergone at the time of presentation and if it is undergone later in the course of the disease, and it allows for an unequivocal diagnosis. In addition, pentostatine may induce objective responses in approximately two-thirds of patients.

In conclusion SMZL $\pm$ VL is an indolent lymphoproliferative disease that allows a long survival with up to two-thirds of patients alive 5 years after diagnosis. In our experience, a wise combination of morphologic and immunophenotypic evaluation of the PB, along with bone marrow examination, can lead to the correct diagnosis. Although it is effective in ameliorating blood cell counts and abdominal discomfort, splenectomy may induce worsening of bone marrow infiltration.[25] Therefore, a conservative approach seems pref-

TABLE 5
Series of Patients with Splenic Marginal Zone Lymphoma With or Without Circulating Villous Lymphocytes

Characteristics	Mulligan et al., 1991[17]	Trussard et al., 1996[14]	Chacon et al., 2002[15]	Parry-Jones et al., 2004[13]	Thieblemont et al., 2002[16]	Arcaini et al., 2004[12]
No. of patients	50	100	60	129	81	34
Median age (yrs)	68	70	63	69	66	59
Male:female ratio	1.77	7.0	1.7	0.9	0.95	0.9
Splenomegaly (%)	—	76	73	77	—	97
Hepatomegaly (%)	—	0	—	12	—	—
Lymphadenopathy (%)	—	0	17	16	—	21
Hemoglobin < 11 g/dL (%)	—	16[a]	28	35	54	29
Leukocyte count > 30.000/μL (%)	—	—	—	18	17	—
Lymphocytes > 4000/μL (%)	—	76	58	75	41	15
Platelets < 100.000/μL (%)	—	15	22	17	22	29
Monoclonal component (%)	—	28[b]	13	22	42	26
Bone marrow involvement (%)	—	—	83	100[c]	95	88
Watch-and-see (%)	28	32	—	27	24	20
Splenectomy (%)	36	28	48	22	31	18
Splenectomy and chemotherapy (%)	—	6	48	17	28	32
Splenic irradiation (%)	14	7	3	7	—	—
Chemotherapy (%)	44	19	—	28	16	24
Overall survival (%)						
3 yrs	82.0	—	—	—	—	—
5 yrs	—	78.0	65.0	72.0	—	—
Median overall survival (yrs)	—	—	—	—	10.5	—[d]

[a] Hemoglobin < 10 g/dL.
[b] Data concerning 80 patients.
[c] Data concerning 93 patients.
[d] The median overall survival was not reached at 3 years.

erable, and we suggest postponing splenectomy until the disease progresses. However, the questions regarding the prognostic stratification and the best therapeutic strategy remain unanswered and need to be addressed in properly designed, prospective trials.

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